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Muscarinic agonists block five different potassium conductances in guinea-pig sympathetic neurones

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Muscarinic excitation of sympathetic ganglion cells has usually been thought to result from inhibition of an outward K^+ current, the M current, although in other neurones several conductances have been shown to be blocked by muscarinic agonists. We report that, as well as resting K^+ conductance, all of four different K^+ conductances, two voltage-dependent (M currents and A currents) and two calcium-dependent (responsible for slow and very slow afterhyperpolarizations), present in different sub-types of guinea-pig sympathetic neurones, are inhibited by the muscarinic agonists, bethanechol and muscarine. All of these effects increase neurone excitability and can lead to repetitive discharge.

Introduction Mammalian sympathetic ganglion cells exhibit prolonged responses to acetylcholine (ACh) that are insensitive to (+)-tubocurarine, but sensitive to atropine (e.g. Kobayashi & Libet, 1970). ACh released naturally (e.g. by reflexes from chemoreceptors, Jänig *et al.*, 1983) can activate muscarinic receptors, evoking discharges in some postganglionic neurones. We present evidence that excitation produced by muscarinic agonists in three different sub-types of guinea-pig sympathetic ganglion cell occurs by blockade of five different K^+ conductances.

Methods Paravertebral ganglia L4–L6, distal lobes of inferior mesenteric ganglia (IMG) or coeliac ganglia were dissected from young guinea-pigs (180–250 g), and intracellular recordings made *in vitro* as described in detail previously (Cassell *et al.*, 1986). Tetrodotoxin and tetraethylammonium (TEA) were not used, so that suprathreshold depolarizations in voltage clamped cells initiated brief 'action currents' corresponding to uncontrolled action potentials (see Cassell *et al.*, 1986). Solutions containing muscarine (10 μM, Sigma) or bethanechol (10–100 μM, Sigma) were normally left in contact with the preparation for at least 15 min. Lower concentrations produced barely detectable effects.

Results In all neurones, the first effect of the drugs was a decrease in the amount of injected current needed to initiate an action potential, accompanied by an increase in cell input resistance. Then, over a few minutes, the membrane depolarized (1–18 mV) and often action potentials were initiated spontaneously. The neurones were then repolarized and maintained at their original resting potentials (−57–−68 mV) by passing steady current (0.01–0.25 nA). Under these conditions, cell resistance tended to return towards control values. All effects of agonists were completely reversed in control solution within 10 min. Bethanechol was applied to ≥ 4 cells of each type, and muscarine ≥ 1. Atropine (0.1 μM) completely abolished all drug effects without detectable direct actions.

Resting conductance was decreased by 1–63% ($n = 32$). The effect doubled with bethanechol concentration between 10 μM (−25% ± 3% s.e.mean, $n = 6$) and 100 μM (−53% ± 4%, $n = 7$). Steady state I/V relations in control solutions and in the presence of muscarinic agonists always intersected close to −90 mV, implying a decreased K^+ conductance. There was no significant effect on anomalous rectification (when present) ($n = 23$), or on the TEA-sensitive rectifier ($n = 4$).

Neurones of all types became more excitable, the same current initiating more action potentials (Figure 1), and the discharge pattern of phasic neurones became tonic (Figure 1A, D; see Brown & Constanti, 1980; Cassell *et al.*, 1986). However, responses elicited under voltage clamp indicated that the underlying conductance changes differed between neurone types.

In phasic neurones of the kind in paravertebral ganglia (Cassell *et al.*, 1986), a major effect was a decrease in I_M (Constanti & Brown, 1981; see Figure 1A). The reduction in outward current attributable to I_M was the same in bethanechol 10 to 100 μM.

In tonic neurones of the IMG, a dominant conduc-

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tance is the transient K^+ current I_A (Cassell *et al.*, 1986), which was reduced in either amplitude or time course (or both) by both agonists ($n = 8$ tonic neurones, Figure 1B; $n = 5$, 3 of other types).

These neurones have Ca-activated K^+ conductances causing afterhyperpolarizations that last several hundred ms. After an 'action current', an exponential outward tail current, $I_{Ca,K1}$ is recorded at -60 mV ($\tau \approx 100$ ms, Cassell *et al.*, 1986). Both amplitude and τ were reduced by up to 50% by either agonist (Figure 1C), on average by similar proportions, the reductions being greater in phasic ($-20\% \pm 7\%$, $n = 8$) than in tonic neurones ($-12\% \pm 4\%$, $n = 9$).

