Inactivation of Hypersensitive Neurons, pages 129-136

SPREADING EXCITATION AND DEPRESSION IN HIPPOCAMPAL SLICES - MECHANISMS AND MODIFICATION OF NON-SYNAPTIC SYNCHRONIZATION

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INTRODUCTION

Signal transmission through chemical synapses is the major way of communication between nerve cells. It is, however, not the only way: Fluctuations in extracellular ions evoked by nervous activity can influence neighbouring neurones, gap junctions between nerve cells allow direct current flow from one cell into another and, if there is a relatively high extracellular resistance, action currents of significant amplitude flow through neighbouring cells to ground thereby influencing the excitability of the latter. In 1942 Arvanitaki first used the term ephaptic interaction to describe transmission between two touching axons of Sepia, but similar phenomena had been described earlier (Jasper and Monnier, 1938; Katz and Schmitt, 1940). The high extracellular resistance at the axon cap of the Mauthner cell directs this cells action currents through nerve endings and smaller neurones resulting in inhibitions (Furukawa and Furshpan, 1963; Korn and Faber, 1975). A simi- lar inhibitory action also occurs on cerebellar Purkinje cells when an action potential invades the basket cell axon plexus (Korn and Axelrad, 1980). In the hippocampus, the lamellar organisation and common orientation of the principal neurones allow the generation of large field potentials during synchronous activity (Andersen et al., 1971). As externally imposed electric fields of a few mV/mm, much smaller than such endogenous fields, alter neuronal excitability (Jefferys, 1981), it is hardly surprising that field effects can synchronize a large population of neurones, especially in the CA 1 area

TABLE 1. Agents and actions

Source	Drug	action on field bursts
		acceleration: +
Fluka	Acetylcholine (ACh)	slowing: −
Fluka	Adenosine	+
Sigma	Adenosine-deaminase	−
Fluka	4-Aminopyridine	−
----	Antiepileptics (Rose et al. 1986)	0
Fluka	Atropine (muscarinic antagonist)	+
Sigma	8-bromo3'5'cyclic AMP (cyclic AMP)	+
Fluka	Caffeine	+
Fluka	Carbamylcholine (Carbachol)	0
SKF	Cimetidine (H2 antagonist)	−
Ciba-Geigy	L-baclofen	−/+
Fluka	Dopamine (DA)	−
Fluka	Dinitrophenol	0
Fluka	Eserine (Cholinesterase-blocker)	−
Calbiochem	Imidazoleacetic acid (IMA)	+
SKF	Impromidine (H2 agonist)	+
Sigma	Isoprenaline (beta-antagonist)	+
Fluka	Histamine (HA)	+
Fluka	Lithium ions (1-2 mM)	0
Sigma	Mepyramine (pyrilamine, H1 antagonist)	+
SKF	4-Methylhistamine (H2,H1 agonist, 4-MH)	0
Ciba-Geigy	Methysergide (5-HT antagonist)	0
SKF	Metiamide (H2 antagonist)	+
Sigma	Muscarine (muscarinic agonist)	−
Sigma	Neuropeptide Y	0
Fluka	Nicotine	no effect:
Sigma	Nitrobenzylthioinosine (Ad.uptake blocker)	−
Fluka	Noradrenaline (NA)	+
Sigma	Phenylephrine (alpha-antagonist)	0
Sigma	Pilocarpine (muscarinic agonist)	+
Sigma	Propranolol (beta-antagonist)	0
Sigma	Prostaglandine E2	+
Sigma	Scopolamine (muscarinic antagonist)	0
Fluka	Serotonin (5-HT)	−
Calbiochem	Tele-methylhistamine (T-MH)	(+)
J.P.Green	Tele-methyl-imidazoleacetic acid (MIA)	−
Fluka	Theophylline	+
SKF	Thiazolethylamine (H1, THEA)	−
Knoll	Verapamil, D600 30 μM	0/−
Sigma	VIP (peptide)	+

Generally, as the frequency increased, the bursts became shorter and more synchronized. Among the several antiepileptics tested, carbamazepine was the most powerful depressant of field bursts presumably because it antagonized repetitive firing (Hood et al., 1983). In nonsubmerged slices, where bursts are smaller and much less synchronized, the firing frequency of single cell action potentials showed the same sensitivity to drugs (Rose et al., 1986). What determines the interburst interval? Electrogenic pumps or other long lasting afterhyperpolarizations could be responsible. The recordings with K sensitive electrodes revealed that a new burst began about at the time when the Ko had returned to normal. The long lasting gK(Ca) has a duration of several seconds in hippocampal pyramidal cells but the distance between bursts is often 10 times longer.

CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE (gK(Ca))

Among the drugs tested in low Ca high Mg on field bursts were several transmitters or modulators which have been shown to interfere with gK(Ca) (Benardo and Prince, 1982; Madison and Nicoll, 1982; Haas and Konnerth, 1983; Haas and Greene, 1986). A striking parallel was observed: All substances which blocked accommodation of firing and the long lasting afterhyperpolarization (AHP) depending on gK(Ca) accelerated the bursts at comparable concentrations. Among these were acetylcholine (muscarine), histamine (H2), noradrenaline (beta), dopamine at 100 µM, 8-bromocyclic AMP, VIP, PGE1, caffeine, theophylline (Haas et al. 1984). On the other hand adenosine (A1) and dopamine at low concen

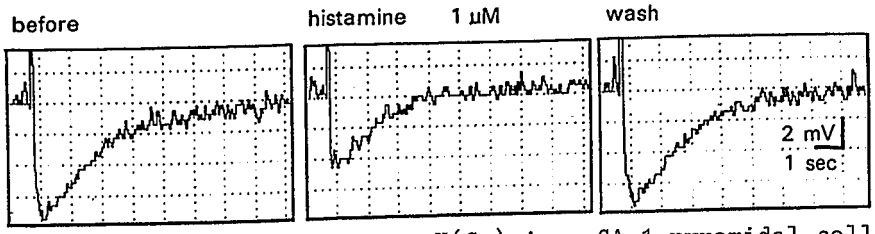

Figure 2. Histamine blocks gK(Ca) in a CA 1 pyramidal cell of the rat hippocampus in vitro. A long lasting afterhyperpolarization (AHP) follows a short burst of five action potentials (out of scale). During bath application of histamine amplitude and duration of AHP are reduced.

The "spontaneous" field bursts which develop in low Ca high Mg medium when chemical synapses are inactivated, provide a sensitive and easy measure for postsynaptic drug effects. This has added significantly to our understanding of the mechanism of action of transmitters and modulators.

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the hippocampus (Jefferys and Haas, 1982; Taylor and Dudek, 1982, 1984a, b; Haas and Jefferys, 1984; Yim et al., 1986). Simultaneous intra- and extracellular recordings allow demonstration of the transmembrane potential as the difference between these: a significant depolarizing transmembrane potential is build up at the soma level by the current generated by action potentials in neighbouring cells (Taylor and Dudek, 1982; Jefferys and Haas, 1984). Although first demonstrated in hippocampal slices in vitro, this phenomenon occurs in situ as well (Taylor et al. 1984; Yim et al. 1986). Pyramidal cells become hyperexcitable when they are exposed to low Ca high Mg solutions, which quickly block synaptic transmission, and entrain themselves in spontaneous synchronous discharges. These rhythmical events, termed field bursts or spreading excitation (Haas and Jefferys, 1984), can persist very regularly over many hours.

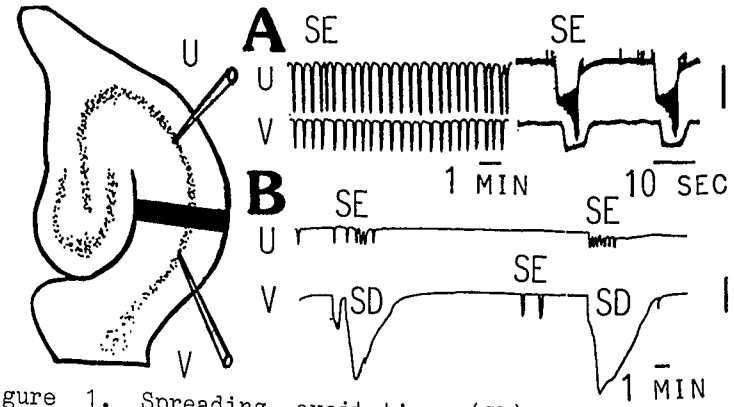

Figure 1. Spreading excitation (SE) and depression (SD) registered extracellularly with two different electrodes (U,V) in hippocampal slice of a rat. A: without lesion, field bursts (SE) occur almost synchronously at the two sites U and V. B: with a knifecut lesion (indicated by black bar), field bursts occur at site U about synchronous with SD at site V indicating a transmission of excitation across the lesion. Downwards is negative in all figures. Voltage calibration 10 mV.

