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Neue Aspekte der Kreislaufregulation bei unterschiedlichen Anaesthesieverfahren

Habilitationsschrift

Zur Erlangung der Venia Legendi
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Veröffentlichungen

Die vorliegende Schrift fasst die Ergebnisse aus sieben thematisch verbundenen Arbeiten (Originalarbeiten siehe Anhang) zusammen. Sie führt zunächst in die Thematik ein, im zweiten und dritten Abschnitt werden die Methoden und wichtigsten Ergebnisse dargestellt. Methodische Details und alle erhobenen Daten lassen sich den beigefügten Originalarbeiten entnehmen. Abschließend werden die wichtigsten Ergebnisse im Kontext der aktuellen Literatur diskutiert.

Publikation 1 **Picker, O.,** A. Schindler, T.W.L. Scheeren (2000): "Accuracy and reproducibility of long-term implanted transit-time ultrasound flowprobes in dogs." Intensive Care Medicine **26**: 601-607.

Publikation 2 **Picker, O.,** A.W. Schindler, T.W.L. Scheeren (2001): "Endogenous endothelin and vasopressin support blood pressure during epidural anesthesia in conscious dogs." Anesthesia & Analgesia **93**: 1580-1586.

Publikation 3 **Picker, O.,** L.A. Schwarte, P. Roth, J. Greve, T.W.L. Scheeren (2004): "Comparison of the role of endothelin, vasopressin and angiotensin in arterial pressure regulation during sevoflurane anaesthesia in dogs." British Journal of Anaesthesia **92**: 102-108.

Publikation 4 **Picker, O.,** T.W.L. Scheeren, J.O. Arndt (2001): "Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity." British Journal of Anaesthesia **87**: 748-754.

Publikation 5 **Picker, O.,** L.A. Schwarte, A.W. Schindler, T.W.L. Scheeren (2003): "Desflurane increases heart rate independent of sympathetic activity in dogs." European Journal of Anaesthesiology **20**: 945-951.

Publikation 6 **Picker, O.,** A.W. Schindler, L.A. Schwarte, B. Preckel, W. Schlack, T.W.L. Scheeren, V. Thämer (2002): "Xenon increases total body oxygen consumption during isoflurane anaesthesia in dogs." British Journal of Anaesthesia **88**: 546-554.

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Inhaltsverzeichnis

1. Einleitung

In Deutschland werden pro Jahr ca. acht Millionen Anaesthesien durchgeführt. Der überwiegende Teil dieser Anaesthesien wird dabei als Inhalationsanaesthesie oder als rückenmarksnahe Leitungsanaesthesie, d.h. entweder als Spinal- oder auch als Periduralanaesthesie durchgeführt. Den erwünschten Wirkungen einer Ausschaltung des Bewusstseins und/oder der Schmerzempfindung stehen einige unerwünschte Effekte gegenüber, die vor allem im Zusammenhang mit der Regulation von Herzfrequenz und Blutdruck stehen. Die Kenntnis dieser unter Anaesthesiebedingungen veränderten Kreislaufregulation ist für die Interpretation einer Kreislaufdepression und damit auch für eine adäquate Prophylaxe und Therapie von besonderer Bedeutung.

In den dieser Schrift zugrunde liegenden Arbeiten geht es um neue Aspekte der Kreislaufregulation sowohl während rückenmarksnaher Leitungsanaesthesien als auch bei Inhalationsanaesthesien. Besonderes Augenmerk wurde dabei gelegt auf die Rolle der vasokonstriktorisch wirkenden Peptide Endothelin und Vasopressin bei der Blutdruckstabilisierung während beider Anaesthesieformen sowie auf die Bedeutung des parasympathischen Nervensystems für die Herzfrequenzregulation während einer Inhalationsanaesthesie.

1.1. Grundlagen der Messungen

Voraussetzung für die Beurteilung der Kreislauf-Regulationssysteme während verschiedener Anaesthesieformen ist zunächst die Kenntnis ihrer physiologischen Funktion unter Grundumsatzbedingungen im Wachzustand. Daher war es für die eigenen Experimente

notwendig, wache, trainierte, chronisch instrumentierte Hunde wiederholt zu untersuchen (wach/Anaesthesie), so dass jedes Tier als seine eigene Kontrolle diente. Dabei stellt die Messung des Herzzeitvolumens für derartige Untersuchungen insofern eine Besonderheit dar, als mit den in der Klinik üblichen Indikatorverteilungsverfahren (z.B. Thermodilutionsmethode) weder eine kontinuierliche noch eine direkte Messung des Blutflusses möglich ist, die darüber hinaus noch eine Ungenauigkeit von etwa ± 20 % aufweisen (28,56,62).

Für Untersuchungen an chronisch instrumentierten Tieren gilt die kontinuierliche Messung des Herzzeitvolumens mittels Ultraschall-Transitzeit-Verfahren als die Methode mit der größtmöglichen Präzision (16). Allerdings liegen bislang keine Untersuchungen vor über die Genauigkeit des Transitzeit-Verfahrens bei längerer Anwendung, d.h. bei Implantationsdauern von mehr als drei Monaten. Da aber im Rahmen der eigenen Experimente wesentlich längere Untersuchungszeiträume erforderlich waren und die Messung des Herzzeitvolumens eine Kernvariable darstellt, war zunächst die Beantwortung der Frage notwendig, ob das Ultraschall-Transitzeit-Verfahren für die kontinuierliche Flussmessung sowohl am wachen Tier als auch während Anaesthesie unter dem Gesichtspunkt der Langzeitanwendung geeignet ist.

1.2. Kreislaufregulation bei unterschiedlichen Anaesthesieverfahren

Während rückenmarksnaher Leitungsanaesthesien als auch während Inhalationsanaesthesie wird die Kreislaufregulation beeinträchtigt. Unter physiologischen Bedingungen (im Wachzustand) stabilisieren hauptsächlich das sympathische Nervensystem und das Renin-Angiotensin-System den Blutdruck, dagegen wird während einer rückenmarksnahen Leitungsanaesthesie das sympathische Nervensystem zumindest teilweise ausgeschaltet und

damit auch das Renin-Angiotensin-System nicht mehr aktiviert, um weitere Blutdruckabfälle zu verhindern (19,35,36). Somit sind während einer rückenmarksnahen Leitungsanaesthesie zwei wesentliche blutdruckunterstützende neurohumorale Systeme ausgeschaltet. Zur Kompensation eines Blutdruckabfalles bzw. zur Aufrechterhaltung eines ausreichenden Blutdruckniveaus wird während einer hohen rückenmarksnahen Leitungsanaesthesie das endogene Peptid Vasopressin freigesetzt (7), vermutlich vermittelt über Druckrezeptoren im Carotissinus und Aortenbogen (50,51,52,53). Allerdings bleibt auch nach Blockade der Vasopressinrezeptoren ein für die Organperfusion ausreichendes Blutdruckniveau erhalten, so dass eine Beteiligung weiterer vasokonstriktorisch wirkender Substanzen an der Blutdruckhomöostase während einer rückenmarksnahen Leitungsanaesthesie zu vermuten ist. Eine wesentliche Rolle könnte in diesem Zusammenhang dem Peptid Endothelin zukommen, welches 1988 als ein hoch wirksamer, vom Endothel freigesetzter Vasokonstriktor entdeckt wurde (64). Da Endothelin bereits unter physiologischen Ruhebedingungen zur Aufrechterhaltung des Blutdruckes beiträgt (17,29,61) und erhöhte Endothelin-Plasmaspiegel gefunden wurden nach Blutdruckabfällen im Rahmen von Blutverlusten oder beim septischen Schock (8,32,41), erschien die Hypothese gerechtfertigt, dass Endothelin auch zur Blutdruckstabilisierung während einer rückenmarksnahen Leitungsanaesthesie beiträgt.

Ungeklärt ist die Bedeutung des Endothelins auch für die Blutdruckregulation während einer Inhalationsanaesthesie. Dabei könnten sich in Abhängigkeit von der Konzentration des Inhalationsanaesthetikums sowohl Gemeinsamkeiten als auch Unterschiede zur Periduralanaesthesie ergeben. Die Inhalationsanaesthesie führt dosisabhängig ebenfalls zu einem Blutdruckabfall (40) sowie zu einer Sympathikolyse, die allerdings erst bei hohen Anaesthetikakonzentrationen nachweisbar ist (33,42). Darüber hinaus reduzieren Inhalationsanaesthetika dosisabhängig die Empfindlichkeit des arteriellen Baroreflexes, die

für die Vasopressinfreisetzung von Bedeutung ist (10,38,63). Demnach könnte in Abhängigkeit von der Anaesthetikakonzentration neben Vasopressin auch Endothelin als Reservesystem zur Blutdruckstabilisierung während Inhalationsanaesthesie beitragen. Somit ergibt sich insgesamt die Frage nach der Bedeutung des Endothelins bei der Blutdruckregulation sowohl während Regional-, als auch während Inhalationsanaesthesien.

Abgesehen von den Effekten auf die Blutdruckregulation erhöhen volatile Anaesthetika konzentrationsabhängig die Herzfrequenz (2,25,47). Dabei steigt die Herzfrequenz unter allen Substanzen mit zunehmender Anaesthesietiefe an, wobei unklar geblieben ist, warum dieser Effekt zwischen den verschiedenen Substanzen unterschiedlich stark ausgeprägt ist (12,13,34,47). Dies ist umso erstaunlicher, als die Inhalationsanaesthetika das Herzzeitvolumen und den Blutdruck in einem vergleichbaren Ausmaß reduzieren (40). Von den meisten Autoren wird der Herzfrequenzanstieg während einer Inhalationsanaesthesie durch eine Aktivierung des sympathischen Nervensystems erklärt, wobei allerdings für lediglich zwei der fünf gebräuchlichen volatilen Anaesthetika (Desfluran und Isofluran) eine Aktivierung sympathischer Fasern nachgewiesen werden konnte (11,12,13). Eine solche *Aktivierung* des sympathischen Nervensystems ist jedoch nur bei niedrigen Konzentrationen der Anaesthetika nachweisbar; demgegenüber führen hohe Konzentrationen (> 1,5 MAC; minimale alveoläre Konzentration) sowohl von Desfluran als auch von Isofluran wie auch der übrigen Inhalationsanaesthetika zu einer vollständigen *Hemmung* der Sympathikusaktivität (33,42). Gerade unter diesen hohen Konzentrationen ist aber der Herzfrequenzanstieg bei allen Inhalationsanaesthetika am stärksten ausgeprägt, so dass eine Beteiligung des sympathischen Nervensystems an dem Herzfrequenzanstieg während einer tiefen Inhalationsanaesthesie unwahrscheinlich ist. Im Gegensatz zu den Effekten der Inhalationsanaesthetika auf das sympathische Nervensystem ist bislang wenig bekannt über

deren Wirkung auf das parasympathische Nervensystem, insbesondere die kardiale Vagusaktivität, die sich durch Analyse der Herzfrequenzvariabilität erfassen lässt (49). Es wurde deshalb die These geprüft, dass die frequenzsteigernde Wirkung volatiler Anaesthetika auf einer Vagushemmung beruht.

In diesem Zusammenhang von Interesse sind nicht nur die halogenierten Inhalationsanaesthetika, sondern auch das Edelgas Xenon, welches für Anaesthesiezwecke besonders geeignet zu sein scheint (27). Bislang sind allerdings die Effekte von Xenon auf die Kreislaufregulation unbekannt. Xenon könnte bezüglich der Kreislaufregulation insofern eine Sonderstellung einnehmen, als auch unter hohen Konzentrationen dieses Inhalationsanaesthetikums, im Gegensatz zu allen anderen volatilen Anaesthetika, eine bemerkenswerte Kreislaufstabilität beobachtet wurde (18,26,45).

Somit stellte sich die Frage, welche Rolle das autonome Nervensystem bei der Herzfrequenzregulation während einer Inhalationsanaesthesie mit konventionellen volatilen Anaesthetika sowie mit dem Edelgas Xenon spielt.

Neben den Inhalationsanaesthetika könnte auch das von den Nervenzellen produzierte Stickstoffmonoxid (n-NO) modulierend auf das autonome Nervensystem wirken. Eine Beteiligung von n-NO an der Herzfrequenzregulation ist zu vermuten, da NO-Synthasen in Kreislaufzentren gefunden wurden (60) und außerdem an Mäusen ohne endotheliales NO (e-NOS knockout-Mäuse) eine zusätzliche Hemmung der NO-Synthase (n-NOS) wie bei intakten Tieren eine Bradykardie auslöste, obwohl der Blutdruck konstant blieb (22). Daher wurde untersucht, welchen Einfluss neuronales NO auf das autonome Nervensystem und damit die Herzfrequenzregulation eines Warmblüters unter physiologischen Bedingungen, d.h. im Wachzustand, hat.

1.3. Fragestellungen

Die vorliegende Habilitationsschrift fasst eigene tierexperimentelle Untersuchungen zu neuen Aspekten der Kreislaufregulation während verschiedener Anaesthesieverfahren zusammen. Besonderes Augenmerk wurde dabei gelegt auf die Bedeutung der vasokonstrikorischen Peptide Endothelin und Vasopressin bei der Blutdruckstabilisierung sowie auf die Rolle des parasympathischen Nervensystems für die Herzfrequenzregulation während einer Inhalationsanaesthesie.

1.3.1. Grundlagen der Messmethoden: Ist die Messung des Herzzeitvolumens mittels Ultraschall-Transitzeit-Verfahren hinreichend genau und für die Langzeitanwendung geeignet?

Publikation 1 **Picker, O.**, A. Schindler, T.W.L. Scheeren (2000): "Accuracy and reproducibility of long-term implanted transit-time ultrasound flowprobes in dogs." Intensive Care Medicine **26**: 601-607.

1.3.2. Bedeutung blutdruckstabilisierender Systeme bei unterschiedlichen Anaesthesieverfahren

Publikation 2 **Picker, O.,** A.W. Schindler, T.W.L. Scheeren (2001): "Endogenous endothelin and vasopressin support blood pressure during epidural anesthesia in conscious dogs." Anesthesia & Analgesia **93**: 1580-1586.

Publikation 3 **Picker, O.,** L.A. Schwarte, P. Roth, J. Greve, T.W.L. Scheeren (2004): "Comparison of the role of endothelin, vasopressin and angiotensin in arterial pressure regulation during sevoflurane anaesthesia in dogs." British Journal of Anaesthesia **92**: 102-108.

1.3.3. Autonomes Nervensystem und Herzfrequenzregulation unter den Bedingungen einer Inhalationsanaesthesie

Publikation 4 **Picker, O.,** T.W.L. Scheeren, J.O. Arndt (2001): "Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity." British Journal of Anaesthesia **87**: 748-754.

Publikation 5 **Picker, O.,** L.A. Schwarte, A.W. Schindler, T.W.L. Scheeren (2003): "Desflurane increases heart rate independent of sympathetic activity in dogs." European Journal of Anaesthesiology **20**: 945-951.

Publikation 6 **Picker, O.,** A.W. Schindler, L.A. Schwarte, B. Preckel, W. Schlack, T.W.L. Scheeren, V. Thämer (2002): "Xenon increases total body oxygen consumption during isoflurane anaesthesia in dogs." British Journal of Anaesthesia **88**: 546-554.

1.3.4. Beziehung zwischen Vagusaktivität, Stickstoffmonoxid und Herzfrequenzregulation unter physiologischen Bedingungen

Publikation 7 **Picker, O.,** T.W.L. Scheeren, J.O. Arndt (2001): "Nitric oxide synthases in vagal neurons are crucial for the regulation of heart rate in awake dogs." Basic Research in Cardiology **96**: 395-404.

2. Material und Methoden

Voraussetzung für die Beurteilung der Kreislaufverhältnisse während verschiedener Anaesthesieformen ist die Kenntnis ihrer physiologischen Funktion unter Grundumsatzbedingungen. Daher wurden alle Versuche an trainierten, chronisch instrumentierten Hunden sowohl im Wachzustand (Grundumsatz) als auch während Anaesthesie durchgeführt. Da sowohl die Instrumentierung vor Beginn der eigentlichen Versuche als auch die verwandten Messmethoden und Interventionen in allen Versuchen ähnlich waren, wird die Methodik zusammenfassend dargestellt. Das jeweilige Versuchsprogramm und die Analyse der Daten ist den beiliegenden Publikationen zu entnehmen.

2.1. Versuchstiere

Die Versuche wurden durchgeführt an wachen, gesunden, trainierten Hunden (Foxhounds beiderlei Geschlechts, Körpergewicht 26 – 35 kg). Die Experimente erstreckten sich insgesamt über einen Zeitraum von fünf Jahren und waren von der Bezirksregierung Düsseldorf genehmigt. Die Versuchstiere befanden sich unter Aufsicht des Tierschutzbeauftragten der Heinrich-Heine-Universität und waren über den gesamten Untersuchungszeitraum gesund.

2.2. Vorbereitende Operationen

In zwei vorbereitenden Operationen (Vorverlagerung von Carotisarterien sowie Implantation eines Flussaufnehmers um die Pulmonalarterie) wurden die Voraussetzungen geschaffen für

die eigentlichen Experimente an wachen Tieren. Diese Eingriffe wurden unter aseptischen Bedingungen in Allgemeinanaesthesie (Enfluran/Lachgas und Fentanyl) vorgenommen.

Bei der ersten Operation wurden beide Carotisarterien aus ihrer natürlichen Umgebung isoliert und in Hautlappen eingenäht (Anlage so genannter Carotisschlingen (59)). Dadurch waren Kanülierungen der Arterie zum Zwecke der Blutdruckmessung und Blutprobenentnahme technisch einfach und schmerzfrei durchführbar.

In einem zweiten Eingriff (etwa drei Wochen nach Vorverlagerung der Carotisarterien) wurde durch eine Thorakotomie im 4. Interkostalraum links ein Ultraschall-Transitzeit-Flussaufnehmer um die Pulmonalarterie implantiert. Das zur Übermittlung des Flussaufnehmersignals notwendige Verbindungskabel wurde zwischen den Schulterblättern nach außen geführt und durch eine Thoraxweste geschützt.

Parallel zu den vorbereitenden Operationen wurden die Hunde abgerichtet, so dass sie sich an die Experimentatoren und Räumlichkeiten gewöhnten und während der mehrere Stunden dauernden Experimente ruhig und ohne Zwang auf dem Untersuchungstisch ausharrten. Bis zum Beginn der eigentlichen Experimente waren die Operationswunden, in der Regel innerhalb von zwei Wochen, vollständig ausgeheilt.

2.3. Messmethoden und Interventionen

Vor Beginn der eigentlichen Experimente wurde ein Oberflächen-EKG angelegt sowie intravasale Katheter gelegt zur Messsung des Blutdruckes in der Aorta und im rechten Vorhof. Der arterielle Blutdruck wurde kontinuierlich in der Aorta ascendens über einen flüssigkeitsgefüllten Katheter gemessen. Dazu wurde an jedem Versuchstag eine der beiden vorgelagerten Aa. carotidea kanüliert (Vygon Leader Cath, Durchmesser 1,2 mm, 18 G, Länge 100 mm). Über eine Hinterlaufvene wurde ein weiterer Katheter unter

Röntgenkontrolle vorgeschoben zur Messung des Druckes im rechten Vorhof sowie zur zentralvenösen Applikation der Testsubstanzen. Alle Drücke wurden mittels Elektromanometrie gemessen (Druckwandler P23 ID, Gould Statham, USA). Die Elektromanometer wurden auf die Höhe des Dornfortsatzes des 7. Halswirbels adjustiert und vor Beginn der Versuche mit einer Quecksilbersäule geeicht. Alle koninuierlich gemessenen Variablen wurden während der Versuche fortlaufend nach analog-digital-Wandlung (MACLab, Adinstruments, Castle Hill, Australien) des Originalsignals auf der Festplatte eines Computers zur späteren Analyse aufgezeichnet.

2.3.1. Herzzeitvolumen

Das Herzzeitvolumen (HZV) wurde mit Hilfe des Ultraschall-Transitzeit-Verfahrens (Transonic, USA) als Blutfluss in der Pulmonalarterie gemessen. Hierzu wurden in vorbereitenden Operationen Flusswandler (20 – 24 mm, Transonic) chronisch um den Pulmonalarterienstamm implantiert. Die Flusswandler wurden geeicht sowohl vor Implantation (in vitro, Durchströmung mit definierten Flüssen einer entgasten Kochsalzlösung über eine Rollerpumpe) als auch wiederholt während der Experimente (in vivo) über das Fick'sche Prinzip als Quotient von Sauerstoffverbrauch und arterio-gemischtvenöser Sauerstoffgehaltsdifferenz ($AVDO_2$). Der Sauerstoffverbrauch wurde gemessen als Sauerstoffaufnahme ($\dot{V}O_2$, STPD)* mit Hilfe der indirekten Kalorimetrie unter Verwendung eines DeltatracII® Metabolic Monitor (Datex-Engström, Helsinki, Finnland). Die $AVDO_2$ wurde bestimmt aus der Differenz des Sauerstoffgehalts ($LexO_2Con$, Lexington Instruments, Waltham, USA) zeitgleich abgenommenen Blutes in der Aorta und der Pulmonalarterie.

* STPD: „Standard Temperature, Pressure, Dry", d.h. bezogen auf physikalische Standardbedingungen (Temperatur von 273 K, Barometerdruck von 760 mmHg und Trockenheit).

Während der Experimente wurde das Flusssignal kontinuierlich aufgezeichnet unter Verwendung eines integrierten 100 Hertz Tiefpass-Filters.

2.3.2. Herzfrequenzvariabilität

Da eine direkte Ableitung der Nervenaktivität von Herzvagus bzw. Sympathikus über einen langen Zeitraum bei chronisch instrumentierten Tieren nicht möglich ist, wurde die Herzfrequenzvariabilität als Maß der Aktivität des autonomen Nervensystems entsprechend definierter Standards (49) untersucht: Dazu wurde unter „steady state“-Bedingungen das Original EKG-Signal über einen Zeitraum von fünf Minuten ausgewertet. Dabei wurde besonders darauf geachtet, dass innerhalb der auszuwertenden Zeitspanne keine Extrasystolen oder Bewegungsartefakte auftraten. Die Herzfrequenzvariabilität wurde anschließend computerunterstützt ausgewertet (CHART, AdInstruments, Castle Hill, Australien). Als Maß der Gesamtaktivität des autonomen Nervensystems wurde zum einen die Standardabweichung aufeinander folgender RR-Zacken in der Zeitdomäne bestimmt, zum anderen in der Frequenzdomäne nach fast-Fourier Transformation des Originalsignals die Aktivität im Hochfrequenz- (HF, 0,15 - 0,5 Hz) und Niederfrequenzbereich (LF, 0,04 - 0,15 Hz). Die Aktivität im Hochfrequenzbereich gilt dabei als ein spezifisches Maß der Vagusaktivität, während die Aktivität im Niederfrequenzbereich sowohl Vagus- als auch Sympathikusaktivität repräsentiert (3,43,49). Zusätzlich wurde als Maß für die autonome Balance das Verhältnis von Hochfrequenz- zu Niederfrequenzaktivität bestimmt.

2.3.3. Sauerstoffverbrauch

Da bekanntlich die Stoffwechselaktivität, erkennbar am Sauerstoffverbrauch des Gesamtsorganismus, sowohl unter Grundumsatzbedingungen als auch während Anaesthesie

als Primärgröße das Herzminutenvolumen bestimmt (40), wurde zusätzlich in den eigenen Experimenten der Sauerstoffverbrauch der Tiere ermittelt und die Beziehung zwischen $\dot{V}O_2$ und HZV berechnet. Die Sauerstoffaufnahme ($\dot{V}O_2$, STPD) wurde mit Hilfe der indirekten Kalorimetrie unter Verwendung eines DeltatracII® Metabolic Monitor gemessen (Datex-Engström, Helsinki, Finnland). Vor jedem Experiment wurden die Gassensoren nach 30-minütiger Aufwärmzeit geeicht mit Raumluft und einem Eichgas bestehend aus 95 % O_2 und 5 % CO_2 sowie für die Sauerstoffverbrauchsmessung durch Verbrennung von 5 ml reinen Ethanols in einer speziellen Brennkammer (Alkohol-Verbrennungstest, Datex-Engström) überprüft.

2.3.4. Plasmakonzentrationen

Die Plasmakonzentrationen von Big-Endothelin und Endothelin sowie Vasopressin wurden während der entspechenden Versuche unter Kontrollbedingungen sowie am Ende jeder Intervention unter „steady state“-Bedingungen bestimmt. Vasopressin-Plasma-konzentrationen wurden mittels Radio-Immunoassay (^{125}I Vasopressin, Bühlmann Laboratory AG, Allschwill, Schweiz) gemessen, während Endothelin- sowie Big-Endothelin-Plasmakonzentrationen als Enzym-Immunoassay analysiert wurden (Endothelin: R&D Systems, Minneapolis, USA; Big-Endothelin: Biomedica, Wien, Austria).

2.3.5. Periduralanaesthesie

Am Morgen vor Beginn der eigentlichen Versuche wurde den Hunden in kurzfristiger Sedierung (Propofol 2 - 3 $mg \cdot kg^{-1}$) ein Periduralkatheter gelegt. Dazu wurde in Höhe des 3. oder 4. Lendenwirbels der Periduralraum punktiert und ein Katheter bis auf Höhe des 10. Brustwirbels vorgeschoben. Die Lage des Katheters wurde nach Kontrastmittelinjektion

röntgenologisch überprüft. Nach Anlage des Periduralkatheters erlangten die Tiere binnen Minuten das Bewusstsein vollständig wieder und liefen für mindestens 60 Minuten frei umher. Während der eigentlichen Versuche erhielt jeder Hund nach einer Kontrollperiode (Grundumsatzbedingungen) zur vollständigen Sympathikolyse über den Periduralkatheter 9 - 13 ml Lidocain 1 % injiziert (Dosis individuell angepasst), gefolgt von einer konstanten Infusion mit 6 ml/h. Dabei wurde besonders beachtet, dass an ein und demselben Tier in aufeinander folgenden Experimenten die Position des Katheters und die Dosis des Lokalanaesthestikums identisch waren. Eine vollständige Sympathikolyse wurde angenommen, wenn die folgenden Kriterien erfüllt waren: 1. Vorfall des sympathisch innervierten 3. Augenlides (Nickhautvorfall), 2. sensorische Blockade bis zum Nackenbereich bei zusätzlicher motorischer Blockade der Hinterläufe und partieller Blockade der Vorderläufe, 3. Änderung des physiologischen Atemmusters zu einer zwerchfellbetonten Atmung bei Blockade der Interkostalmuskulatur und 4. Ausbleiben einer sympathischen Vasokonstriktion nach Simulation eines Blutdruckabfalles durch kurzzeitiges (45 s) Abklemmen beider Carotisarterien (19,35).

2.3.6. Inhalationsanaesthesie

Nach einer Kontrollperiode (wache Tiere) wurden die Tiere anaesthesiert (Propofol 3 - 4 $mg \cdot kg^{-1}$) und kontrolliert beatmet. Die Inhalationsanaesthetika wurden in equipotenter Konzentration, d.h. als Vielfaches der substanzspezifischen MAC (minimale alveoläre Konzentration) der Beatmungsluft zugeführt. Als MAC-Werte gelten für Halothan 0,8 Vol %, Isofluran 1,4 Vol %, Enfluran 1,6 Vol %, Sevofluran 2,0 Vol % und Desfluran 7,0 Vol %, und zwar beim Hund ebenso wie beim Menschen. Das Edelgas Xenon wurde in den

Konzentrationen 50 % und 70 % der Atemluft zugemischt, entsprechend einem MAC-Wert von 0,4 und 0,6 (14).

2.3.7. Rezeptorblockade

Um die Bedeutung der Hormonsysteme für die Blutdruckregulation während verschiedener Anaesthesieverfahren zu erfassen, wurde deren Wirkung durch Injektion von spezifischen Rezeptorantagonisten aufgehoben. Zur Blockade des Endothelin-Systems wurde Tezosentan (3 $mg \cdot kg^{-1}$ initial, gefolgt von 3 $mg \cdot kg^{-1} \cdot h^{-1}$) injiziert, ein Endothelin-Rezeptorantagonist mit hoher Affinität zu ET_A- und ET_B-Rezeptoren (9). Die Vasopressin-Rezeptoren wurden durch Injektion eines selektiven V_{1A}-Rezeptor Anatgonisten, [*d*$(CH_2)_5$Tyr(Me^2)]AVP, in einer Dosierung von 40 $\mu g \cdot kg^{-1}$ blockiert (23).

2.4. Versuchsprogramme und Statistik

Alle Versuche wurden in randomisierter Reihenfolge durchgeführt, wobei durch wiederholtes Untersuchen eines Hundes jedes Tier als seine eigene Kontrolle diente. Zwischen aufeinander folgenden Experimenten an ein und demselben Tier wurde mindestens ein Intervall von einer Woche eingehalten. Eine detaillierte Aufstellung der jeweiligen Versuchsprogramme sowie der angewandten Statistiken ist den Publikationen zu entnehmen.

3. Ergebnisse

3.1. Grundlagen der Messmethoden: Ist die Messung des Herzzeitvolumens mittels Ultraschall-Transitzeit-Verfahren hinreichend genau und für die Langzeitanwendung geeignet?

Publikation 1 **Picker, O.,** A. Schindler, T.W.L. Scheeren (2000): "Accuracy and reproducibility of long-term implanted transit-time ultrasound flowprobes in dogs." Intensive Care Medicine **26**: 601-607.

Die Ultraschall-Flussaufnehmer waren im Mittel 22 Monate um die Pulmonalarterie implantiert (6 - 47 Monate). Das mittels Ultraschall-Flussaufnehmern gemessene Herzzeitvolumen korrelierte in jedem einzelnen Tier eng mit dem nach dem Fick'schen Prinzip ermittelten Herzzeitvolumen (Abbildung 1). Allerdings unterschätzte das Ultraschall-Transitzeit-Verfahren das tatsächliche Herzzeitvolumen (HZV_{Fick}) in etwa der Hälfte der Tiere um bis zu 38 %. Diese Differenz blieb über den gesamten Untersuchungszeitraum unabhängig von der absoluten Größe des Herzzeitvolumens konstant, so dass

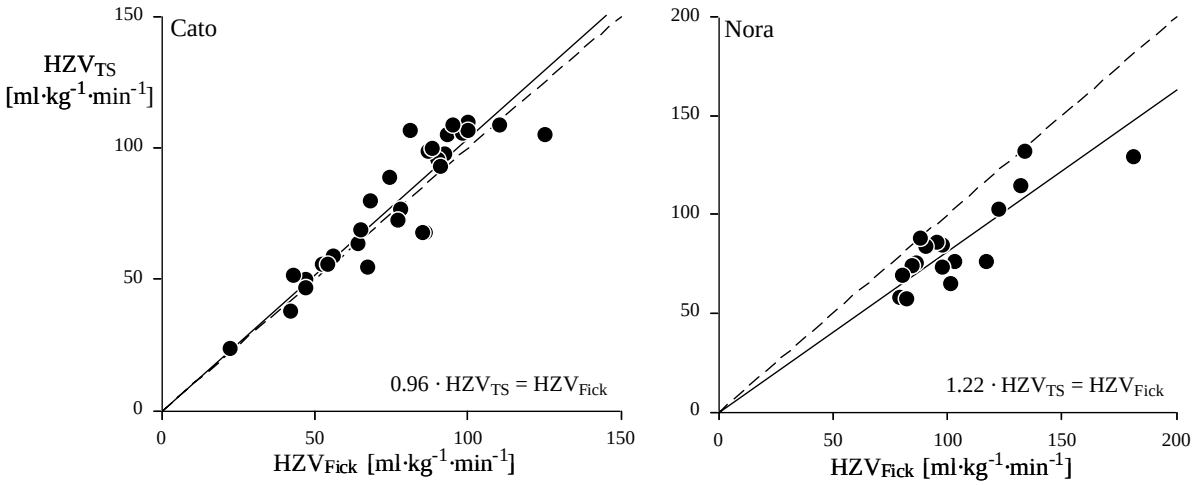

Abbildung 1: Korrelation (—) und Identitätslinie (---) zwischen dem Herzzeitvolumen bestimmt mittels Ultraschall-Transitzeit-Verfahren (HZV_{TS}) und mittels Fick'schem Prinzip (HZV_{Fick}) als Quotient aus Sauerstoffaufnahme und arterio-gemischtvenöser Sauerstoffgehaltsdifferenz an zwei Hunden mit Implantationsdauern von 47 Monaten (Cato) und 15 Monaten (Nora).
Während beim Hund Cato HZV_{Fick} und HZV_{TS} übereinstimmten, unterschätzte beim Hund Nora das Ultraschall-Transitzeit-Verfahren das HZV_{Fick} permanent um 22%.

Korrekturfaktoren ermittelt werden konnten. Nach individueller Korrektur (Abbildung 2) bestimmte das Ultraschall-Transitzeit-Verfahren das Herzzeitvolumen verlässlich (mittlere Differenz –1,1 $ml \cdot kg^{-1} \cdot min^{-1}$).

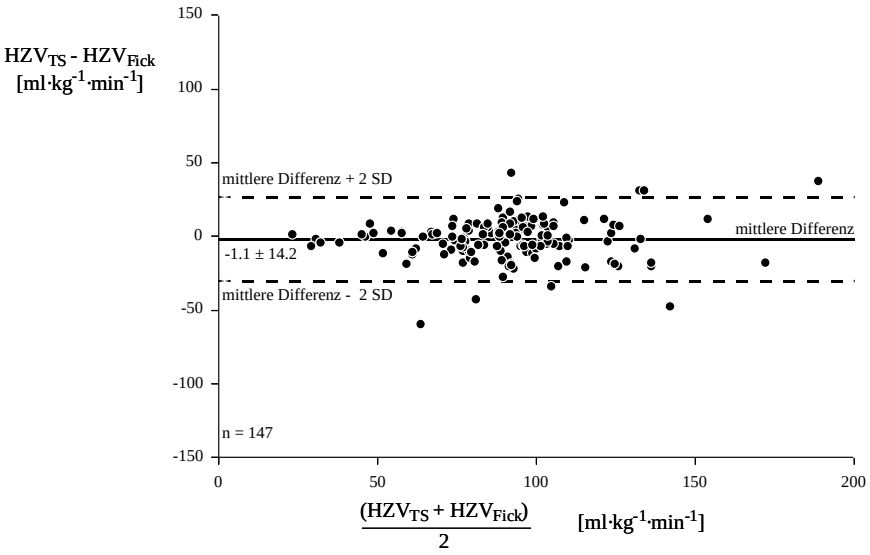

Abbildung 2: Bland & Altmann-Beziehung des Herzzeitvolumens bestimmt mittels Ultraschall-Transitzeit-Verfahren (HZV_{TS}) und mittels Fick'schem Prinzip (HZV_{Fick}) als Differenz und Mittelwert von HZV_{TS} und HZV_{Fick}.
Nach individueller Korrektur bestimmte das Ultraschall-Transitzeit-Verfahren das tatsächliche HZV (HZV_{Fick}) sehr genau (mittlere Differenz -1,1 $ml \cdot kg^{-1} \cdot min^{-1}$).

