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Cholinergic disinhibition in hippocampal slices of the rat

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In hippocampal slices of the rat maintained in vitro the addition of carbachol to the perfusion medium caused a depolarization accompanied by a conductance decrease and a block of inhibitory postsynaptic potentials in CA1 pyramidal cells. During tetrodotoxin poisoning a smaller depolarization with conductance decrease was observed. Atropine blocked the effects of carbachol.

The muscarinic excitatory action of acetylcholine (ACh) on cortical neurons, first reported by Krnjević and Phillis[15], has been analyzed by many investigators[3,5–7,10,13,14]. Intracellular recordings have revealed a depolarization accompanied by an increase in neuron input resistance[3,10,16]. Recently a disinhibitory action of ACh in the hippocampus has also been described[17]. The conductance decrease produced by cholinergic drugs could therefore be due to two different mechanisms: one on the pyramidal cells themselves and a second, indirect one removing a tonic inhibition. I have now measured the inhibitory postsynaptic potentials (IPSPs) and their associated conductance change in order to study the latter mechanism and I have investigated the direct effects of carbachol in hippocampal slices poisoned with tetrodotoxin (TTX).

Transverse slices, 450 μm thick, from the hippocampus of young male Wistar rats (100–150 g) were maintained in a perfusion chamber[12] at 32 °C. Conventional techniques were used for intracellular recording with potassium-acetate- or chloride-filled electrodes. For the measurement of membrane resistance, apart from constant current pulses, an alternating current was injected at 10 Hz and 10–20 traces superimposed to allow monitoring of the membrane permeability during IPSPs[9]. Carbamylcholine chloride (carbachol, Fluka), atropine sulfate (Fluka) and tedrodotoxin (TTX, Sanyo) were added to the perfusion fluid by injection into the inlet tubing at high concentrations. Although carbachol was effective at a final concentration of 10^{-7} M the standard dose was 10^{-5} M. Carbachol and acetylcholine (Fluka, 10^{-4} M, pH 7.4) were also applied locally by diffusion from a micropipette held in a separate manipulator. Intracellular recordings were obtained from 21 pyramidal neurons with resting potentials more negative than 65 mV, action potentials larger than 80 mV and input resistances higher than 30 MΩ.

When carbachol at concentrations of 10^{-6}–10^{-4} M was added to the perfusion fluid, an initial hyperpolarization up to 5 mV was always followed by a depolarization

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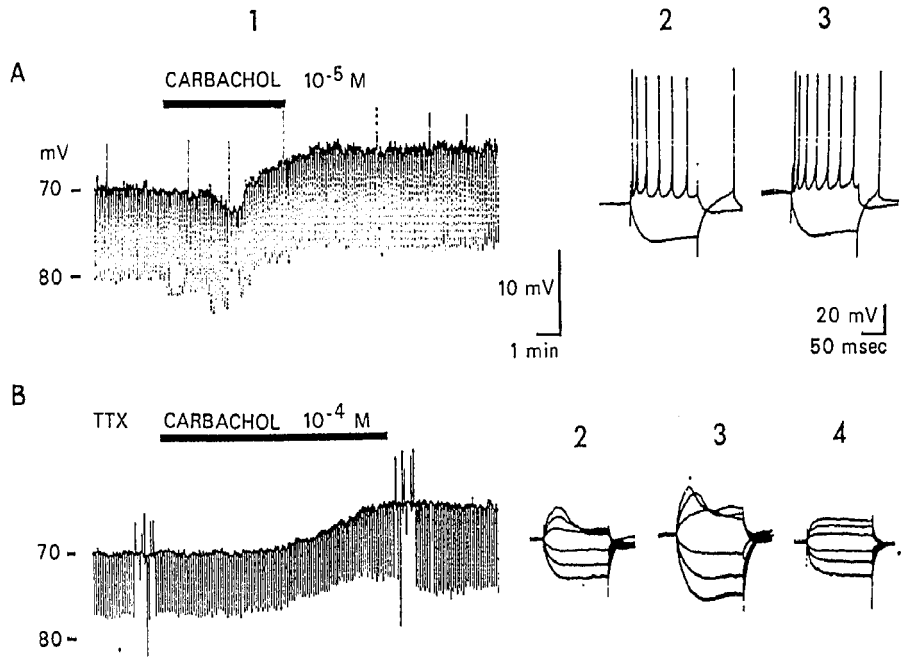

