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Presynaptic M_1 muscarinic cholinoceptors mediate inhibition of excitatory synaptic transmission in the hippocampus in vitro

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The effects of the cholinoceptor agonist, carbachol (CCh), were examined in the rat hippocampal slice preparation. Intracellular recordings from CA1 pyramidal neurones revealed that CCh (1–3 μM) inhibited excitatory postsynaptic responses evoked by stimulation of the Schaffer collateral/commissural pathway while, at the same time, direct excitability was enhanced. Extracellularly, CCh produced a concentration-dependent reduction of the amplitude of the field excitatory postsynaptic potential (field EPSP) recorded in the CA1 apical dendritic region. The muscarinic receptor antagonist, pirenzepine, competitively antagonized the effects of CCh on the field EPSP with a pA_2 of 7.4. These results confirm earlier reports of a presynaptic inhibitory action of CCh in the hippocampal CA1 region and provide strong evidence that this effect is mediated by muscarinic receptors of the M_1 subtype.

The classification of muscarinic receptors into two broad categories on the basis of their high (M_1) and low (M_2) affinities for the antagonist, pirenzepine [9], has prompted a number of attempts to establish the subtypes mediating the electrophysiological responses to muscarinic agonists throughout the brain [4–6, 14–16, 19]. In hippocampal CA1 pyramidal neurones, both the membrane depolarization and blockade of I_{AHP} induced by carbachol (CCh) have been attributed to an action at M_1 receptors, whereas inhibition of the M-current may result from M_2 receptor activation [4]. In addition to these postsynaptic effects, muscarinic agonists can also reduce the synaptically-evoked excitation of CA1 neurones by a presynaptic mechanism [11, 18]. Unfortunately, attempts to classify this latter response in terms of receptor subtype have yielded conflicting reports: both M_2 [4] and M_1 [16] receptors have been implicated. However, since neither of these earlier studies employed formal quantitative pharmacological procedures, conclusions from them must remain tentative. We

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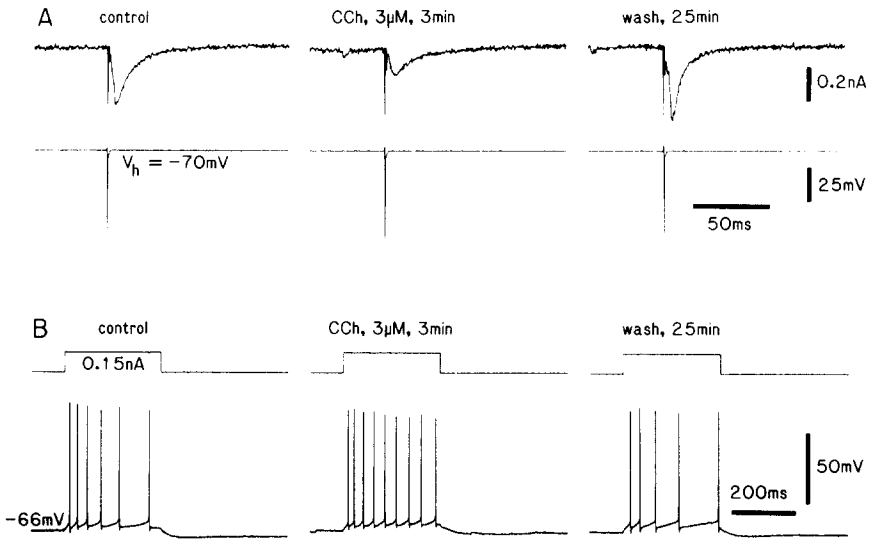

Fig. 1. Intracellular recordings from a single CA1 pyramidal neurone (RMP = −66 mV). A: CCh (3 μM) reversibly reduced the EPSC evoked by electrical stimulation of the SCC pathway (holding potential, V_H = −70 mV). To facilitate comparisons of the EPSCs in the absence and presence of drug, the small inward current induced by CCh has been subtracted from the centre trace. B: effect of CCh (3 μM) on spike trains generated by depolarizing current injection (0.15 nA, 300 ms). During drug application, the membrane potential was manually clamped to −66 mV to compensate for the small depolarization induced by CCh in this cell (5 mV). Note the reversible enhancement by CCh of discharge frequency together with a reduction of the afterhyperpolarization. Records in B were obtained immediately after the corresponding voltage-clamp measurements shown in A.

have therefore re-examined the identity of the presynaptic muscarinic receptor underlying inhibition of excitatory transmission in CA1 using full Schild analysis [2] of the antagonistic effects of pirenzepine on the CCh-induced suppression of the field excitatory postsynaptic potential (EPSP).