3.2. Bedeutung blutdruckstabilisierender Systeme bei unterschiedlichen Anaesthesieverfahren

Publikation 2 **Picker, O.,** A. W. Schindler, T.W.L. Scheeren (2001): "Endogenous endothelin and vasopressin support blood pressure during epidural anesthesia in conscious dogs." Anesthesia & Analgesia **93**: 1580-1586.

Publikation 3 **Picker, O.,** L.A. Schwarte, P. Roth, J. Greve, T.W.L. Scheeren (2004): " Comparison of the role of endothelin, vasopressin and angiotensin in arterial pressure regulation during sevoflurane anaesthesia in dogs." British Journal of Anaesthesia **92**: 102-108.

Während im Wachzustand der Blutdruck nur nach Gabe des Endothelin-Rezeptorblockers abfiel, war der Blutdruckabfall nach Anlage einer rückenmarksnahen Leitungsanaesthesie oder während einer Inhalationsanaesthesie stärker ausgeprägt in Gegenwart einer Endothelin- oder Vasopressin-Rezeptor-Blockade (Abbildung 3). Das Herzzeitvolumen blieb unter einer

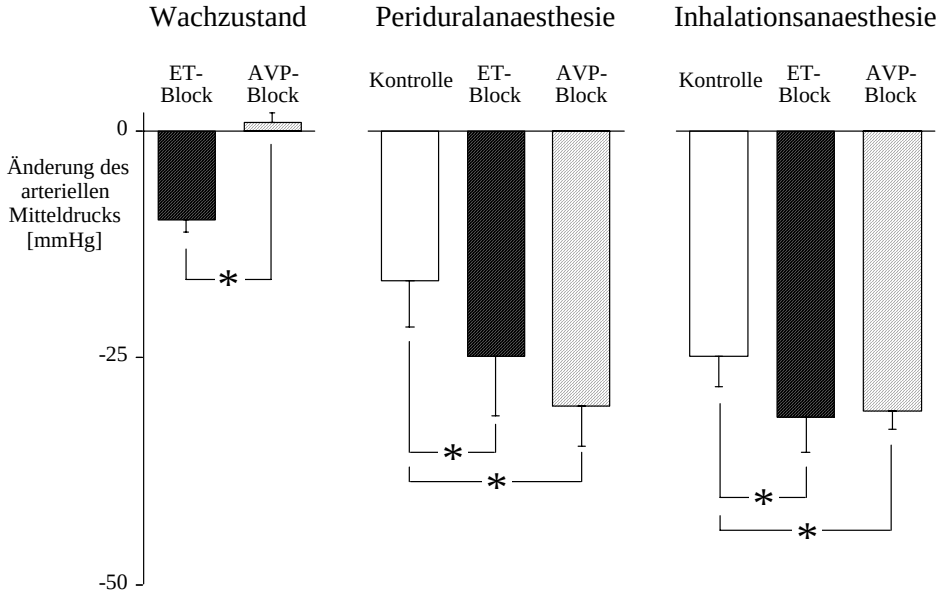

Abbildung 3: Verhalten des arteriellen Mitteldruckes im Wachzustand, nach Anlage einer Periduralanaesthesie oder während einer Inhalationsanaesthesie mit Sevofluran 1 MAC. Die Versuche wurden durchgeführt in Gegenwart eines Endothelin- (ET-Block) oder eines Vasopressin-Rezeptor-Antagonisten (AVP-Block), sowie unter Kontrollbedingungen (Periduralanaesthesie bzw. Inhalationsanaesthesie allein).
Mittelwerte ± Standardfehler. * bedeuten $p < 0,05$.
Die stärkere Abnahme des arteriellen Mitteldrucks in Gegenwart einer ET- bzw. AVP-Rezeptorblockade während beider Anaesthesieformen deutet darauf hin, dass sowohl Endothelin als auch Vasopressin zur Blutdruckaufrechterhaltung während Inhalationsanaesthesie und Periduralanaesthesie beitragen.

rückenmarksnahen Leitungsanaesthesie nahezu unverändert, wohingegen es während einer Inhalationsanaesthesie konzentrationsabhängig abfiel. Diese Effekte waren unabhängig von der Gegenwart oder Abwesenheit einer Endothelin- oder Vasopressin-Rezeptor-Blockade. Sowohl im Wachzustand als auch während beider Anaesthesieformen blieben die Plasmakonzentrationen von Endothelin und Big-Endothelin unverändert, während die Plasmakonzentrationen von Vasopressin parallel zum Blutdruckabfall unter Anaesthesie anstiegen. Demnach tragen sowohl Endothelin als auch Vasopressin zur Blutdruckstabilisierung während beider Anaesthesieformen bei, allerdings wird ein für die Organperfusion ausreichendes Blutdruckniveau ausschließlich über eine zusätzliche Vasopressinfreisetzung gewährleistet.

3.3. Autonomes Nervensystem und Herzfrequenzregulation unter den Bedingungen einer Inhalationsanaesthesie

Publikation 4 **Picker, O.,** T.W.L. Scheeren, J.O. Arndt (2001): "Inhalation anesthetics increase heart rate by decreasing cardiac vagal activity." British Journal of Anaesthesia **87**: 748-754.

Publikation 5 **Picker, O.,** L.A. Schwarte, A.W. Schindler, T.W.L. Scheeren (2003): "Desflurane increases heart rate independent of sympathetic activity in dogs." European Journal of Anaesthesiology **20**: 945-951.

Publikation 6 **Picker, O.,** A.W. Schindler, L.A. Schwarte, B. Preckel, W. Schlack, T.W.L. Scheeren, V. Thämer (2002): "Xenon increases total body oxygen consumption during isoflurane anaesthesia in dogs." British Journal of Anaesthesia **88**: 546-54.

Die Tiere hatte im Wachzustand niedrige Herzfrequenzen (~75 min^{-1}) sowie eine hohe Herzfrequenzvariabilität als Zeichen der überwiegenden Vagusaktivität. Im Einklang hiermit führte eine pharmakologische Sympathektomie (ß-Rezeptor-Blockade) am wachen Tier zu keiner Änderung der Herzfrequenz, während eine Vagusblockade (Atropin) die Herzfrequenz auf etwa 145 min^{-1} steigerte. Die Anaesthesie mit konventionellen Inhalationsanaesthetika

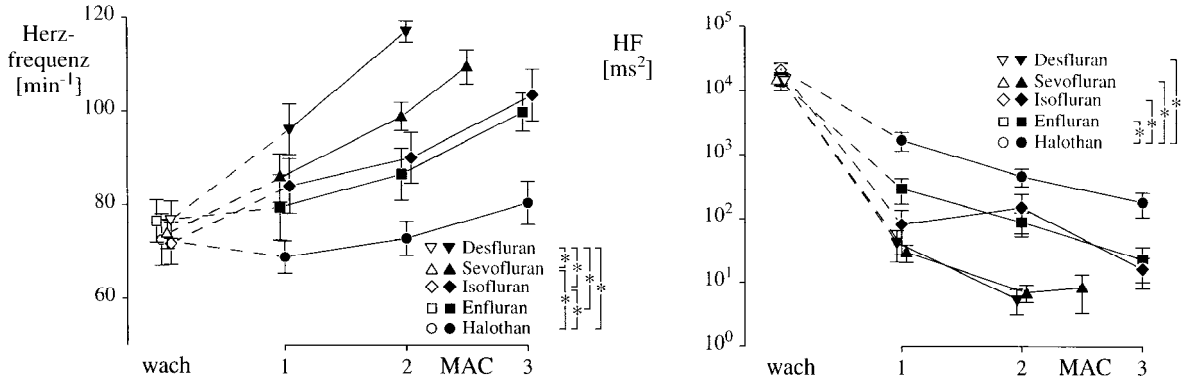

Abbildung 4: Verhalten der Herzfrequenz und der Vagusaktivität, gemessen als Herzfrequenzvariabilität in der Hochfrequenzdomäne (HF) während einer Inhalationsanaesthesie (1-3 MAC) mit fünf verschiedenen Inhalationsanaesthetika. Mittelwerte ± Standardfehler von sieben Hunden.
* bedeuten $p < 0,05$ zwischen den Gruppen.
Der Herzfrequenzanstieg ging in allen Gruppen mit einer Hemmung der Vagusaktivität einher.

(Desfluran, Sevofluran, Isofluran, Enfluran und Halothan) führte dosisabhängig zu einem Anstieg der Herzfrequenz und zu einem Abfall der Vagusaktivität (Abbildung 4). Dabei spiegelten sich substanzspezifische Unterschiede bezüglich des Herzfrequenzanstieges im Verhalten der Vagusaktivität wider. Der Herzfrequenzanstieg während einer Desfluran-Anaesthesie war auch nach einer pharmakologischen Sympathikusblockade uneingeschränkt vorhanden, wohingegen eine Vagusblockade diesen vollständig verhinderte. Im Gegensatz zu diesen substanzspezifischen Effekten auf die Herzfrequenz und die Vagusaktivität senkten alle konventionellen Inhalationsanaesthetika konzentrationsabhängig sowohl den Sauerstoffverbrauch als auch das Herzzeitvolumen. Während einer Inhalationanaesthesie mit dem Edelgas Xenon war jedoch die Beziehung von $\dot{V}O_2$ und HZV insofern verändert, als bei gleichbleibendem HZV der $\dot{V}O_2$ anstieg. Mit Blick auf die Herzfrequenzregulation korrelierte auch während einer Xenonanaesthesie sowie bei einer Inhalationsanaesthesie mit den konventionellen Anaesthetika die Herzfrequenz invers mit der Vagusaktivität. Demnach scheint für die Herzfrequenzeinstellung während beider Formen der Inhalationsanaesthesie die jeweilige Vagusaktivität entscheidend zu sein.

3.4. Beziehung zwischen Vagusaktivität, Stickstoffmonoxid und Herzfrequenzregulation unter physiologischen Bedingungen

Publikation 7 **Picker, O.,** T.W.L. Scheeren, J.O. Arndt (2001): "Nitric oxide synthases in vagal neurons are crucial for the regulation of heart rate in awake dogs." Basic Research in Cardiology **96**: 395-404.

Eine Hemmung der NO-Synthase mit dem unspezifischen NO-Synthase-Hemmer L-NAME führte bei wachen Hunden dosisabhängig zu einer ausgeprägten Bradykardie, gefolgt von einem Abfall des HZV, sowie zu einer Vagusaktivierung und Vasokonstriktion. Die Dosis-Wirkungs-Beziehungen der Herzfrequenz- und Vaguseffekte von L-NAME wiesen eine etwa 3-fach niedrigere ED_{50} (halbmaximale Wirkdosis) auf im Vergleich zu den vasokonstriktorischen Wirkungen (Abbildung 5). Nach pharmakologischer Blockade des

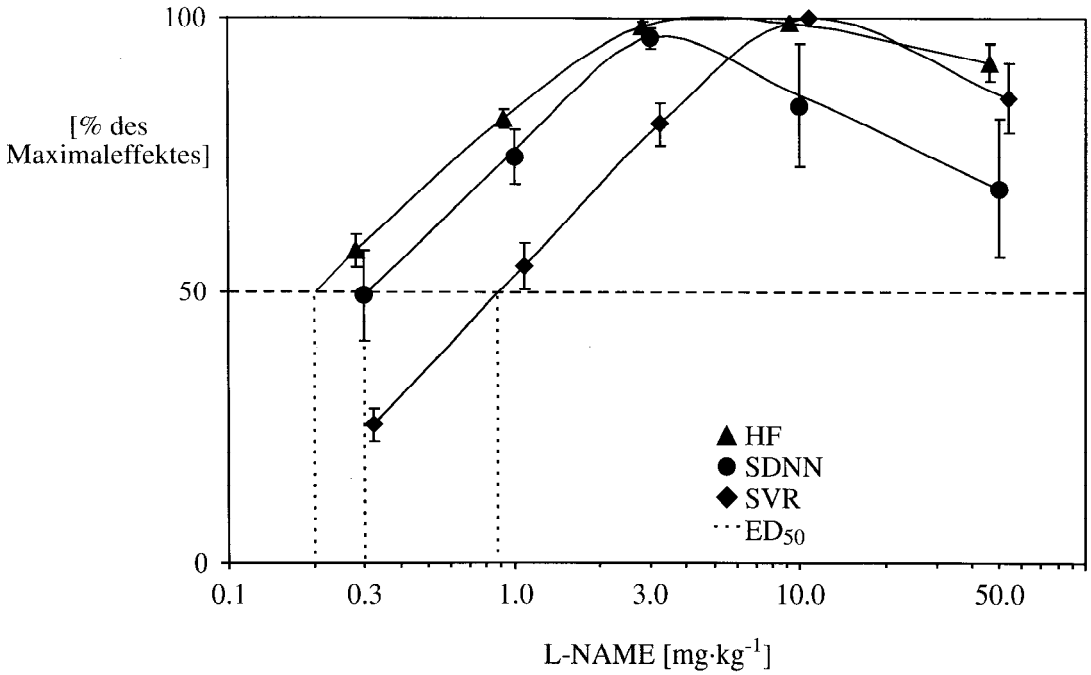

Abbildung 5: Abfall der Herzfrequenz (HF) sowie Anstieg des systemischen Gefäßwiderstandes (SVR) und der Vagusaktivität, gemessen als Standardabweichung der RR-Abstände (SDNN) an wachen Hunden während einer Hemmung der NO-Synthase mit L-NAME (0,3 – 50 $mg \cdot kg^{-1}$). Dargestellt sind die Änderungen der Variablen in % des Maximaleffekts.
Mittelwerte ± Standardfehler von sieben Hunden. ED_{50}: Halbmaximale Wirkdosis
Die Dosis-Wirkungsbeziehungen der Herzfrequenz- und Vaguseffekte (SDNN) unterscheiden sich signifikant ($p < 0.05$) von denen des Gefäßwiderstandes mit einer 3-fach niedrigeren ED_{50}.

gesamten autonomen (sympathischen und parasympathischen) Nervensystems (Hexamethonium) führte L-NAME zu einer unverminderten Vasokonstriktion, ohne dass eine Bradykardie auftrat bzw. der Vagus aktiviert wurde. Daraus ist zu schließen, dass zum einen L-NAME direkt die Vagusaktivität beeinflusst, und zum anderen dass NO an der Herzfrequenzregulation beteiligt ist.

4. Diskussion

4.1. Grundlagen der Messmethoden: Ist die Messung des Herzzeitvolumens mittels Ultraschall-Transitzeit-Verfahren hinreichend genau und für die Langzeitanwendung geeignet?

Die Prämisse dieser Untersuchungen war, dass eine Eichung der Ultraschall-Transitzeit-Flussaufnehmer mit dem Fick'schen Prinzip, also als Quotient von Sauerstoffverbrauch und arterio-gemischtvenöser Sauerstoffgehaltsdifferenz, hinreichend genau ist. Durch die direkte Messung des Sauerstoffgehaltes (Lex-O_2-Con) mit einer Genauigkeit von ± 1 % (57) und die Bestimmung des Sauerstoffverbrauchs mittels indirekter Kalorimetrie und einer Genauigkeit von 4 % (24,46,54) lässt sich ein Fehler für die Eichung der Flussaufnehmer von maximal 10 % errechnen. Diese Präzision liegt damit deutlich über der von herkömmlichen Indikatorverdünnungsverfahren, die eine Fehlerbreite von etwa ± 20 % aufweisen (20,28,56,58,62). Unsere Ergebnisse zeigen, dass bei der Hälfte aller untersuchten Tiere beide Verfahren gut übereinstimmten, und zwar unabhängig von der absoluten Höhe des Herzzeitvolumens. Bei der anderen Hälfte der Tiere allerdings unterschätzte das Ultraschall-Transitzeit-Verfahren das tatsächliche Herzzeitvolumen um bis zu 38 %. Ursächlich hierfür ist wahrscheinlich ein Abweichen des Flussaufnehmers von der Gefäßlängsachse, welches bedingt ist durch ein nicht achsengerechtes Einwachsen des Messkopfes. Im Rahmen von Re-Operationen, die bei Funktionsverlust der Messgeräte durchgeführt wurden, konnte bei den Tieren mit der größten Differenz zwischen beiden Verfahren ein Abweichen des Flusskopfes aus der Längsachse beobachtet werden. Daher konnten individuelle Korrekturfaktoren ermittelt werden, danach stimmten beide Verfahren sehr genau überein, mit einer mittleren Differenz von lediglich -1,1 $ml \cdot kg^{-1} \cdot min^{-1}$.

Demnach ist das Ultraschall-Transitzeit-Verfahren nach in vivo-Kalibration geeignet, das Herzzeitvolumen über einen langen Untersuchungszeitraum präzise zu bestimmen.

4.2. Bedeutung blutdruckstabilisierender Systeme bei unterschiedlichen Anaesthesieverfahren

Die vorliegenden Ergebnisse zeigen, dass sowohl während Inhalationsanaesthesie als auch während rückenmarksnaher Leitungsanaesthesie die endogenen Peptidhormone Endothelin und Vasopressin zur Blutdruckstabilisierung beitragen.

Unter physiologischen Ruhebedingungen, d.h. im Wachzustand, wird der Blutdruck von einer Vielzahl verschiedener Systeme stabilisiert, und zwar insbesondere durch das sympathische Nervensystem und das Renin-Angiotensin-System. Die Bedeutung von Vasopressin und insbesondere Endothelin bei der Blutdruckaufrechterhaltung unter physiologischen Bedingungen hingegen war lange Zeit unklar. Im Einklang mit der Literatur (29,61) zeigen die eigenen Ergebnisse, dass Endothelin auch zur Blutdruckaufrecherhaltung unter physiologischen Bedingungen beiträgt, da im Wachzustand eine Endothelinrezeptor-Blockade den Blutdruck um etwa 10 mmHg senkte. Demgegenüber spielt Vasopressin bei der Blutdruckregulation unter physiologischen Bedingungen offenbar keine Rolle (6,15), da eine Blockade der Vasopressin-Rezeptoren zu keiner Änderung des Blutdruckes führte.

Im Unterschied zu den physiologischen Bedingungen wacher Hunde tragen während verschiedener Anaesthesieformen sowohl Endothelin als auch Vasopressin zur Blutdruckaufrechterhaltung bei. In den eigenen Experimenten fiel der Blutdruck nach Endothelinrezeptor-Blockade sowohl während Inhalations- als auch während rückenmarksnaher Leitungsanaesthesie um etwa den gleichen Betrag (10 mmHg) stärker ab als ohne vorhergehende Rezeptor-Blockade. Demnach ist Endothelin bei der

Blutdruckstabilisierung während beider Anaesthesieverfahren beteiligt, und zwar im gleichen Ausmaß wie im Wachzustand. Unabhängig von der Intervention und dem Ausmaß des Blutdruckabfalls blieben allerdings die Big-Endothelin- und Endothelin-Konzentrationen unverändert, als Ausdruck einer fehlenden Aktivierung des Systems (31). Demnach trägt Endothelin zwar zur Blutdruckaufrechterhaltung während Inhalations- und rückenmarksnaher Leitungsanaesthesie bei, allerdings wird das System nicht zusätzlich aktiviert, um weitere Blutdruckabfälle zu verhindern.

In Gegenwart einer Vasopressin-Rezeptor-Blockade fiel der Blutdruck während Inhalations- und rückenmarksnaher Leitungsanaesthesie stärker ab als im Wachzustand. Parallel zum Blutdruckabfall stiegen während aller Interventionen die Vasopressinspiegel im Plasma an, und zwar proportional zum Ausmaß der Blutdrucksenkung. Die Freisetzung von Vasopressin wird dabei vermutlich über arterielle Barorezeptoren im Aortenbogen und Carotissinus vermittelt (52), da Anstiege der Vasopressinkonzentration nach Denervation dieser Rezeptoren ausblieben (51). Das Herzzeitvolumen scheint allerdings für die Freisetzung von Vasopressin während verschiedener Anaesthesieformen von untergeordneter Bedeutung zu sein, da sowohl während rückenmarksnaher Leitungsanaesthesie (bei unverändertem HZV) als auch bei Inhalationsanaesthesie (bei stark abgefallenem HZV) die Vasopressinspiegel anstiegen. Demnach stellt das Vasopressin-System nicht nur während physiologischer Bedingungen, sondern auch während Inhalations- und rückenmarksnaher Leitungsanaesthesie ein Reservesystem der Blutdruckregulation dar, das jedoch erst bei deutlichen Blutdruckabfällen aktiviert wird.

4.3. Autonomes Nervensystem und Herzfrequenzregulation unter den Bedingungen einer Inhalationsanaesthesie

Die vorliegenden Ergebnisse zeigen, dass unter physiologischen Bedingungen, d.h. im Wachzustand, die Herzfrequenz durch die Vagusaktivität bestimmt wird und Änderungen der Herzfrequenz durch Änderungen der Vagusaktivität ausgelöst werden.

Im Gegensatz dazu wurden die Herzfrequenzanstiege während einer Inhalationsanaesthesie bislang über eine Aktivierung des sympathischen Nervensystems erklärt, insbesondere während einer Anaesthesie mit dem neuen Inhalationsanaesthetikum Desfluran (11). Da Desfluran andererseits die Atemwege reizt, könnte auch eine Stimulation so genannter „irritant receptors“ für den Herzfrequenzanstieg von Bedeutung sein (48). Bei hohen Anaesthetikakonzentrationen werden jedoch sowohl die Symapthikusaktivität (33) als auch die Nervenaktivität der „irritant receptors“ gehemmt (30), so dass beide Effekte nicht für die gerade unter hohen Anaesthetikakonzentrationen besonders deutlich ausgeprägten Tachykardien verantwortlich sein können. Direkte Effekte volatiler Anaesthetika auf das Herz als Auslöser der Herzfrequenzanstiege können ebenfalls ausgeschlossen werden, da diese Pharmaka am isolierten Herzen zu einer Bradykardie führen (5,44). Somit verbleibt eine Hemmung der Vagusaktivität als einzige Erklärung für die Herzfrequenzeffekte der Inhalationsanaesthetika, die in den eigenen Experimenten durch eine Analyse der Herzfrequenzvariabilität auch nachgewiesen werden konnte. Darüber hinaus war das Ausmaß der Vagushemmung umgekehrt proportional zum Ausmaß der Herzfrequenzeffekte, d.h. die Herzfrequenz korrelierte unter allen Bedingungen invers mit der Vagusaktivität. Dies galt ebenso für das besonders frequenzsteigernde Anaesthetikum Desfluran, das auch nach pharmakologischer Sympathektomie (ß-Rezeptoren-Blockade) die Herzfrequenz unverändert steigerte. Nach Vagusblockade (Atropin) blieb dagegen ein Frequenzanstieg vollständig aus.

Dabei werden höchst wahrscheinlich die substanzspezifischen Effekte der konventionellen Inhalationsanaesthetika vermittelt über eine substanzspezifisch unterschiedliche Vagushemmung. Diese Interpretation basiert auf den Ergebnissen, dass alle Substanzen sowohl den Blutdruck, als möglichen Auslöser für Reflextachykardien, als auch das Herzzeitvolumen und den $\dot{V}O_2$ gleichartig senken und somit Änderungen der Haemodynamik nicht ursächlich sein können für die substanzspezifischen Effekte der Inhalationsanaesthetika auf die Herzfrequenz. Während einer Inhalationanaesthesie mit dem Edelgas Xenon allerdings war die Beziehung von $\dot{V}O_2$ und HZV verschoben, d.h. bei unverändertem HZV stieg der $\dot{V}O_2$ an, so dass die haemodynamische Stabilität während einer Xenonanaesthesie durch einen erhöhten $\dot{V}O_2$ erkauft wird. Die Herzfrequenz korrelierte auch während einer Xenonanaesthesie sowie bei einer Inhalationsanaesthesie mit den konventionellen Anaesthetika invers mit der Vagusaktivität. Somit unterscheiden sich prinzipiell weder die Herzfrequenzregulation noch die Regulation des Herzzeitvolumens während Inhalationsanaesthesie mit den konventionellen volatilen Anaesthetika oder dem Edelgas Xenon.

4.4. Beziehung zwischen Vagusaktivität, Stickstoffmonoxid und Herzfrequenzregulation unter physiologischen Bedingungen

Die eigenen Ergebnisse zeigen, dass Stickstoffmonoxid modulierend auf die Vagusaktivtät wirkt und eine wesentliche Rolle bei der Herzfrequenzregulation eines Warmblüterorganismus unter physiologischen Bedingungen, d.h. im Wachzustand, spielt. Diese Interpretation basiert auf der Beobachtung, dass eine Hemmung der NO-Synthase mit L-NAME bereits bei 3-fach niedrigeren Dosierungen eine Vagusaktivierung mit Bradykardie auslöste als eine Vasokonstriktion. Diese Unterschiede der halbmaximalen Wirkdosen (ED_{50})

legen die Vermutung nahe, dass die vasomotorischen- bzw. Herzfrequenz-/Vaguseffekte von L-NAME über unterschiedliche Isoformen der NO-Synthase vermittelt werden. Da das vom Endothel über e-NOS freigesetzte NO an der Regulation des Gefäßtonus beteiligt ist (4,37) und sich bei e-NOS knockout Mäusen durch L-NAME keine Vasokonstriktion auslösen lies, bei allerdings uneingeschränkten Effekten auf die Herzfrequenz (22), werden demnach die Herzfrequenz-/Vaguseffekte von L-NAME über eine andere Isoform der NO-Synthase, vermutlich die neuronale NO-Synthase (n-NOS), vermittelt.

Diese Interpretation steht auch insofern mit den eigenen Ergebnissen im Einklang, als nach Blockade der Vagus- und Sympathikusaktivität (Hexamethonium) L-NAME keine Bradykardie mehr auslöste, ein vasokonstriktorischer Effekt jedoch unverändert nachweisbar war. Darüberhinaus blieb eine Bradykardie nach Vagotomie (39,55) sowie an isolierten Herzen aus (1,21). Außerdem führte L-NAME bereits in niedriger Dosierung (0,3 $mg \cdot kg^{-1}$) zu einer deutlichen Bradykardie (Herzfrequenzabfall von ~ 80 auf 55 min^{-1}), einer Dosis, bei der kein Blutdruckanstieg als möglicher Auslöser für eine baroreflektorisch vermittelte Bradykardie zu beobachten war. Demnach sind für die Herzfrequenzeffekte von L-NAME weder ein Blutdruckanstieg mit nachfolgender Aktivierung der Baroreflexe noch das e-NOS Gen notwendig. Daraus lässt sich die These ableiten, dass endogenes NO, freigesetzt von neuronalen NO-Synthasen, die zentrale Vagusaktivtät hemmt und somit beteiligt ist an der Herzfrequenzeinstellung des Warmblüterorganismus unter physiologischen Bedingungen.

5. Zusammenfassung

Die vorliegenden Experimente dienten der Klärung bislang unbekannter Fragen der Kreislaufregulation während verschiedener Anaesthesieverfahren im Vergleich zum Wachzustand unter Grundumsatzbedingungen. Im Einzelnen wurde untersucht, welche Bedeutung den endogenen Peptidhormonen Endothelin und Vasopressin bei der Aufrechterhaltung des Blutdrucks während rückenmarksnaher Leitungsanaesthesie und Inhalationsanaesthesie zukommt sowie die Effekte von endogenem NO, konventionellen Inhalationsanesthesika und des Edelgases Xenon auf die Vagusaktivität und deren Bedeutung für die Herzfrequenzregulation. Dazu wurden Versuche durchgeführt an chronisch instrumentierten Hunden, die sowohl im Wachzustand unter Grundumsatzbedingungen als auch während verschiedener Anaesthesieverfahren (Periduralanaesthesie / Inhalationsanaesthesie) wiederholt untersucht wurden. Vor Beginn der eigentlichen Versuche wurde den Tieren zur kontinuierlichen Messung des Herzzeitvolumens ein Ultraschall-Transitzeit-Flussaufnehmer um die Pulmonalarterie implantiert. Die Genauigkeit dieser Flussaufnehmer während einer Langzeitimplantation (6 - 47 Monate) wurde im Rahmen einer Voruntersuchung überprüft, mit dem Ergebnis, dass diese nach individueller Eichung des Herzzeitvolumens sehr genau über einen langen Zeitraum messen. Zusätzlich wurden bei den Tieren zur kontinuierlichen Blutdruckmessung beide Carotisarterien isoliert und in Hautschlingen eingenäht.

Die Aussagen stützen sich auf die Analyse der hämodynamischen Variablen, insbesondere des arteriellen Blutdrucks und auf die Messung der Plasmaspiegel von Big-Endothelin, Endothelin und Vasopressin. Verglichen wurden die Werte im Wachzustand unter Grundumsatzbedingungen mit denen während Peridural- sowie Inhalationsanaesthesie jeweils

mit und ohne Blockade des Endothelin- (Tezosentan) oder des Vasopressin-Systems ([*d*(CH_2)$_5$Tyr(Me^2)]AVP). Als Maß der autonomen Aktivität, insbesondere der Vagusaktivität, wurde die Herzfrequenzvariabilität bestimmt sowohl in der Zeitdomäne (Standardabweichung der RR-Abstände) als auch in der Frequenzdomäne (Aktivität im Hoch- und Niederfrequenzbereich). Untersucht wurde der Einfluss der verschiedenen Interventionen (konventionelle Inhalationsanaesthetika, Xenonanaesthesie, NO) auf die Beziehung von Vagusaktivität und Herzfrequenz. Dabei wurde neben dem arteriellen Blutdruck besonders das Herzzeitvolumens sowie der Sauerstoffverbrauch des Gesamtorganismus berücksichtigt, der als Primärgröße überwiegend das Herzzeitvolumen bestimmt.

Die Ergebnisse zeigen, dass eine Endothelin-Rezeptorblockade sowohl im Wachzustand als auch während Peridural- oder Inhalationsanaesthesie zu einem Blutdruckabfall führt und somit Endothelin zur Aufrechterhaltung des Blutdrucks während beider Anaesthesieverfahren wie im Wachzustand beiträgt. Demgegenüber senkte die Vasopressin-Rezeptorblockade den Blutdruck nur während Peridural- oder Inhalationsanaesthesie. Die Plasmakonzentrationen von Big-Endothelin und Endothelin blieben unverändert während aller Interventionen, wohingegen die Vasopressinkonzentration parallel zum Blutdruckabfall unter Anaesthesie anstiegen. Das Herzzeitvolumen blieb während der Versuche in Periduralanaesthesie weitgehend unverändert und fiel während Inhalationsanaesthesie konzentrationsabhängig ab. Demnach tragen beide Systeme (Endothelin und Vasopressin) zur Aufrechterhaltung des Blutdrucks während beider Anaesthesieverfahren bei, allerdings wird nur endogenes Vasopressin im Sinne eines Reservesystems zusätzlich freigesetzt, um eine stärkere Hypotension zu vermeiden. Die Freisetzung von Vasopressin wird dabei ausgelöst durch das arterielle Blutdruckniveau und ist unabhängig von der Größe des Herzzeitvolumens.

Weiterhin zeigen die Ergebnisse, dass im Wachzustand unter Grundumsatzbedingungen die Herzfrequenz überwiegend durch die Vagusaktivität bestimmt wird und endogenes NO, vermutlich vermittelt über die neuronale NO-Synthase (n-NOS), an der Herzfrequenzregulation beteiligt ist. Auch während einer Inhalationsanaesthesie mit konventionellen Anaesthetika wird die Herzfrequenz durch die Vagusaktivität bestimmt, und unterschiedliche Effekte der Inhalationsanaesthetika auf die Herzfrequenz beruhen vermutlich auf substanzspezifischen Vagushemmungen. Diese Interpretation basiert auf dem Ergebnis, dass sämtliche Anaesthetika konzentrationsabhängig zu einem Abfall der Vagusaktivität und einem Anstieg der Herzfrequenz führten und beide Variablen unter allen Bedingungen invers miteinander korrelierten.

Im Gegensatz zu diesen substanzspezifischen Effekten auf die Herzfrequenz und die Vagusaktivität senkten alle konventionellen Inhalationsanaesthetika konzentrationsabhängig sowohl den Sauerstoffverbrauch als auch das Herzzeitvolumen gleichartig. Während einer Inhalationanaesthesie mit dem Edelgas Xenon war jedoch die Beziehung von Sauerstoffverbrauch und Herzzeitvolumen derart verändert, als bei gleichbleibendem Herzzeitvolumen der Sauerstoffverbrauch anstieg. Mit Blick auf die Herzfrequenzregualtion korrelierte auch während einer Xenonanaesthesie die Herzfrequenz invers mit der Vagusaktivität. Demnach wird die Regulation der Herzfrequenz im Wachzustand, während konventioneller Inhalationsanaesthesie oder einer Anaesthesie mit dem Edelgas Xenon durch die Vagusaktivität bestimmt.

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8. Anhang: Publikationen

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EXPERIMENTAL

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Accuracy and reproducibility of long-term implanted transit-time ultrasound flow probes in dogs

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Abstract *Objective:* To assess the accuracy and reproducibility of long-term implanted ultrasound transit-time flow probes for measuring cardiac output.
Design: Prospective animal study.
Settings: Animal research laboratory in a university department.
Animals: Eleven anaesthetised dogs, 24–34 kg.
Measurements and results: Flow probes (16–24 mm S-series, Transonic) were implanted around the pulmonary artery for a mean duration of 22 months (range 6–47 months). Comparisons (n = 147) were made between cardiac output thus obtained and that measured by the direct Fick principle using oxygen uptake (Deltatrac II Metabolic Monitor) and the arterial to mixed venous oxygen content difference measured by a galvanic cell (Lex-O_2-Con-TL). Measurements were made either during baseline conditions or during pharmacologically altered cardiac output (range 22–180 ml · kg^{-1} · min^{-1}). Regardless of the intervention, the two methods yielded the same results in half of the dogs. In the others, however, cardiac output was underestimated by the flow probes by up to 38 % (probably because of non-perpendicular position of the probe towards the vessel). This difference was constant for the whole range of cardiac output studied and remained constant over the entire observation period for each individual dog, so that a correction factor was used. Thereafter, the mean difference between the two methods was –1.1 ml · kg^{-1} · min^{-1} with a precision (SD) of 14.2 ml · kg^{-1} · min^{-1} for all experiments.
Conclusions: After in vivo calibration, ultrasound transit-time flow probes measure cardiac output precisely for several years, regardless of the intervention.

Key words Cardiac output · Comparison of methods · Ultrasound transit time · Fick principle

Introduction

The accurate and continuous determination of cardiac output is of major interest in many experimental settings using chronically instrumented animals. To achieve this goal ultrasound transit-time flow probes have been advocated. However, the accuracy and reproducibility of these probes is unknown when they are implanted over a long period. We therefore evaluated cardiac output determination by this method and compared the results with those obtained from the direct Fick principle in dogs in which the flow probes were implanted for 6–47 months (mean duration of 22 months).

Transit-time flow probes consist of a U-reflector positioned around the vessel and a probe body screwed above. They emit a plane of ultrasound that transects the whole cross-section of the vessel. The transit time of the ultrasonic beam is measured in both the upstream

and downstream directions, and the difference between the integrated transit times is determined across the whole cross-section of the vessel. By these means, volume flow is measured independently of vessel diameter. It is claimed by the producer that the flow measurement is unaffected by minor misalignments of the vessel within the flow probe and is independent of the flow profile [1]. However, there are only few reports about the accuracy of these flow probes, and in most studies such experiments have been conducted immediately after, or within a short period after, implantation [2, 3].