Fig. 1. Effect of carbachol bath application on CA1 pyramidal cells. A1: membrane potential, negative (downward) deflections are from hyperpolarizing intracellular current injection (0.5 nA). These pulses are reduced by the slow frequency response of the pen recorder. A2, 3: responses to intracellular current injection (± 0.5 nA, superimposed) before (2) and during (3) carbachol action. B: as A but in a different cell recorded in the presence of 10^{-6} mg/ml TTX. B4: 45 min after addition of atropine (10^{-6} M). Time of perfusion is indicated by black bars.

of up to 25 mV (11 cells). This bipolar action has previously been described[3,6,15,16]. Similar effects were observed when carbachol and acetylcholine were applied by diffusion from micropipettes lowered to the apical dendritic region (50–100 μm from the pyramidal cell somata). In 3 such experiments the effects of ACh and carbachol recovered completely within 5–20 min after withdrawal of the pipette while recovery after bath application of carbachol was only partial even after 2 h. During the initial hyperpolarization the input resistance was unaffected or decreased but was increased during the depolarization. The onset and offset of this resistance increase was usually delayed with respect to the depolarization by up to several minutes. Depolarization and resistance increase but not the initial hyperpolarization also occurred in 4 experiments conducted in preparations poisoned with TTX (10^{-6} g/ml) (Fig. 1B). Atropine (10^{-5} M for 20–40 min) reversed the actions of carbachol in 7 of 8 slices tested, including two during TTX.

Seven neurons were recorded with potassium-chloride-filled electrodes. Carbachol (10^{-5} M) produced larger and only depolarizing actions (10.4 ± 5.4 (S.D.) mV vs 6.3 ± 5.2 mV) in all of these cells with a shorter latency (1.5 ± 0.5 (S.D.) min vs 3.7 ± 2.1 min) than in the neurons recorded with potassium acetate-filled electrodes. After injection of chloride ions spontaneous depolarizing potentials, presumably IPSPs evoked by interneuron firing[1] were readily observed. Size and frequency of these potentials were enormously increased during the first minutes when carbachol was

added to the perfusion medium but later on they were reduced or undetectable even during artificial hyperpolarization of the cell to the original resting potential. Considering the time course of diffusion into slices which are not completely submerged[8,11] and the absence of an initial hyperpolarization when recording was with KCl-filled electrodes or during TTX poisoning, the most likely explanation for the initial hyperpolarization is an indirect effect, presumably an excitation of inhibitory interneuron by pyramidal cells laying deeper in the slice and being first affected by the drug (the recorded cells were not deeper than 200 μm in the 450 μm thick slices). This could also explain the initial increase and the delayed decrease in conductance. As carbachol diffuses in the whole slice the direct action on interneurons appears: probably a reduction in their excitability or the inhibitory transmission onto pyramidal cells.

IPSPs recorded after electrical stimulation of the stratum radiatum or the alveus[2] were averaged before and during perfusion with carbachol. In 3 of 8 cells they were markedly reduced (by more than 50%), in the other 5 cells they were slightly reduced or unchanged in spite of the depolarization and the conductance decrease. These latter effects lead to an increase of the actual IPSP size and will therefore often mask a real reduction of IPSPs. In an attempt to study the effectivity of and the conductance change produced by the IPSP, a 10 Hz alternating current was injected through the recording pipette[9]. The amplitude of this alternating current was chosen large enough to discharge action potentials on its rising phases so that the inhibition of action potential firing by IPSPs[1] could be observed along with the conductance changes. Such an experiment is illustrated in Fig. 2. In all 4 cells investigated in this way (2 with stratum radiatum and 2 with alveus stimulation) the conductance change