Transverse slices of hippocampus (thickness 500 μm), prepared from adult male Sprague–Dawley rats, were stored in an artificial cerebrospinal fluid (CSF) containing (in mM): NaCl 124, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 22, D-glucose 10, gassed at room temperature with 95% O_2/5% CO_2. After at least 1 h, one slice was transferred to the recording chamber and submerged in continuously flowing artificial CSF at 30–33°C. A concentric stimulation electrode, placed in the Schaffer collateral/commissural (SCC) pathway, was used to evoke synaptic excitation of neurones in area CA1 (stimulation frequency: 0.05 Hz). For the intracellular experiments glass electrodes containing 4 M potassium acetate (pH 7.2; resistance 60–90 MΩ) were used. Voltage-clamp measurements of excitatory postsynaptic currents (EPSCs) were made using a switched current- and voltage-clamp amplifier (npi SEC 1L, switching frequency 18–20 kHz, 25% duty cycle) (see Ref. 17). To minimize contamination of EPSCs by inhibitory inputs, synaptic currents evoked by low stim-

ulation intensities were recorded at a holding potential (V_H) corresponding to the reversal potential of the γ-aminobutyric acid A ($GABA_A$)-mediated, chloride-dependent inhibitory postsynaptic current (IPSC; -67 to -72 mV) [1]. At this potential the amplitude of the IPSC should be negligible. A late, potassium-dependent IPSC was not observed at the low stimulation intensities used. For the extracellular experiments glass micropipettes containing 4 M NaCl (resistance 2–5 MΩ) were used to record field EPSPs in the apical dendritic region of area CA1. Care was taken to adjust the stimulation current so that the field EPSPs were free from contaminating population spikes. Cumulative dose–response curves to CCh were constructed for each slice, allowing 15 min equilibration at each concentration. Following completion of the control dose-response curve, the slice was superfused with drug-free solution for 30 min. (Only slices in which the field EPSP amplitude returned to within $\pm 5\%$ of the initial control value were included in subsequent antagonism studies.) The slice was then superfused with pirenzepine (0.1, 0.3 or 1 μM) for 45 min, after which the dose–response curve to CCh was repeated. Only one antagonist concentration was applied to each slice. Concentration–response curves were fitted by non-linear regression (weighted by actual distance). Antagonism data were analysed by the method of Arunlakshana and Schild [2], dose-ratios being derived for each slice and then pooled. Grouped data are expressed as mean $\pm$ S.E.M.

Firstly, the effects of CCh on the excitability of single CA1 neurones (resting membrane potential, RMP > -65 mV, input resistance >50 MΩ; $n=4$) were investigated. In agreement with others, CCh (1–3 μM) induced small membrane depolarizations (5–8 mV), increased input resistance (9–15%), blocked the adaptation of firing seen during prolonged depolarizing current injection and reduced the afterhyperpolarization associated with a spike train. In contrast, synaptically-driven responses were attenuated: both EPSPs and excitatory postsynaptic currents (EPSCs) evoked by low intensity stimulation of the SCC pathway were reversibly reduced. An example of the action of CCh on one cell is shown in Fig. 1.

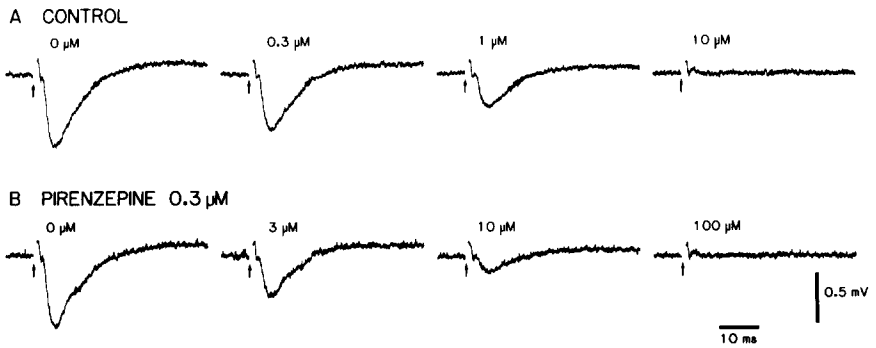

Fig. 2. Concentration-dependent reduction of the extracellularly recorded field EPSP by CCh in a single slice in the absence (A) and presence (B) of pirenzepine (0.3 μM). CCh concentrations (μM) are shown above the traces (averages of 3–5 sweeps). CCh was applied cumulatively (see text) and the records taken at 15 min following the start of each application. Arrows signify point of electrical stimulation.