In this study we describe an extensive evaluation of the accuracy and reproducibility of long-term implanted transit-time flow probes around the pulmonary artery in dogs and we will show that in some animals the flow probes underestimate cardiac output by up to 38 % but, after correcting for this error, measurements remain stable and reliable for up to 47 months.

Methods

The data derive from 147 measurements in 11 dogs (foxhounds of both sexes weighing 24–34 kg, studied with approval of the District Governmental Animal Investigation Committee). The dogs were housed in an animal research department and fed a standard chow (medium adult 1, Royal Canin, Aimargues, France) and water was available ad libitum.

Implantation of flow probes

Anaesthesia was induced by intravenous thiopental sodium ($10\ mg \cdot kg^{-1}$) and maintained by 1.5 vol.% of enflurane in a mixture of oxygen and nitrous oxide (30:70 %; flow rate $3\ l \cdot min^{-1}$; tidal volume $10\ ml \cdot kg^{-1}$; respiration rate 10–12 breaths $\cdot\ min^{-1}$). Analgesia was provided repetitively with fentanyl (0.1 mg) when necessary during the operation and with piritramid postoperatively (7.5 mg, every 4–6 h). Lincomycin ($20\ mg \cdot kg^{-1}$, twice daily) was administered intraoperatively and for 1 week postoperatively to prevent wound infections.

To implant the flow probes (16–24 mm S-series with a silicone shielded U-reflector, Transonic) on the pulmonary artery, a thoracotomy through the left fourth intercostal space was performed and the probe was carefully implanted on the trunk of the pulmonary artery. To achieve this the U-reflector was positioned around the vessel after pulmonary artery and aorta had been carefully separated. The probe body was placed above the reflector and fixed by two screws. A probe size was chosen that neither compressed the vessel wall nor left too much space between vessel and the reflector, to avoid kinking. Thereafter, the pericardium was closed again and the cable was fixed at the inner thoracic wall in a loop. The end of the cable was guided through the thorax and subcutaneously tunnelled towards the scapulae where it was left subcutaneously. After at least 3 weeks of recovery, the cable was exteriorised by a short skin incision and connected to the flowmeter (T 101, Transonic) prior to each experiment.

After the end of the study all dogs were killed, and their hearts and the adjacent vessels including the ultrasound flowmeter excised to identify morphological and functional changes of the vessels or the flowmeters.

Additional measurements

Several weeks prior to the implantation of the flow probes both carotid arteries were exteriorised in skin loops for blood pressure recording and blood sampling [4]. For the experiments anaesthesia was induced either with pentobarbital ($20–25\ mg \cdot kg^{-1}$ followed by a continuous infusion of $2–4\ mg \cdot kg^{-1} \cdot h^{-1}$) or with propofol ($3–4\ mg \cdot kg^{-1}$) and maintained with a volatile anaesthetic (enflurane 1.5 MAC). Arterial pressure was measured through a catheter which was advanced through the carotid artery into the thoracic aorta. A pulmonary artery catheter was advanced through a peripheral vein to deliver drugs, to collect mixed venous blood and to measure central venous and pulmonary artery pressures. All catheters were connected to pressure transducers (Statham P-23ID) which were calibrated daily against a mercury manometer and referenced to the atmosphere at the midchest level. Heart rate (HR) was derived from a standard ECG registered from surface electrodes.

All variables were recorded on an eight channel polygraph (model RS 3800, Gould, Cleveland, Ohio) and simultaneously stored on the hard disc of a conventional personal computer for further analysis after analogue to digital conversion with a rate of 400 Hz (POWERLAB, ADInstruments, Castle Hill, Australia).

Calibration of transit-time probes

The flow probes were calibrated in vitro by a constant flow set-up and in vivo by the Fick principle as described below. The in vivo experiments were performed either under baseline conditions (anaesthetised dogs) or during pharmacological interventions to vary cardiac output over a wide range. Cardiac output was increased with norepinephrine ($0.2–0.4\ \mu g \cdot kg^{-1} \cdot min^{-1}$), dopexamine ($1–6\ \mu g \cdot kg^{-1} \cdot min^{-1}$) and nitroglycerine ($10–40\ \mu g \cdot kg^{-1} \cdot min^{-1}$) and decreased by volatile anaesthetics (enflurane, isoflurane or sevoflurane, 1–3 MAC).

In vitro calibration of transit-time probes

The flow probes were calibrated prior to implantation with the help of a gravity-fed constant flow set-up as recommended by the producer [5]. A segment piece of exteriorised dog aorta was mounted within a water bath, lined with cotton wool to absorb acoustic echoes and the probe was positioned around it. The inlet and outlet of the aorta were connected with silicone tubes which were in a horizontal position for at least 50 cm to avoid turbulent flow. A constant flow of degassed saline was achieved with a reservoir bag of 20 l mounted about 0.5–1 m above the organ bath. Flow through the in vitro system was quantified by collecting the outflowing fluid into a measuring cylinder during exactly 1 min and flows thus obtained were compared to those measured by the transit-time probe. Variations in saline flow, from $1–12\ l \cdot min^{-1}$ were achieved by an adjustable external occluder fixed on the inlet tube.

In vivo calibration of transit-time probes

In vivo calibration of flow probes was performed with the help of the direct Fick principle from oxygen uptake ($\dot{V}O_2$, STPD) and the arterial to mixed venous oxygen content difference ($C_{(a-\bar{v})}O_2$). $\dot{V}O_2$ was measured continuously by indirect calorimetry with a Deltatrac II Metabolic Monitor (Datex-Engstrom Division, Instrumentarium, Helsinki, Finland). Before each experiment, the gas sensors were calibrated with air and a gas mixture containing

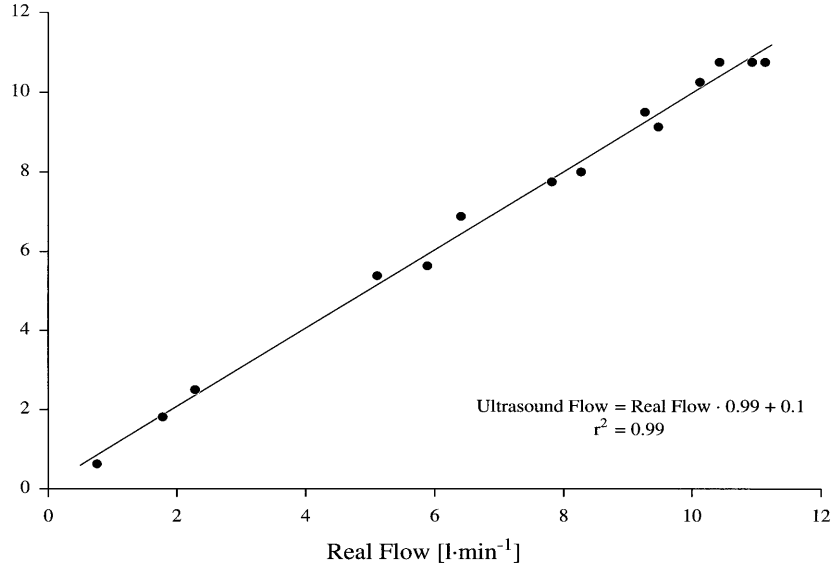

Fig. 1 In vitro calibration of a single flow probe with a gravity-fed constant flow set-up. Note the close correlation ($r^2 = 0.99$) between real flow and that measured by ultrasound flowmetry

95 % O_2 and 5 % CO_2, after a warm-up period of at least 30 min, when minute-by-minute variations in $\dot{V}O_2$ had stabilised at less than 5 %. The measurement of $\dot{V}O_2$ was calibrated by burning 5 ml of pure ethanol (alcohol burning test kit, Datex-Engstrom).

During spontaneous breathing in pentobarbital anaesthetised dogs, $\dot{V}O_2$ was measured with a flow through technique (canopy mode). A plastic hood was fixed above the dog's head and upper trunk allowing room air to enter at the edges and air was sucked continuously by the constant flow generator of the Deltatrac II for analysis. Hood volume of approximately 70 l and a flow generator rate of 40 $l \cdot min^{-1}$ resulted in a time constant of 1.75 min.

During controlled ventilation (volatile anaesthetics), $\dot{V}O_2$ was measured directly from the respiratory gases. The expired air was collected and fed to the mixing chamber of the Deltatrac II (respiration mode, a collection technique with a time constant of 1 min). Care was taken to prevent air leakage in the respiratory system, particularly around the endotracheal tube. $\dot{V}O_2$ measurements were corrected for the dilution of the respiratory gas concentrations caused by the addition of the anaesthetics [6].

The arterial to mixed venous oxygen content difference ($C_{(a-\bar{v})}O_2$) was determined by collecting arterial and mixed venous blood simultaneously. Oxygen content was measured in duplicate with a galvanic cell (Lex-O_2-Con-TL, Lexington Instruments, Waltham, USA) and an accuracy of ± 0.75 %. The instrument was calibrated by injecting a standard volume of dry air. Temperature and barometric pressure corrections were made using a calibration chart supplied by the manufacturer. In our study, calibration of the galvanic cell was considered satisfactory when five consecutive readings for dry air were within ± 0.1 vol.% of the estimated oxygen content. The oxygen content of dry air was measured repeatedly throughout the experiments to ensure that the galvanic cell was air-tight and that the electrical system was functioning properly.

Data analysis

All results are presented as means ± standard error of the mean (SEM). Comparisons between the cardiac output measurements by the transit-time ultrasound flow probes and the Fick principle were performed using the method of Bland and Altman [7] with bias and precision. To compare the in vitro measurements, linear regression analysis was used.

Results

In vitro calibration of transit-time probes

Flow determined by the ultrasound transit-time flow probes was exactly the same as true flow obtained from the saline outflow (Fig. 1). The results correlated closely over a wide range with $r^2 = 0.99 \pm 0.005$. The regression line $y = mx + c$, with y = transit-time blood flow, m = calibration coefficient, x = true flow and c = zero offset, was calculated. Values of m were 0.94 ± 0.07 and the corresponding values of c were 0.067 ± 0.17 ml $\cdot$ $kg^{-1} \cdot min^{-1}$.

In vivo calibration of transit-time probes

Cardiac output determined by the ultrasound flow probes was compared to that obtained from the Fick principle in each dog and the results of all baseline measurements over the entire observation period are depicted in Fig. 2. During baseline conditions, i. e. without any intervention to increase or decrease cardiac output, the flow probes and the Fick principle yielded nearly the same results in some dogs (dogs 1, 3–6), whereas in other animals the flow probes underestimated real flow, as determined by the Fick principle. This underestimation was sometimes rather large, e. g. in one dog only 62 % of real flow was measured. To compensate for this underestimation a correction factor was used if the difference between the two methods was larger than 10 %.

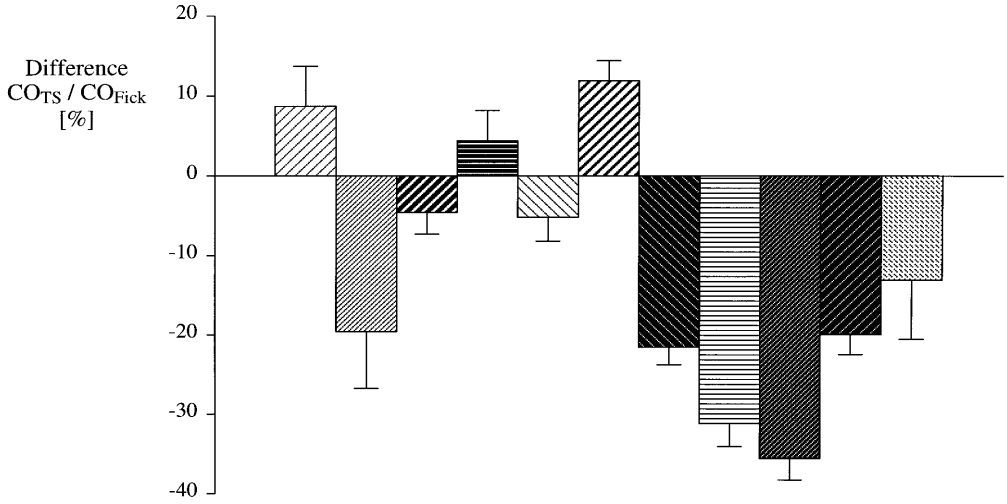

Fig. 2 Difference between cardiac output measured by the ultrasound flow probes compared to the Fick principle under baseline conditions (no interventions to alter cardiac output). Whereas in half the dogs the two methods yielded the same results, cardiac output was underestimated by the flow probes by up to 38 % in the other dogs. Values are means ± SEM, each bar representing all baseline measurements for one dog

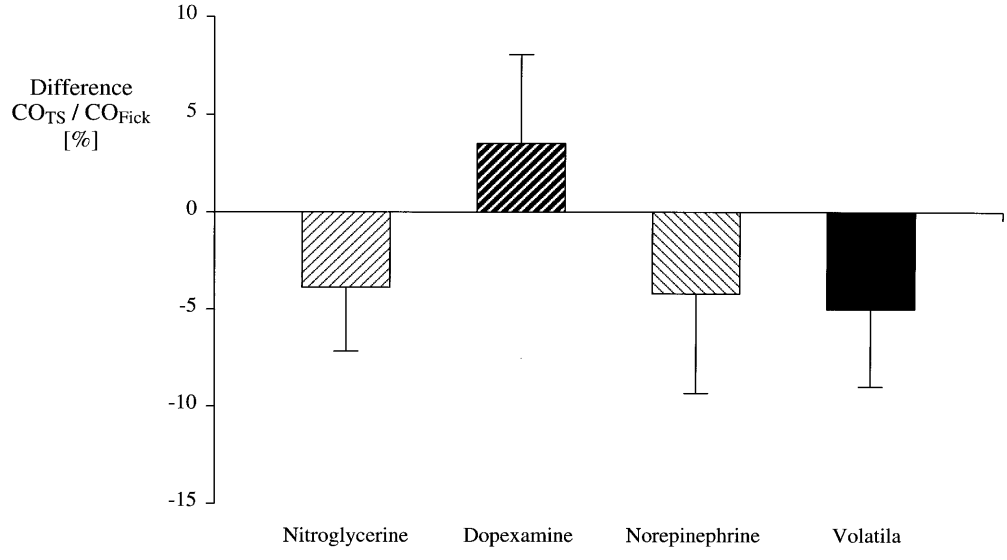

Fig. 3 Cardiac output measured by the Fick principle and ultrasound transit-time flow probes during pharmacological interventions. Cardiac output was increased by nitroglycerine ($n = 12$), dopexamine ($n = 13$) and norepinephrine ($n = 10$) and decreased by volatile anaesthetics ($n = 25$). Cardiac output is measured precisely by the flow probes even during induced changes in cardiac output. Values are means ± SEM

The results after correction of the individual difference between the two methods were compared by the statistical method for assessing the agreement between two methods as described by Bland & Altman. All cardiac output values ($n = 87$) obtained during baseline conditions over the entire observation period ranging from 6 to 47 months showed no mean difference (0.3 ± 12.1 ml · kg^{-1} · min^{-1}) between the two methods, indicating a sufficient correction of the individual animal.

After the baseline measurements, pharmacological interventions were performed to increase cardiac output either by adrenergic stimulation (dopexamine, $n = 13$ and norepinephrine, $n = 10$) or reflexively by lowering arterial pressure (nitroglycerine, $n = 12$). Moreover, cardiac output was reduced by using increasing concentrations of volatile anaesthetics ($n = 25$). Thereby cardiac output was varied between 22 and 180 ml · kg^{-1} · min^{-1}. Note that both methods yielded cardiac output values which differed by less than 5 % regardless of the intervention (Fig. 3).

The results for all measurements during baseline conditions as well as during the interventions are shown in Fig. 4. Again there was almost no difference (–1.1 ± 14.2 ml · kg^{-1} · min^{-1}) between the two methods. The time course of the individual differences between the two methods is exemplified in Fig. 5 for two dogs, one of them without any correction (A) and another dog after correction of flow signals derived by ultrasound transit time by a factor of 1.26 (B). The figure shows two results, first that in both animals the individual differences for a single experiment were in the range of ± 20 % and, second, that these differences vary

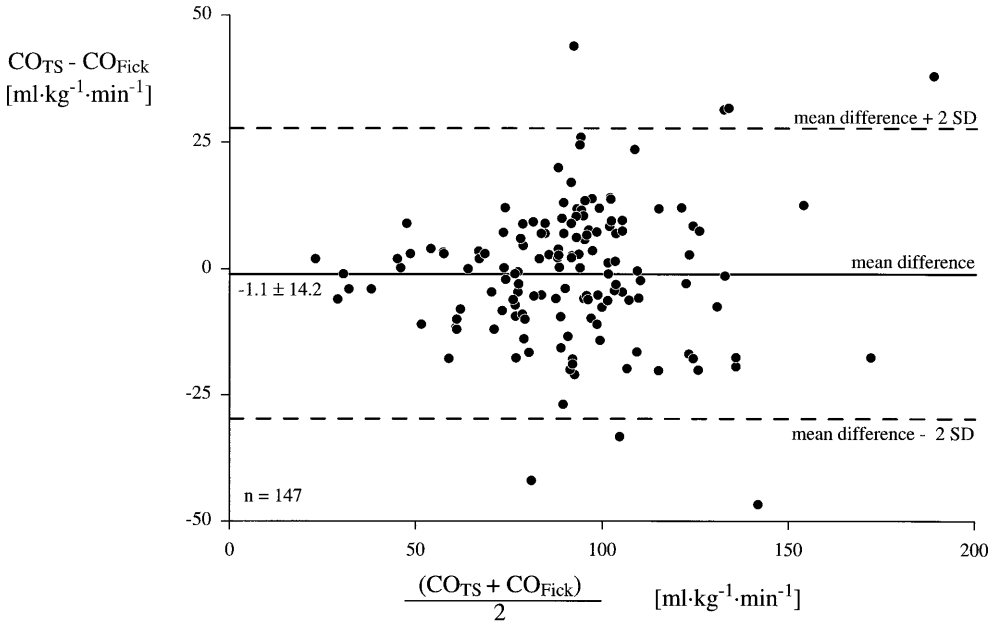

Fig. 4 Cardiac output measured by the Fick principle and ultrasound transit-time flow probes during baseline conditions and during pharmacological interventions. Bias and precision of both methods, after a correction for the individual difference, were obtained. Note that both methods then yielded exactly the same flows, regardless of the intervention

around zero without any time relation. In general, the mean difference between the two methods over time for each dog ranged from –9.7 to 3.7 % with a coefficient of variation of the individual correction factors of 12.8 ± 1.3 % (mean ± SD). Thus, ultrasound flow probes measure cardiac output precisely after in vivo calibration for several years, regardless of the intervention.

Complications and post-mortem findings

Three dogs included in our study died because of complications with the flow probes. Two of these died because of pulmonary artery rupture, one dog about 2 weeks after the implantation and the other dog about 6 months after rethoracotomy and changing of the flow probe. The third dog died of kinking of the flow probe 14 months after implantation, probably because the flow probe chosen was too large in relation to vessel size. In all the other dogs no macroscopic evidence of vessel damage, kinking or lumen occlusion was found. All flow probes were totally encapsulated without any signs of destruction of the reflector or the probe body. Implantation duration on the pulmonary artery ranged from 2 weeks (pulmonary artery rupture, not included in the study) to more than 47 months and the dogs tolerated the flow probes without any sign of discomfort.

Discussion

This study was performed to evaluate the accuracy and reproducibility of long-term implanted ultrasound transit-time flow probes around the pulmonary artery in dogs. We provide experience over a mean period of 22 months (range 6–47 months), a duration of implantation which has never been reported before.

Our main finding was that the ultrasound flow probes need to be recalibrated in vivo despite accurate calibration before implantation, but when a correction for the difference is applied they measure cardiac output precisely over a long period.

Critique of methods

Our results rely on the premise that the direct Fick principle measures cardiac output precisely. This method calculates cardiac output according to the formula $CO = \frac{\dot{V}O_2}{C_{(a-\bar{V})}O_2}$ and is based on the assumption that $\dot{V}O_2$ and $C_{(a-\bar{V})}O_2$ are in a steady state [8]. To achieve this goal values were only obtained when minute by minute variations in $\dot{V}O_2$ were less than 5 %. Moreover, it is necessary to measure both variables as accurately as possible to increase the precision of the measurement. We therefore measured the arterial and mixed venous oxygen content directly rather than calculating it from blood samples as a product of multiple single variables (oxygen content = 1.34 Hb · SaO_2 + 0.028 PO_2) with individual errors which would multiply according to the law of error propagation.

For every measurement we calculated the maximum error according to the law of error propagation: the accuracy of the oxygen content analyser is ± 1 % for a sin-

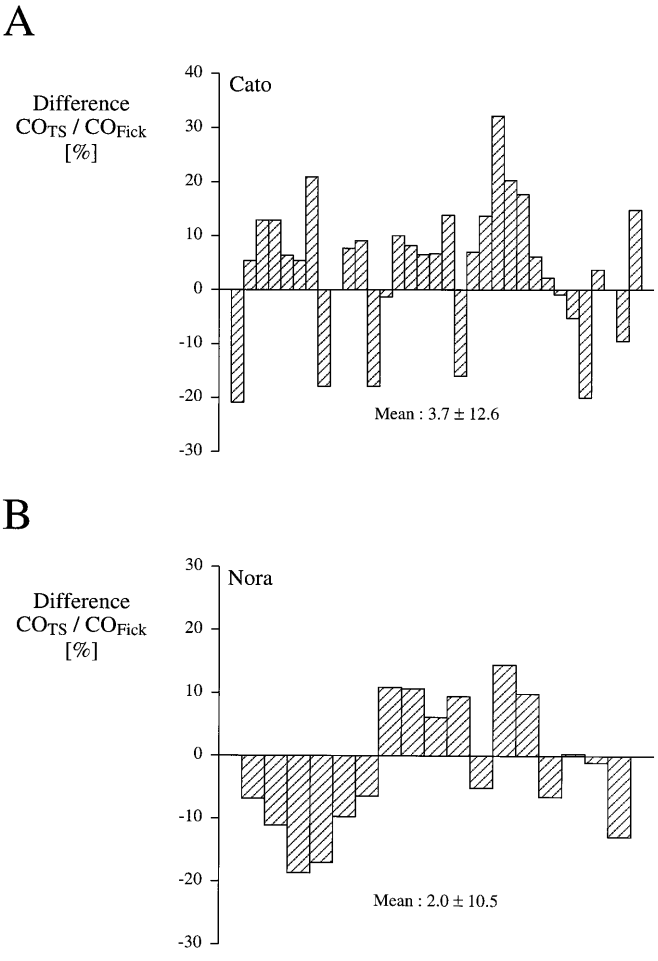

Fig. 5 Difference between cardiac output measured by the Fick principle and ultrasound transit-time flow probes for two dogs over the entire observation period, one of them without any correction (A) and another dog after correction of the flow signals derived by ultrasound transit time by a factor of 1.26 (B). Each bar represents a single experiment. Flow probes were implanted for 47 months (A) and 37 months (B)

gle measurement and ± 0.75 % for the mean of two measurements [9]. Hence, the 95 % confidence interval for an arterial to mixed-venous sample is within ± 1.5 % (± 2 SD) of the measured value and the error of the calculated arteriovenous oxygen content difference sums up to 6 % (2 × 3 %). $\dot{V}O_2$was measured with the Deltatrac II and a precision of 4 %. The precision is independent of the collection mode, flow through or canopy [10, 11, 12] and not influenced by the addition of volatile anaesthetics, if a correction for the exhaled concentration of the anaesthetic is made [6]. Thereby the resultant maximum error of the cardiac output determination by the Fick principle sums up to 10 % (6 % + 4 %) which is sufficiently precise to calibrate the ultrasound flowmeters and to serve as a reference method.

Of note, lung oxygen consumption, which is not detected by the Fick measurements but is detected with the Deltatrac II, is negligible in healthy dogs [13].

Discussion of results

Transit-time flow probes are widely used for the measurement of regional blood flow in many organs as well as of cardiac output. However, there are only a few studies which have examined their accuracy after chronic implantation when ingrowth of fibrous tissue has occurred within the acoustic window and around the flow probes. Only relatively short periods of about 3 months have been observed [2]. In accordance with other studies [2, 14] the in vitro calibration yielded a close correlation between the real flow and that measured by the ultrasound flow probes, indicating good in vitro accuracy. However, the in vivo calibrations differed from those results because the flowmeters underestimated real flow in about half the dogs, whereas in the others the Fick principle and flowmeters agreed to an acceptable degree (< 10 %). An underestimation of real flow has also been observed for probes mounted on the aorta [15] or the portal vein [16] and has even been observed in sheep with pulmonary artery flow probes [2].

In contrast to these results are findings of a good agreement between cardiac output measured by ultrasound flowmetry and a reference method in acute and in chronic experiments [17, 18]. However, most of these comparisons were made with indicator dilution methods, which sometimes overestimate cardiac output and have poor precision [19, 20, 21, 22, 23] and are therefore unsuitable for the calibration of a more precise method like ultrasound flowmeters. The inconsistent results from the literature as well as our discrepancies might be explained by a non-perpendicular fit of the probe to the vessel, which is likely because it was not present in all the dogs and, when this occurred, the difference was stable over several years. We can only speculate about the mechanism for this underestimation: the ultrasonic beam intersects the vessel twice on its reflective pathway. During each intersection, the transit time of the beam is modified by a vector component which is directly proportional to the flow. However, if any deviation from this perpendicular fit occurs this vector is less modified, resulting in a smaller modification and a lower calculated flow despite the fact that the true flow is unchanged. Thereby a non-perpendicular fit of the flow towards the vessel results in an underestimation of true cardiac output. Additionally, after the end of the study, during reoperation of the dog with the largest underestimation of real cardiac output, we could verify a non-perpendicular position of the probe towards the vessel axis. In conclusion, ultrasound flow probes underestimate

cardiac output not per se but only if a non-perpendicular fit of the probes occurs.

After correction for the difference the two methods agree well during baseline conditions as well as during pharmacologically induced changes in cardiac output over a wide range. We therefore conclude that ultrasound transit-time flowmeters measure cardiac output over a wide range of cardiac output precisely, regardless of the intervention.

In recent years the manufacturer has developed the "A probes" which should overcome the problems of underestimating cardiac output occurring with the "S probes" by illuminating the vessel with four crystals. Although the relative accuracy of the "A probes " tends to be higher, it is advocated that they also be calibrated in vivo and then they have the same accuracy as the "S probes" (2%) according to the manufacturer. "A probes" were recently validated in vivo using a calibrated roller pump for right to left atrium bypass, and even in this semi in vitro experiment the difference between aortic flow and roller pump were in the range we observed with our in vivo measurements with the "S probes" [3].

We have only minor experience with the "A probes": we implanted one in one dog only, but it became dislodged from the pulmonary artery only a few weeks after implantation. Reimplantation was not possible because aorta and pulmonary artery had totally grown together and could not be separated adequately. In contrast to the "A probe", we reimplanted new "S probes" in several dogs by only exchanging the probe body and leaving the reflector in place without any complications, even after several years. From our point of view, therefore, we can not advocate the use of "A probes" if long-lasting implantation is required.

Taken together, we have shown that transit-time ultrasound flow probes need to be calibrated in vivo but, thereafter, they measure cardiac output precisely over several years in a wide range of cardiac output, independent of the intervention.

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REGIONAL ANESTHESIA
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Endogenous Endothelin and Vasopressin Support Blood Pressure During Epidural Anesthesia in Conscious Dogs

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We studied whether endogenous endothelin, like endogenous vasopressin, helps to maintain blood pressure during high epidural anesthesia when efferent sympathetic drive is diminished. On different days, six awake dogs underwent each of the following five interventions: blockade of vasopressin V_{1a} receptors using [$d(CH_2)_5Tyr(Me^2)$]AVP, (40 μg/kg) or endothelin receptors using tezosentan (3 mg/kg followed by 3 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) with or without epidural anesthesia (1% lidocaine, intraindividual dose did not differ between experiments), and epidural saline ($n = 5$). The effects of endothelin- or vasopressin-receptor blockade were analyzed (means $\pm$ SEM) and compared by an analysis of variance for repeated measures (paired Student's t-test, α-adjusted, $P < 0.05$). Vasopressin-receptor blockade decreased blood pressure (10 $\pm$ 2 mm Hg) only in the presence of epidural anesthesia, whereas endothelin-receptor blockade reduced blood pressure both in the presence and absence of epidural anesthesia (12 $\pm$ 3 versus 10 $\pm$ 1 mm Hg). During baseline and each intervention, plasma concentrations of vasopressin and big-endothelin were measured and compared by a Wilcoxon's rank sum test; $P < 0.05$. Vasopressin concentrations increased during epidural anesthesia and after additional endothelin receptor blockade, but big-endothelin concentrations remained unchanged during each intervention. We conclude that vasopressin acts as a reserve system, as it stabilizes blood pressure specifically during epidural anesthesia, whereas the unchanged concentrations of big-endothelin indicate that the endothelin system is not specifically activated to support blood pressure during epidural anesthesia.

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During resting conditions the sympathetic nervous system and the renin-angiotensin system predominate in the maintenance of blood pressure. The blood concentrations of the vasoactive peptides endothelin and vasopressin are small, so they can be characterized as a reserve system of blood pressure control. In contrast, during high epidural anesthesia widespread sympathetic blockade occurs, leading to a depression of the renin-angiotensin system (1,2), thus eliminating the two major vasoconstrictor mechanisms. Accordingly, during epidural anesthesia, endogenous vasopressin and endothelin are capable of maintaining blood pressure. Although the release of endogenous vasopressin is a key mechanism to maintain blood pressure during epidural anesthesia (2), the role of endothelin is unknown. However, in view of the increased endothelin concentrations during hypotensive challenges induced by hypovolemia (3), mesenteric ischemia (4), or pharmacologically (5), it is likewise plausible that endothelin contributes to the maintenance of blood pressure during epidural anesthesia. Therefore, we hypothesized that during epidural anesthesia the release of endogenous endothelin supports blood pressure in a similar fashion as vasopressin. To test this we studied the cardiovascular response to blockade of either endothelin or vasopressin receptors in combination with epidural anesthesia in awake dogs and compared these effects with those elicited by a sole blockade of either endothelin or vasopressin receptors.

Methods

The data derive from 29 experiments on 6 trained dogs (foxhounds weighing 28–35 kg) studied with approval of the District Governmental Animal Investigation Committee and treated in accordance with the Guiding Principles on the Care and Use of animals of the American Physiologic Society.

Several weeks before the experiments the dogs were operated under general anesthesia (enflurane/nitrous oxide + fentanyl) and sterile conditions. For blood pressure recording and blood sampling, both carotid

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arteries were exteriorized in skin loops. Ultrasound transit-time flow transducers (T101, Transonic Systems, Ithaca, NY) were implanted around the pulmonary artery for the continuous recording of cardiac output (CO) and calibrated *in vivo* at least three weeks after implantation as previously described (6). During convalescence the dogs were trained to lie quietly and unrestrained on their right side and to become familiar with the experimenters and the laboratory.

During the experiments the following variables were recorded continuously on an eight-channel polygraph (RS 3800, Gould Inc., Cleveland, OH) and simultaneously stored on the hard disk of a conventional personal computer for further analysis after analog-to-digital conversion with a rate of 1 kHz per channel.

Mean arterial pressure (MAP) and central venous pressure (CVP) were measured electromanometrically (Statham P-23ID; Elk Grove, IL) through catheters in the carotid artery and in the superior caval vein. Correct position of the central venous catheter, which was advanced from the animal's hind limb, was checked by fluoroscopy and adequacy of the venous pressure curve. The electromanometers were referenced to the processus spinosus of the seventh vertebra and calibrated with a mercury manometer. CO was measured continuously and systemic vascular resistance (SVR) was calculated as quotient of (MAP − CVP) and CO. We also measured (intermittently) arterial blood gas tensions, O_2 saturation, and pH (ABL3®, Radiometer, Copenhagen, Denmark).

To determine the plasma concentrations of vasopressin, endothelin, and big-endothelin, arterial blood samples were collected during baseline and at the end of each intervention (epidural anesthesia, receptor blockade) in chilled EDTA-tubes and placed immediately in crushed ice. Within 10 min plasma was separated by centrifugation and stored at −20°C until analysis. Vasopressin was measured by means of radioimmunoassay (^{125}I Vasopressin, Bühlmann Laboratories AG, Allschwil, Switzerland) using rabbit anti-vasopressin antiserum. The minimum detectable dose was calculated to be 0.35 ng/L. Of note, vasopressin concentrations could not be measured after injection of the V_{1a}-receptor antagonist because the blocker interferes with the test assay. Endothelin and big-endothelin, a sensitive measure of endothelin system activation (7), were measured by means of enzyme immunoassay (Biomedica, Boston, MA) using immunoaffinity purified polyclonal capture antibody and a monoclonal detection antibody. The detection limit for endothelin and big-endothelin is 0.05 pmol/L and 0.025 pmol/L, respectively.

At least 2 h before each experiment an epidural catheter was introduced percutaneously into the epidural space (usually between L5 and L6) through a 16-gauge Tuohy needle under sterile conditions during short-term sedation with propofol (3–4 mg/kg). The catheter was advanced rostrally into the epidural space and sutured to the skin. Exact position of the catheter tip (approximately T_{10}) was verified by fluoroscopy with contrast medium, and care was taken that in successive experiments the catheter position did not vary for more than one intervertebral space in one animal.

Lidocaine 1% was injected into the epidural space; the volume injected depending on the dog's length and individual spread of nerve block. Although the volume of lidocaine differed slightly between dogs (9–13 mL), it was identical in each animal in successive experiments. Cranio-caudal spread of epidural anesthesia was assessed as described previously (1,2) by paresis of the nictitating membrane of the eyes, absence of blood pressure increase to bilateral occlusion of the exteriorized carotid arteries (duration 45 s), sensory blockade up to the lower neck region, complete motor blockade of the hind limbs at the end of the experiments, and changed mode of respiration from a thoracic to a diaphragmatic pattern, indicating at least partial blockade of the intercostal musculature.

Endothelin was prevented from acting at its receptors by injecting tezosentan (3 mg/kg, followed by 3 $mg \cdot kg^{-1} \cdot h^{-1}$, Actelion Ltd., Allschwil, Switzerland), an endothelin receptor antagonist with high affinity to ET_A and ET_B receptors (8). Vasopressin(V_{1a})-receptors were blocked by [*d*$(CH_2)_5$Tyr(Me^2)]AVP (V-2255; Sigma Chemicals) (9) at a dose of 40 μg/kg.

Completeness of receptor blockade was assessed by injecting 2.5 and 5 μg of endothelin-1 (E-7764; Sigma Chemicals) or 200 and 400 mU of Arg-Vasopressin (V-0377; Sigma Chemicals), respectively, after the experiments. MAP increased after injection of 5 μg of endothelin-1 by 22 ± 1 mm Hg and remained constant (1 ± 3 mm Hg) after previous receptor blockade. In parallel, 400 mU vasopressin increased MAP by 20 ± 2 mm Hg without and −2 ± 2 mm Hg after blocking of the vasopressin receptors.