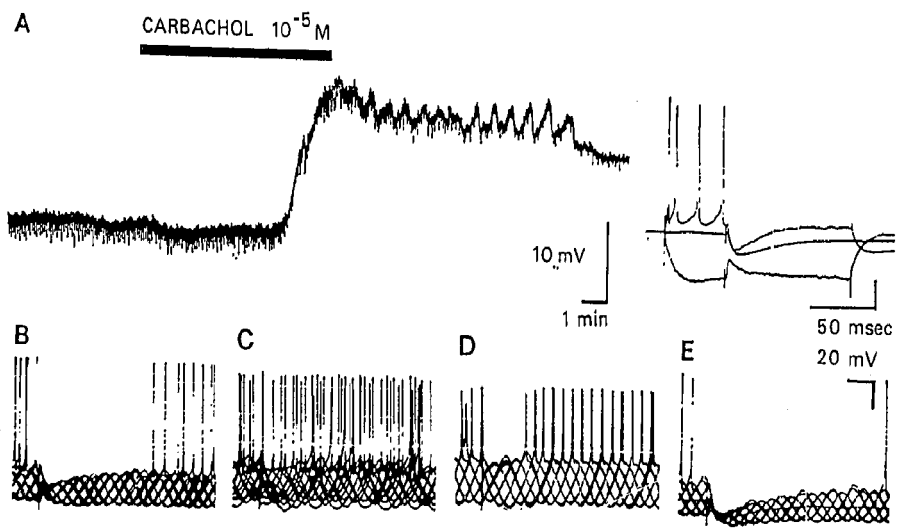

Fig. 2. Effect of carbachol on inhibition in a CA1 pyramidal cell. A: membrane potential and IPSPs evoked by stimulation of radiatum fibers at 3 different voltage levels. Superimposed records with and without $\pm$ 1 nA current injection. B–E: IPSPs with injection of an alternating current of 10 Hz. The width of the envelope formed by 10 superimposed traces is proportional to the membrane resistance at any time during the sweep. B: before, C: during carbachol. D: 5 min after adding atropine 10^{-4} M. E: after atropine (10^{-6} M) had been in the bath for 60 min.

and the pause in action potential firing produced by the IPSP were markedly reduced or blocked. Atropine (10^{-6} M) reversed the disinhibition in 3 cells with a long latency of onset (at least 30 min) and prevented the effect of carbachol in 2 cells.

These results demonstrate that the cholinergic drug carbachol acts by depolarization and increasing membrane resistance directly on pyramidal cells in the hippocampus, as indirect effects involving the firing of action potentials are abolished by TTX. The smaller effect seen during TTX suggests that normally a second mechanism contributes to the depolarization. An initial hyperpolarization which was not present during TTX poisoning and when the chloride equilibrium potential was shifted by injection of chloride ions is possibly produced by a transiently increased firing of inhibitory interneurons. The conductance increase caused by these cells may still be operating when the recorded cell is reached by the drug and could therefore explain the delay in the conductance decrease. The delay in onset and the long duration of the effects of carbachol might be partly due to slow diffusion into and from the slices of the cholinesterase resistant drug but are also likely to represent involvement of an intracellular process induced by the drug–receptor interaction[3].

