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Muscarinic receptors mediating suppression of the M-current in guinea-pig olfactory cortex neurones may be of the M_2-subtype

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Guinea-pig olfactory cortical neurones *in vitro* were voltage clamped by means of a single intracellular microelectrode technique. Hyperpolarizing voltage commands from holding potentials between −40 to −50 mV produced slow inward current relaxations reflecting deactivation of the M-current (I_M). I_M was reversibly suppressed by 30 μM muscarine or carbachol; this suppression was insensitive to pirenzepine (up to 300 nM) but was inhibited by gallamine (10–20 μM) or 4-diphenylacetoxy-N-methylpiperidine (100, 500 nM), suggesting the involvement of the M_2-type muscarinic receptor.

Introduction There has been much interest recently in the possible subdivision of muscarinic receptors into M_1 and M_2 subtypes, based on their relative affinities for the competitive antagonist pirenzepine (Hammer *et al.*, 1980; Hammer & Giachetti, 1982). M_1-receptors exhibit a high binding affinity towards pirenzepine ($K_D \sim 10–20$ nM) and are found predominantly on neural tissue (e.g. autonomic ganglia), whereas the M_2-type show a low affinity towards this agent ($K_D \sim 200–800$ nM), and are considered to exist on both neural tissue and peripheral organs such as heart and ileal smooth muscle (see Hammer & Giachetti, 1984). A further subclassification of the peripheral M_2-receptor has also been suggested in view of the fact that certain skeletal neuromuscular blockers e.g. gallamine, show more selective anti-muscarinic effects on the heart (Nedoma *et al.*, 1985), whereas antagonists such as 4-diphenyl-acetoxy-N-methylpiperidine (4-DAMP) show a relatively higher affinity for the ileal muscarinic receptor (Barlow *et al.*, 1976). Recent intracellular studies on mammalian central neurones have suggested that both excitatory and inhibitory actions of cholinoceptor agonists can be mediated via M_2-muscarinic receptors (Egan & North, 1985; 1986; McCormick & Prince, 1986a). In the present study, we have attempted to characterize the muscarinic receptor subtype underlying the slow muscarinic depolarization of single olfactory cortical neurones *in vitro*. Under voltage clamp, a persistent, time-and-voltage-dependent potassium current, the M-current (I_M) exists at holding potentials more positive than −60 mV; this current can be specifically suppressed by muscarinic agonists (Constanti & Galvan, 1983b). We show here that the neuronal receptor mediating this suppression of I_M is relatively insensitive to pirenzepine, and therefore may be of the M_2- subtype.

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Methods Intracellular recordings were made from guinea-pig olfactory cortical neurones *in vitro* (23–25°C) using rostro-caudal slices of olfactory cortex (~500 μm thick; Constanti & Sim, 1984) cut with an Oxford vibratome. Slices were held between two nylon meshes (completely submerged) and superfused with oxygenated Krebs solution containing (mM): NaCl 118, KCl 3, $CaCl_2$ 1.5, $NaHCO_3$ 25, $NaH_2PO_4.2H_2O$ 1.2, $MgCl_2.6H_2O$ 1 and D-glucose 11; (bubbled with 95% O_2: 5% CO_2, pH 7.4). Microelectrodes were filled with 4M K acetate (50–80 MΩ) and coupled to an Axoclamp-2, single microelectrode current/voltage clamp preamplifier (2–3 kHz switching frequency, 30% duty cycle: see Constanti & Galvan, 1983a, for method of optimal adjustment). Sampled membrane currents (filtered at 300 Hz, low pass 48db per octave) and voltage were recorded on a Gould Brush 2400 pen recorder. All drugs (BDH Analar, or Sigma) were prepared in Krebs solution and applied via the superfusate (bath-exchange time ~30 s). 4-DAMP methylbromide was a gift from Dr R. B. Barlow (Bristol). The results described below are based on data pooled from five neurones (average impalement time ~3 h).

Results Figure 1 shows typical membrane currents recorded from a single olfactory cortex neurone, voltage clamped at a holding potential of −45 mV. On applying a 10 mV (500 ms) negative voltage jump from this potential, the membrane current showed an initial (instantaneous) step followed by a slow inward relaxation to a steady level. Stepping back to −45 mV