All experiments were performed with the awake dogs in the basal metabolic state (food withheld for 12 h with free access to water) and under standardized experimental conditions (dogs lying on their right side, lightly dimmed laboratory at thermoneutral temperature for dogs of 24°C) always beginning at 8 AM. During the experiments the dogs remained unrestrained on their right side on a cushioned table.

After connecting the animals to the recording system, we waited for approximately 30 min until all variables had reached a steady state as the animals calmed down. The actual experiments started with baseline measurements for a further 30 min. Thereafter, the dogs were randomly assigned to one of the following interventions:

1. Endothelin receptor blockade alone ($n = 6$). Tezosentan was injected to assess the role of endothelin during resting conditions.
2. Vasopressin receptor blockade alone ($n = 6$). The V_{1a}-blocker was injected to assess the role of vasopressin during resting conditions.
3. Epidural anesthesia followed by endothelin receptor blockade ($n = 6$). Lidocaine 1% (9–13 mL) was injected into the epidural space over 5 min and the data were recorded for further 30 min, i.e., for a time sufficient to allow full spreading of epidural blockade. Thereafter, the endothelin receptor antagonist was injected as described above.
4. Epidural anesthesia followed by vasopressin receptor blockade ($n = 6$). After epidural injection of lidocaine 1% as described above, the V_{1a}-blocker was injected IV.
5. Sham epidural anesthesia ($n = 5$). Saline (9–13 mL) was injected into the epidural space to exclude time-related changes during the experiments for the duration of 30 min.

After these interventions all variables were recorded for further 20 min. An interval of at least 2 wk was interspaced between successive experiments in one and the same animal.

Data for all results are given as means ± SEM. Comparisons were made by an analysis of variance for repeated measures (ANOVA), followed by a paired Student's *t*-test and α-adjusted according to the Bonferroni procedure. The change in blood pressure after vasopressin or endothelin receptor blockade was compared by a paired Student's *t*-test. Plasma concentrations of endothelin, big-endothelin and vasopressin were compared by Wilcoxon's rank sum test. A P value < 0.05 was considered statistically significant.

Results

In general, endogenous endothelin maintains MAP during resting conditions and during epidural anesthesia, whereas endogenous vasopressin stabilizes MAP only during epidural anesthesia. This is detailed in Figure 1 in which the time course of MAP is summarized over the entire experimental period. First, baseline measurements apparently did not differ between study groups, which is a prerequisite for meaningful comparison. During resting conditions (Fig. 1A), MAP declined for approximately 10 mm Hg after endothelin receptor blockade, whereas it remained unchanged after vasopressin receptor blockade. During epidural anesthesia (Fig. 1B) MAP decreased similarly in the V_{1a}- and Tezosentan group after injection of the respective blocker. In detail, starting from the same baseline, MAP decreased for approximately 20 mm Hg with the onset of epidural anesthesia and

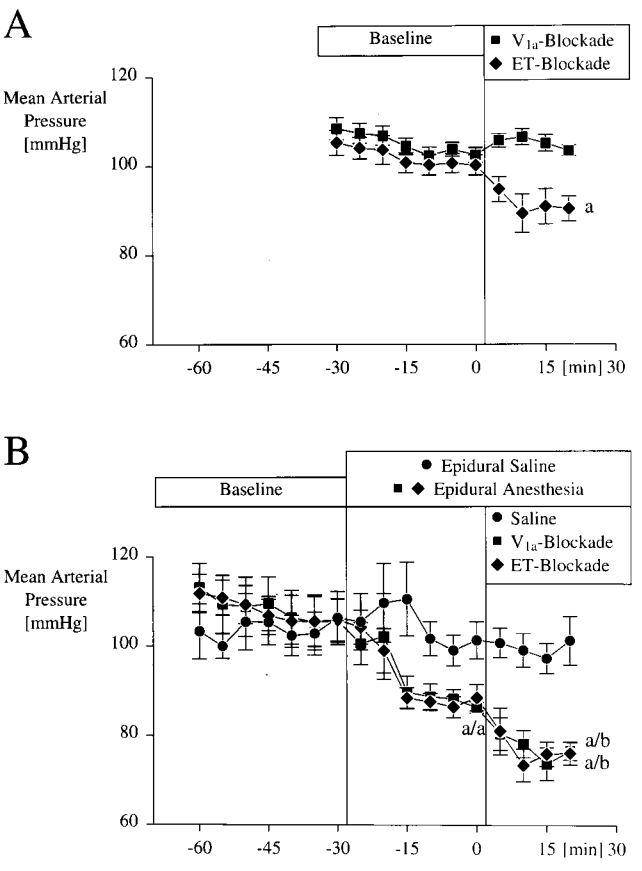

Figure 1. Time course of blood pressure during physiological conditions (A) and during epidural anesthesia (B) after injection of either an endothelin or a vasopressin receptor antagonist. Means ± SEM from six awake dogs in each group and five dogs in the "epidural saline" group. a indicates $P < 0.05$ against baseline; b indicates $P < 0.05$ against epidural anesthesia.

for a further 10 mm Hg during injection of the blockers. In contrast, MAP was almost unchanged throughout the experiment during sham epidural anesthesia (epidural saline).

Vasopressin, big-endothelin, and endothelin plasma concentrations (Table 1) mirror the course of MAP. Vasopressin concentrations increased during epidural anesthesia, and increased further as MAP decreased after additional endothelin receptor blockade. In contrast, big-endothelin concentrations remained unchanged during all interventions and only endothelin concentrations increased after injection of tezosentan (with or without epidural anesthesia).

The effects on MAP elicited by endothelin receptor blockade in the presence or absence of epidural anesthesia are summarized in Table 2, and it is apparent that endothelin supports MAP during both conditions, whereas vasopressin supports MAP only during epidural anesthesia. After the injection of the endothelin receptor blocker, MAP decreased from 100 ± 2 mm Hg to 90 ± 3 mm Hg in the absence of epidural anesthesia and from 88 ± 3 mm Hg to 76 ± 3 mm Hg

Table 1. Plasma Concentrations

	Endothelin (fmol/mL)	big-Endothelin (fmol/mL)	Vasopressin (ng/L)
ET-Blockade ($n = 6$)			
Baseline	0.4 ± 0.1	0.6 ± 0.2	0.9 ± 0.2
ET-Blockade	3.8 ± 0.8*	0.4 ± 0.1	1.2 ± 0.2
Baseline	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.2
EPID	0.4 ± 0.1	0.3 ± 0.1	6.1 ± 3.1*
EPID + ET-Blockade	5.7 ± 1.5*†	0.2 ± 0.1	12.0 ± 3.9*†
V_{1a}-Blockade ($n = 6$)			
Baseline	0.1 ± 0.0	0.2 ± 0.2	0.9 ± 0.2
V_{1a}-Blockade	0.2 ± 0.0	0.2 ± 0.1	NM
Baseline	0.2 ± 0.1	0.5 ± 0.3	0.8 ± 0.2
EPID	0.3 ± 0.1	0.2 ± 0.1	1.5 ± 0.4*
EPID + V_{1a}-Blockade	0.1 ± 0.0	0.2 ± 0.1	NM
Sham epidural ($n = 5$)			
Baseline	0.2 ± 0.1	1.3 ± 0.6	1.0 ± 0.2
Sham EPID	0.2 ± 0.1	1.2 ± 0.7	1.0 ± 0.2
Sham EPID + saline	0.1 ± 0.1	1.1 ± 0.6	0.6 ± 0.3

Values are mean ± SEM.
ET = endothelin; V_{1a} = vasopressin; EPID = epidural anesthesia; NM = not measurable.
* $P < 0.05$ versus baseline; † $P < 0.05$ versus EPID.

Table 2. Hemodynamic Variables

	MAP (mm Hg)	CO ($mL \cdot kg^{-1} \cdot min^{-1}$)	CVP (cm H_2O)	HR (bpm)	SVR ($mm\ Hg \cdot min \cdot L^{-1}$)
ET-Blockade					
Baseline	100 ± 2	91 ± 3	3 ± 1	79 ± 3	35 ± 3
ET-Blockade	90 ± 3*	91 ± 6	3 ± 1	87 ± 6	32 ± 2*
Baseline	106 ± 5	81 ± 7	4 ± 1	71 ± 5	43 ± 5
EPID	88 ± 3*	81 ± 4	2 ± 2	90 ± 5*	34 ± 2*
EPID + ET-Blockade	76 ± 3*†	70 ± 7†	2 ± 3	87 ± 3	35 ± 4
V_1-Blockade					
Baseline	102 ± 2	90 ± 7	2 ± 1	80 ± 2	37 ± 3
V_{1a}-Blockade	103 ± 1	88 ± 9	3 ± 1	71 ± 5*	39 ± 4
Baseline	106 ± 5	86 ± 8	4 ± 1	79 ± 6	40 ± 6
EPID	87 ± 1*	76 ± 5	1 ± 1	94 ± 5	35 ± 3
EPID + V_{1a}-Blockade	79 ± 3*†	79 ± 5	1 ± 1	96 ± 5*	30 ± 3*†
Sham epidural ($n = 5$)					
Baseline	106 ± 6	87 ± 4	3 ± 1	64 ± 1	39 ± 4
Sham EPID	101 ± 4	85 ± 5	2 ± 1	61 ± 3	39 ± 4
Sham EPID + saline	101 ± 6	86 ± 6	3 ± 1	62 ± 2	38 ± 4

Values are mean ± SEM.
MAP = mean arterial pressure; CO = cardiac output; CVP = central venous pressure; HR = heart rate; SVR = systemic vascular resistance; ET = endothelin; V_{1a} = vasopressin; EPID = epidural anesthesia.
* $P < 0.05$ versus baseline; † $P < 0.05$ versus EPID.

in its presence ($P < 0.05$ for both conditions). Although this decrease in MAP during resting conditions was mainly a result of a 10% decrease of SVR from 35 ± 3 $mm\ Hg \cdot min \cdot L^{-1}$ to 32 ± 2 $mm\ Hg \cdot min \cdot l^{-1}$ ($P < 0.05$), a 10% reduction of CO from 81 ± 4 $mL \cdot kg^{-1} \cdot min^{-1}$ to 70 ± 7 $mL \cdot kg^{-1} \cdot min^{-1}$ mainly accounted for the decrease of MAP during epidural anesthesia ($P < 0.05$). In contrast, V_{1a} receptor blockade decreased MAP from 87 ± 1 to 79 ± 3 mm Hg only in the presence of epidural anesthesia; this was a result of a reduction of SVR ($P < 0.05$) at an unchanged CO. The injection of the V_{1a} receptor blocker during resting conditions did not change any of these hemodynamic variables.

For comparison of the effect size on MAP elicited by the blockers, relative changes to the respective control are given in Figure 2. MAP did not change after V_{1a} receptor blockade but did so in all the other groups to a similar extent.

Discussion

Our results show that during epidural anesthesia both endogenous endothelin and vasopressin contribute to

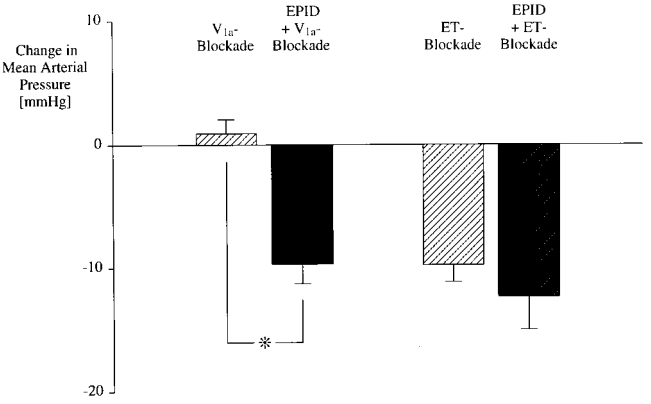

Figure 2. Changes in blood pressure from the respective baseline values after injection of the vasopressin- (V_{1a}) or the endothelin (ET) receptor antagonist. Values are means ± SEM from six awake dogs. Note that blood pressure deceased to almost the same extent after endothelin or vasopressin receptor blockade during epidural anesthesia (EPID) as well as after sole endothelin receptor blockade.

the same extent to the maintenance of MAP, whereas during resting conditions mainly endothelin supports MAP. However, the increase in vasopressin plasma concentrations during epidural anesthesia and the unchanged big-endothelin concentrations indicate that endogenous vasopressin supports MAP specifically during epidural anesthesia.

Our conclusion rests primarily on the tenable premises of a sufficient blockade of either the endothelin or the vasopressin system and the presence of almost complete and comparable sympathetic denervation in the animals.

There is little information in the literature about the amount of tezosentan required to obtain complete blockade of the endothelin receptors in dogs. However, like others (10) we observed a substantial increase in MAP after IV injection of endothelin-1, whereas in our experiments MAP remained unchanged after the same dose of endothelin-1 during endothelin receptor blockade. Moreover, the dosage used in our study is nearly identical to that used by others to completely block endothelin receptors in dogs (11,12).

Vasopressin is a very potent vasoconstrictor even at "physiological" concentrations. These effects are mediated via V_{1a} receptors that are selectively blocked by the antagonist used in our study (13). This is additionally confirmed by the absence of any vasopressor response after injection of 200–400 mU of arg-vasopressin in the presence of the antagonist used here, whereas without this antagonist blood pressure increased by 20 mm Hg with a corresponding decrease in heart rate (2). Therefore, from a methodological point of view, the chosen dosages of the vasopressin and the endothelin antagonists used in our study should have been appropriate to block the respective receptors sufficiently.

Epidural anesthesia almost eliminated sympathetic activity as already discussed (see Methods). Sympatholysis should have been nearly complete, but even if sympathetic activity was not completely blocked this effect was identical in each individual animal, and it would only reduce the quantity but not the quality of the effects.

Thus, our study design was appropriate to detect changes in MAP related to the injection of either the endothelin or the vasopressin receptor blocker in the presence or absence of epidural anesthesia.

The endothelins are a group of potent vasoconstrictive peptides that is comprised of 21 amino acids. Until now three different isoforms (endothelin 1–3) have been identified, of which endothelin-1 is the original endothelin that is produced exclusively by endothelial cells. Within the synthesis of endothelin-1 several precursor peptides are involved, including big-endothelin, which is further cleaved to endothelin-1. The physiological role of endogenous endothelin in the maintenance of blood pressure is unclear because, for instance, in anesthetized dogs endothelin receptor blockade did not change blood pressure (14). However, it is now accepted that endogenous endothelin does contribute to blood pressure regulation under resting conditions (15), as blockade of its receptors decreased blood pressure in healthy volunteers (16) as well as in awake dogs (17). Consistent with our experiments, this was accompanied by a decrease in SVR as a measure of vasomotor tone. This may be explained by the fact that resistance arteries, which are the main determinant of vasomotor tone, are particularly sensitive to the effects of endothelin (18). Compared with the vasoactive properties of endothelin, vasopressin is less potent but nevertheless causes vasoconstriction even at "physiological" concentrations (19,20). However, under these conditions, most of its direct vascular actions are buffered by baroreflexes and are only unmasked after baroreceptor denervation (19) or after destruction of the central nervous system (21). In accordance with our results, sole blockade of the V_{1a}-receptors failed to exert demonstrable cardiovascular effects not only in dogs (22) but also in humans (23). Thus, endogenous endothelin, but not vasopressin, contributes substantially to maintain blood pressure during resting conditions.

These resting conditions differ substantially from the situation during epidural anesthesia. Concomitant with the reduction of sympathetic tone during epidural anesthesia the renin angiotensin system does not counterbalance the hypotension associated with epidural anesthesia, as seen by the absence of any increase in renin concentration during epidural anesthesia (1,2). Thus, during epidural anesthesia, endothelin as well as vasopressin could act as a reserve system for blood pressure control. The role of vasopressin was unmasked by the injection of the V_{1a}-blocker, which elicited a reduction in SVR resulting in a substantial

decrease of MAP at an unchanged CO. This is in accordance with the view that during hypotensive challenges vasopressin is released, as indicated by the increase in vasopressin plasma concentrations in our experiments not only during epidural anesthesia but also during hemorrhage (24,25), when it acts as a vasopressor (26). Vasopressin plasma concentrations increased in our experiments and thus mirrored the reduction of MAP, as indicated by the largest vasopressin concentrations during a combination of epidural anesthesia and endothelin receptor blockade in association with the lowest MAP. Several receptor sites, like cardiopulmonary afferents, are sensitive to heart volume (24) as well as arterial baroreceptors (27) and thus are involved in the control and release of endogenous vasopressin. Nevertheless, the increased vasopressin concentrations in our study were most probably driven by unloading of arterial baroreceptors, as shown by the absence of this effect after sinoaortic denervation during graded hypotension (27) in contrast to an unchanged increase in vasopressin after sole cardiopulmonary denervation.

During epidural anesthesia, the role of endogenous endothelin to support MAP seems to be similar to vasopressin. Again, this was unmasked after injection of the respective antagonist, which reduced MAP slightly larger in the presence of epidural anesthesia than in the absence. However, there are substantial differences compared to the effects of vasopressin. Whereas MAP decreased after injection of the vasopressin blocker only in the presence of epidural anesthesia, endothelin receptor blockade elicited almost the same reduction in MAP in the presence as well as in the absence of epidural anesthesia. Surprisingly, endothelin did not support MAP to a much larger extent during epidural anesthesia than during resting conditions, as did vasopressin. This is in accordance with the unchanged concentrations of big-endothelin during the experiments, whereas the increase in endothelin concentration after endothelin receptor blockade most probably indicates displacement of endothelin from the receptors by tezosentan (15). This interpretation is likewise supported by the unchanged endothelin and big-endothelin concentrations during epidural anesthesia with concomitant vasopressin receptor blockade, although the reasons for the unchanged endothelin concentrations are unclear. In contrast to these results, hypotensive challenges induced pharmacologically (5), by hypovolemia (3), or by mesenteric ischemia (4) elicited increases in endothelin plasma concentrations, indicating that probably the integrity of the sympathetic nervous system is required to elicit increases in endothelin concentrations to counterbalance hypotensions. Nevertheless, endothelin receptor blockade elicited a decrease in MAP during epidural anesthesia and during resting conditions. This reduction in MAP was almost the same during both interventions, but was caused mainly by a decrease in CO during epidural anesthesia in contrast to a decrease in SVR during resting conditions.

In addition to the physiological understanding of blood pressure control during epidural anesthesia, the results of our study are of potential interest to clinicians because endothelin- and perhaps vasopressin receptor antagonist are on the rise as antihypertensive drugs. Accordingly, if an epidural anesthesia is performed within patients receiving such drugs, they are likely to expect a more severe hypotension.

Regardless of these speculations, we showed for the first time that during epidural anesthesia both endothelin and vasopressin contribute to the same extent to the maintenance of MAP, whereas during resting conditions endothelin, but not vasopressin, supports MAP. However, the different response of the endothelin and vasopressin plasma concentrations indicate that the endothelin system does not specifically support MAP during epidural anesthesia, whereas vasopressin acts as a reserve system for blood pressure control because it stabilizes MAP specifically during epidural anesthesia.

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BJA

Comparison of the role of endothelin, vasopressin and angiotensin in arterial pressure regulation during sevoflurane anaesthesia in dogs

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Background. In this study we aimed to clarify the role of endothelin in arterial pressure regulation during anaesthesia with increasing concentrations of sevoflurane (1–3 MAC) and compare it with those of vasopressin and angiotensin.

Methods. After an awake control period, on different days, six dogs underwent each of the following four interventions: sevoflurane anaesthesia alone (1–3 MAC), sevoflurane after block of either endothelin receptors using tezosentan (3 mg kg^{-1} followed by 3 mg kg^{-1} h^{-1}), vasopressin V_{1a} receptors using [$d(CH_2)_5Tyr(Me^2)$]AVP (40 μg kg^{-1}) or angiotensin receptors using losartan (6 mg kg^{-1} h^{-1}). Plasma concentrations of endothelin, big endothelin, vasopressin and renin were measured. Effects of sevoflurane in the presence and absence of the respective receptor block were analysed and compared using analysis of variance for repeated measures (ANOVA followed by Fisher's PLSD (protected least significant difference) ($P<0.05$)).

Results. Mean arterial pressure decreased in a dose-dependent manner with sevoflurane during all interventions. At 1 MAC, this decrease was greatest during angiotensin receptor block (mean (SEM), –41 (3) mm Hg), intermediate during vasopressin and endothelin receptor block (–31 (4) and –30 (2) mm Hg respectively), and least during sevoflurane alone (–24 (3) mm Hg). The course of systemic vascular resistance mirrored the course of arterial pressure, while cardiac output did not differ between groups. Plasma concentrations of endothelin, big endothelin and renin did not change during any intervention, whereas vasopressin concentration increased from ~0.5 to 40 ng $litre^{-1}$ at 3 MAC as arterial pressure decreased in all groups.

Conclusions. At 1 MAC, angiotensin attenuated the decrease in arterial pressure during sevoflurane anaesthesia more than endothelin and vasopressin. However, at higher MAC only vasopressin was specifically activated to partly compensate for the arterial pressure decrease.

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Since the discovery of the endothelins[1] as the most potent vasoconstrictor peptides, there has been much effort to clarify their role in physiological and pathophysiological situations. Endothelin is involved in the local regulation of vasomotor tone and thus arterial pressure in the awake state in dogs.[2] Increased concentrations of endothelin occur during pathophysiological conditions such as septic shock in rats,[3] essential and pulmonary hypertension and cardiac failure,[4] and block of endothelin receptors has been suggested as a therapeutic option in these conditions.[5] As endothelin receptor antagonists are on the rise as antihypertensive drugs,[6] understanding the role of the endothelin system during anaesthesia is essential. For instance, during regional anaesthesia endothelin contributes to the maintenance of arterial pressure in dogs,[7] and the increased plasma concentrations of endothelin during pharmacologically induced hypotension in dogs[8] indicate that the endothelin system is activated during severe hypotension.

However, regarding inhalation anaesthesia, it is unknown whether and to what extent endogenous endothelin contributes to the maintenance of arterial pressure and whether the endothelin system is specifically activated to counterbalance further hypotension. In addition to endothelin, the endogenous peptides angiotensin and vasopressin should be considered, as both have been shown to be involved in arterial pressure regulation during inhalation anaesthesia in rats[9] and humans.[10 11]

Therefore, we studied whether endogenous endothelin is involved in the regulation of arterial pressure during inhalation anaesthesia, and compared the role of endothelin with those of angiotensin and vasopressin. For this purpose, we studied the effects of endothelin receptor block on haemodynamics during inhalation anaesthesia with sevoflurane compared with sevoflurane alone or sevoflurane after vasopressin or angiotensin receptor block.

Methods

The data were from 24 experiments on six trained dogs (Foxhounds of both sexes, weighing 28–35 kg) studied with approval of the District Governmental Animal Investigation Committee (Bezirksregierung Düsseldorf).

Several weeks before the experiments the dogs were operated under general anaesthesia (enflurane/nitrous oxide+fentanyl) and sterile conditions. For arterial pressure recording, baroreflex testing and blood sampling, both carotid arteries were exteriorized in skin loops. Ultrasound transit-time flow transducers (T101; Transonic Systems, Ithaca, NY) were implanted around the pulmonary artery for continuous recording of cardiac output (CO) and calibrated *in vivo* at least 3 weeks after implantation, as described previously.[12] During convalescence the dogs were trained to lie quietly and unrestrained on their right side and to become familiar with the experimenters and the laboratory.

During the experiments the following variables were recorded continuously on an eight-channel polygraph (model RS 3800; Gould, Cleveland, OH, USA) and stored simultaneously on the hard disk of a conventional personal computer for further analysis after analogue-to-digital conversion at the rate of 1 kHz per channel.

Mean arterial pressure (MAP), carotid sinus pressure (CSP) and central venous pressure (CVP) were measured electromanometrically (Statham P-23ID, Elk Grove, IL, USA) through catheters in the carotid artery and in the superior vena cava. Correct position of the central venous catheter, which was advanced from the animal's hind limb, was checked by fluoroscopy and adequacy of the venous pressure curve. Arterial pressure in the sinus of the carotid artery (CSP) was measured with a second catheter, which was advanced rostrally through the second exteriorized carotid artery. The electromanometers were referenced to the processus spinosus of the seventh cervical vertebra and calibrated with a mercury manometer. CO was measured continuously (see above), and systemic vascular resistance (SVR) was calculated as the quotient of (MAP–CVP) and CO.

To assess whether potential differences in arterial pressure between groups are modified by the arterial baroreflex, we measured the sensitivity of the carotid baroreflex sensitivity (BRS) as described previously.[13] Both carotid arteries were occluded simultaneously for 45 s with self-made external cuff occluders, resulting in a decrease in CSP and an increase in heart rate. BRS was calculated as the quotient of changes in RR interval (ms) and CSP (BRS=ΔRR/ΔCSP).

We also measured intermittently arterial blood gas tensions, oxygen saturation and pH (ABL3®; Radiometer, Copenhagen, Denmark).

During the respective experiments, endothelin was prevented from acting at its receptors by injecting tezosentan (3 mg kg^{-1} followed by 3 mg kg^{-1} h^{-1}; Actelion, Actelion Ltd, Allschwil, Switzerland), an endothelin receptor antagonist with high affinity for endothelin receptors ET_A and ET_B.[14] Vasopressin (V_{1a}) receptors were blocked by [*d*(CH_2)$_5$Tyr(Me^2)]AVP (V-2255; Sigma Chemicals, Taufkirchen, Germany)[15] at a dose of 40 μg kg^{-1}. Angiotensin-receptor block was achieved by infusing losartan (6 mg kg^{-1} h^{-1}; MSD Sharp and Dohme, Haar, Germany), an angiotensin II receptor antagonist.[16]

Completeness of receptor block was assessed during control experiments in awake animals by injecting 2.5 and 5 μg of endothelin–1 (E-7764; Sigma Chemicals), arginine-vasopressin 200 and 400 mU (V-0377; Sigma Chemicals) or angiotensin II 10 and 20 ng kg^{-1} (A-9525; Sigma Chemicals). After injection of endothelin-1 5 μg, MAP increased by 22 (1) mm Hg but remained constant (1 (3) mm Hg) after previous receptor block. Similarly, vasopressin 400 mU increased MAP by 20 (2) mm Hg but remained constant at –2 (2) mm Hg after block of the vasopressin receptors. Angiotensin II at a dose of 20 ng kg^{-1} increased MAP by 15 (3) mm Hg but remained constant after losartan (1 (2) mm Hg).

To determine the plasma concentrations of vasopressin, endothelin, big endothelin and renin, arterial blood samples were collected at baseline and at the end of each intervention in chilled EDTA (ethylenediamine tetraacetate) tubes, which were immediately placed on crushed ice. Within 10 min, plasma was separated by centrifugation and stored at 20°C until analysis. Endothelin and big endothelin, a sensitive measure of activation of the endothelin system,[17] were measured by enzyme immunoassay (endothelin, R&D Systems, Minneapolis, MN, USA; big endothelin, Biomedica, Vienna, Austria). Vasopressin was measured by radioimmunoassay (Bühlmann Laboratories, Allschwil, Switzerland). Renin concentrations were measured with a chemiluminescence immunoassay on a highly automated platform (Nichols Advantage®; Nichols Institute Diagnostics, San Clemente, CA, USA). Endothelin concentrations were not measured once tezosentan had been injected, as increased endothelin concentrations after

tezosentan are caused by receptor displacement rather than by activation of the endothelin system.[7] Vasopressin concentrations could not be determined once the vasopressin blocker had been injected because of cross-reactivity in the vasopressin immunoassay.

All experiments were carried out with awake dogs in basal metabolic state (food withheld for 12 h with free access to water) under standardized experimental conditions (dogs lying on their right side, lightly dimmed laboratory at a thermoneutral temperature for dogs (24°C)), always beginning at 8 a.m. During the experiments, dogs remained unrestrained on their right side on a cushioned table.

After connecting the animals to the recording system, we waited for ~30 min until haemodynamic variables had reached a steady state as the animals calmed down. The actual experiments started with baseline measurements for 30 min. Thereafter, the dogs were randomly assigned to one of the following four interventions. To maintain a minimum perfusion pressure during high concentrations of sevoflurane in the presence of the respective blockers, sevoflurane concentrations were restricted to maintain MAP above 35 mm Hg. Therefore, the highest sevoflurane concentrations were 2.5 MAC in the vasopressin–sevoflurane (AVP) group and 2.0 MAC in the angiotensin–sevoflurane (AT) group (see below).

In the sevoflurane (control) group (n=6), after baseline measurement in awake dogs, anaesthesia was induced with propofol 3 mg kg^{-1} and a tracheal tube was inserted. Thereafter, the animals' lungs were ventilated with oxygen-enriched air ($F_{I_{O_2}}$ 0.3) at a constant rate and, if necessary, tidal volume was adjusted to maintain normocarbia at higher MAC. Sevoflurane was added and immediately adjusted to an end-tidal concentration of 1 MAC (2 vol%)[18] then to 2 and eventually to 3 MAC. Each anaesthetic concentration was maintained for 30 min, which was sufficiently long for the inspiratory and end-tidal concentrations to equilibrate.

In the endothelin–sevoflurane (ET) group (n=6), the endothelin receptor blocker was injected in awake animals to assess the role of endothelin during resting conditions. Thereafter, the same protocol was repeated, as during control conditions.

In the vasopressin–sevoflurane (AVP) group (n=6), the V_{1a} blocker was injected in awake animals to assess the role of vasopressin during resting conditions. Thereafter, the same protocol was repeated, as during the control condition. Sevoflurane concentrations applied were 1, 2 and 2.5 MAC.

In the angiotensin–sevoflurane (AT) group (n=6), the angiotensin receptor blocker was injected in awake animals to assess the role of the renin–angiotensin system during resting conditions. Thereafter, the same protocol was repeated as during control conditions. Sevoflurane concentrations applied were 1, 1.5, and 2 MAC.

To ensure complete elimination of sevoflurane and blockers, an interval of at least 1 week was interspaced between successive experiments in the same animal.

Data analysis and statistics

Data for all results are given as mean (SEM). Comparisons within each group were made using analysis of variance (ANOVA) for repeated measures. Comparisons between groups were made with the results obtained during the control condition and at 1 and 2 MAC of sevoflurane using repeated-measures ANOVA. If appropriate, Fisher's PLSD (protected least significant difference) test was applied and statistical significance was assumed if P<0.05.

Results

Baseline measurements did not differ between study groups. With the onset of anaesthesia, arterial pressure decreased in all experimental groups to differing degrees: most in the AT group, less during AVP block and least during control conditions and in the ET group (P<0.05) (Fig. 1). At the end of the experiments, MAP values decreased to similar values (AT group: 34 (3), AVP group: 31 (1), control: 36 (3) and ET group: 33 (2) mm Hg); however, these values were reached at different MAC values: 2 MAC in the AT group, 2.5 in the AVP group and 3 MAC during control conditions and the ET group. Cardiac output decreased similarly in all experimental groups, with the exception of the AT group, in which cardiac output was reduced more (P<0.05). Changes in SVR resembled the course of MAP. SVR at low sevoflurane concentrations (1 MAC) was lowest in the AT group, and higher during the other interventions.

Above 1 MAC, SVR did not decrease further with the exception of the AVP group, in which SVR was reduced concentration-dependently to at least 20 (1) mm Hg $litre^{-1}$ min, indicating that, at high sevoflurane concentrations, only vasopressin is released to counterbalance a further decrease in SVR in order to partly compensate for a further arterial pressure reduction.

Plasma concentrations of vasopressin (Fig. 2), big endothelin, endothelin and renin yielded similar results. Whereas the concentrations of renin, endothelin and big endothelin were unchanged during each of the study conditions (data not shown), vasopressin concentrations increased from ~0.5 to ~40 ng $litre^{-1}$ at 3 MAC of sevoflurane in all groups. For comparison of the effect on MAP between groups elicited by sevoflurane in the presence of the respective blockers, changes in arterial pressure at 1 MAC of sevoflurane in comparison with the respective controls were calculated. Sevoflurane 1 MAC was chosen for comparison, because at this anaesthetic concentration the vasopressin system was not activated, so that a meaningful comparison of the effects of the blockers during anaesthesia was feasible, i.e. without the compensatory actions of increased vasopressin concentrations. With the transition from awake to 1 MAC of sevoflurane, arterial pressure decreased least with sevoflurane given alone (–24 (3) mm Hg), to an intermediate degree in the presence of endothelin and vasopressin receptor block (–31 (4) and –30

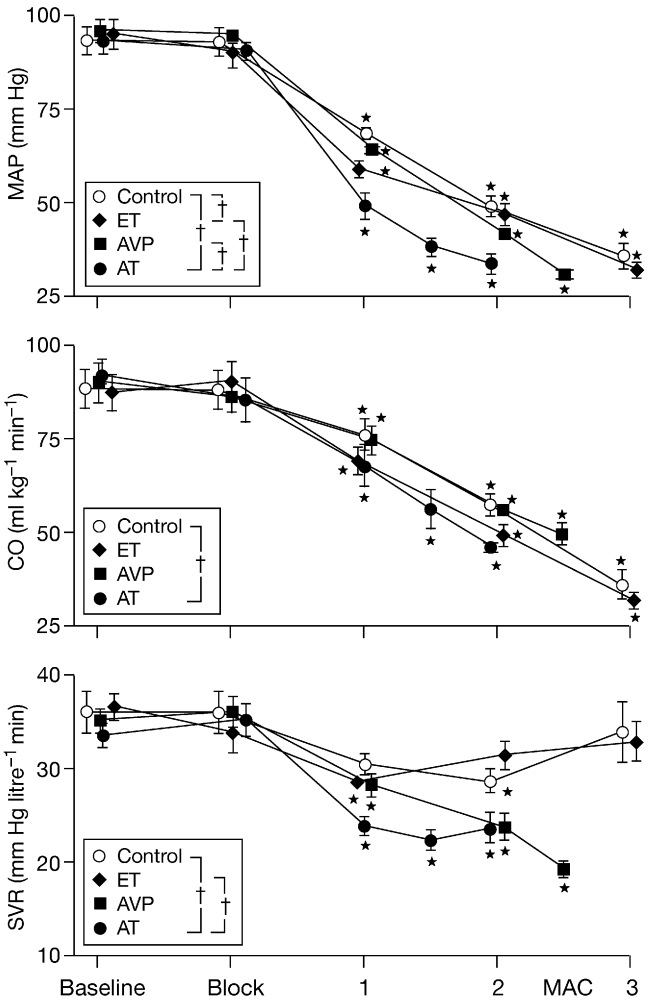

Fig 1 Time course of arterial pressure (MAP), cardiac output (CO) and systemic vascular resistance (SVR) in the awake state and during sevoflurane anaesthesia (1–3 MAC) alone or after pretreatment with an endothelin (ET), vasopressin (AVP) or angiotensin (AT) receptor antagonist. Mean (SEM) from six dogs in each group. Asterisks indicate $P<0.05$ compared with control conditions; crosses indicate $P<0.05$ for comparisons between groups (values included: control, 1 and 2 MAC).

