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PRELIMINARY NOTES

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OPPOSITE ACTIONS OF MUSCARINIC AND NICOTINIC AGENTS ON HIPPOCAMPAL DENDRITIC NEGATIVE FIELDS RECORDED IN RATS

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Summary - In rats under urethane anaesthesia, microiontophoretic application of muscarinic or nicotinic agents, in the pyramidal layer of the hippocampus, enhanced the population spike (s) evoked by fimbrial stimulation. In contrast, muscarinic and nicotinic agents had an opposite action on on the dendritic field potentials, they respectively reduced and enhanced the negative fields (field EPSP) recorded in the apical dendrites. These effects were antagonized by muscarinic and nicotinic antagonists, respectively.

Recent studies suggest that acetylcholine (ACh) has a presynaptic action in the hippocampus. Thus, local application of ACh in the dendritic region reduces the excitatory post-synaptic potential (EPSP) and this effect appears to be mediated by a reduction of excitatory transmitter release (Hounsgard, 1978; Valentino and Dingledine, 1981). Furthermore, local application of ACh on pyramidal somata produces a reduction of the intracellularly recorded inhibitory post-synaptic potential (IPSP) and its associated conductance increase without significantly altering the effects of local applications of GABA (Ben-Ari, Krnjević, Reinhardt and Ropert, 1981b). These observations suggest that, in addition to its conventional post-synaptic action, i.e. decrease in potassium conductance (Dodd, Dingledine and Kelly, 1981; Ben-Ari *et al.*, 1981b), ACh modulates both the excitatory and inhibitory inputs to the pyramidal cells. We have now compared *in situ* the pharmacology of cholinergic agents upon the somatic and dendritic field potentials.

METHODS

Male adult (250 g) rats were anaesthetized with urethane (2.5-3 g/kg, i.p.) and placed in a stereotaxic frame. A pair of glass-coated metal electrodes (tip diameter < 70 μm, intertip distance of 1 mm) were inserted at an angle of 30° in the rostro-caudal plane and used to stimulate the ventral hippocampal commissure and fimbria (i.e. Ben-Ari, Krnjević, Reiffenstein and Reinhardt, 1981a). The recording microelectrodes were introduced vertically to the CA1 field at a mid-septo-temporal level through the overlying cortex. To record the somatic or dendritic field potentials and eject various agents, multibarreled (7) pipettes (4-6 μm) were used. The central barrel contained NaCl (3M) or pontamine sky blue dissolved in acetate and the side barrels contained, in various permutations: GABA (1.0M at pH 4.5): AChCl (0.5-1.0M, pH 4); muscarine (0.1M); acetyl-β-methylcholine chloride (0.1M); carbamyl-choline chloride (CARB)(0.1M); atropine (1 mM in NaCl 165 mM); scopolamine (1 mM in NaCl 165 mM); dimethyl-phenyl-piperazine (DMPP)(0.1M); tetra-methyl-ammonium (1 M); d-tubocurare (10 mM in NaCl 165 mM). The agents were applied at regular intervals, usually 20 or 40s on and 40s off. The positions of the electrodes were checked histologically.