SPREADING EXCITATION AND DEPRESSION IN LOW Ca++ HIGH Mg++

A field burst is often indistinguishable from the initial excitatory phase of spreading depression (Leao, 1972

Haas and Jefferys, 1984). The extracellular potassium concentration [K]o raises during but usually not before a burst (to ca. 10 mM) and falls back to normal within 10-30 sec after termination. Occasionally however, [K]o raises further (upto ca. 30 mM), the cells' input resistances fall to a very low level for tens of seconds and a wave of potassium moves through the tissue at a speed of a few mm per sec. This slow propagation can be explained by potassium diffusion and redistribution (Gardner-Medwin, 1983; Haas and Jefferys, 1984; Konnerth et al., 1984). Spreading excitation arises frequently at the subicular end of a hippocampal slice and moves at a much higher speed (upto 12 cm per sec) through the tissue, evidently synchronized by field interactions (Jefferys and Haas, 1982). This fast propagation is most easily observed in non-submerged slices, which are more susceptible to field effects and to accumulations of potassium than slices which are completely submerged in perfusion fluid (Rose et al., 1986). Field bursts are often followed by a small positive potential corresponding to a membrane hyperpolarization which seems to contribute to the regulation of burst frequency. The only condition that accelerated and prolonged the bursts was hypoxia which often lead to spreading depression. Dinitrophenol and azide which might be expected to impair the energy supply both slowed the bursts, presumably through increasing intracellular calcium level and consequently gK(Ca). Blocking energy dependent pumps by ouabain, lithium and low temperature and raising the pH accelerated the bursts, acidification slowed them (Haas and Jefferys, 1984).

MODULATION BY TRANSMITTERS AND DRUGS

The original rationale for exposing slices to low Ca high Mg solutions had been to study neuronal properties in synaptic isolation, to determine the actions of transmitters and drugs without interference from indirect effects mediated by other neurones: The regular field bursts, stable over many hours provide ,indeed, a simple and sensitive test system for investigating the pharmacology of hippocampal principal neurones. We have examined a large number of transmitters, modulators and neuroactive drugs (Table 1). Interestingly, the frequency rather than duration or intensity of the bursts was most affected by the drugs added to the perfusion fluid.

trations reduced the burst frequency and increased the long lasting AHP. The parallelity of these effects could depend on changes in membrane resistance, as all drugs with a postsynaptic inhibitory action associated with an increased conductance (GABA, taurine, imidazoleacetic acid, baclofen, serotonin, adenosine) depressed the bursting, and vice versa drugs which block tonic (potassium- or chloride-, Avoli and Agopyan, 1986) conductances accelerated the firing. Only 4-aminopyridine, the drug that blocks the A-current, reduced rather than enhanced the burst firing frequency. This effect may be attributable to the marked increase in refractoriness caused by 4-AP (Haas et al. 1983). However, several of these drugs are very selectively working on gK(Ca) and it seems likely that their action in low Ca high Mg and in normal solution occurs through an interference with intracellular Ca level. A change in Ca binding capacity of proteins close to the membrane could in this way indirectly regulate the potassium currents.

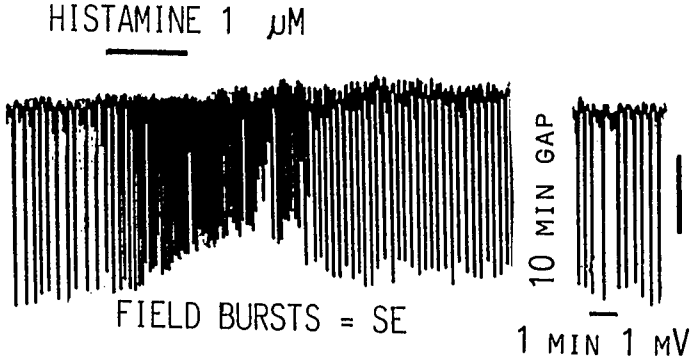

Figure 3. Histamine accelerates field bursts. Every downstroke represents the negative potential shift caused by one burst. Histamine was present at 1 uM in the perfusion fluid during time indicated by bar above trace.

CONCLUSION

While synaptic mechanisms are normally responsible for neuronal synchronization (see Schwartzkroin, 1983; Traub and Wong, 1982), a variety of non-synaptic mechanisms such as field interactions and [K]o-fluctuations can lead to hypersynchronous discharges as well. These may be prominent only in pathological situations but are likely to have a significant role to play in physiological conditions as well.

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