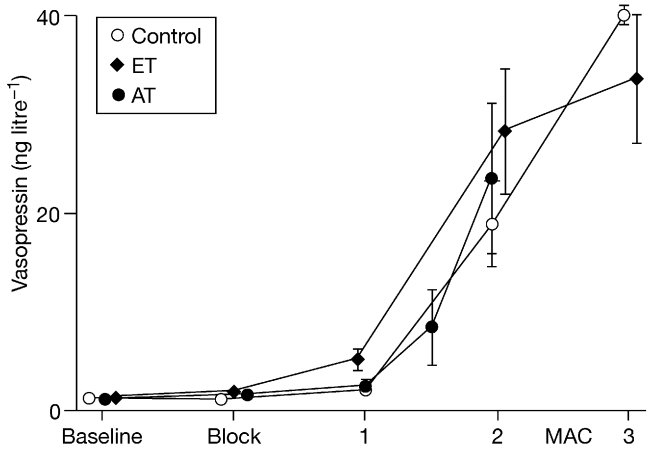

Fig 2 Plasma concentrations of vasopressin in the awake state and during sevoflurane anaesthesia (1–3 MAC) alone or after pretreatment with an endothelin (ET) or angiotensin (AT) receptor antagonist. Mean (SEM) from six dogs in each group. $P<0.05$ for all vasopressin plasma concentrations when sevoflurane concentration was $\geq$2 MAC in all groups compared with control conditions, with no difference between groups.

Table 1 pH, blood gas tensions and arterial oxygen saturation (SAT) in the awake state and during increasing concentrations of sevoflurane (1–3 MAC) in the presence and absence of endothelin, vasopressin and angiotensin receptor block. Mean (SEM) from six dogs. *$P<0.05$ compared with control conditions

	Control	Endothelin receptor block	Vasopressin receptor block	Angiotensin receptor block
pH				
Control	7.34 (0.01)	7.34 (0.01)	7.34 (0.01)	7.32 (0.01)
Block		7.35 (0.01)	7.34 (0.01)	7.34 (0.01)
1 MAC	7.34 (0.01)	7.34 (0.01)	7.35 (0.01)	7.34 (0.01)
1.5 MAC				7.31 (0.02)
2 MAC	7.30 (0.01)	7.31 (0.01)	7.33 (0.01)	7.29 (0.01)
2.5 MAC			7.30 (0.01)	
3 MAC	7.27 (0.01)*	7.27 (0.01)*		
P_{O_2} (mm Hg)				
Control	105 (5)	108 (4)	103 (4)	104 (10)
Block		106 (1)	102 (2)	113 (7)
1 MAC	107 (7)	101 (4)	100 (2)	107 (9)
1.5 MAC				99 (3)
2 MAC	102 (9)	105 (10)	97 (3)	105 (9)
2.5 MAC			93 (2)	
3 MAC	95 (4)	91 (6)		
SAT (%)				
Control	96 (1)	96 (1)	96 (1)	94 (2)
Block		96 (1)	96 (1)	96 (1)
1 MAC	96 (1)	96 (1)	96 (1)	96 (1)
1.5 MAC				95 (1)
2 MAC	95 (1)	95 (1)	95 (1)	95 (1)
2.5 MAC			94 (1)	
3 MAC	94 (1)	93 (1)		
P_{CO_2} (mm Hg)				
Control	37 (1)	37 (1)	37 (1)	38 (2)
Block		36 (1)	38 (1)	37 (1)
1 MAC	37 (1)	37 (1)	36 (1)	37 (1)
1.5 MAC				39 (1)
2 MAC	40 (1)	39 (1)	38 (1)	40 (1)
2.5 MAC			39 (1)	
3 MAC	41 (1)	41 (1)		

(2) mm Hg respectively), and most after angiotensin receptor blocker (–41 (3) mm Hg).

The changes in arterial pressure were not related to differences in the sensitivity of the arterial baroreflex, either during awake conditions or during sevoflurane anaesthesia, as baroreflex sensitivity (which was ~7 ms mm Hg^{-1} during baseline conditions and in the presence of the blockers) was almost completely suppressed during anaesthesia, with no difference between groups.

Oxygen saturation, pH, P_{O_2} and P_{CO_2} were in the physiological range during awake conditions and remained so during sevoflurane anaesthesia up to 3 MAC, except for pH, which decreased at higher MAC, resulting mainly from the slightly increased P_{CO_2} (Table 1).

Discussion

Our results show that, during sevoflurane anaesthesia (1–3 MAC), endogenous endothelin, renin and vasopressin attenuate the decrease in MAP. However, the increase in plasma vasopressin concentrations during high sevoflurane concentrations while big endothelin, endothelin and renin concentrations remain unchanged indicates that only endogenous vasopressin is specifically activated to partly compensate for a further decrease in MAP.

Discussion of the methods

Our conclusions rest upon a sufficient block of the endothelin–, vasopressin– or renin–angiotensin system and comparable conditions in the same dog during repetitive experiments. The endothelin and vasopressin receptor block has been discussed in detail previously.[7] Losartan is a specific angiotensin II receptor antagonist and is appropriate for the elimination of the vasoconstriction elicited by the renin–angiotensin system as it antagonizes the final mediator of this vasoconstrictor system. The dosage used in our study is comparable to that used by others in conscious dogs.[2 16] Moreover, angiotensin II at the dose of 20 ng kg^{-1} increased MAP by ~15 mm Hg, an effect which was abolished after losartan pretreatment in control experiments (data not shown).

Therefore, from a methodological point of view, the chosen dosages of the vasopressin, endothelin and angiotensin receptor antagonists should have been appropriate to block the respective receptors sufficiently.

Discussion of the results

Hypotension during inhalation anaesthesia may be caused by several factors, e.g. by a decrease in cardiac output and systemic vascular resistance. In our experiments, both factors contributed significantly to the reduction of arterial pressure, except at 3 MAC, where SVR reverted to control levels. During angiotensin-receptor block cardiac output was reduced more when compared with the control group, which may partially explain the fact that the lowest arterial pressure occurred in the AT group, but not the differences between the other experimental groups. Changes in baroreflex sensitivity can likewise be excluded as a cofactor because it was almost completely suppressed during sevoflurane anaesthesia at 1 MAC. Therefore, differences in arterial pressure between groups in our study are related mainly to different levels of vasoconstriction (systemic vascular resistance).

The physiological role of endogenous endothelin in the regulation of arterial pressure has long been unclear; for instance, in anaesthetized dogs endothelin receptor block did not change arterial pressure.[19] However, it is now accepted that endogenous endothelin does contribute to arterial pressure regulation under resting conditions, because block of its receptors decreased arterial pressure in healthy volunteers[20] and awake dogs.[2 7] Consistent with our experiments, this was accompanied by a small decrease in SVR, a measure of vasomotor tone. This may be explained by the fact that resistance arteries, which are the main determinants of vasomotor tone, are particularly

sensitive to the effects of endothelin.[21] In contrast, the role of endothelin in partly compensating for hypotension is unclear, but the fact that endogenous endothelin is activated during severe hypotension[8] indicates a role for endothelin in the stabilization of arterial pressure also during inhalation anaesthesia. This, however, is only partially confirmed by our own study because the decrease in arterial pressure attributable to the administration of 1 MAC sevoflurane was larger in the presence of endothelin receptor block compared with sevoflurane alone, but concentrations of neither big endothelin nor endothelin increased with severe hypotension. Thus, endothelin attenuates the drop in arterial pressure during sevoflurane anaesthesia to some extent, but the endothelin system is not specifically activated to compensate for further hypotension.

Likewise, renin–angiotensin partly compensated for the decrease in MAP during inhalation anaesthesia with sevoflurane, but to an even greater extent, as arterial pressure decreased most in the presence of angiotensin receptor block (–41 (3) mm Hg). This result is in accordance with other experiments in which hypotension was more severe during isoflurane anaesthesia after an angiotensin II receptor blocker was given in rats[9] and humans.[11] Again in parallel to the endothelin system, even the renin–angiotensin system is not additionally activated to compensate further for arterial pressure reductions, as indicated by the unchanged renin concentrations during all interventions. This, however, contrasts with other experiments in which renin activity increased during 1.5 MAC of desflurane and isoflurane anaesthesia.[10] However, activation of the renin–angiotensin system depends mainly on the integrity of the sympathetic nervous system, which is activated only during anaesthesia with isoflurane and desflurane,[22] whereas all other inhalation anaesthetics concentration-dependently suppress sympathetic activity.[23] Nevertheless, the renin–angiotensin system was the main contributor to arterial pressure in our experiments.

In contrast, vasopressin shows different properties and contributes little to arterial pressure during physiological conditions. Most of its direct vascular actions are buffered by baroreflexes and are only unmasked after baroreceptor denervation[24] or after destruction of the central nervous system.[25] In accordance with our results, block of only the V_{1a} receptors failed to demonstrate cardiovascular effects in dogs[26] and humans.[27] During low concentrations of sevoflurane, i.e. at 1 MAC, vasopressin concentrations did not increase, indicating that arterial pressure has to fall below a certain level before vasopressin release is activated. Accordingly, at 1 MAC, a concentration at which the vasopressin system is not activated, the additional arterial pressure decrease during receptor block (angiotensin and endothelin) results solely from the respective receptor block, which was the rationale for our decision to compare arterial pressure effects at this MAC. At higher MAC, however, vasopressin concentrations increased while arterial pressure decreased. This is in accordance with the view that, during hypotensive challenges, vasopressin release is activated, as indicated by the increase in plasma vasopressin concentrations, not only in our experiments during inhalation anaesthesia but also during epidural anaesthesia[7] and during haemorrhage.[28] [29] The control and release of endogenous vasopressin depends on cardiopulmonary afferents being sensitive to heart volume[28] and on arterial baroreceptors.[30] The absence of vasopressin release after sinoaortic denervation during graded hypotension,[30] in contrast to an unchanged increase in vasopressin after sole cardiopulmonary denervation, indicates that sinoaortic receptors are the main regulators of vasopressin release. This observation contrasts in part with our own, in which the sensitivity of the arterial baroreflex was almost eliminated at 1 MAC of sevoflurane. However, this result indicates only that the suppressed regulation of heart rate (baroreflex) is independent of the vasopressin-mediated regulation of arterial pressure to prevent severe hypotension.

Clinical implications

The results of our study are of potential interest to clinicians because antagonists of endothelin, angiotensin and perhaps vasopressin receptors are already commonly used or are on the rise as antihypertensive drugs. Accordingly, if inhalation anaesthesia is performed in patients receiving such agents, they are likely to expect severe hypotension, which has already been shown in patients receiving an angiotensin receptor antagonist.[11] However, this effect should be less for vasopressin and endothelin blockers. Moreover, our study provides additional information about which of these blockers should be continued (or not) before inhalation anaesthesia is performed.

Regardless of this speculation, we have shown for the first time that during inhalation anaesthesia with sevoflurane the endogenous vasoconstrictors endothelin, angiotensin and vasopressin compensate to different extents for the drop in arterial pressure in dogs. However, only vasopressin is additionally released to counteract a further decrease in arterial pressure, and thus to avoid more severe hypotension.

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LABORATORY INVESTIGATION

Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity in dogs

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Inhalation anaesthetics decrease heart rate in isolated hearts but mostly increase heart rate in the intact organism, although most inhibit sympathetic drive. Differences in the degree of increase in heart rate between agents may be related to differences in their vagolytic action. To test this hypothesis, we studied the effects of halothane (H), isoflurane (I), enflurane (E), sevoflurane (S) and desflurane (D) [1–3 MAC (minimum alveolar concentration)] on heart rate and heart rate variability (HRV) as a measure of cardiac vagal activity in seven dogs. HRV was analysed in the time domain as the standard deviation of the RR interval (SDNN) and in the frequency domain as power in the high-frequency (HF, 0.15–0.5 Hz) and low-frequency (LF, 0.04–0.15 Hz) ranges. Heart rate increased with anaesthetic concentration and there were corresponding decreases in SDNN, HF power and LF power. Heart rate increased most with D (+40 beats min^{-1}), least with H (+8 beats min^{-1}) and to an intermediate extent with S, I and E. SDNN and HF power, as measures of vagal activity, changed in the opposite direction and decreased in the same order as heart rate increased. However, SDNN and HF power correlated significantly with heart rate [r=–0.81 (0.04) and –0.81 (0.03) respectively] and were independent of the anaesthetic and its concentration (P<0.05). Consistent with our hypothesis, these results suggest that differences between agents in the degree of increase in heart rate are explained by differences in their vagolytic action.

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Inhalation anaesthetics increase heart rate (HR) *in vivo* both in animals[1–3] and humans,[4 5] but decrease heart rate in isolated hearts.[6 7] In the intact organism, HR increases with increasing anaesthetic concentration, i.e. with the depth of anaesthesia but, curiously and for unknown reasons, HR increases more with the halogenated ethers, in particular desflurane, than with the strained-chain hydrogencarbon halothane.[3 4 8 9] Activation of the sympathetic nervous system may explain the increase in HR during desflurane or isoflurane anaesthesia, which has been shown to be associated with increases in spike traffic in sympathetic nerves innervating skeletal muscle in humans.[8–10] This effect, however, is more pronounced during transient than during steady-state conditions.[10] For the other inhalation anaesthetics, this explanation is at variance with the large body of evidence showing inhibition rather than activation of sympathetic drive,[11] particularly at deeper levels of anaesthesia.[12]

Under physiological conditions, HR is determined primarily by cardiac vagal activity,[13] but surprisingly little is known about its response to anaesthesia. Halothane decreases spike traffic in efferent cardiac vagal nerves,[14] but corresponding information is lacking for other inhalation anaesthetics. By the use of HR variability (HRV) as an indicator of cardiac vagal activity, vagal inhibition has also been shown for halothane anaesthesia in dogs and for isoflurane anaesthesia in humans.[15 16] This fragmentary information suggests that cardiac vagal activity may also determine HR during inhalation anaesthesia and that differences between agents in the degree of increase in HR may reflect differences in their vagolytic action. We tested

this hypothesis in dogs by looking at the effects of five currently used inhalation anaesthetics [1–3 MAC (minimum alveolar concentration)] on HR and HRV as an indicator of cardiac vagal activity.

Methods

The data derive from seven trained dogs (Foxhounds of both sexes, weighing 24–34 kg) studied with approval of the District Governmental Animal Investigation Committee. Each dog received, in random order, each of five anaesthetics [halothane (H), enflurane (E), isoflurane (I), desflurane (D) and sevoflurane (S)], with an interval of at least 1 week between successive experiments in the same animal, so that each dog served as its own control.

Several weeks before the experiments, the dogs were operated under general anaesthesia (enflurane/nitrous oxide+fentanyl) and aseptic conditions. For arterial blood pressure recording and blood sampling, both carotid arteries were exteriorized in skin loops.[17] Ultrasound transit-time flow transducers were implanted around the pulmonary artery through a left-sided thoracotomy for the continuous recording of cardiac output. During convalescence, the dogs were trained to lie quietly and unrestrained on their right side and to become familiar with the experimenters and the laboratory.

The following variables were recorded continuously on an eight-channel polygraph (model RS 3800; Gould, Cleveland, OH, USA) and stored simultaneously on the hard disk of a conventional personal computer for further analysis after analogue-to-digital conversion at the rate of 1000 Hz.

Heart rate and RR interval

HR and RR interval (heart period) were determined from a standard ECG (surface electrodes) that was used for triggering a rate meter, which provided a continuous recording of the RR interval.

Arterial blood pressure

Arterial blood pressure was measured electromanometrically (Statham P-23ID, Elk Grove, USA) through a catheter in the carotid artery. The electromanometer was calibrated with a mercury manometer and referenced to the processus spinosus of the 7th vertebra while the animals were lying on their right side. Mean arterial pressure was measured by integrating the original pressure signal.

Cardiac output

Blood flow through the pulmonary artery was measured continuously with an ultrasound transit-time system (T101; Transonic Systems, Ithaca, NY, USA). Each flow transducer (20–24 mm S-series with silicone-shielded U-reflector; Transonic Systems) was calibrated *in vitro* before implantation and *in vivo* at least 3 weeks after implantation by the Fick principle from oxygen consumption ($\dot{V}_{O_2}$), measured by indirect calorimetry (Deltatrac II®), and the arterial–mixed venous oxygen content difference ($C(a-\bar{v})_{O_2}$), measured with a galvanic cell (Lex-O_2-Con-TL, Lexington Instruments, Waltham, USA), giving high precision, as described previously.[18]

Respiratory rate

Respiratory rate was measured continuously with a mercury-in-Silastic gauge mounted around the animal's thorax.

Heart rate variability

HRV, an indicator of the activity of the autonomous nervous system, was studied as described.[19] The original ECG signal, free of aberrant ECG complexes and artefacts, was analysed over a period of 5 min during steady-state conditions after each incremental concentration of the respective inhalation anaesthetic (CHART; ADInstruments, Castle Hill, Australia). HRV was analysed in the time domain and expressed as the standard deviation of the RR interval (SDNN). In addition, HRV was analysed in the frequency domain and calculated as the activity in the high-frequency (HF, 0.15–0.5 Hz) and the low-frequency (LF, 0.04–0.15 Hz) ranges, the former showing exclusively vagal activity and the latter both vagal and sympathetic activity.

During anaesthesia, respiratory gases and vapour concentrations were measured continuously at the endotracheal tube orifice by infrared spectroscopy (Capnomac; Ultima, Datex-Engstrom, Finland). We also intermittently determined arterial blood gas tensions, oxygen saturation and pH (ABL3; Radiometer, Copenhagen, Denmark).

All experiments were carried out with awake dogs in the basal metabolic state (food was withheld for 12 h with free access to water) and under standardized experimental conditions [slightly dimmed laboratory lighting; thermoneutral temperature for dogs (24°C)].[20] During the experiments, which always began at 8 a.m., the dogs remained unrestrained on a cushioned table. To ensure complete elimination of the inhalation anaesthetics, there was an interval of at least 1 week between successive experiments.

After connecting the animals to the recording system, a 30 min stabilization period was commenced. Each experiment began with baseline measurements occupying 30 min, while the awake animals breathed spontaneously. After the insertion of an endotracheal tube (intravenous injection of propofol 3 mg kg^{-1}), the animals' lungs were ventilated with air at a constant rate and, if necessary, tidal volume was adjusted to maintain normocarbia. In the case of desflurane, the vapour was fed together with oxygen-enriched air (30% oxygen in nitrogen) to prevent hypoxia. The anaesthetics were added and immediately adjusted to an end-tidal concentration of 1 MAC (30 min for 1 MAC to minimize

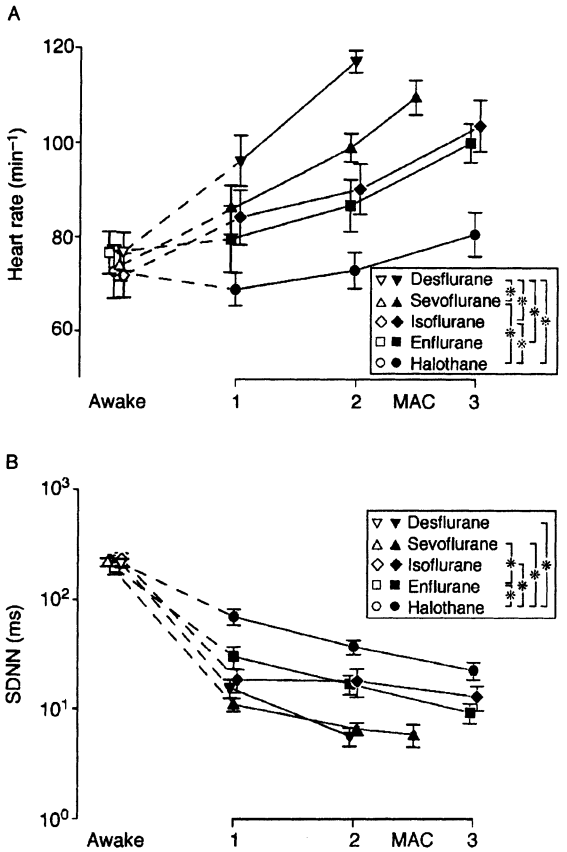

Fig 1 Heart rate (A) and heart rate variability (B) analysed in the time domain (SDNN) during baseline conditions (awake, open symbols) and during anaesthesia with increasing concentrations of five inhalation anaesthetics (closed symbols). Values are mean and SEM for seven dogs. Heart rate increased and SDNN decreased with increasing anaesthetic concentration ($P<0.05$); *indicates differences between the anaesthetics.

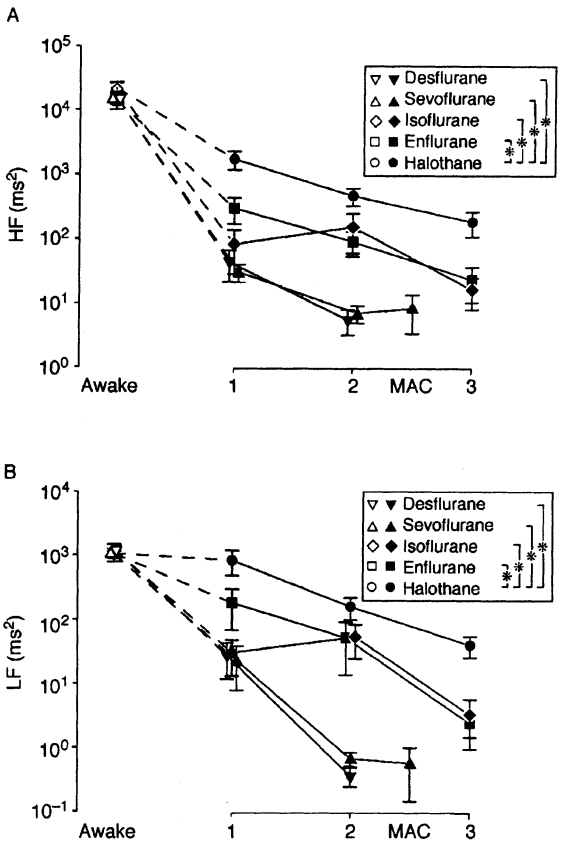

Fig 2 Heart rate variability analysed in the frequency domain [HF (A) and LF (B)] during baseline conditions (awake, open symbols) and during anaesthesia with increasing concentrations of five inhalation anaesthetics (closed symbols). Values are mean and SEM for seven dogs. HF and LF power decreased with the anaesthetic concentration ($P<0.05$) with differences between the anaesthetics. *HF and LF power decreased most during increasing concentrations of sevoflurane and desflurane, and least during halothane anaesthesia.

interaction with propofol) and then to 2 and eventually to 3 MAC (for 20 min). Exposure times were sufficient for the inspiratory and end-tidal concentrations of the anaesthetics to equilibrate.

In addition, we repeated the experiments with D in the presence of β-receptor blockade (propranolol 2 mg kg^{-1} initially, followed by 1 mg kg^{-1} h^{-1}) in two dogs, to exclude the contribution of the sympathetic nervous system to the effects of D on heart rate.

In agreement with the literature, MAC values were assumed to be 0.8, 1.6, 1.4, 2.0 and 7.0 vol% for halothane,[21] enflurane, isoflurane,[22] sevoflurane and desflurane[23] respectively, and the anaesthetics were delivered with conventional vaporizers (Dräger, Lübeck, Germany). Because of limitations of the vaporizers, anaesthetic concentrations had to be restricted to 2.5 and 2.0 MAC for sevoflurane and desflurane respectively.

Results for concentration–effect relationships are given as mean (SEM). Comparisons for heart rate, SDNN and HF and LF power were made by analysis of variance for repeated measures with anaesthetic as between factor. Individual comparisons were made by Fisher's PLSD if appropriate. $P<0.05$ was considered statistically significant.

After logarithmic transformation of the results, linear correlation coefficients were calculated between HRV (SDNN, HF and LF power) and HR for all experiments in one dog and the following null hypothesis was tested: HR during inhalation anaesthesia is independent of vagal activity (SDNN and HF power). For this purpose, the individual correlation coefficients were calculated and compared using a sign test. The null hypothesis was rejected and statistical significance assumed when $P<0.05$.

Results

In general, HR increased and was associated with a decrease in HRV during inhalation anaesthesia. These effects

Table 1 Haemodynamic variables and gas exchange. MAP=mean arterial pressure. Values are mean (SEM) for seven dogs

Agent	MAC	MAP (mmHg)	Cardiac output (ml kg^{-1} min^{-1})	Respiratory rate (min^{-1})	P_{CO_2} (mmHg)	P_{O_2} (mmHg)	pH
Isoflurane	0	94 (3)	108 (6)	21 (2)	33 (1)	98 (2)	7.39 (0.01)
	1	67 (3)	86 (8)	14 (1)	34 (1)	104 (3)	7.38 (0.01)
	2	46 (3)	64 (8)	14 (1)	34 (1)	104 (4)	7.37 (0.02)
	3	33 (1)	40 (4)	14 (1)	33 (1)	106 (6)	7.38 (0.01)
Desflurane	0	93 (2)	104 (5)	23 (2)	35 (1)	103 (4)	7.38 (0.01)
	1	72 (3)	81 (5)	13 (1)	34 (1)	147 (7)	7.37 (0.02)
	2	54 (5)	58 (7)	13 (1)	36 (1)	143 (4)	7.36 (0.02)
Sevoflurane	0	100 (2)	107 (6)	20 (3)	35 (1)	100 (3)	7.39 (0.01)
	1	69 (3)	75 (7)	13 (1)	34 (1)	96 (1)	7.41 (0.01)
	2	55 (3)	60 (6)	13 (1)	34 (1)	96 (3)	7.40 (0.01)
	2.5	53 (5)	56 (9)	13 (1)	34 (1)	92 (3)	7.41 (0.01)
Halothane	0	95 (2)	101 (5)	20 (1)	34 (1)	107 (4)	7.40 (0.01)
	1	71 (2)	71 (6)	14 (1)	34 (1)	104 (2)	7.39 (0.01)
	2	58 (1)	56 (4)	13 (1)	32 (1)	110 (4)	7.40 (0.02)
	3	40 (2)	35 (4)	12 (1)	34 (2)	106 (5)	7.38 (0.01)
Enflurane	0	103 (1)	107 (9)	22 (2)	35 (1)	106 (4)	7.40 (0.03)
	1	68 (3)	73 (7)	13 (1)	35 (1)	105 (4)	7.38 (0.01)
	2	53 (4)	57 (6)	13 (1)	36 (1)	103 (3)	7.38 (0.01)
	3	40 (3)	40 (4)	14 (1)	34 (1)	105 (5)	7.38 (0.01)

decrease in HRV, but, at equianaesthetic concentrations, these effects differ substantially between agents.

Our conclusions rest primarily on the tenable premise that HRV is a measure of cardiac vagal activity. By definition, cardiac vagal activity is the spike traffic in cardioinhibitory vagal neurones which to date cannot be recorded in the intact organism. As a rule, vagal spike frequency correlates linearly with HR, which, in turn, correlates linearly with the respiratory changes in HR, i.e. the degree of respiratory arrhythmia.[24] The correspondence of these correlations is the rationale for using respiratory changes in HR, expressed as standard deviations, for instance as an index of cardiac vagal activity. This information can be derived simply from continuous recording of beat-to-beat HR (analysis in the time domain). Apart from the vagally mediated HR changes coincident with respiration (respiratory frequency 0.15–0.5 Hz), HR also changes with fluctuations in arterial blood pressure (frequency band 0.05–0.15 Hz), which are associated with changes in sympathetic activity. These two components can be separated by spectral analysis of instantaneous HR (analysis in the frequency domain), and there is agreement that power in the HF band reflects exclusively vagal activity. Power in the LF band, which was primarily thought to reflect only sympathetic activity, has been shown to contain both sympathetic and vagal activity.[19 25 26] Despite these uncertainties, we have included the analysis of the LF range for completeness.

HRV may be influenced by changes in P_{CO_2}, respiratory rate and tidal volume.[27 28] Because the influence of tidal volume is small compared with that of respiratory rate,[27 28] the animals were ventilated at the same rate and only tidal volume was varied to maintain normocarbia. Moreover, HRV is strongly reduced by the transition from the awake state (with spontaneous respiration) to anaesthesia (with controlled ventilation).[29] To avoid such interference, we tested only the results obtained during anaesthesia with controlled ventilation. Thus, HRV at respiratory frequencies (activity in the HF range) is a reliable indicator of cardiac vagal activity in our experiments.

The degree of increase in HR differed substantially between the five inhalation anaesthetics. At equianaesthetic concentrations, the increase was greatest for D (40 beats min^{-1}), least for H (8 beats min^{-1}) and intermediate for S, I and E. This essentially confirms previous observations in dogs[1–3] and humans,[5] although the results in humans seem to be less clear at lower concentrations of inhalation anaesthetics.[8 10] Nevertheless, our experiments show for the first time corresponding differences in the strength of vagolytic activity of inhalation anaesthetics.

In contrast to *in vivo* experiments, studies with inhalation anaesthetics uniformly show decreased HR in isolated hearts or isolated pacemaker cells. *In vivo*, they also inhibit sympathetic activity, which would promote decreases—certainly not increases—in HR. At first glance, D seems to be exceptional because it activates specifically sympathetic fibres supplying peripheral blood vessels more during transient conditions than during steady-state conditions.[10] Nevertheless, D evoked greater changes in HR than isoflurane, whereas norepinephrine concentrations did not differ between agents,[30] so that D is not a general stimulant of the sympathetic nervous system. Moreover, although D

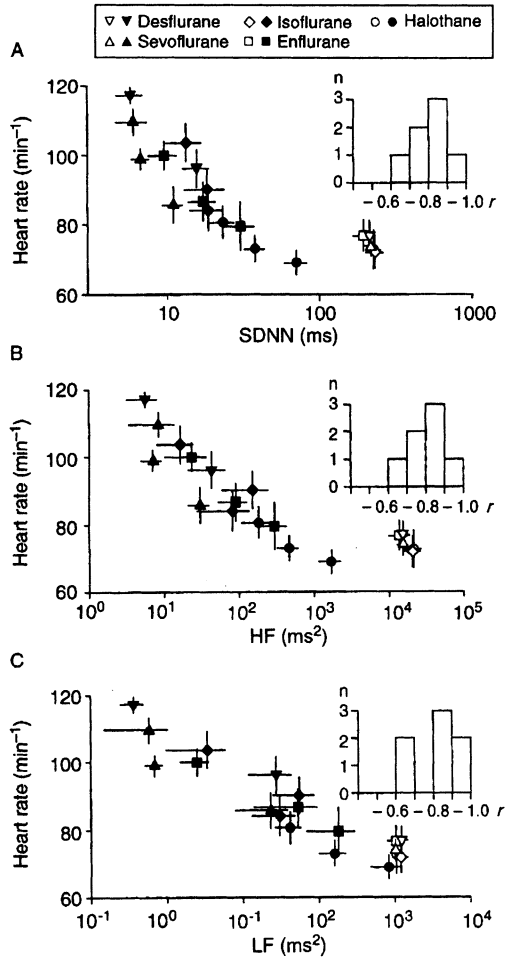

Fig 3 Correlations between heart rate variability [SDNN (A), HF power (B) and LF power (C)] and heart rate for all anaesthetics. Spearman rank correlation coefficients are given in the insets as correlations between heart rate variability and heart rate during anaesthesia (closed symbols) for each individual animal and all anaesthetics. Note that the indices of vagal activity (SDNN and HF power) correlated significantly with heart rate ($P<0.05$). Open symbols show the awake values.

increased with the concentration of anaesthetic but differed markedly between agents. This is shown by the concentration–effect relationships in Figure 1. At baseline (awake, basal metabolic state), the animals always had a low HR (73–77 beat min^{-1}) with strong HRV (193–230 ms), showing the presence of substantial vagal activity before induction of anaesthesia in all groups. During anaesthesia, HR increased with increasing anaesthetic concentration ($P<0.05$) but, at each MAC, HR changed most with D, less with S, I and E, and least with H. Differences in the degree of increase in HR were substantial; for instance, at 2 MAC the HR was only 73 (4) beats min^{-1} in the presence of H but 117 (2) beats min^{-1} in the presence of D and between the two extremes for the other agents.

These HR changes were always associated with changes in HRV in the opposite direction (Fig. 1B). HRV was already substantially reduced, by about 80%, with the loss of consciousness (i.e. on the transition from awake to 1 MAC), and decreased further to a fraction of the baseline value at $\geq$2 MAC ($P<0.05$). Note in particular that, at 2 MAC, HRV reached a minimum in the presence of D and S while HRV was about 10 times greater in the presence of H. Thus, the analysis of HRV in the time domain, which mainly reflects cardiac vagal activity, indicates a vagolytic effect and corresponding opposite changes in HR. These effects differed between agents as they were least for H, greatest for D and S and intermediate for E and I.

Similar results were obtained by more detailed analysis of HRV in the frequency domain (Fig. 2). At baseline, HF power was about 10 times greater than LF power, reflecting the predominance of vagal activity in the awake state. During anaesthesia, both indices of the activity of the autonomic nervous system decreased as MAC increased ($P<0.05$); this effect was least for H, greatest for D and S, and intermediate for E and I. Note also the magnitude of these effects: both HF and LF power decreased by about 99% and reached a minimum at 2 MAC for both S and D.

Thus, analysis of HRV showed that the five inhalation anaesthetics inhibited vagal activity in a concentration-related manner. However, these effects differed between agents as at $\geq$2 MAC vagal activity was strongly reduced in the presence of D and S, less so for E and I, and least for H.

Regardless of these substance-specific differences, HR correlated significantly with the indices of vagal activity during inhalation anaesthesia, independently of the anaesthetic or its concentration (Fig. 3). The correlation coefficients for all experiments in each animal were mostly ≤ -0.8 (see inset in Fig. 3). Thus, vagal activity probably determines and regulates HR during inhalation anaesthesia and, accordingly, the substance-specific differences in the degree of increase in HR are related to the level of vagal activity. This interpretation also applies to D, which increased HR even after β-receptor blockade in two dogs from 62 and 74 beats min^{-1} to 114 and 107 beats min^{-1} at 2 MAC in parallel to a reduction in HF power and SDNN by about 99%.

To help in the interpretation of our observations, additional information is summarized in Table 1. Mean arterial blood pressure and cardiac output decreased substantially as MAC increased but there were no differences between anaesthetics. Respiratory rate was essentially the same during baseline in awake animals, and during anaesthesia respiratory rate was maintained for each animal throughout the experiment.

Discussion

We have shown that inhalation anaesthetics elicit a concentration-related increase in HR with a corresponding

has been shown to release intramyocardial catecholamines,[31] this agent, like the others, decreased HR in a dose-dependent fashion in isolated hearts.[32] In additional experiments we showed that D produced the same tachycardia even after β-receptor blockade.