Although the disinhibitory effect of acetylcholine has been found by Krnjević et al. recently[17], this is the first demonstration of a reduction in the conductance change and the effectivity of the intracellularly recorded IPSPs by a cholinergic drug. The exact mechanism of this disinhibition remains to be investigated. There is good evidence that the spontaneous depolarizing potentials seen with KCl-filled electrodes are spontaneous IPSPs (and own observation). Further experiments are required to determine whether the spontaneous depolarizing potentials appearing early during carbachol perfusion and those present after the initial enormous increase is reduced are still IPSPs. The fact that these potentials are reduced while carbachol is still being applied may be interpreted as a reduction in firing of inhibitory interneurons as it occurs in the thalamus[4]; a quickly desensitizing excitation however, cannot be excluded. The reduction in IPSPs could also be secondary to a presynaptic inhibitory action as described for acetylcholine by Hounsgaard[14]. In accordance with the results of Benardo and Prince[3] is the finding that atropine blocks the effects of carbachol and lowers the input resistant below the original value. In addition it increased the IPSPs beyond the original size indicating also a tonic cholinergic disinhibition in the slice. Nicotinic antagonists were not tested in this study. Therefore, although all effects of carbachol were reduced or blocked by atropine a conclusion cannot be made on whether these effects are purely muscarinic.

1 Alger, B. E. and Nicoll, R. A., Spontaneous inhibitory postsynaptic potentials in hippocampus: mechanism for tonic inhibition, *Brain Research*, 200 (1980) 195–200.
2 Andersen, P., Eccles, J. C. and Loyning, Y., Pathway of postsynaptic inhibition in the hippocampus, *J. Neurophysiol.*, 27 (1964) 608–619.
3 Benardo, L. S. and Prince, D. A., Acetylcholine induced modulation of hippocampal pyramidal neurons, *Brain Research*, 211 (1981) 227–234.
4 Ben-Ari, Y., Dingledine, R., Kanazawa, I. and Kelly, J. S., Inhibitory effects of acetylcholine on neurones in the feline nucleus reticularis thalami, *J. Physiol. (Lond.)*, 261 (1976) 647–671.

5 Bird, S. J. and Aghajanian, J. K., The cholinergic pharmacology of hippocampal pyramidal cells: a microiontophoretic study, *Neuropharmacology*, 15 (1976) 273–282.
6 Biscoe, T. J. and Straughan, D. W., Microelectrophoretic studies of neurons in the cat hippocampus, *J. Physiol. (Lond.)*, 183 (1966) 341–359.
7 Bland, B. H., Kostopoulos, G. K. and Phillis, J. W., Acetylcholine sensitivity of hippocampal formation neurons, *Canad. J. Physiol. Pharmacol.*, 52 (1974) 966–971.
8 Buckle, P. J. and Haas, H. L., Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat, *J. Physiol. (Lond.)*, in press.
9 Dingledine, R. and Langmoen, I. A., Conductance changes and inhibitory actions of hippocampal recurrent IPSPs, *Brain Research*, 185 (1980) 277–287.
10 Dodd, J., Dingledine, R. and Kelly, J. S., The excitatory action of acetylcholine on hippocampal neurones of the guinea-pig and rat maintained in vitro, *Brain Research*, 207 (1981) 109–127.
11 Haas, H. L. and Ryall, R. W., Is excitation by enkephalins of hippocampal neurones in the rat due to presynaptic facilitation or to disinhibition? *J. Physiol. (Lond.)*, 308 (1980) 315–330.
12 Haas, H. L., Schaerer, B. and Vosmansky, M., A simple perfusion chamber for the study of nervous tissue slices in vitro, *J. Neurosci. Meth.*, 1 (1979) 323–325.
13 Herrling, P. L., The membrane potential of cat hippocampal neurons recorded in vivo displays four different reaction-mechanisms to iontophoretically applied transmitter agonists, *Brain Research*, 212 (1981) 331–343.
14 Hounsgaard, J., Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus, *Exp. Neurol.*, 62 (1978) 787–797.
15 Krnjević, K. and Phillis, J. W., Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex, *J. Physiol. (Lond.)*, 166 (1963) 328–350.
16 Krnjević, K., Pumain, R. and Renaud, L., The mechanism of excitation by acetylcholine in the cerebral cortex, *J. Physiol. (Lond.)*, 215 (1971) 247–268.
17 Krnjević, K., Reiffenstein, R. J. and Ropert, N., Disinhibitory action of acetylcholine in the hippocampus, *J. Physiol. (Lond.)*, 308 (1980) 73–74 P.