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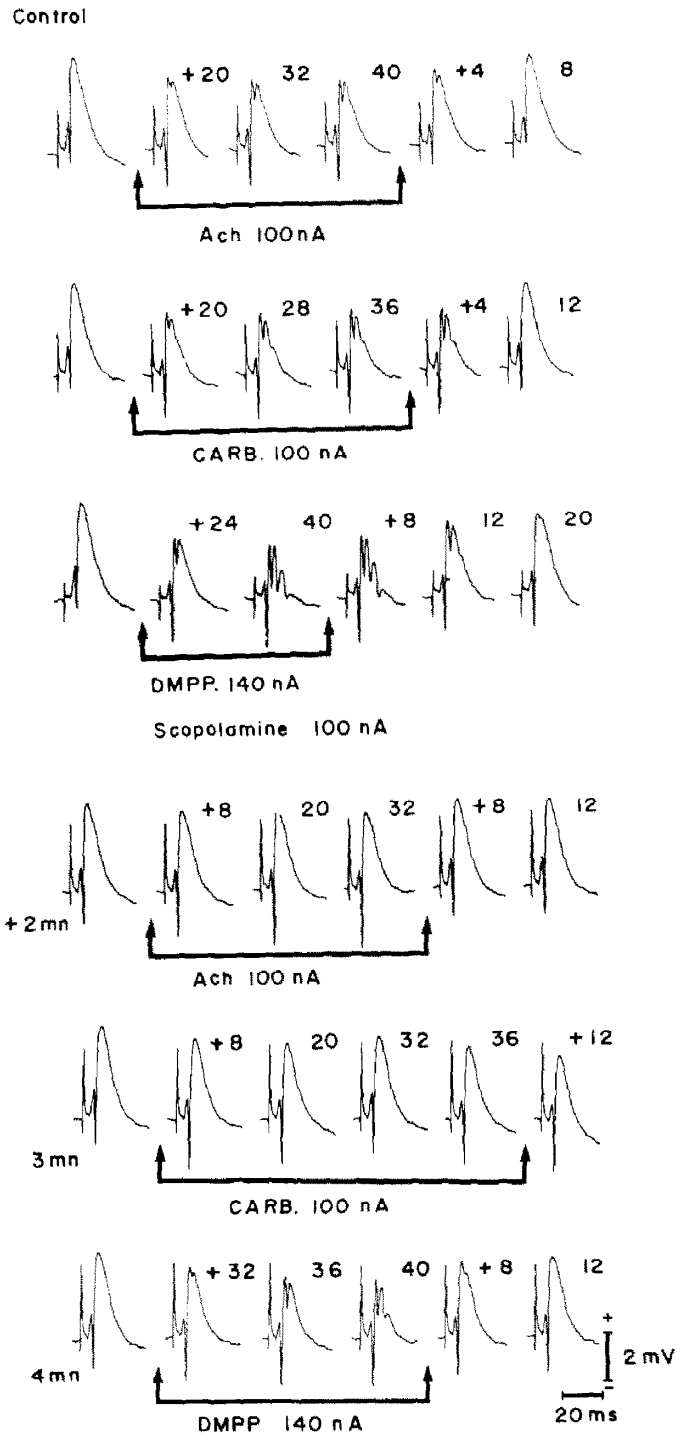

Figure 1. Effects of local application of acetylcholine (ACh), carbamylcholine (CARB) and di-methyl-phenyl-piperazine (DMPP) in the pyramidal layer of CA1. The numbers above each trace indicate the delay (in s) after the beginning or end of the application. Note that the 3 agents induce a reduction of the positive field and an enhancement of the negative population spike (s). Scopolamine considerably reduced the actions of ACh and CARB but not the effects of DMPP. Complete recovery from the blocking action of scopolamine was obtained after 10 min. In this and the following figure single sweeps were digitized and displayed on a polygraph.