Accordingly, during inhalation anaesthesia, cardiac vagal activity is the remainder of the autonomous nervous system participating in the central control of HR. That inhalation anaesthetics inhibit cardiac vagal activity has been shown before for two agents. For instance, halothane decreased both spike traffic in cardioinhibitory neurones[14] and HRV in dogs,[29] as isoflurane does in humans.[15] Our experiments revealed agent-specific differences in the strength of vagolytic action. Regardless of the agent used, the indices of cardiac vagal activity (HRV and HF power) decreased in a dose-related manner, i.e. with the depth of anaesthesia, but the magnitude of this effect increased in the same order as HR increased. At 2 MAC, the indices of cardiac vagal activity decreased to a fraction of the baseline value and had reached a minimum in the presence of either D or S. Vagal activity was still 10 times greater in the presence of H. The corresponding HR was approximately 70 beats min^{-1} in the case of H but nearly 120 beats min^{-1} in the case of D. This is the HR that is seen after complete blockade of cardiac vagal activity either by atropine or by cutting the vagal nerves in both dogs and humans.[33,34] The agreement between the drug-specific changes in both HR and the indices of cardiac vagal activity and the close correlation between the two variables (Fig. 3) suggest that, during inhalation anaesthesia, HR depends primarily on cardiac vagal activity. This interpretation is justified because the positive chronotropic effects of the inhalation anaesthetics are unlikely to have been evoked by the accompanying inhibition of sympathetic drive or direct effects on the heart's pacemaker cells.[6,7,35] Thus, our observations support the hypothesis that the differences between inhalation anaesthetics in the degree of increase in HR result from differences in their vagolytic action.

Of note, cardiac output, which has been shown to depend primarily on $\dot{V}_{O_2}$ during inhalation anaesthesia,[36] decreased uniformly in spite of vagally mediated increases in HR.

We cannot explain why, at the same level of anaesthesia, some anaesthetics inhibit the autonomic nervous system more than others. Anaesthetics at high concentrations may stimulate irritant receptors of the airways,[4,37] although the spike traffic from these receptors decreased in a dose-related manner—to the greatest extent for sevoflurane,[38] which is generally accepted as a non-pungent and non-irritating anaesthetic. Accordingly, stimulation of irritant receptors can explain only the transient tachycardia during the induction phase of desflurane anaesthesia, not the continuous increase in HR that occurs at deeper levels of anaesthesia, which are known to suppress any cardiovascular response to manipulation of the airways. It is also unlikely that differences in baroreflex activity would account for the differences in HR between the various inhalation anaesthetics. All suppress baroreflex activity to a similar extent[39–42] and decreased arterial blood pressure to a similar extent in our own experiments.

One limitation of this study is that the autonomic balance in dogs differs from that observed in humans. Whereas vagal activity predominates in the control of HR in dogs, as also shown in this study, the sympathetic nervous system makes a larger contribution than vagal activity in humans.[19] But regardless of these differences, the principle responses of the autonomous nervous system do not differ between the two species. Accordingly, although autonomic balance is not identical in humans and dogs, our conclusions should, with caution, also apply to humans.

HRV reflects the activity of cardioinhibitory neurones in the brainstem. These neurones constitute the final common pathway for all vagally mediated HR changes, not only those of reflex origin via irritant receptors or baroreceptors, for instance, but also those of cortical origin, and hence the state of awareness. It is also of interest that inhalation anaesthetics decrease somatosensory function uniformly, but at the same concentration inhibit the activity of the autonomic nervous system in an agent-specific manner.

Regardless of these general considerations, our experimental findings are consistent with the hypothesis that the differences in the degree of increase in HR between five inhalation anaesthetics are explained by differences in their vagolytic action.

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Original Article

Desflurane increases heart rate independent of sympathetic activity in dogs

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Summary

Background and objective: Desflurane has been shown to increase sympathetic activity and heart rate (HR) in a concentration-dependent manner. Nevertheless, desflurane, like all other volatile anaesthetics, increased HR in parallel to vagal inhibition in a previous study. Therefore, our hypothesis is that desflurane elicits tachycardia by vagal inhibition rather than by activation of the sympathetic nervous system.

Methods: Six dogs were studied awake and during desflurane anaesthesia (1 and 2 MAC) alone, after pre-treatment with propranolol ($2\,mg\,kg^{-1}$ followed by $1\,mg\,kg^{-1}\,h^{-1}$), or after pre-treatment with atropine ($0.1\,mg\,kg^{-1}$ followed by $0.05\,mg\,kg^{-1}\,h^{-1}$). The effects on HR and HR variability were compared by an analysis of variance ($P < 0.05$). HR variability was analysed in the frequency domain as power in the high- (0.15–0.5 Hz, vagal activity) and low-frequency range (0.04–0.15 Hz, sympathetic and vagal activity).

Results: HR increased during 2 MAC of desflurane from about 60 (awake) to $118 \pm 2\,beats\,min^{-1}$ (mean ± SEM) in controls and to $106 \pm 3\,beats\,min^{-1}$ in dogs pre-treated with propranolol. In contrast, pre-treatment with atropine increased HR from 64 ± 2 to $147 \pm 5\,beats\,min^{-1}$ (awake) and HR decreased to $120 \pm 5\,beats\,min^{-1}$ after adding desflurane. High-frequency power correlated inversely with HR ($r^2 = 0.95/0.93$) during desflurane alone and in the presence of β-adrenoceptor blockade, with no significant difference between regression lines. There was no correlation between these variables during atropine/desflurane.

Conclusions: The increase in HR elicited by desflurane mainly results from vagal inhibition and not from sympathetic activation.

Keywords: ANAESTHETICS, INHALATION; PARASYMPATHETIC NERVOUS SYSTEM; SYMPATHETIC NERVOUS SYSTEM.

In physiological circumstances heart rate (HR) is primarily determined by cardiac vagal activity [1]. In a previous study we showed that this fundamental principle also applies during anaesthesia with all volatile anaesthetics currently in use, including desflurane [2]. Accordingly, the increase in HR during inhalation anaesthesia is mediated by vagal inhibition. However, desflurane is exceptional since it is the only anaesthetic, which uniformly increases sympathetic activity to skeletal muscles in human beings [3–5] and kidneys in dogs at low concentrations [6]. Activation of the sympathetic nervous system could, in parallel to vagal inhibition, contribute to the increase in HR commonly observed during desflurane anaesthesia.

In contrast, desflurane decreases renal sympathetic nerve activity at higher concentrations [6] when its tachycardic effect is greatest [2]. Although desflurane has been shown to release intramyocardial catecholamines [7], norepinephrine plasma concentrations do

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not change during desflurane anaesthesia [8]. Desflurane, like all other volatile anaesthetics, decreases HR in a concentration-dependent fashion in isolated hearts [9].

Our aim was to clarify the role of the sympathetic nervous system on the HR increase during desflurane anaesthesia. For this purpose, we studied the effects of desflurane on HR in intact dogs and after blockade of sympathetic or vagal activities, respectively.

Methods

Six trained dogs (foxhounds of both sexes, weighing 24–34 kg) were studied with approval of the District Governmental Animal Investigation Committee. Several weeks before the actual experiments the dogs were operated upon under general anaesthesia (enflurane/nitrous oxide + fentanyl) and aseptic conditions. Both carotid arteries were exteriorized in skin loops [10] for arterial pressure recording and blood sampling. Ultrasound transit-time flow transducers were implanted around the pulmonary artery through a left-sided thoracotomy for continuous recording of cardiac output. During the convalescence the dogs were trained to lie quietly and unrestrained on their right side and to become familiar with the experimenters and the laboratory.

The following variables were recorded continuously on an eight-channel polygraph (model RS 3800®; Gould Inc., Cleveland, OH, USA) and simultaneously stored on a personal computer for further analysis after analogue-to-digital conversion with a rate of 1000 Hz (CHART®; ADInstruments, Castle Hill, Australia).

HR and respiratory rate (RR) intervals were determined from a standard electrocardiography (ECG), using surface electrodes, which triggered a rate meter providing a continuous recording of the heart periods (RR intervals).

Arterial pressure was measured electromanometrically (Statham P-23ID®; Elk Grove, IL, USA) in the ascending aorta through a catheter advanced via the carotid artery. The transducer was calibrated with a mercury manometer and referenced to the spinus process of the seventh cervical vertebra with the animals lying on the right side. Mean arterial pressure (MAP) was measured by integration of the original pressure signal. Blood pressure (BP) in the sinus of the carotid artery was measured by a second catheter that was advanced rostrally through the second exteriorized carotid artery.

Cardiac output: Blood flow through the pulmonary artery was measured continuously with an ultrasound transit-time system (T101®; Transonic Systems Inc., Ithaca, USA). Each flow transducer (20–24 mm S-series with silicone shielded U-reflector, Transonic) was calibrated *in vitro* prior to implantation and *in vivo* at least 3 weeks after implantation as previously described [11].

RR was measured continuously by a mercury-in-silastic-gauge (self-made) mounted around the animal's thorax.

HR variability, an indicator of the activity of the autonomous nervous system, was studied as recommended [12]. The original ECG signal, free of aberrant ECG complexes and artefacts, was analysed over a period of 5 min during steady-state conditions after each incremental change in desflurane concentration (CHART®). HR variability was analysed in the frequency domain and calculated as activity in the high-frequency (HF, 0.15–0.5 Hz) and the low-frequency (LF, 0.04–0.15 Hz) range, the former showing exclusively vagal activity and the latter both vagal and sympathetic activity [12].

To assess whether potential differences in BP between groups could trigger HR changes, we measured the sensitivity of the carotid baroreflex as described previously [13]. Both carotid arteries were simultaneously occluded for 45 s with self-made external cuff occluders, resulting in a decrease in carotid sinus pressure (CSP) and an increase in HR. Carotid baroreflex sensitivity (BRS) was calculated as the quotient of changes in HR (RR intervals) and in CSP ($BRS = \Delta RR/\Delta CSP$).

During anaesthesia, respiratory gases and vapour concentrations were measured continuously at the endotracheal tube orifice by infra-red spectroscopy (Capnomac®; Ultima SV, Datex-Engström, Helsinki, Finland). We also determined intermittently arterial blood-gas tensions, O_2 saturation, and pH (ABL3®; Radiometer, Copenhagen, Denmark).

All experiments were carried out with the dogs awake in basal metabolic state (food withheld for 12 h and free access to water) and under standardized conditions (lightly dimmed laboratory at thermoneutral temperature for dogs of 24°C) [14]. During the studies, which always began at 08:00 h the dogs remained unrestrained on a cushioned table. The following three experiments were performed in each animal in a randomized order. At least 1 week was allowed between successive experiments to ensure complete elimination of the administered drugs.

Control group (n = 6)

After connecting the dogs to the recording system, we waited about 30 min until all variables had reached a steady state. The actual experiments started with baseline measurements for a further 30 min with the dogs awake and breathing spontaneously. Following the insertion of an endotracheal tube (intravenous (i.v.) injection of propofol 3 mg kg^{-1}; Diprivan® 1%, Fresenius Kabi, Bad Homburg, Germany) the lungs

were ventilated with 30% of O_2 in N_2 at a constant rate of 14 breaths min^{-1}. If necessary, tidal volume was adjusted to maintain normocarbia. Desflurane (Suprane®; Baxter, Munich, Germany) was added and immediately adjusted to an end-tidal concentration of 1 MAC (7.0 volumes per cent [15]) for the duration of 30 min, and then to 2 MAC for 20 min. The exposure times were sufficiently long for the inspiratory and end-tidal concentrations of the anaesthetics to equilibrate. The exposure time of 30 min for 1 MAC was chosen to allow for the rapid redistribution phase of propofol (half-life of the α-phase of about 2 min [16]) and thus to minimize interaction with propofol.

Propranolol group (n = 6)

After baseline measurements propranolol (P 0884®; Sigma, Taufkirchen, Germany) was injected i.v. (2 mg kg^{-1}, followed by 1 mg kg^{-1} h^{-1} continuous infusion) to achieve sympathetic blockade. Thereafter, the same experimental program was repeated as in the control group. The completeness of receptor blockade was assessed by an i.v. injection of orciprenalin 0.5 μg kg^{-1} at the end of the experiments. This dose increased HR by about 15 min^{-1} and decreased arterial pressure by about 25 mmHg during pilot experiments without preceding receptor blockade and was without any detectable effect on haemodynamic variables after β-adrenoreceptor blockade, as tested in each experiment.

Atropine group (n = 6)

After baseline measurements, atropine was injected i.v. (0.1 mg kg^{-1}, followed by 0.05 mg kg^{-1} h^{-1}) to achieve vagal blockade. Thereafter, the same experimental program as in the control group was repeated. Completeness of receptor blockade was assessed by the absence of any HR change after i.v. injection of atropine 1.0 mg at the end of each experiment.

The results of the concentration–effect relations are given as mean ± SEM. Comparisons for HR, HF, F and LF-power were made by an analysis of variance (ANOVA), followed by Fisher's PLSD test if appropriate. $P < 0.05$ was considered significant. After logarithmic transformation of the results, linear correlation coefficients were calculated between HR variability (HF-power) and HR and the regression lines in the presence and absence of propranolol were compared by an *F*-test for differences between regression lines. $P < 0.05$ was considered significant.

Results

During baseline conditions (awake dogs) the HR was identical in all groups (about 60 beats min^{-1}). It was almost unchanged after propranolol (64 ± 2 beats min^{-1}) whereas atropine increased the HR to 147 ± 5 beats min^{-1} ($P < 0.0001$). With the transition from the awake state to 1 MAC desflurane anaesthesia (Fig. 1), the HR increased identically in the control and propranolol-treated dogs to 107 ± 3 and 108 ± 2 beats min^{-1}, respectively ($P < 0.0001$ for both groups). On the contrary, in the atropine-treated dogs the HR was reduced to 120 ± 5 beats min^{-1} ($P = 0.0006$). At 2 MAC of desflurane, HR increased further during control conditions to 118 ± 2 beats min^{-1} ($P = 0.0049$) with no difference to the atropine group (114 ± 3 beats min^{-1}). In the propranolol dogs, HR did not increase further with the deepening of desflurane anaesthesia from 1 to 2 MAC. Yet, HR was about 12 beats min^{-1} lower in the absence of sympathetic activity ($P = 0.0066$). Completeness of vagal blockade in the atropine-treated dogs can be assumed since a further dose of atropine 1 mg did not change HR (+1 ± 1 beats min^{-1}). Nor did orciprenalin 0.5 μg kg^{-1} cause any changes in HR or arterial pressure (0 ± 1 beats min^{-1} and −1 ± 2 mmHg) in the propranolol dogs.

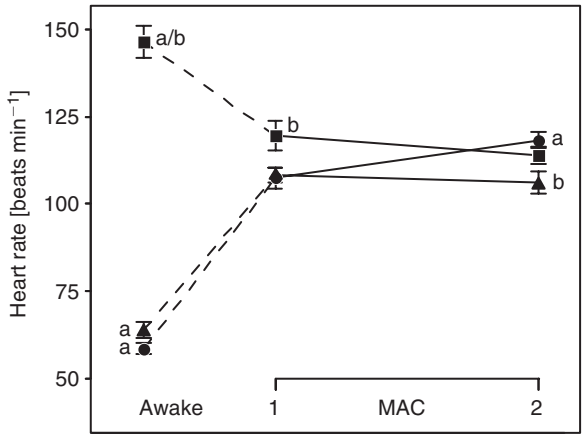

Figure 1.
*Heart rate in the awake state and during desflurane anaesthesia (1 and 2 MAC) in control dogs (○●), after β-adrenoceptor blockade with propranolol (△▲), and after parasympathetic blockade with atropine (□■). Values are mean ± SEM from six dogs. (**a**) P < 0.05 compared to 1 MAC within each group; (**b**) P < 0.05 compared to the control group.*

The increased HR during desflurane anaesthesia in control and propranolol-treated dogs were always associated with changes in vagal activity (HF-power) in the opposite direction (Fig. 2, panel a). HF-power decreased markedly, without any difference between the two groups. In contrast, HF-power was entirely suppressed in the atropine group before the addition of desflurane, indicating successful blockade of vagal activity to the heart. LF-power decreased almost identically during desflurane in the control and

propranolol animals (Fig. 2, panel b). In the atropine dogs, LF-power was reduced in the awake state compared to control conditions and decreased further during desflurane anaesthesia.

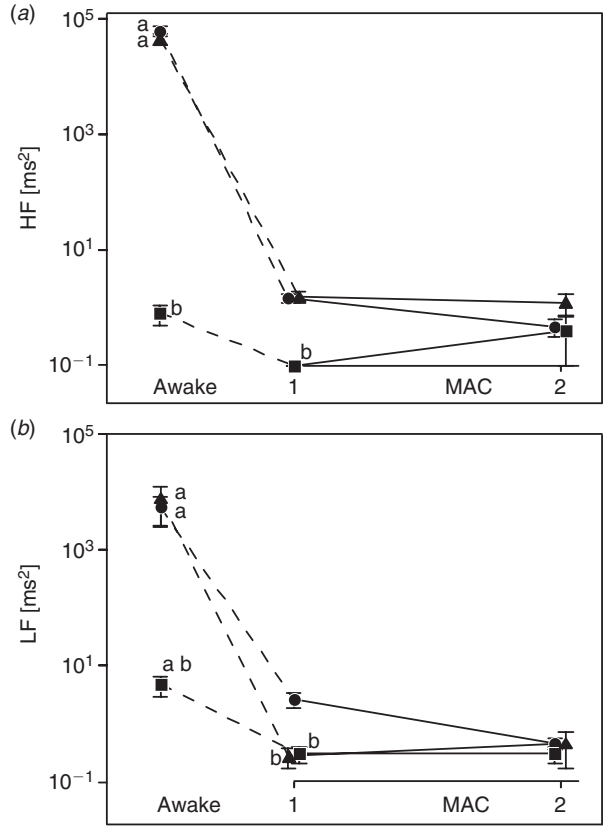

Figure 2.
*Heart rate variability analysed in the frequency domain (HF (**a**) and LF (**b**)) in the awake state and during desflurane anaesthesia (1 and 2 MAC) in control dogs (○●), after β-adrenoceptor blockade with propranolol (△▲), and after parasympathetic blockade with atropine (□■). Values are mean ± SEM from six dogs. (**a**) $P < 0.05$ compared to 1 MAC within each group; (**b**) $P < 0.05$ compared to the control group.*

As already indicated in Figures 1 and 2, HR correlated closely with vagal activity (HF-power) during desflurane anaesthesia, independent of the presence or absence of sympathetic activity or the anaesthetic concentration (Fig. 3). The correlation coefficients were 0.93 and 0.95 and the regression lines did not differ between the controls and the propranolol-treated dogs. In contrast, in the atropine-treated animals HR did not correlate with HF-power either in the awake state or during desflurane anaesthesia.

Additional information is summarized in Table 1. Sympathetic blockade *per se* did not alter any of the haemodynamic variables nor gas exchange, whereas MAP and cardiac output increased after parasympathetic blockade. During desflurane anaesthesia, particularly at 2 MAC, MAP and cardiac output were

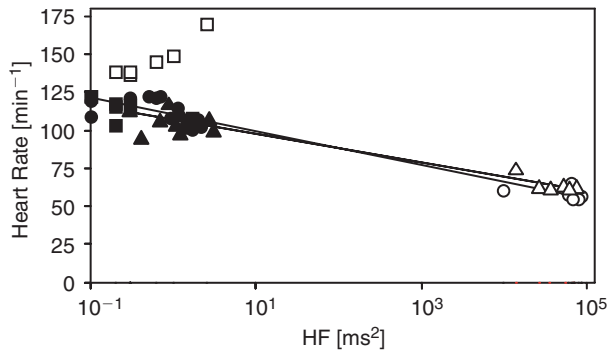

Figure 3.
Correlations between vagal activity and HR in the awake state (○△□) and during desflurane anaesthesia (●▲■) in control dogs (○●; $r^2 = 0.95$), after β-adrenoceptor blockade with propranolol (△▲; $r^2 = 0.93$), and after parasympathetic blockade with atropine (□■). Vagal activity was determined by the analysis of HR variability in the HF domain (HF-power). Note that vagal activity (HF-power) correlated significantly with HR ($P < 0.05$) in the controls and β-adrenoceptor blockade dogs and did not differ between the groups. After parasympathetic blockade the two variables did not correlate.

Table 1. Haemodynamics, baroreflex sensitivity (BRS), and gas exchange.

Group	Condition	MAP (mmHg)	Cardiac output ($mL\,kg^{-1}\,min^{-1}$)	$PaCO_2$ (kPa)	PaO_2 (kPa)	pH	BRS ($ms\,mmHg^{-1}$)
Control	Awake	92 (2)	86 (7)	4.9 (0.1)	13.5 (0.6)	7.36 (0.01)	14 (4)
	1 MAC	76 (3)	96 (6)	5.1 (0.1)	18.9 (0.3)	7.35 (0.01)	0 (0)
	2 MAC	63 (4)	79 (3)	5.4 (0.1)	16.5 (0.6)	7.30 (0.01)	0 (0)
Propranolol	Awake	91 (3)	86 (9)	5.0 (0.1)	13.5 (0.6)	7.31 (0.01)	13 (1)
	Block	93 (3)	85 (11)	4.8 (0.1)	13.5 (0.6)	7.32 (0.01)	10 (2)
	1 MAC	63 (1)*	92 (8)	4.8 (0.1)	18.9 (0.6)	7.30 (0.01)	0 (0)
	2 MAC	42 (3)*	50 (4)*	5.2 (0.2)	16.6 (0.6)	7.26 (0.01)	0 (0)
Atropine	Awake	97 (5)	87 (13)	4.8 (0.1)	13.1 (0.7)	7.29 (0.01)	9 (3)
	Block	109 (5)*†	108 (12)†	4.8 (0.2)	14.4 (0.7)	7.32 (0.01)	1 (0)
	1 MAC	79 (8)	92 (11)	5.2 (0.2)	16.3 (1.5)	7.28 (0.01)	0 (0)
	2 MAC	57 (2)†	66 (7)*	4.9 (0.2)	16.1 (0.8)	7.27 (0.01)	0 (0)

MAP: mean arterial pressure. Values are mean (SEM) for six dogs. *$P < 0.05$ vs. control experiments; †$P < 0.05$ vs. the propranolol group.

lower in the propranolol-treated dogs. Carotid baroreflex sensitivity did not differ between groups in the awake state and during anaesthesia with desflurane. Carotid baroreflex sensitivity was zero during desflurane anaesthesia at 1 and 2 MAC in all groups, indicating that decreases in CSP are not compensated for by changes in HR. The respiratory rate was similar at baseline in all awake dogs, and during anaesthesia respiratory rate was kept constant throughout the experiments.

Discussion

We have shown that desflurane elicits tachycardia with a corresponding decrease in HR variability, both in the presence and absence of sympathetic activity. Parasympathetic blockade *per se* increased HR with no further increase in the presence of desflurane. Thus, HR changes during desflurane anaesthesia are independent of the sympathetic nervous system and most probably result from vagal inhibition.

Our conclusions imply that a sufficient degree of blockade of sympathetic and parasympathetic activity had been achieved. We also make the tenable assumption that HR variability is a measure of autonomic activity, particular vagal activity.

Sympathetic activity was blocked by propranolol in a dose of $2\,mg\,kg^{-1}$ initially, followed by $1\,mg\,kg^{-1}\,h^{-1}$. This β-adrenoceptor antagonist blocks both β_1- and β_2-receptors and has no agonistic effects on these receptors [17]. The chosen dosage is comparable to that used by others to block sympathetic activity in dogs [18]. The completeness of β-adrenoceptor blockade in our experiments was verified by the administration of a β-agonist (orciprenalin $0.5\,\mu g\,kg^{-1}$), which did not cause any changes in HR or arterial pressure. This dose has been shown previously in pilot studies to cause an increase in HR of about $15\,beats\,min^{-1}$ and a decrease in arterial pressure of about 25 mmHg in animals without preceding β-adrenoceptor blockade.

Vagal activity to the heart was blocked by atropine $0.1\,mg\,kg^{-1}$, followed by $0.05\,mg\,kg^{-1}\,h^{-1}$. Completeness of receptor blockade can be assumed from two observations: First, in pilot experiments a further dosage increase did not change HR in these dogs. Moreover, 1 mg atropine given at the end of each experiment in this study did not induce any change in HR.

Autonomic activity is the spike traffic in sympathetic neurons and cardio-inhibitory vagal neurons. Direct recording from these nerves cannot be made in the intact organism, in particular if repetitive experiments in one and the same animal are performed. However, as a surrogate for direct nerve recordings, beat-to-beat changes in HR, termed HR variability, are used as indices of autonomic activity. Spectral analysis of instantaneous HR (analysis in the frequency domain) in a frequency range coincident with respiration (respiratory frequency 0.15–0.5 Hz, HF) is believed to exclusively reflect vagal activity [12]. HR also changes with fluctuation in arterial pressure (frequency band 0.04–0.15 Hz, LF), which was believed to reflect sympathetic activity only, but has been shown to contain both sympathetic and vagal activity [12,19,20]. This view is supported by our own experiments in which LF-power was reduced by the parasympathetic blockade indicating that the information included in LF-power does not consist of only sympathetic activity.

HR variability may be influenced by changes in $PaCO_2$, respiratory rate, and tidal volume [21,22]. Since the influence of tidal volume is small compared to that of respiratory rate [21,22], the animals were ventilated at the same rate and only tidal volume was varied (up to 20%) to maintain normocarbia. Accordingly, our methods were able to detect differences in the HR changes during desflurane anaesthesia related to the presence and absence of sympathetic and vagal activity, respectively.

HR increases during desflurane anaesthesia have been observed in man [3] as well as in dogs [2,6]. In parallel to HR, desflurane increases sympathetic activity, which has been shown particularly for fibres supplying peripheral muscles [3]. These effects are more pronounced during transient increases in desflurane concentrations compared to steady-state conditions [23]. The transient component of the HR increase at desflurane concentrations of about 1 MAC seems to be influenced by the sympathetic nervous system as evidenced by the fact that β-adrenoceptor blockade attenuates this response [23]. In addition, direct recordings from renal nerves in dogs have revealed that sympathetic activity was increased only at desflurane concentrations up to 1 MAC, whereas it was strongly reduced at concentrations above 1 MAC [6]. These results are not in accordance with our own, since we found a strong reduction in LF-power at 1 MAC, although LF-power tended to be higher in the control experiments compared to the β-adrenoceptor blocked group. However, these differences in LF-power are obviously of only minor importance for HR adjustment during desflurane anaesthesia, since HR was identical in both controls and propranolol-treated dogs at 1 MAC. Moreover, HR correlated closely with all measures of autonomic activity and the regression lines did not differ between animals with or without β-blockade at either 1 or 2 MAC (Fig. 3). Since sympathetic inhibition, as indicated by the marked reduction in LF-power, cannot explain tachycardia, vagal inhibition remains the only rationale for the HR increase during desflurane anaesthesia. This is also

supported by the fact that HR did not increase in dogs pre-treated with atropine, i.e. in the absence of vagal activity. In fact HR rather decreased at 1 MAC in these dogs which can only be explained by suppression of the remaining sympathetic activity.

At 2 MAC of desflurane, HR did not differ between controls and dogs given atropine. HR was slightly lower in the β-blocked dogs, i.e. in the absence of sympathetic activity. One might speculate that this difference results from sympathetic activation, which is most unlikely, since direct nerve recordings uniformly have yielded strongly suppressed sympathetic activity at this concentration [6] and LF-power in our own experiments did not differ between groups. Accordingly, the difference in HR at 2 MAC of desflurane probably does not result from sympathetic activation, although we have no tenable alternative explanation. It is obvious that cardiac output and BP were lower in the absence of sympathetic activity (β-blocked dogs) compared to desflurane alone only at 2 MAC of desflurane. With due caution, we may speculate that cardiac vagal afferents located in the ventricles could be activated during conditions with compromised circulation leading to an additional slowing of HR [24]. This phenomenon has been observed during bleeding experiments, in which HR decreased in parallel to further reductions in arterial pressure once the pressure fell below a certain level [25,26].

Desflurane has previously been shown to release intramyocardial catecholamines *in vitro* [7]. However, like all other inhalational agents desflurane decreases HR in a concentration-dependent fashion in isolated hearts [9] and it has not been shown to change systemic plasma catecholamine concentrations [8]. Thus, during desflurane anaesthesia *in vivo*, intramyocardial catecholamine release should only make a minor contribution to the HR adjustment. It is also unlikely that baroreflex activation triggers tachycardia, since desflurane like all other volatile anaesthetics suppresses the baroreflex sensitivity [27,28] and this was already substantially reduced at 1 MAC in our own experiments.

Our study suggests, that although sympathetic activation of the muscles and the kidneys has been observed during desflurane anaesthesia, the increase in HR most probably has a different explanation. We found similar degrees of tachycardia during desflurane anaesthesia in dogs in the presence or absence of β-adrenoceptor blockade making a substantial contribution of the sympathetic nervous system unlikely. In contrast, once vagal activity to the heart was blocked with atropine, desflurane did no longer increase HR. Thus, HR adjustment during desflurane anaesthesia seems to result from vagal inhibition which is in accordance with other inhalation anaesthetics [2]. From a clinical point of view, there is no reason to avoid desflurane in patients in whom sympathetic activation to the heart is undesirable.

Acknowledgements

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Xenon increases total body oxygen consumption during isoflurane anaesthesia in dogs

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Background. This study was designed to examine whether the coupling between oxygen consumption ($\dot{V}O_2$) and cardiac output (CO) is maintained during xenon anaesthesia.

Methods. We studied the relationship between $\dot{V}O_2$ (indirect calorimetry) and CO (ultrasound flowmetry) by adding xenon to isoflurane anaesthesia in five chronically instrumented dogs. Different mixtures of xenon (70% and 50%) and isoflurane (0–1.4%) were compared with isoflurane alone (1.4% and 2.8%). In addition, the autonomic nervous system was blocked (using hexamethonium) to study its influence on $\dot{V}O_2$ and CO during xenon anaesthesia.

Results. Mean (SEM) $\dot{V}O_2$ increased from 3.4 (0.1) ml kg^{-1} min^{-1} during 1.4% isoflurane to 3.7 (0.2) and 4.0 (0.1) ml kg^{-1} min^{-1} after addition of 70% and 50% xenon, respectively ($P<0.05$), whereas CO and arterial pressure remained essentially unchanged. In contrast, 2.8% isoflurane reduced both, $\dot{V}O_2$ [from 3.4 (0.1) to 3.1 (0.1) ml kg^{-1} min^{-1}] and CO [from 96 (5) to 70 (3) ml kg^{-1} min^{-1}] ($P<0.05$). $\dot{V}O_2$ and CO correlated closely during isoflurane anaesthesia alone and also in the presence of xenon ($r^2=0.94$ and 0.97, respectively), but the regression lines relating CO to $\dot{V}O_2$ differed significantly between conditions, with the line in the presence of xenon showing a 0.3–0.6 ml kg^{-1} min^{-1} greater $\dot{V}O_2$ for any given CO. Following ganglionic blockade, 50% and 70% xenon elicited a similar increase in $\dot{V}O_2$, while CO and blood pressure were unchanged.

Conclusions. Metabolic regulation of blood flow is maintained during xenon anaesthesia, but cardiovascular stability is accompanied by increased $\dot{V}O_2$. The increase in $\dot{V}O_2$ is independent of the autonomic nervous system and is probably caused by direct stimulation of the cellular metabolic rate.

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Metabolic regulation contributes to the adjustment of the circulation in order to meet tissue oxygen demand, and manifests itself as a linear relationship between blood flow (i.e. cardiac output [CO]), and oxygen consumption ($\dot{V}O_2$) during physiological conditions (e.g. physical exercise) in dogs[1] and humans.[2] We have recently shown that this fundamental principle is preserved during inhalation anaesthesia with the five volatile anaesthetics currently in use, unless the anaesthetic concentration exceeded 2 MAC.[3] This finding implies that increasing anaesthetic depth reduces $\dot{V}O_2$ and CO in parallel. We have also shown that the decrease in CO during inhalation anaesthesia is mainly a consequence of reduced metabolic rate rather than a direct side-effect of the anaesthetic.[3]

Recently, the noble gas xenon has been the subject of widespread interest because it has minimal effects on the cardiovascular system, leading to haemodynamic stability.[4] This cardiovascular stability has been explained by the fact that xenon does not alter myocardial function in humans[5] and animals[6] or in isolated hearts.[7] This haemodynamic stability with an unchanged CO despite an increase in anaesthetic depth is in contrast to findings with other volatile anaesthetics, which reduce $\dot{V}O_2$ and CO in parallel.[3]

We therefore questioned whether, and to what extent, xenon alters $\dot{V}O_2$ and whether metabolic regulation of blood flow is maintained during xenon anaesthesia. To test this, we studied the relationship between CO and $\dot{V}O_2$ during different levels of xenon anaesthesia added to an isoflurane baseline in chronically instrumented dogs and compared the effects of xenon with those elicited by isoflurane.

Methods

The data derive from 15 experiments on five trained dogs (foxhounds of both sexes weighing 24–34 kg) studied with the approval of the District Governmental Animal Investigation Committee. Each dog was assigned to each of the three intervention groups with an interval of at least 1 week between successive experiments in the same animal, so that each dog served as its own control.

Surgery

Several weeks before the experiments, the dogs were operated on under general anaesthesia (enflurane/nitrous oxide + fentanyl) and aseptic conditions. For blood pressure recording and blood sampling, both carotid arteries were exteriorized in skin loops.[8] Ultrasound transit-time flow transducers were implanted around the pulmonary artery through a left-sided thoracotomy for continuous recording of CO.

Measurements

Cardiac output

Blood flow through the pulmonary artery was measured continuously with an ultrasound transit-time system (T101, Transonic Systems, Ithaca, NY, USA). Each flow transducer (20–24 mm S-series with silicone shielded U-reflector; Transonic Systems) was calibrated *in vitro* before implantation and *in vivo* at least 3 weeks after implantation, using the Fick principle from $\dot{V}O_2$ and the arterial-to-mixed venous oxygen content difference ($C(a-\bar{v})_{O_2}$) measured with a galvanic cell (Lex-O_2-Con-TL®, Lexington Instruments, Waltham, USA), resulting in high precision, as described previously.[9]

Oxygen uptake ($\dot{V}O_2$)

$\dot{V}O_2$ (at standard temperature [273 K], pressure [760 mmHg] and dry P_{H_2O} 0 mm Hg]) was measured continuously by indirect calorimetry with a Deltatrac II® Metabolic Monitor (Datex-Engstrom Division, Instrumentarium Corp., Helsinki, Finland). Before each experiment, the gas sensors were calibrated with air and a gas mixture containing 95.0 (0.05)% O_2 and 5.0 (0.03)% CO_2, and the measurement of $\dot{V}O_2$ was calibrated by burning 5 ml pure ethanol (alcohol burning test kit, Datex-Engstrom, Helsinki, Finland).

Burning of alcohol was also repeated in the presence of 50 and 70% xenon to ensure that the high density of xenon did not alter the flow constant of the built-in flow generator. In addition, baseline stability of the gas sensors was tested by feeding xenon to the mixing chamber of the Deltatrac II to check if xenon alters O_2 and CO_2 measurements.

During spontaneous breathing in awake dogs, $\dot{V}O_2$ was measured with a flow-through technique (canopy mode), as described previously,[10] which calculated $\dot{V}O_2$ from the difference of inspired and expired oxygen concentration and the constant gas flow through the built-in flow generator. For this purpose, a transparent plastic canopy was fixed above the dog's head and upper trunk, allowing room air to enter at the edges as air was sucked continuously through the Deltatrac II for analysis. A canopy volume of approximately 70 litres and a flow generator rate of about 40 litre min^{-1} resulted in a system time constant of 1.75 min.