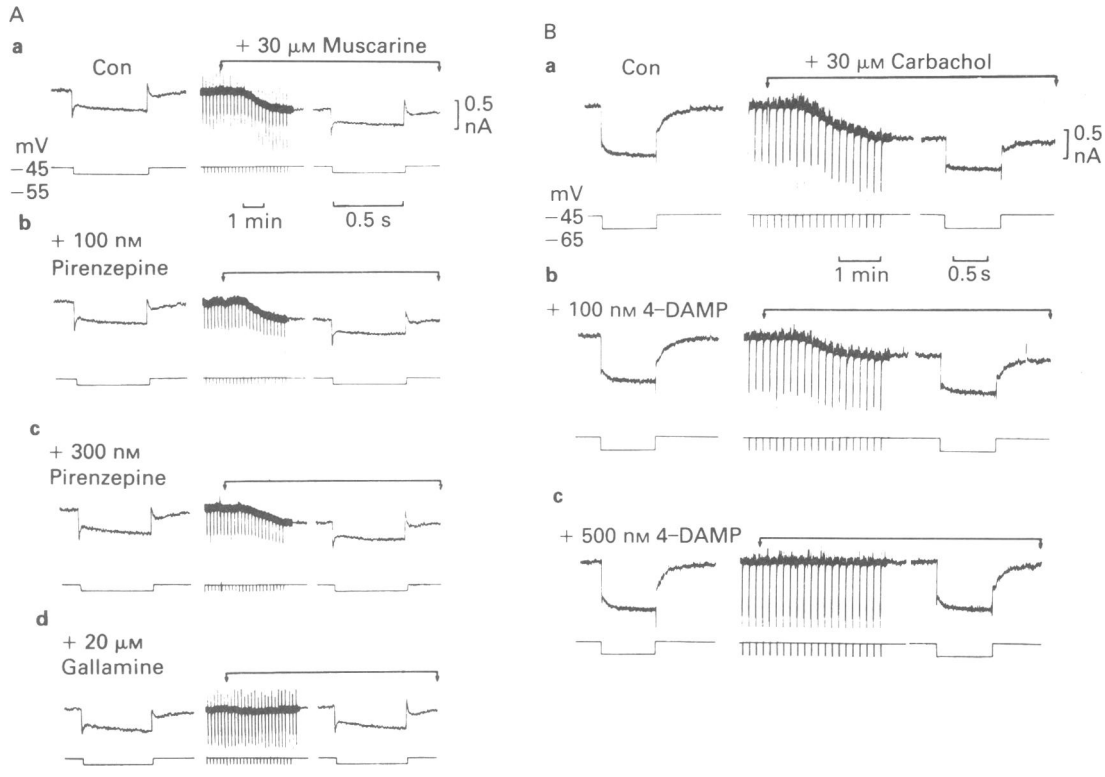

Figure 1 (A) Pirenzepine does not prevent the inhibition of the M-current (I_M) by muscarine. (a–d): Continuous chart record of clamp currents measured in a single olfactory cortex neurone under voltage clamp (holding potential = −45 mV). In each row, upper traces show membrane current; lower traces, voltage. (a): Inhibition of I_M by 30 μM muscarine recorded in control Krebs solution (Con). Record shows initially, the inward current relaxation in response to a −10 mV (500 ms) voltage jump (0.1 Hz). Chart speed was then slowed during drug application and speeded up after 3 min in muscarine. The effect of muscarine was still apparent in 100 nM (b) or 300 nM pirenzepine (c) but was abolished in the presence of 20 μM gallamine (d). Muscarine applications were followed by a 15 min washout period (not shown). Pirenzepine and gallamine were applied for 20 min and 10 min respectively, before testing muscarine. (B) 4–DAMP blocks the inhibition of I_M by carbachol (different neurone; V_{hold} = −45 mV). (a) Inhibition of I_M and inward shift in holding current induced by 30 μM carbachol in control solution (Con) (voltage jumps = −20 mV, 800 ms, 0.1 Hz). (b) Effect of carbachol was partially blocked after 10 min in 100 nM 4-diphenyl-acetoxy-N-methylpiperidine (4-DAMP), and abolished after 10 min in 500 nM DAMP (c). No recovery from antagonism was obtained after a 30 min wash. Each carbachol application was followed by a 25 min washout period (not shown).

evoked a smaller instantaneous step, followed by a decaying inward relaxation to the holding current level. These slow relaxations reflect the deactivation and reactivation respectively, of a 'background' outward K^+ current, I_M (Constanti & Galvan, 1983b).

Bath-application of (±)-muscarine (30 μM) (Figure 1Aa) induced a slow inward shift in holding current and a clear (reversible) reduction in the I_M relaxation amplitude revealed during the negative voltage command. Under current-clamp, this action would be manifest as a slow membrane depolarization and increase in cell input resistance. The muscarinic suppression of I_M was not blocked by low concentrations of pirenzepine (up to 300 nM), pre-applied for at least 20 min (Figure 1A b,c), whereas gallamine (20 μM), a 'cardioselective' M_2-receptor antagonist (Hammer & Giachetti, 1984; Nedoma *et al.*, 1985), produced a clear inhibition of the action of muscarine (Figure 1Ad). Higher concentrations of pirenzepine (500 nM–1 μM) did, however, exhibit an anti-muscarinic effect (not illustrated).

Further support for an M_2-mediated inhibition of I_M was obtained with 4-DAMP. Figure 1Ba shows (in a different cell) that the inward current and I_M

suppression induced by 30 μM carbachol could be partially reduced then completely antagonized in the presence of 100 and 500 nM 4-DAMP respectively. Pirenzepine, gallamine or 4-DAMP applied alone, had no notable effect on I_M or on the holding current level.

Discussion These preliminary results provide the first evidence obtained under voltage clamp, that the receptor mediating muscarinic inhibition of the M-current on a mammalian cortical neurone may be of the M_2-subtype. In view of the relatively slow recovery of I_M relaxations to their control amplitude after washout of muscarinic agonists, it was impractical to construct dose-response relationships for muscarinic suppression of I_M on the same cell. We could not therefore, at present, estimate K_D values for the muscarinic blocking action of the antagonists on single cells, nor can we speculate on their underlying modes of action.

Since Brown *et al.* (1980) showed that pirenzepine was a potent inhibitor of slow muscarinic (I_M-mediated?) depolarizations in rat ganglia, the notion has developed that the slow cholinergic depolarization of central pyramidal neurones mediated by I_M suppression may also involve an M_1-receptor (see McCormick & Prince, 1986b). Our results clearly indicate that unlike the ganglionic M_1-receptor, the cortical muscarinic receptor linked with I_M shows a low sensitivity to pirenzepine, but is sensitive to 'cardioselective' and 'ileum-selective' muscarinic M_2 antagonists, suggesting that the cortical 'M_2' receptor may be different from the cardiac and ileal receptor types. The availability of more specific M_2-antagonists in the future should help to establish any fundamental differences between central neuronal and peripheral M_2-receptors.

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