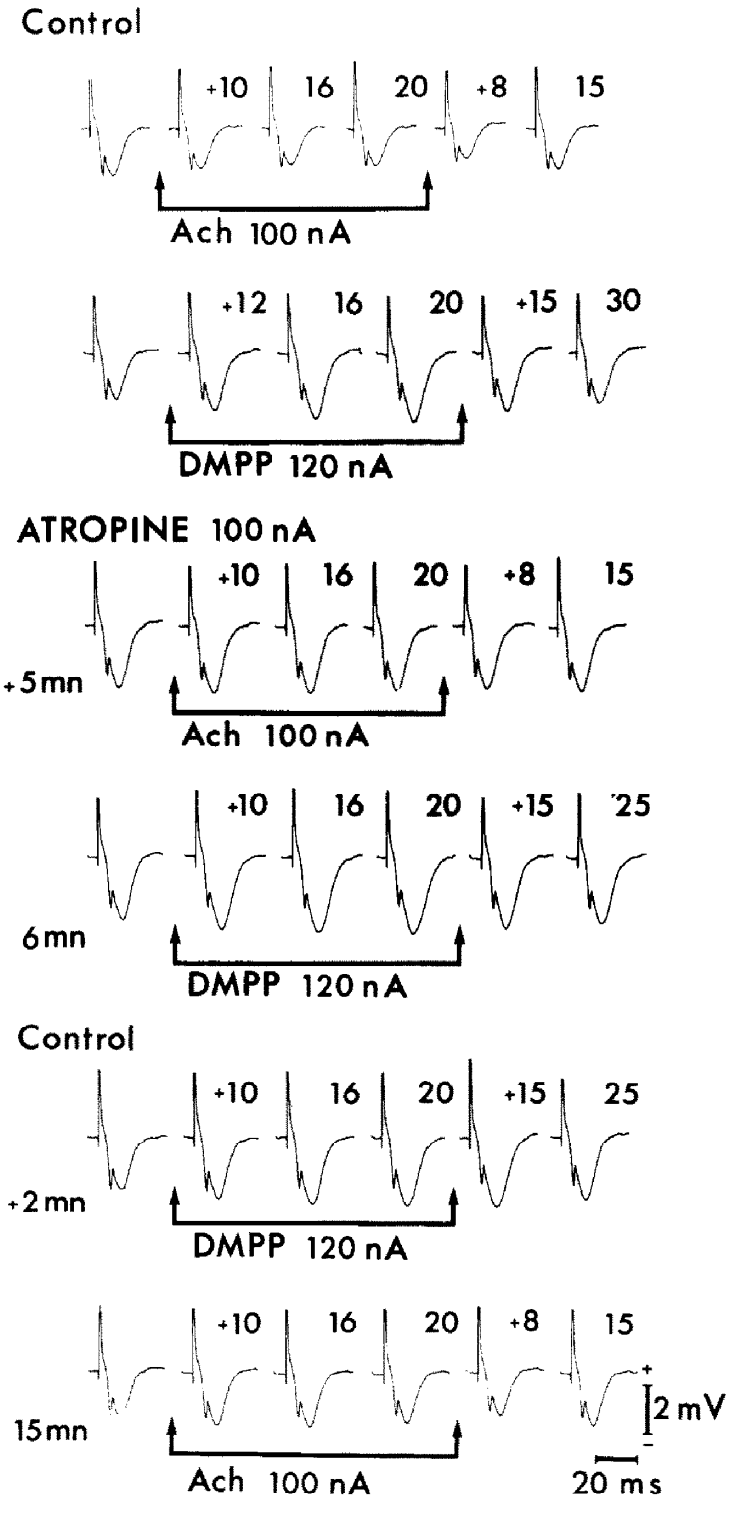

Figure 2. Opposite effects of local application of a muscarinic and nicotinic agent in the dendritic layer of CA1. ACh reduces the EPSP whereas DMPP enhances it. Note that the depressant action of ACh is blocked by atropine, complete recovery occurred after approximately half an hour.

RESULTS AND DISCUSSION

In agreement with the results of recent studies performed in the same preparation (Krnjević, Reiffenstein and Ropert, 1981), local application of ACh in the soma consistently produced a reduction of the positive field (extracellular inhibitory post-synaptic potential, IPSP) and an increase in the population spike (s). This effect was dose-dependent (20 to 100 nA), had a swift onset (typically 2-6 sec.) and offset (5-10 sec.) and was restricted exclusively to the immediate vicinity of the pyramidal layer (i.e. Krnjević *et al.*, 1981). In 14 rats, local application of muscarinic agents (notably CARB, Fig. 1), or muscarine itself (not shown), or nicotinic agents (in particular DMPP, Fig. 1), produced a similar disinhibitory action. However, the actions of the latter (an notably DMPP) had a slower time course and required repeated larger applications. These effects were pharmacologically specific since, (in 9 rats) muscarinic antagonists (such as scopolamine or atropine) significantly reduced the actions of muscarinic agents, whereas nicotinic antagonists (notably curare) reduced the actions of DMPP or TMA and not that of the muscarinic agonists.

Local application of ACh in the apical dendrites consistently reduced the size of the field EPSP. The action of ACh had a swift onset (a maximal effect was usually obtained toward the end of a 20 sec. application, i.e. Fig. 1) and recovery took 10-20 sec.; the magnitude of the actions of ACh was highly variable, and even large currents (i.e. 100 nA, see Fig. 2) did not produce a reduction of more than 50% of the control fields (n = 24 rats). In 5 cases, the depth profile showed the maximal effects of ACh to be restricted to an area (220-250 μm from the pyramidal layer) in which the maximal field EPSPs were recorded (this corresponds to the synaptic activation through the Schaffer's collaterales).

In 14 rats, muscarinic agents (notably muscarine) consistently produced a similar depression of the field EPSP. In contrast, nicotinic substances (notably DMPP, Fig. 2) produced an opposite effect, i.e. an increase in the amplitude of the field EPSP. This effect had a slow onset (usually more than 15 sec.) and recovery took more than 15-20 sec. As shown in Figure 2, these effects were pharmacologically specific since the inhibitory action of muscarinic agents (including ACh or muscarine) were reduced or completely blocked by local application of atropine (or scopolamine), whereas local ejection of curare readily depressed the actions of nicotinic agents without blocking the effects of muscarinic agents. Similar observations were made in 8 cases.

In conclusion, muscarinic and nicotinic agents produced similar effects when applied at the soma and exerted opposite effects when applied in the apical dendrites. The muscarinic action on the EPSP appears to be mediated by a presynaptic decrease in the release of excitatory transmitter (i.e. Hounsgard, 1978; Valentino and Dingledine, 1981). In contrast, the mechanism of action of nicotinic agents remains to be elucidated; it is perhaps releveant to emphasise that in the sympathetic system the cholinergic modulation of noradrenaline release is also exerted vai a dual control system, wherein 'muscarinic' is inhibitory and 'nicotinic' excitatory (Loffelholz, 1980; Muscholl, 1979).

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