During anaesthesia and controlled ventilation $\dot{V}O_2$ was measured directly from the respiratory gases. The expired air was collected and fed to the mixing chamber of the Deltatrac II (respiration mode, a collection technique with a time constant of 1 min). As a cross-check of the Deltatrac II measurements, we also intermittently measured $\dot{V}O_2$ from the product of CO and $C(a-\bar{v})_{O_2}$ (pulmonary artery catheter). In agreement with others,[11,12] the precision of this device was 3.5% (average coefficient of variation) and the accuracy was 0.1 ml min^{-1}, with 95% confidence intervals of –4.8 to 5.0 ml min^{-1}.

Arterial pressure

Arterial pressure was measured electromanometrically (Statham P-23ID, Elk Grove, USA) through a catheter in the carotid artery. The electromanometer was calibrated with a mercury manometer and referenced to the processus spinosus of the 7th vertebra while the dogs were lying on its right side. Mean arterial pressure (MAP) was measured by integration from the original pressure signal.

Heart rate and RR intervals

HR and RR were determined from a standard ECG (surface electrodes) used for triggering a rate meter, which provided a continuous recording of the heart periods (RR intervals).

All variables were recorded continuously on an eight-channel polygraph (model RS 3800, Gould Inc., Cleveland, OH, USA) and simultaneously stored on the hard disk of a conventional personal computer for further analysis after analog-to-digital conversion with a rate of 400 Hz. During anaesthesia, respiratory gases and vapour concentrations were measured continuously at the endotracheal tube orifice by infrared spectroscopy (Capnomac® Ultima SV, Datex-Engstrom, Helsinki, Finland). We also intermittently determined arterial blood gas tensions, oxygen saturation, and pH (ABL3®, Radiometer, Copenhagen, Denmark).

Derived variables

Heart rate variability

HR variability (HRV), an indicator of the activity of the autonomic nervous system, was studied as recommended.[13] For this purpose, the original ECG signal, free of aberrant

ECG complexes and artefacts, was analysed during the last 5 min of each intervention (CHART®, ADInstruments, Castle Hill, Australia). HRV was analysed in the frequency domain and calculated as activity in the high frequency (HF: 0.15–0.5 Hz) and low frequency (LF: 0.04–0.15 Hz) range, the former showing predominantly vagal activity and the latter mainly sympathetic activity.[13] Autonomic balance was assessed by calculating the quotient of power in the high frequency (nuHF) and low frequency (nuLF) range, respectively, divided by total power (sum of HF and LF power).[13]

Experimental protocol

All experiments were carried out with awake dogs in the basal metabolic state (food withheld for 12 h with free access to water) and under standardized experimental conditions (lightly dimmed laboratory at thermoneutral temperature for dogs of 24°C).[14] To ensure complete elimination of the inhalation anaesthetic, successive experiments were performed at least 1 week apart.

After instrumentation and connecting the animal to the recording system, we waited for 30 min until all variables had reached steady state. The experiments started with baseline measurements for a further 30 min while the animal breathed spontaneously. Following the insertion of the endotracheal tube (intravenous injection of propofol 3 mg kg^{-1}), the animal's lungs were ventilated with 25% oxygen in nitrogen (tidal volume about 10 ml kg^{-1} and a rate of 14 bpm to maintain normocarbia). Isoflurane was added and adjusted to an end-tidal concentration of 1.4% (1 MAC).[15] We then waited 30 min, in order to minimize interaction with propofol. During this equilibration period, a pulmonary artery catheter was advanced from the animal's hind limb to obtain mixed venous blood samples. Thereafter, the following experiments were performed.

Oxygen uptake during xenon anaesthesia (n=5)

To evaluate whether xenon alters oxygen uptake, the following three mixtures were administered to each dog, but in a sequence which was randomized for each dog: FE′iso = 1.4% + FIXE = 50%, FE′iso = 1.4% + FIXE = 70%, FE′iso = 2.8%. The randomization resulted in two of the six possible sequences being used in two dogs each and one in another dog. Each gas mixture was maintained for 20 min to reach steady state. Before the end of the experiment, the animal was ventilated again with isoflurane 1.4% (1 MAC) in air to check whether $\dot{V}O_2$ and CO returned to control values.

Metabolic regulation of CO during xenon anaesthesia (n=5)

In a second series of experiments on the same animals, the interventions of group 1 were repeated (with randomization leading to one of the six possible sequences being used in three dogs and one in two dogs) and extended by two additional mixtures: FE′iso = 0.7% + FIXE = 50%, and FIXE = 70%, always in that sequence. The total of five different mixtures was again administered between two periods of FE′iso = 1.4%. Thus, we studied the dogs under a total of four interventions in the presence of xenon and under two different interventions with isoflurane, alone plus the awake state.

Oxygen uptake during ganglionic blockade (n=5)

After completion of groups 1 and 2, we studied the same animals again in order to see whether the increase in $\dot{V}O_2$ is of central or peripheral origin. For this purpose, hexamethonium, a ganglionic blocking agent, 7.5 mg kg^{-1} was injected before induction of anaesthesia, followed by continuous infusion of 7.5 mg kg^{-1} h^{-1}. Thereafter, the following two mixtures were administered to each dog: FE′iso = 1.4% + FIXE = 50%, FE′iso = 1.4% + FIXE = 70%, with randomization leading to one sequence being used in four dogs and the other in one dog.

Data analysis and statistics

Results are given as mean (SEM) and were compared using a paired *t* test. The resulting *P* values were corrected for multiple testing according to the Bonferroni procedure. In the case of repeated experiments in one animal, the results from individual dogs were averaged. CO was regressed on $\dot{V}O_2$ during the awake state and isoflurane anaesthesia, as well as during anaesthesia in the presence of xenon, and results were compared using an *F* test for differences between regression lines. The slopes of the individual relationships between $\dot{V}O_2$ and CO are given as mean slope and confidence interval. The effects on HR, MAP, systemic vascular resistance and $C(a-\bar{v})_{O_2}$ were compared by an analysis of variance for repeated measures (ANOVA), followed by Fisher's protected least significant difference test[16] if appropriate. Statistical significance was assumed when $P<0.05$.

Results

In general, $\dot{V}O_2$ increased significantly while CO remained essentially constant when xenon was added to isoflurane baseline anaesthesia. In detail, with the addition of xenon (50% and 70%) during isoflurane anaesthesia, $\dot{V}O_2$ increased from 3.4 (0.1) to 4.0 (0.1) and to 3.7 (0.2) ml kg^{-1} min^{-1} ($P<0.05$), respectively, whereas CO remained essentially unchanged (Fig. 1). In contrast, with the transition from awake to 1 MAC and eventually 2 MAC isoflurane alone, $\dot{V}O_2$ decreased by about 25% from 4.1 (0.2) ml kg^{-1} min^{-1} (awake) to 3.1 (0.1) ml kg^{-1} min^{-1} (2 MAC isoflurane). In parallel, CO decreased by about 40% from 121 (6) ml kg^{-1} min^{-1} (awake) to 70 (3) ml kg^{-1} min^{-1} (2 MAC isoflurane).

To answer the question of whether metabolic regulation of blood flow is maintained during xenon anaesthesia, we analysed the relationship between CO and $\dot{V}O_2$ in the presence and absence of xenon (Fig. 2). In both conditions, CO increased linearly with $\dot{V}O_2$ ($r^2=0.97$ and 0.94, respect-

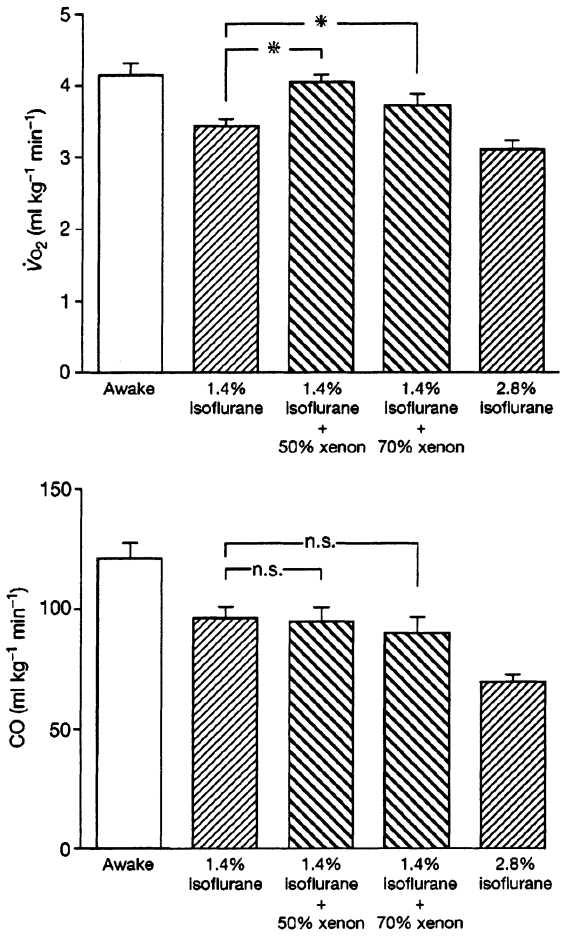

Fig 1 Oxygen consumption ($\dot{V}O_2$) and cardiac output (CO) in the awake state, during isoflurane anaesthesia 1.4% and 2.8% and after adding 50% and 70% xenon to an isoflurane baseline. Data are mean±SEM from five dogs. Note the significant increase in $\dot{V}O_2$ (*P<0.05) in the presence of xenon compared with 1.4% isoflurane, whereas CO remained essentially unchanged. n.s., not significant.

ively) and the regression lines differed significantly (P<0.05). For any given CO, $\dot{V}O_2$ was 0.3–0.6 ml kg^{-1} min^{-1} greater in the presence of xenon. Conversely, CO is 14–21 ml kg^{-1} min^{-1} lower for a given $\dot{V}O_2$ when xenon is present.

To distinguish whether the increase in $\dot{V}O_2$ results from a central or peripheral effect, we analysed HRV to assess sympathovagal balance (see Fig. 3) and, in additional experiments, added xenon to the respiratory gases after ganglionic blockade (pretreatment with hexamethonium). After increasing anaesthetic depth with xenon or isoflurane, nuHF tended to increase and nuLF tended to decrease, indicating a shift towards vagal activation.

After ganglionic blockade (Fig. 4), $\dot{V}O_2$ increased during the addition of 50% or 70% xenon to 1.4% isoflurane anaesthesia from 3.4 (0.2) ml kg^{-1} min^{-1} to 4.0 (0.2) and 3.7 (0.3), respectively, whereas CO remained almost unchanged. Thus, in the presence or absence of ganglionic blockade, xenon elicited similar effects on $\dot{V}O_2$ and CO, albeit at a lower CO (compare with Fig. 1).

$C(a-\bar{v})_{O_2}$ tended to increase from 3.7 (0.2) ml 100 ml^{-1} during 1 MAC isoflurane anaesthesia to 4.1 (0.5) ml 100 ml^{-1} and finally 4.4 (0.5) ml 100 ml^{-1} with the addition of 50% and 70% xenon, respectively (Table 1). Nevertheless, $C(a-\bar{v})_{O_2}$ increased with increasing anaesthetic depth in a parallel manner in the presence and absence of xenon – except for 70% xenon alone (Fig. 5). However, the error bars show that, at any given MAC, the differences between isoflurane and xenon (with or without isoflurane) are not significant. It is worthy of note that HR (105 [5] beats min^{-1} during 1.4% isoflurane), decreased on the addition of xenon 50% and 70% to 95 (2) and 92 (2) beats min^{-1}, respectively, which was in parallel with vagal activation, as indicated from the analysis of HRV (Fig. 3). Arterial pressure, however, did not change on the addition of xenon to 1.4% isoflurane anaesthesia.

Discussion

Our experiments show that, within the range of conditions studied, adding xenon to isoflurane baseline anaesthesia increases $\dot{V}O_2$. This increase in $\dot{V}O_2$ is independent of the autonomic nervous system and is probably caused by an increase in the cellular metabolic rate. Furthermore, metabolic regulation of blood flow is maintained during xenon anaesthesia, as shown by the linear relationship between CO and $\dot{V}O_2$. Accordingly, haemodynamic stability when adding xenon to isoflurane baseline anaesthesia is accompanied by an increase in the whole body metabolic rate ($\dot{V}O_2$).

Critique of methods

Attempts to compare the effects of different anaesthetics on CO and $\dot{V}O_2$ rest primarily on the precision of the measurement methods. This question is of particular interest because $\dot{V}O_2$ during xenon anaesthesia has not been measured before.

$\dot{V}O_2$ was measured with the Deltatrac II at a precision of 3.5%. The precision is independent of the collection mode, flow through, or canopy,[12 17 18] and is not influenced by the addition of volatile anaesthetics if a correction for the exhaled concentration of the anaesthetic is made.[19] Moreover, the four times greater density of xenon[4] compared with air did not alter the flow constant of the flow generator, and xenon did not influence oxygen – or carbon dioxide – measurements in our experiments. Accordingly, measurements of $\dot{V}O_2$ using a Deltatrac II were sufficiently precise to evaluate $\dot{V}O_2$ during anaesthesia with xenon in relationship to isoflurane.

CO was measured by ultrasound transit-time flow probes placed around the pulmonary artery. These probes had been

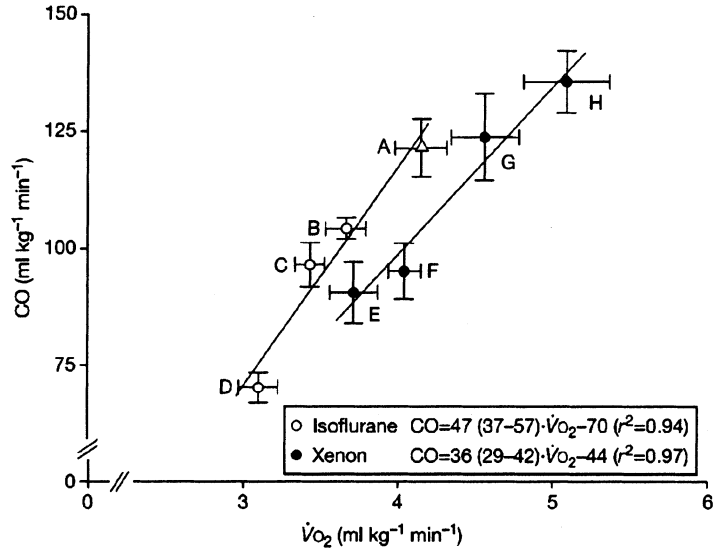

Fig 2 Relationship between cardiac output (CO) and oxygen consumption ($\dot{V}O_2$) in the awake state (A) and during isoflurane anaesthesia (1.4% [B, C] and 2.8% [D]) in comparison with xenon anaesthesia (70% and 50% xenon with 1.4% isoflurane [E, F], 50% xenon with 0.7% isoflurane [G] and 70% xenon alone [H]). Values are given as mean±SEM from five dogs. Note the close relationship between $\dot{V}O_2$ and CO during the awake state and during isoflurane anaesthesia (open circles), as well as in the presence of xenon (closed circles), with different regression lines ($P<0.05$).

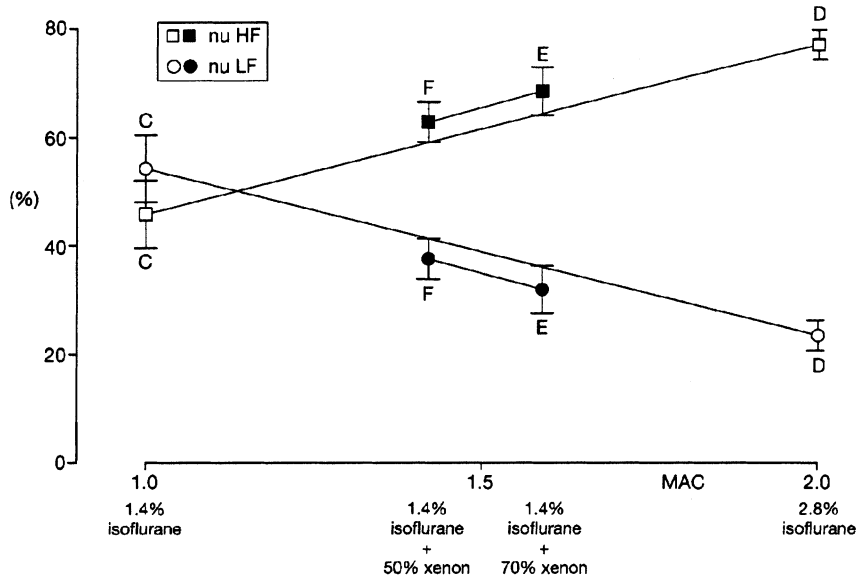

Fig 3 Sympathovagal balance analysed as heart rate variability in the frequency domain and expressed as high and low frequency normalized units (nuHF [squares] and nuLF [circles], respectively) during isoflurane anaesthesia ([open symbols] 1.4% [C], 2.8% [D]) and after adding xenon (70% and 50% [closed symbols] to 1.4% isoflurane [E, F]). Values are mean±SEM from five dogs. Note the parallel shift towards vagal activation (nuHF) when the anaesthetic depth is increased by adding xenon or by increasing the isoflurane alone.

calibrated *in vitro* by a given saline flow and, after implantation, by the Fick principle from $\dot{V}O_2$ and $C(a-\bar{v})_{O_2}$. Implantation around the pulmonary artery was chosen to obtain the entire cardiac output, which cannot be measured with flow probes placed around the aorta because coronary flow is not detected. These probes have been shown to continuously measure CO precisely over several years.[9]

The accuracy of our three independent measurement methods ($\dot{V}O_2$, CO and $C(a-\bar{v})_{O_2}$) can be cross-checked

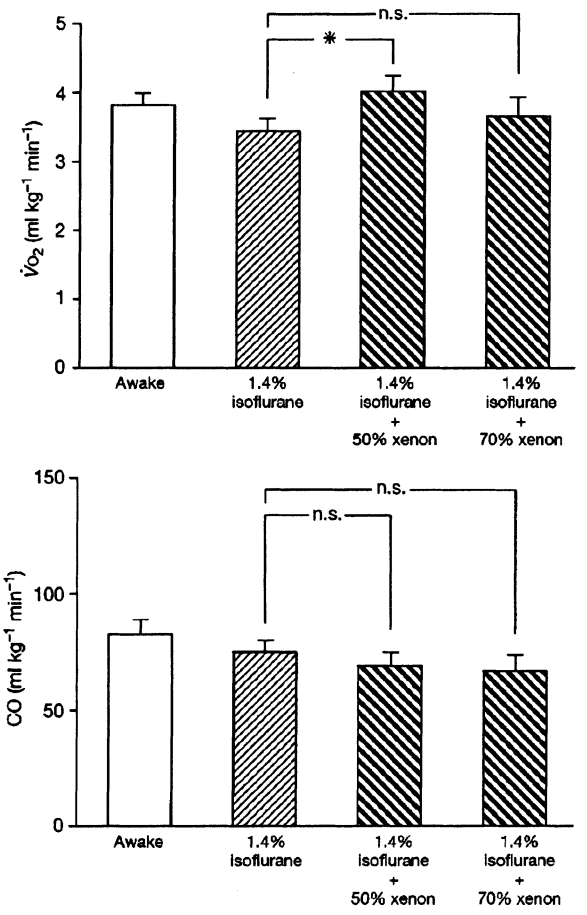

Fig 4 Oxygen consumption ($\dot{V}O_2$) and cardiac output (CO) after ganglionic blockade with hexamethonium in the awake state, during 1.4% isoflurane anaesthesia alone and after adding 50% and 70% xenon to isoflurane baseline anaesthesia. Data are mean±SEM from five dogs. Note that $\dot{V}O_2$ increased ($P<0.05$), whereas CO remained almost unchanged (n.s., not significant), as in the absence of ganglionic blockade (compare with Fig. 1).

using the Fick equation. Adding 50% xenon to 1.4% isoflurane did not change CO, so that changes in $\dot{V}O_2$ and $C(a-\bar{v})_{O_2}$ should balance each other. In fact, $\dot{V}O_2$ and $C(a-\bar{v})_{O_2}$ increased by 18% and 9%, respectively, confirming that CO was an essentially unchanged (calculation would yield 108%), with only 8% difference between independent measurement (ultrasound flowmetry) and calculation. This accuracy is likewise confirmed by the mean difference between measured and calculated $C(a-\bar{v})_{O_2}$ values, which was only 3.9 (3.1)%.

Propofol, needed for inserting the endotracheal tube, may have influenced the effects of the inhalation anaesthetics. However, the plasma concentration of propofol should have decayed to a fraction of the initial peak within 10 min because of redistribution (half-life of the α-phase of about 2 min) and, thereafter, more gradually as elimination continues (half-life of the γ-phase of about 4 h).[20] Moreover, in pilot experiments, all dogs resumed their normal activity and behaviour within 15 min after the injection of a single dose of propofol. Accordingly, the additive anaesthetic effects of propofol should have been small, and comparable for all interventions.

The dosage of hexamethonium used in our study was appropriate to eliminate the influence of the autonomic nervous system, as indicated not only from the literature[21] but also from our own experiments, in which arterial pressure and HR did not change after 45 s of bilateral carotid artery occlusion. In contrast, before hexamethonium administration, arterial pressure increased by about 40 mm Hg and HR by 20 beats min^{-1}. Thus, our methods should have been appropriate for deriving reliable measurements.

Interpretation of results

Metabolic regulation of blood flow manifests itself as a linear relationship between CO and $\dot{V}O_2$, during both physiological conditions[1] and inhalation anaesthesia.[3] In

Table 1 Haemodynamic variables and blood gas tensions in the awake state and during combinations of xenon and isoflurane anaesthesia in the intact dog and after ganglionic blockade with hexamethonium. Data are mean (SEM) from 15 experiments in five dogs. $^*P<0.05$ *vs* awake; $^\dagger P<0.05$ *vs* 1.4% isoflurane (1st). HR, heart rate; MAP, mean arterial pressure; SVR, systemic vascular resistance; Pa_{O_2} and Pa_{CO_2}, arterial oxygen and carbon dioxide gas tensions, respectively; Sa_{O_2}, arterial oxygen saturation; $C(a-\bar{v})_{O_2}$, mixed venous oxygen content difference

	Isoflurane (vol %)	Xenon (vol %)	HR (beats min^{-1})	MAP (mm Hg)	SVR (mm Hg litre^{-1} min)	Pa_{O_2} (mm Hg)	Pa_{CO_2} (mm Hg)	pH	Sa_{O_2} (%)	$C(a-\bar{v})_{O_2}$ (ml 100 ml^{-1})
Intact autonomic nervous system	Awake		82 (3)	98 (3)	25 (1)	94 (2)	37 (1)	7.35 (0.01)	95 (1)	
	1.4 (1st)		105 (5)*	63 (3)*	20 (1)*	123 (4)*	37 (1)	7.33 (0.01)	97 (1)	3.7 (0.2)
	1.4 (2nd)		110 (4)*	71 (2)*†	21 (1)*	126 (4)*	41 (1)	7.31 (0.01)	97 (1)	3.7 (0.2)
	2.8		102 (1)*	42 (2)*†	19 (1)*	119 (7)*	41 (1)	7.30 (0.01)	97 (1)	5.1 (0.5)†
		70	110 (4)*	115 (3*†	26 (1)†	120 (2)*	44 (1)	7.29 (0.02)	97 (1)	4.1 (0.4)
	0.7	50	107 (7)*	85 (3)*†	21 (2)*	116 (3)*	43 (1)	7.29 (0.02)	96 (1)	3.3 (0.2)
	1.4	50	95 (2)*	63 (4)*	21 (1)*	121 (6)*	41 (1)	7.31 (0.01)	97 (1)	4.1 (0.5)
	1.4	70	92 (2)†	65 (5)*	23 (1)*†	121 (5)*	42 (1)	7.29 (0.01)	97 (1)	4.4 (0.5)
Plus hexamethonium	Awake		137 (4)	88 (3)	36 (3)	83 (4)	40 (2)	7.34 (0.01)	93 (1)	
	1.4		117 (4)*	61 (3)*	27 (1)*	124 (7)*	38 (1)	7.34 (0.01)	97 (1)	3.8 (0.4)
	1.4	50	112 (4)*	55 (3)*	27 (3)*	117 (5)*	42 (1)	7.30 (0.01)	97 (1)	4.0 (0.4)
	1.4	70	111 (3)*†	52 (3)*†	26 (3)*	115 (5)*	43 (2)	7.29 (0.01)	96 (1)	4.0 (0.4)

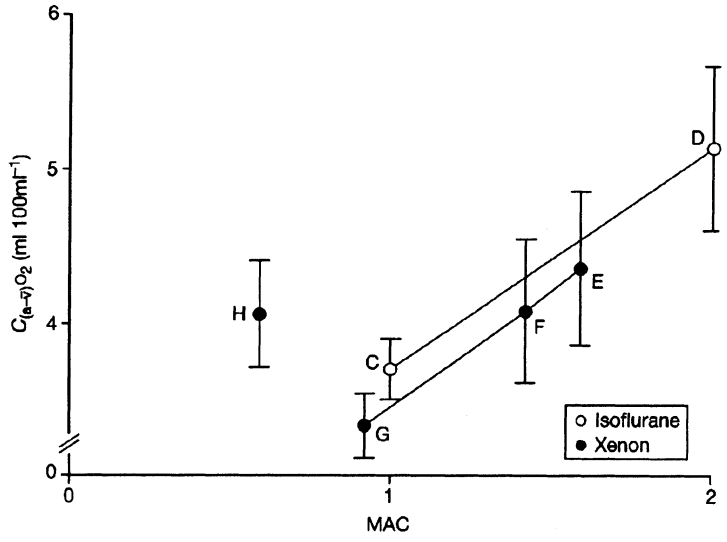

Fig 5 Arterial to mixed venous oxygen content difference ($C(a-\bar{v})_{O_2}$) during isoflurane anaesthesia (1.4% [C] and 2.8% [D]) in comparison with xenon anaesthesia (70% and 50% xenon with 1.4% isoflurane [E, F], 50% xenon with 0.7% isoflurane [G] and 70% xenon alone [H]) plotted against combined MAC. Data are mean±SEM from five dogs. Note that in the range above 1 MAC, $C(a-\bar{v})_{O_2}$ tended to increase with increasing anaesthetic depth in a parallel manner in the presence and absence of xenon.

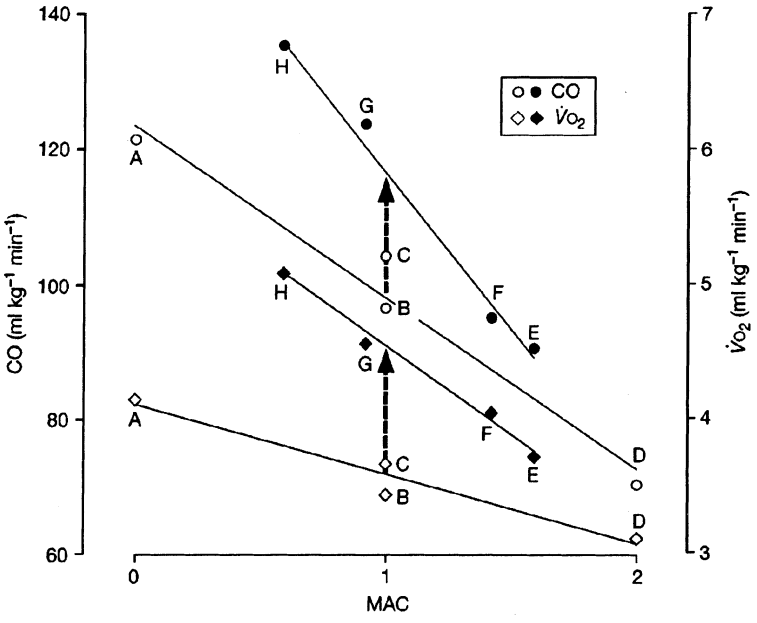

Fig 6 Oxygen consumption ($\dot{V}O_2$ [diamonds]) and cardiac output (CO [circles]) in relation to the anaesthetic depth (MAC), in the awake state (A) and during isoflurane anaesthesia (1.4% [B, C] and 2.8% [D]) in comparison with xenon anaesthesia (70% and 50% xenon with 1.4% isoflurane [E, F], 50% xenon with 0.7% isoflurane [G] and 70% xenon alone [H]). Values are means from five dogs. Both variables correlate closely in the absence (open symbols) and presence (closed symbols) of xenon. Note that substituting xenon for parts of the isoflurane (see arrows) would lead to an increase in CO and $\dot{V}O_2$, with the effects of xenon tending to decrease as MAC increases.

this context, $\dot{V}O_2$ is considered the independent variable and thus determines CO, and not vice versa.[3] In our experiments during inhalation anaesthesia with isoflurane, CO and $\dot{V}O_2$ decreased from the awake state (basal metabolic conditions) to 2 MAC (points A–D in Fig. 2). This relationship was linear, with a slope of CO *vs* $\dot{V}O_2$ of 47, confirming our previous observations.[3] In that study,[3] we could also show that the relationship between CO and $\dot{V}O_2$ did not differ

significantly between the five most commonly used volatile anaesthetics.[3]

In contrast to the effects of these volatile anaesthetics, increasing anaesthetic depth from 1 MAC isoflurane with xenon by about 0.5 MAC (MAC value of 119% in dogs[22]) increased $\dot{V}O_2$ while CO remained essentially unchanged. Cardiovascular stability during xenon anaesthesia has generally been observed in healthy individuals,[23,24] as well as in dogs with dilated cardiomyopathy.[6] Moreover, xenon had only minimal effects on myocardial contractility *in vivo*[5,25] and maintained cardiovascular stability during surgical stimulation.[26] However, total body oxygen consumption, the main determinant of CO, has not been measured during xenon anaesthesia before.

Increases in $\dot{V}O_2$ could be related to either an increase in efferent sympathetic activity or a direct stimulating effect on the cellular metabolic rate. To test the contribution of the autonomic nervous system, we repeated the experiments after autonomic blockade. The increase in $\dot{V}O_2$ during xenon anaesthesia was identical after ganglionic blockade, thus excluding increased sympathetic activity and suggesting a direct effect on cellular metabolic rate. However, there are no studies of the interaction between xenon and the molecular mechanisms of metabolism, and explanations of this phenomenon are beyond the scope of our experiments. The absence of sympathetic contribution to the increase in $\dot{V}O_2$ is confirmed by the shift towards vagal activation, as indicated from the analysis of HRV in the experiments with the intact autonomic nervous system. Similar effects of xenon on the autonomic nervous system were previously observed in humans.[27] In conclusion, xenon increases $\dot{V}O_2$ most likely by directly stimulating the cellular metabolic rate.

Only myocardial oxygen consumption has been previously studied in detail during xenon anaesthesia, but this did not change either *in vivo*[25] or in isolated hearts.[7] However, myocardial oxygen consumption contributes only 10–15% to total body $\dot{V}O_2$, and changes in myocardial oxygen consumption may not necessarily parallel changes in total body $\dot{V}O_2$

When anaesthetic depth changed, $\dot{V}O_2$ and CO were linearly related in the presence of xenon, much like in the presence of volatile anaesthetics. However, the regression lines for xenon with and without isoflurane, and for isoflurane alone, differed significantly (Fig. 2). At any given CO, $\dot{V}O_2$ was greater in the presence of xenon. If, in addition, CO and $\dot{V}O_2$ are plotted against MAC, at least one more interpretation can be obtained (Fig. 6). Over the range of anaesthetic depths studied (below 2 MAC), substituting xenon for a proportion of the isoflurane (see arrows) would lead to an increase in CO and $\dot{V}O_2$, with the effects of xenon tending to decrease as MAC increases. However, this interpretation has to be drawn with caution since it depends on the MAC of xenon, which has only been measured once in dogs[22] and, in contrast to the other inhalation anaesthetics, differed by a factor of two between dogs and humans. It is also worth noting that HR decreased slightly during xenon anaesthesia in parallel with vagal activation, a phenomenon which has likewise been shown for the volatile anaesthetics.[28] Thus, changes in HR during xenon anaesthesia are most likely caused by vagal activation. This interpretation is in accordance with the absence of this effect in isolated hearts.[7] Accordingly, regulation of HR during xenon anaesthesia apparently does not differ from that during isoflurane anaesthesia.

In summary, adding xenon to isoflurane baseline anaesthesia increases $\dot{V}O_2$, while haemodynamics, including CO, are essentially unchanged. Metabolic regulation of blood flow is maintained at a higher tissue oxygen extraction rate. When xenon is substituted for a proportion of the isoflurane, both $\dot{V}O_2$ and CO are increased.

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ORIGINAL CONTRIBUTION

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Nitric oxide synthases in vagal neurons are crucial for the regulation of heart rate in awake dogs

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Abstract Nitric oxide synthase (NOS) inhibitors elicit bradycardias independent of the endothelium (e-NOS) or increases in blood pressure. Therefore, this bradycardia could be mediated by other NOS isoforms, most likely that of the nervous system (n-NOS). If so, heart rate variability (HRV) as a measure of vagal activity should be an indicator of the activity of n-NOS in vagal neurons. To test this, we studied the dose-effect relations of L-NAME (0.3 – 50 mg·kg^{-1}) on heart rate (HR), HRV and systemic vascular resistance (SVR) in seven awake dogs. HRV was analyzed in the time domain as standard deviation of the RR-intervals (SDNN) and in the frequency domain as power in the high (0.15 – 0.5 Hz) and low (0.04 – 0.15 Hz) frequency range. The effects of HR and SDNN reached their maxima at a dose of 3 mg·kg^{-1} and had their ED_{50} at 0.27 ± 0.03 mg·kg^{-1} and 0.43 ± 0.1 mg·kg^{-1}, respectively, whereas SVR had its maximum at 10 mg·kg^{-1} and ED_{50} at 0.86 ± 0.11 mg·kg^{-1} ($p < 0.05$). HF-power (vagal activity) predominated compared to LF-power (mainly sympathetic activity) during baseline as well as after L-NAME. The effects on HR and HRV were absent after ganglionic blockade (hexamethonium), whereas the effects on SVR remained unchanged. Thus, NO exerts a powerful restraining activity on vagal neurons and plays a key role in the adjustment of heart rate in awake resting animals with prevailing vagal activity.

Key words Autonomic nervous system – heart rate – heart rate variability – nitric oxide – vasoconstriction

Introduction

Inhibitors of nitric oxide synthases (NOS) elicit not only vasoconstriction but also profound bradycardia (14, 25, 28). This bradycardia is widely held to result from vagal activation via baroreflexes in response to the initial increase in blood pressure subsequent to the blockade of NOS of the endothelium (e-NOS). Thus, inhibition of e-NOS is thought to be the prime mover for the bradycardic action (48). However, it is equally possible that the bradycardia may be initiated independently of baroreflexes by the blockade of NO synthases in the nervous system (n-NOS), which exist not only in brain regions involved in cardiovascular control (47) but also in sympathetic and parasympathetic nerves innervating the heart (23, 39, 40, 47) and its pacemaker cells (39). This view is supported by observations in mice lacking the e-NOS gene, in which NOS inhibitors no longer elicit increases in blood pressure but still cause profound bradycardia (24).