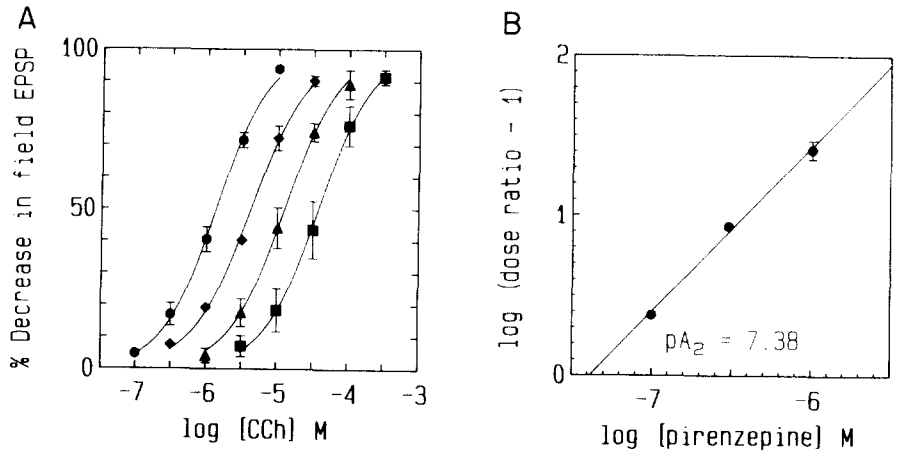

Fig. 3. A: pooled concentration–response curves to CCh in the absence (●) and presence of 0.1 (◆), 0.3 (▲) and 1.0 μM (■) pirenzepine. Each curve was obtained from 3 to 12 slices (correlation coefficient $r \geqslant 0.998$ in each case). B: Schild plot for the antagonism by pirenzepine of the effects of CCh on the field EPSP ($r = 0.998$).

Superfusion of slices with CCh (0.1–3 μM) produced consistent, non-desensitizing reductions in the amplitude of the field EPSP: responses were stable after 10–12 min of drug application and readily reversible upon wash-out. A cumulative dosing schedule was therefore adopted in subsequent experiments, allowing 15 min at each concentration of CCh before progressing to the next. An example of the effect of CCh on the field EPSP from a single slice is shown in Fig. 2 and a summary from all slices studied is shown in Fig. 3A (control curve; $EC_{50} = 1.43 \pm 0.15$ μM; $n = 12$).

In the final series of experiments the antagonistic effects of pirenzepine on the CCh-induced depression of the field EPSP were studied. Pirenzepine (0.1, 0.3 or 1 μM) produced parallel rightward displacements of the CCh concentration–response curve (Fig. 3A). A Schild plot constructed from these data is shown in Fig. 3B ($pA_2 = 7.38 \pm 0.06$; slope $= 1.03 \pm 0.04$).

In confirmation of earlier reports [11, 18], the present data provide evidence that CCh is able to depress excitatory synaptic transmission in the SCC/CA1 pathway by a presynaptic mechanism. Thus, although the direct excitability of CA1 neurones was enhanced by CCh, synaptically-evoked responses (EPSC and EPSP) were consistently depressed. That this latter action was mediated at a presynaptic locus is considerably strengthened by the fact that input resistance was invariably increased by CCh, an effect which would tend to potentiate synaptically-driven responses rather than diminish them. Extracellularly, CCh induced a concentration-dependent, pirenzepine-sensitive reduction of the amplitude of the field EPSP. The pA_2 value of 7.38 for the antagonism by pirenzepine of this depressant action of CCh, is in close agreement with recent binding data for the interaction of pirenzepine with both cortical ($pK_i = 7.7$; [7]) and hippocampal ($pK_i = 7.43$; [3]) M_1 receptors labelled with [^{3}H]pirenzepine.

That this presynaptic cholinergic mechanism may have some physiological relevance is supported by a recent report from Herreras et al. [10], who found that the potent inhibitory effect of sensory stimulation on SCC pathway-evoked CA1 field potentials in the anaesthetized rat was completely abolished by muscarinic, but not by GABAergic, antagonists. The morphological basis for a cholinergic innervation of the apical dendritic region of area CA1 is already firmly established [12].

In conclusion, our findings provide strong evidence that the presynaptic muscarinic receptor mediating depression of synaptic transmission in the SCC pathway is of the M_1 subtype. In this respect it is interesting that Williams and Constanti [19], using similar pharmacological techniques, have attributed the muscarinic depression of the synaptically-evoked potential in the olfactory cortex slice preparation also to an action at presynaptic M_1 receptors.

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