Central vagal tone appears to be the dominant mediator of the bradycardic action of NOS inhibitors just for the absence of these effects in vagotomized animals (33, 42) and likewise in isolated hearts (1, 21). Also, muscarinic and β-adrenergic pharmaceuticals produce the

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same effects on the beating rate of isolated atria, regardless whether they derive from animals lacking the e-NOS gene or not (45). Thus, contrary to earlier suggestions (11), e-NOS is not obligatory for the autonomic control of heart rate.

Together these observations suggest that the NOS-inhibitor-induced bradycardia is primarily a manifestation of the activity of n-NOS in vagal neurons of the brain, and therefore should be characterized by a particular dose-effect relationship. To test this hypothesis we determined the effects of N-ω-nitro-arginine-methyl-ester (L-NAME), a nonselective NOS inhibitor, on heart rate and heart rate variability as a measure of vagal activity (22, 46) as well as on vascular flow resistance as a measure of vasomotor tone in a placebo-controlled study on awake dogs. These experiments will show for the first time that the dose-dependent bradycardia occurs in parallel with vagal activation at smaller doses of L-NAME than those which cause vasoconstriction. Thus, NO in the central nervous system exerts a powerful restraining activity on vagal neurons and plays a key role in the adjustment of heart rate in awake resting animals.

Methods

The data derive from 26 experiments on seven trained dogs (Foxhounds of both sexes weighing 27 – 34 kg) studied with approval of the District Governmental Animal Investigation Committee. Each dog was randomly assigned to each of the following experiments (L-NAME, placebo, hexamethonium, and D-NAME) with an interval of at least two weeks between successive experiments in one animal, so that each dog served as its own control.

Surgery

Several weeks before the actual experiments the dogs were operated under general anesthesia (enflurane/nitrous oxide + fentanyl) and aseptic conditions. For blood pressure recording and blood sampling, both carotid arteries were exteriorized in skin loops (44). Ultrasound transit-time flow transducers were implanted around the pulmonary artery through a left-sided thoracotomy for the continuous recording of cardiac output. During reconvalescence the dogs were trained to lie quietly and unrestrained on their right side and to become familiar with the experimenters and the laboratory.

Measurements

Heart rate (HR) and RR intervals (RR)

HR and RR were determined from a standard ECG (surface electrodes) used for triggering a rate meter which provided a continuous recording of the heart periods (RR intervals).

Arterial pressure and central venous pressure (CVP)

Both pressures were measured electromanometrically (Statham P-23ID) through catheters in the carotid artery and the right atrium. The electromanometers were referenced to the processus spinosus of the 7th vertebra and calibrated with a mercury manometer. Mean arterial pressure (MAP) was measured by integration from the original signal.

Cardiac output (CO)

Blood flow through the pulmonary artery was measured continuously with an ultrasound transit-time system (T101, Transonic Systems, Ithaca, NY). Each flow transducer (20 – 24 mm S-series with silicone shielded U-reflector, Transonic) was calibrated in vitro prior to implantation and in vivo at least three weeks after implantation by the Fick principle from oxygen consumption ($\dot{V}O_2$) and the arterial to mixed venous oxygen content ($C_{(a-\bar{v})}O_2$) measured with a galvanic cell (Lex-O_2-Con-TL®), resulting in high precision, as previously described (31).

Systemic vascular resistance (SVR)

SVR, a measure of vasomotor tone, was calculated online as the quotient of the arterial to venous pressure gradient (MAP – CVP) and cardiac output after analog-digital signal conversion (POWERLAB®, ADInstruments, Castle Hill, Australia).

Respiratory rate

Respiratory rate was measured continuously by a mercury-in-silastic-gauge mounted around the animal's thorax.

Oxygen consumption ($\dot{V}O_2$)

$\dot{V}O_2$ was measured continuously by indirect calorimetry with a DeltatracII® Metabolic Monitor (Datex-Engstrom Division, Instrumentarium Corp., Helsinki, Finland).

All variables were recorded continuously on an eight-channel polygraph (model RS 3800, Gould Inc., Cleveland, OH) and simultaneously stored on the hard disk of a conventional personal computer for further analysis after analog-to-digital conversion at a rate of 1000 Hz.

Derived variables

Heart rate variability (HRV)

HRV, an indicator of the activity of the autonomous nervous system, was studied as recommended (41). For this purpose, the original ECG signal, free of aberrant ECG complexes and artifacts, was analyzed for the duration of 5 min as recommended (30) during baseline conditions and between the 20th and the 30th min after the injection of each incremental dose of L-NAME (CHART®, ADInstruments, Castle Hill, Australia). HRV was analyzed in the time domain and expressed as the standard deviation of the RR intervals (SDNN). In addition, HRV was analyzed in the frequency domain and calculated as the activity in the high (HF, 0.15 – 0.5 Hz) and the low (LF, 0.04 – 0.15 Hz) frequency range, the former showing exclusively vagal activity and the latter mainly sympathetic activity. To analyze the sympathovagal balance, power spectra in the high and low frequency range were normalized by calculating their respective percentage of total power (i.e., high + low frequency power) and expressed as normalized units for the high and the low frequency range (nuHF and nuLF).

We also measured intermittently arterial blood gas tensions, O_2 saturation, and pH (ABL3, Radiometer, Copenhagen, Denmark).

Program of experimentation

All experiments were carried out with the awake dogs in the basal metabolic state (food withheld for 12 hours with free access to water) and under standardized experimental conditions (dogs lying on their right side, slightly dimmed laboratory at thermoneutral temperature for dogs of 24 °C) (10) always beginning at 8 a.m. During the experiments the dogs remained unrestrained on their right side on a cushioned table with their head under a transparent plastic hood for measuring $\dot{V}O_2$. To ensure complete elimination of the NOS inhibitor (half-life of about one day) (38), at least two weeks were interspaced between successive experiments.

After connecting the animals to the recording system, we waited for about 30 min until all variables had reached a steady state as the animals calmed down. The actual experiments started with baseline measurements for further 30 min. Thereafter the dogs were randomly assigned to one of the following interventions.

L-NAME (n = 7)

N-ω-nitro-L-arginine methyl ester (L-NAME; Sigma Chemicals, St. Louis, USA, dissolved in 0.9 % saline) was injected cumulatively at increasing doses (0.3/1.0/3.0/10 and 50 mg·kg^{-1}). A period of 30 min was interspaced between successive L-NAME injections so that the experiments lasted for about 3.5 h.

Hexamethonium (n = 6)

To test whether the effects of L-NAME are of central or of peripheral origin, hexamethonium (Sigma Chemicals, St. Louis, USA, dissolved in 0.9 % saline), a ganglionic blocking agent, was injected (7.5 mg·kg^{-1} initially followed by 7.5 mg·kg^{-1}·h^{-1}) 30 min prior to the first L-NAME dose. To confirm that the autonomic blockade was complete and maintained for the duration of the experiments, we tested the blood pressure response to bilateral occlusion of the carotid arteries (duration 45 s) with self-made external occluders placed around each carotid artery. Blood pressure and heart rate increased by 30 mmHg and 20 min^{-1}, respectively during control conditions but both variables remained constant during ganglionic blockade. Thereafter, L-NAME was injected cumulatively at increasing doses between 0.3 and 50 mg·kg^{-1}. These experiments were performed in the same dogs and lasted for about 4 h.

Placebo (n = 7)

To check for time-related effects, identical experiments were performed in the same dogs in which vehicle (NaCl) was injecting instead of L-NAME. These experiments also lasted for 3.5 h.

D-NAME (n = 6)

To test for the stereospecifity of the effects of L-NAME, a single dose of D-NAME (50 mg·kg^{-1}) was injected instead of L-NAME in the same dogs. These experiments lasted for about 1.5 h.

Data analysis and statistics

For showing the time course of the L-NAME and placebo effects, results are given as means ± standard error of the mean (SEM). An intergroup comparison was made between L-NAME and placebo, and in this case *P* values, based on a Wilcoxon's rank sum test, are given for descriptive purposes and are interpreted as effect measures.

For analyzing the relation between heart rate variability and heart period as well as the relation between heart rate and cardiac output during L-NAME injections, linear regression analysis was performed for each dog and experiment. The results (medians and ranges) are given as the slopes and intercepts of these functions.

The dose-effect relations were calculated for each experiment as percent of the maximal effects relative to

baseline by the equation: response $= \frac{-a}{1 + (\text{dosage}/c)^b} + a$,

with a = response at maximum, b = constant related to the slope and c = ED_{50} (half effective dose). For statistical analysis the individual ED_{50} were compared by Wilcoxon's rank sum test. A *P* value of < 0.05 was considered to be significant. The resulting *P* values were α-adjusted for multiple testing according to the Bonferroni procedure.

Results

In general, L-NAME elicited in a dose-dependent fashion profound bradycardia in parallel with a strong increase in heart rate variability and also, though at larger doses, vasoconstriction.

This is shown in Fig. 1, which contrasts the time course of our measurements for both the L-NAME and placebo experiments based on the steady-state values obtained at the end of each intervention period. The following is apparent: First, the baseline measurements do not differ between study groups, but the placebo experiments show a trend for heart rate as well as cardiac output to decrease while all the other variables increase slightly over time. Second, relative to the corresponding time periods of the placebo experiments, heart rate along with cardiac output decrease while arterial blood pressure and vascular flow resistance increase in the presence of L-NAME. Third, all effects grow with the dose of L-NAME and attain their minimum at doses of 3 mg·kg^{-1}, with the exception of systemic vascular resistance and

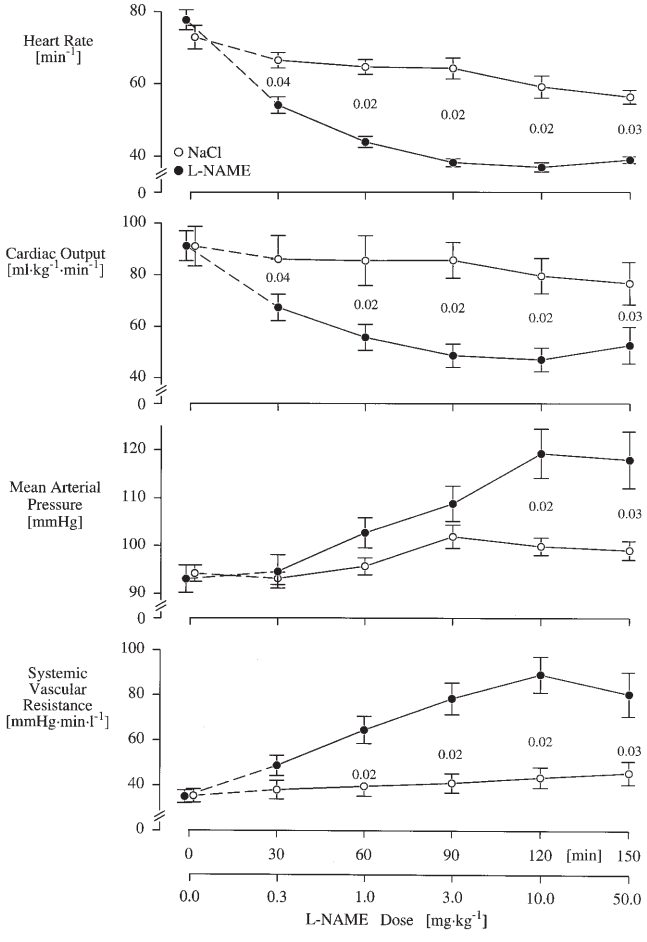


Fig. 1 Hemodynamic effects of increasing doses of L-NAME compared to placebo (NaCl). Means ± SEM from 7 awake dogs. Values between lines are P values. Heart rate and cardiac output decreased already substantially at 0.3 mg·kg^{-1} of L-NAME, whereas blood pressure and systemic vascular resistance started to increased at higher doses. Note also the dose dependency of the L-NAME effects relative to placebo with differences in the maxima.

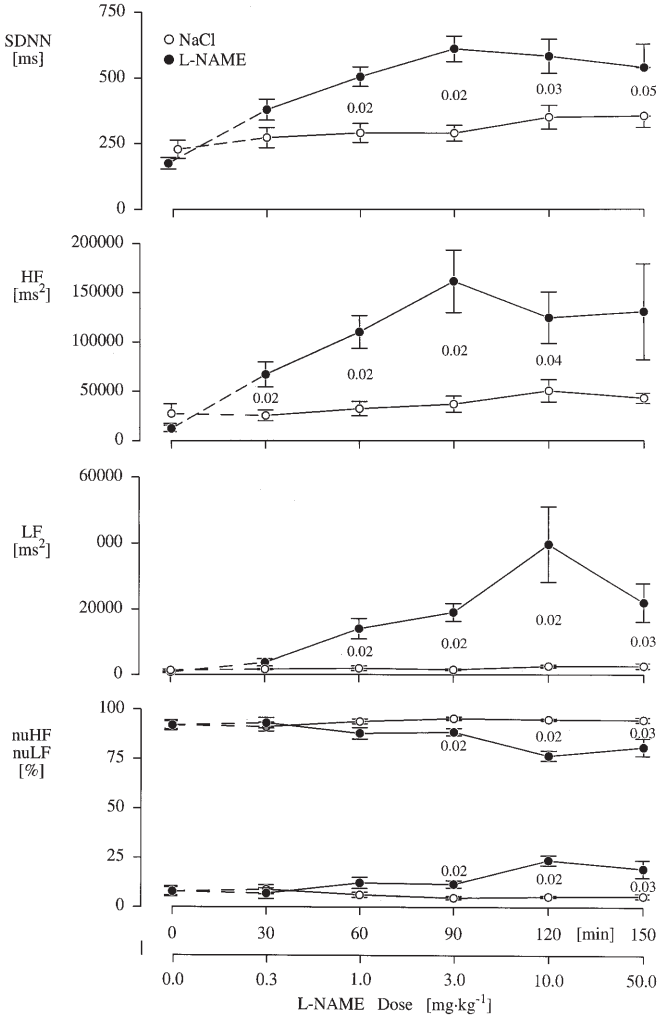


Fig. 2 Heart rate variability during increasing doses of L-NAME compared to placebo (NaCl). Means ± SEM from 7 awake dogs. Values between lines are P values. The measures of heart rate variability both in the time domain (SDNN) and in the frequency domain (HF-power and LF-power) increased dose-dependently and vagal activity (nuHF) always predominated compared to sympathetic activity (nuLF).

Table 1 Cardiovascular variables after hexamethonium/L-NAME and D-NAME injection

	HR [min^{-1}]	SDNN [ms]	CO [$ml \cdot kg^{-1} \cdot min^{-1}$]	MAP [mmHg]	SVR [$mmHg \cdot min \cdot l^{-1}$]
Baseline	81 ± 5	180 ± 37	114 ± 10	96 ± 4	29 ± 3
Hexamethonium	129 ± 4	4 ± 1	113 ± 10	90 ± 5	28 ± 3
0.3 mg·kg^{-1} L-NAME	119 ± 3	5 ± 1	102 ± 10	97 ± 5	33 ± 3
1.0 mg·kg^{-1} L-NAME	108 ± 3	16 ± 4	93 ± 10	125 ± 11	43 ± 4
3 mg·kg^{-1} L-NAME	107 ± 5	15 ± 4	84 ± 9	164 ± 13	61 ± 7
10 mg·kg^{-1} L-NAME	112 ± 4	8 ± 3	84 ± 11	172 ± 12	68 ± 12
50 mg·kg^{-1} L-NAME	112 ± 3	6 ± 2	82 ± 9	151 ± 13	61 ± 11
Baseline	69 ± 10	178 ± 30	97 ± 10	103 ± 4	32 ± 2
50 mg·kg^{-1} D-NAME	62 ± 7	234 ± 40	90 ± 5	109 ± 3	35 ± 1

Heart rate *(HR)*, heart rate variability *(SDNN)*, cardiac output *(CO)* mean arterieal pressure *(MAP)* and systemic vascular resistance *(SVR)* after hexamethonium/L-NAME and D-NAME injection. Means ± SEM, from 6 awake dogs

blood pressure which both attain their maximum at 10 mg·kg^{-1}. Fourth, the effects on heart rate are substantial as it is about halved from 78 ± 3 to 37 ± 1 min^{-1} at 3 mg·kg^{-1} of L-NAME. Fifth, it is noteworthy also that, at the smallest dose of L-NAME, heart rate had already decreased substantially in spite of an unchanged arterial blood pressure.

These hemodynamic changes are accompanied by corresponding changes of heart rate variability in the opposite direction (Fig. 2). SDNN, HF-power and LF-power increase substantially while the dose of L-NAME increases. However, only SDNN and HF-power reach their maximum at 3 mg·kg^{-1} of L-NAME, whereas LF-power reaches its maximum at a dose of 10 mg·kg^{-1} of L-NAME. Vagal activity predominates during baseline (nuHF-power about 92 % and nuLF-power 8 %, respectively) as well as during increasing doses of L-NAME. Scrutiny reveals that, at higher doses of L-NAME (10 mg·kg^{-1}), this ratio is slightly shifted towards nuLF-power, indicating sympathetic activation. Accordingly, at low doses of L-NAME does vagal activity predominates, whereas only at higher doses of L-NAME does additional sympathetic activation occur.

To test whether the effects of L-NAME are of central or peripheral origin, L-NAME was injected after ganglionic blockade with hexamethonium (Table 1). As a result, heart rate increased and heart rate variability decreased after ganglionic blockade but both variables remained almost constant during all doses of L-NAME. In contrast, SVR did not change after injection of hexamethonium, but did so to about the same extent as without previous ganglionic blockade after L-NAME (compare with Fig. 2). Thus, changes in heart rate are of central origin, whereas SVR increased mainly via a peripheral mechanism. It is moreover noteworthy that, in the presence of hexamethonium, blood pressure increased strongly because of lacking bradycardia. Additionally, we tested the stereospecifity of the effects of L-NAME, which is confirmed by the absence of any effect after the injection of 50 mg·kg^{-1} D-NAME (Table 1).

Thus, L-NAME exerts its stereospecific cardiovascular actions in a dose-dependent manner. But, relative to the placebo experiments, the maximum effects on heart rate, heart rate variability and cardiac output occur at smaller doses than the maximum effects on vascular flow resistance and arterial blood pressure.

As the course of heart rate variability analyzed in the time or in the frequency domain was almost identical, it was justified to use only SDNN for the dose-effect ratio which express the L-NAME effects as percent of the respective maxima (Fig. 3). These relations are by and large congruent, i.e., the effects increase with the same dose-effect ratio as the dose is increased, attain a maximum at a certain dose and then decrease slightly (Fig. 3). However, the graphs for heart rate and its variability reach a maxima already at a dose of 3 mg · kg^{-1}, whereas

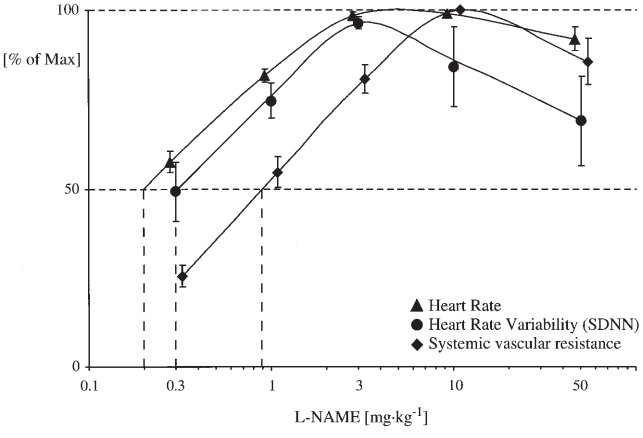
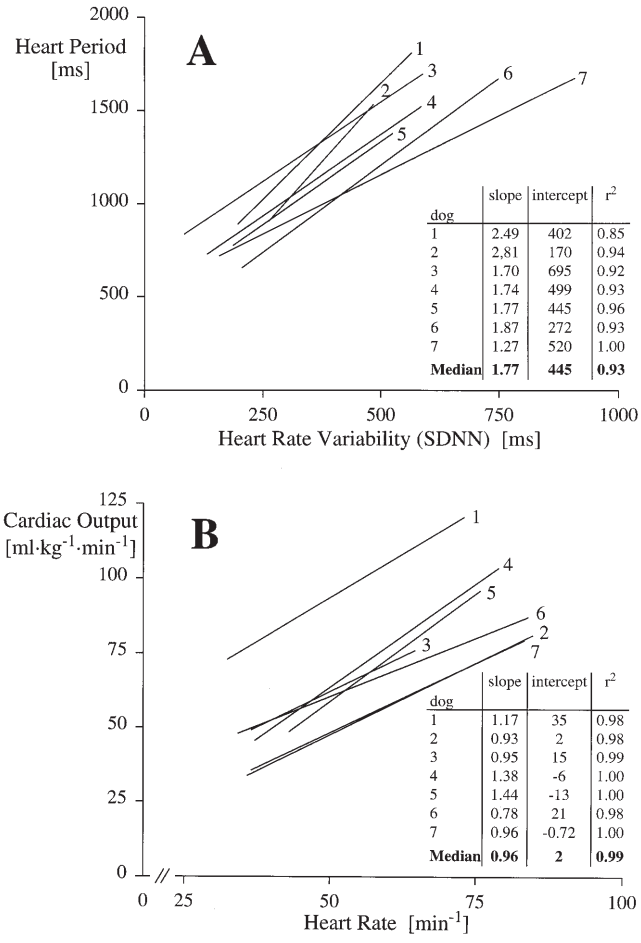


Fig. 3 Dose-effect relations for heart rate, heart rate variability and systemic vascular resistance. Effect percent of the respective maxima (means ± SEM) for seven awake dogs. The ED_{50} for systemic vascular resistance is three times greater ($p < 0.05$) than that for either heart rate or heart rate variability.



dog	slope	intercept	r^2
1	2.49	402	0.85
2	2,81	170	0.94
3	1.70	695	0.92
4	1.74	499	0.93
5	1.77	445	0.96
6	1.87	272	0.93
7	1.27	520	1.00
Median	**1.77**	**445**	**0.93**

dog	slope	intercept	r^2
1	1.17	35	0.98
2	0.93	2	0.98
3	0.95	15	0.99
4	1.38	-6	1.00
5	1.44	-13	1.00
6	0.78	21	0.98
7	0.96	-0.72	1.00
Median	**0.96**	**2**	**0.99**

Fig. 4 Correlations between heart period and heart rate variability (**A**) and for cardiac output and heart rate (**B**). The lines represent data from each dog at baseline and in the presence of L-NAME at doses of 0.3 – 50 mg·kg^{-1}. The correlation coefficients are near unity. Note also that the correlation between heart period and heart rate variability has an intercept near 445 ms corresponding to a heart rate of about 130 min^{-1}.

the graph for vascular flow resistance has its maximum at 10 mg·kg^{-1}. Similarly, the ED50 for the heart rate effect and likewise for heart rate variability are 0.27 ± 0.03 mg·kg^{-1} and 0.43 ± 0.1 mg·kg^{-1}, respectively, whereas the ED_{50} for the systemic vascular resistance is 0.86 ± 0.11 mg·kg^{-1}, a difference which is statistically significant (Wilcoxon's rank sum test, $p < 0.05$). Accordingly, L-NAME is nearly three times more effective on heart rate variability and heart rate than on vasomotor tone.

The detailed analysis of the relations between heart period (the reciprocal of heart rate) and heart rate variability (Fig. 4A) and between cardiac output and heart rate (Fig. 4B) revealed close linear correlations with r^2 near unity. Converting the intercept (445 ms) into heart rate indicates that heart rate would be near 130 min^{-1} if heart rate variability and thus vagal tone would be abolished. It is also apparent (Fig. 4B) that HR is mainly responsible for the decrease in cardiac output.

Finally, $\dot{V}O_2$ during baseline agreed with the basal metabolic rate of awake dogs and did not change compared to placebo throughout the course of our experiments nor did respiratory rate change (Table 2). Hence, the decrease of cardiac output and thus in O_2 delivery is compensated by a corresponding increase in O_2 extraction, i.e., an increase in the arterio-venous O_2 content. However, these effects are not accompanied by changes in blood gas tensions and pH of the arterial blood (Table 3), indicating that the O_2 demand of the tissues is still met.

Discussion

L-NAME, which inhibits both n-NOS and e-NOS, elicits in a dose-dependent fashion bradycardia with a corresponding increase in heart rate variability at smaller doses than it causes vasoconstriction, and the bradycardia and likewise cardiac output correlate linearly with heart rate variability. These observations indicate that n-NOS related to vagal activity mediates the effects on heart rate.

This conclusion rests primarily on the premises that heart rate variability and vascular flow resistance are measures of cardiac vagal activity and vascular smooth muscle tone, respectively.

Table 2 Oxygen consumption ($\dot{V}O_2$), arterio-mixed venous oxygen content difference ($C_{(a-\bar{v})}O_2$) and respiratory rate in the presence of L-NAME compared to placebo

	$\dot{V}O_2$ [ml·kg^{-1}·min^{-1}]		$C_{(a-\bar{v})}O_2$ [ml·dl^{-1}]		Respiratory rate [min^{-1}]	
Dose [mg·kg^{-1}]	L-NAME	Placebo	L-NAME	Placebo	L-NAME	Placebo
0	4.0 ± 0.3	4.0 ± 0.2	4.5 ± 0.5	4.5 ± 0.4	18 ± 2	18 ± 3
0.3	3.7 ± 0.3	3.8 ± 0.2	5.7 ± 0.5	4.7 ± 0.5	15 ± 2	16 ± 3
1	3.4 ± 0.2	3.9 ± 0.2	6.4 ± 0.5	4.8 ± 0.4	14 ± 1	16 ± 3
3	3.5 ± 0.3	3.9 ± 0.2	7.5 ± 0.7	4.7 ± 0.5	14 ± 2	16 ± 3
10	3.5 ± 0.2	3.7 ± 0.1	7.8 ± 0.7	4.9 ± 0.6	13 ± 2	14 ± 2
50	3.4 ± 0.2	3.9 ± 0.2	6.8 ± 0.7	5.4 ± 0.6	13 ± 2	13 ± 2

Means ± SEM, from 7 awake dogs

Table 3 Arterial blood gas tension (pO_2 and pCO_2) and pH during L-NAME and placebo injection

	pO_2 [mmHg]		pCO_2 [mmHg]		pH	
Dose [mg·kg^{-1}]	L-NAME	Placebo	L-NAME	Placebo	L-NAME	Placebo
0	114 ± 6	107 ± 6	35 ± 1	36 ± 1	7.33 ± 0.01	7.32 ± 0.03
0.3	111 ± 5	111 ± 2	35 ± 1	35 ± 1	7.33 ± 0.01	7.32 ± 0.03
1	114 ± 8	110 ± 4	35 ± 1	35 ± 1	7.34 ± 0.01	7.33 ± 0.02
3	99 ± 3	105 ± 3	35 ± 1	36 ± 1	7.34 ± 0.01	7.34 ± 0.01
10	104 ± 4	103 ± 3	34 ± 2	35 ± 1	7.35 ± 0.01	7.35 ± 0.01
50	104 ± 2	102 ± 4	35 ± 2	35 ± 1	7.31 ± 0.02	7.34 ± 0.01

Means ± SEM, from 7 awake dogs

In essence, vagal tone of the heart is the spike traffic in cardioinhibitory neurons, which have their cell bodies in vagal nuclei of the medulla and their axons in the vagal nerves. The activity of these neurons, recordable in anesthetised animals only, correlates linearly with the heart period, the reciprocal of heart rate (20), and this relation can be duplicated by electro-stimulation of the vagal nerves (29). Accordingly, vagal spike activity is a reliable predictor of heart rate, even when the sympathetic nerves are intact (19). Furthermore, in spontaneously breathing animals, cardiac vagal activity also correlates linearly with the respiratory variations of heart rate (respiratory arrhythmia) (19) which is the rationale for the use of heart rate variability, expressed as standard deviation of instantaneous heart period (SDNN), for instance, as an indicator of cardiac vagal tone in awake animals and humans as well (2, 26, 41). In fact, general anesthetics abolish the spike traffic in vagal neurons (16), attenuate heart rate variability, and elicit tachycardias (18). In addition, heart rate is about 130 min^{-1} as in our experiments when vagal tone is completely abolished by cooling of the vagal nerves (19) or pharmacological denervation (4). Apart of the vagally mediated heart rate changes coincident with respiration (respiratory frequency 0.15 – 0.5 Hz), heart rate changes also with fluctuation of arterial blood pressure (frequency band rate 0.05 – 0.15 Hz), which probably reflects changes in sympathetic tone. These two components can be separated from each other by spectral analysis of instantaneous heart rate (analysis in the frequency domain), and there is agreement that power in the high frequency band exclusively reflects vagal activity and power in the low frequency band reflects mainly sympathetic activity (37, 41). We have included this more detailed analysis to also gain information on the autonomic balance in our experiments.

Moreover, the finding that in mice deficient of the n-NOS gene, heart rate variability is reduced compared to wild-type mice, implies a role for n-NOS in the vagal regulation of heart rate (17). Accordingly, heart rate variability is in all likelihood a manifestation of cardiac vagal activity and thus of n-NOS activity in our experiments.

Concerning the function of e-NOS, there is general agreement that its activity, which depends on the shear rate and thus on blood flow in peripheral vessels (8), determines the release of NO and this way modulates vascular smooth muscle tone (9). Consistent with this view, NOS inhibitors increase in a dose-dependent fashion arterial blood pressure subsequent to the increase in vascular flow resistance. We therefore used vascular flow resistance, the quotient of the arterio-venous pressure gradient and cardiac output, as a measure of e-NOS activity in our experiments.

Our premise is therefore tenable that heart rate variability and vascular flow resistance as measures of cardiac vagal activity and vascular smooth muscle tone, respectively, are useful variables for tracing the e-NOS and n-NOS mediated effects of L-NAME.

Our conclusions rest mainly on the analysis of dose-effect relations which depend on the baseline conditions and thus on study design. In general, heart rate results from the balance of vagal and sympathetic drive with the former predominating in the resting awake state in dogs (35) and humans (43). Because of our primary interest in the vagal control of heart rate we studied awake dogs with prevailing vagal activity and correspondingly low heart rates (78 ± 3 min^{-1}) during baseline as a prerequisite for a meaningful comparison of the response of vagal activity between successive experiments in one animal (L-NAME, placebo, D-NAME). Finally, the dose range was appropriate for tracing the full operating range of the involved NO synthases up to the maximum of action, which is a prerequisite for the determination of meaningful ED_{50} values as a basis for a reliable comparison of the bradycardic and vasoconstrictor actions of L-NAME.

Interpretation of data

Our experiments show that the n-NOS mediated effects of L-NAME on cardiac vagal tone and this way on heart rate occur at smaller doses than the e-NOS mediated effects on vascular flow resistance. Both effects attain a

maximum, indicating saturation of the involved binding sites. Why the effects on heart rate and particularly on heart rate variability are attenuated at L-NAME doses greater than 10 $mg \cdot kg^{-1}$ is unclear. But two explanations are at least plausible and in accordance with our results. NOS inhibition on the one hand increased sympathetic activity not only in our experiments (at doses $> 10\ mg \cdot kg^{-1}$), but also in other studies in animals (6, 15) as well as in humans (34), and on the other hand inhibits central vagal influence at the level of the heart (5). Thus, NO exerts a tonic restraint on sympathetic activity because at a high degree of NOS inhibition sympathetic activation occurs.

However regardless of this, the dose-effect relations for heart rate and its variability are shifted to smaller doses (ED_{50} 0.27 $mg \cdot kg^{-1}$ and 0.47 $mg \cdot kg^{-1}$, respectively) relative to the dose-effect relations for vascular flow resistance (ED_{50} 0.87 $mg \cdot kg^{-1}$). This indicates that L-NAME is about three times more effective in its action on heart rate than on vascular smooth muscle, probably because of differences in the affinity of L-NAME for different NOS isoforms.

As pointed out previously, there is general agreement that e-NOS participates in the local control of vascular smooth muscle tone (3, 32). This is shown most convincingly by the loss of the vasoconstrictory action of NOS inhibitors in animals lacking the e-NOS gene (24). However, the almost fully maintained bradycardic action of NOS inhibitors in these animals suggests other isoforms of NOS, most likely n-NOS as mediators for this bradycardia.

This assumption is supported by the linear relationship between the L-NAME-induced bradycardia and heart rate variability (vagal activity) and the absence of this effect after ganglionic blockade in our experiments. Moreover, this bradycardia does not occur in vagotomized animals (33, 42), nor in isolated hearts (1, 21). Accordingly, for a given animal, heart rate variability is a reliable predictor of heart rate even in the presence of L-NAME, if surrounding conditions which could likewise alter heart rate variability, i.e., in particular respiratory rate, do not change.

Cardiac vagal activity originates undoubtedly in vagal nuclei of the medulla which contain NO synthases (47). It is therefore justified to conclude that heart rate variability is the manifestation of the activity of n-NOS in vagal neurons of the brain during L-NAME injection particularly in our experiments in which vagal activity always predominated compared to sympathetic activity (nuHF > nuLF). Consequently, our dose-response relations describing the effects of L-NAME on heart rate and heart rate variability show for the first time the operating range of the n-NOS-dependent influences on cardiac vagal activity and this way on heart rate.

It is additionally noteworthy that, parallel to heart rate, cardiac output decreased to a minimum of 47 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$. This substantial decrease in cardiac output implies a severe decrease of O_2 delivery. Accordingly, the O_2 demand of the tissues is met by a corresponding increase in O_2 extraction, i.e., an increase in the arteriovenous O_2 content difference, so that oxygen consumption did not change.

To what extent NOS in the heart and its pacemaker cells contribute to the bradycardia we cannot say with certainty. But these contributions are probably small for the following reasons. First, heart rate and heart rate variability remained almost constant during L-NAME in our experiments after pretreatment with hexamethonium. Second, a certain ion channel related to pacemaker cell activity in isolated atria responds to NOS inhibitors only when activated with adrenergic drugs mimicking increased sympathetic drive (12, 13). Third, the heart rate effects of NOS inhibitors are similar in mice with and without the e-NOS gene as are the effects of muscarinic and ß-adrenergic drugs on the beating rate of isolated atria from such animals (45). And, fourth, the bradycardia evoked by electric stimulation of the distal end of the cut vagal nerves in anesthetised animals is only slightly attenuated (7) or unchanged (27, 36) in the presence of a NOS inhibitor. Together these observations do not argue for a significant contribution of e-NOS in the heart of awake animals unless the sympathetic tone is elevated.

Finally, L-NAME elicited bradycardia in mice lacking the e-NOS-gene (24) and in our experiments at doses which did not increase blood pressure. Thus, neither an increase in blood pressure with subsequent activation of baroreflexes nor the presence of the e-NOS gene are necessary preconditions for the bradycardic action of NOS inhibitors. Nevertheless, we cannot totally exclude any contribution of baroreflexes to our results, but the strong bradycardia $-25\ min^{-1}$ at an unchanged blood pressure makes this explanation less plausible. Moreover, baroreflexes as well as heart rate variability are used as a measure of vagal activity and are both integrated in the brain. Consequently, NOS in the central nervous system are the most likely candidates as mediators of the heart rate effects in our experiments. The conclusion is therefore justified that, in addition to its sympatholytic properties, NO exerts a powerful restraining activity on vagal neurons of the brain and therefore plays a key role in the physiological adjustment of heart rate in awake resting animals with prevailing vagal activity.

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