A third sub-type of neurone in the coeliac ganglion is characterized by a second Ca-activated K conductance producing an afterhyperpolarization lasting several seconds (McLachlan, 1987; cf. North & Tokimasa, 1983). The prolonged tail current, recorded at -60 mV following an action potential, $I_{Ca,K2}$, was almost completely abolished by either agonist (10 μM, $n = 5$) revealing the diminished $I_{Ca,K1}$ (Figure 1D).

Discussion In bullfrog sympathetic neurones (Adams *et al.*, 1982), muscarinic agonists almost exclusively affect I_M, but little or no I_M can be demonstrated in some mammalian sympathetic neurones (Galvan & Sedlmeir, 1984; Belluzzi *et al.*, 1985; Cassell *et al.*, 1986). Of three sub-types identified in guinea-pigs (Cassell *et al.*, 1986; McLachlan, 1987),

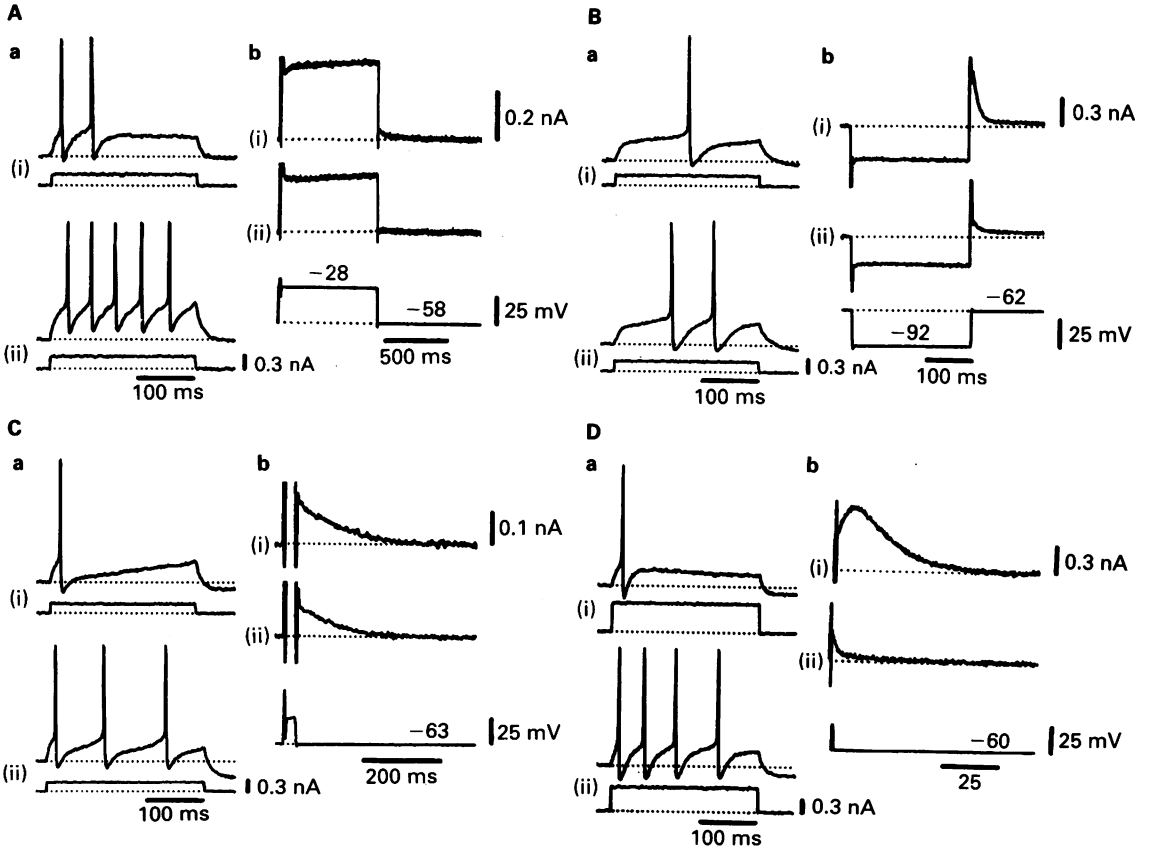

Figure 1 Responses of different guinea-pig sympathetic neurones to application of bethanechol 50 μM (10 μM in D). Records show membrane potential (above) and intracellular current (below) in current clamp (a), and current (above) and voltage (below) in voltage clamp (b), in control solution (i) and in the presence of bethanechol (ii). (A) Phasic neurone of L5 paravertebral ganglion. (B,C) Tonic neurones of the IMG. (D) Phasic ($I_{Ca,K2}$) neurone of the coeliac ganglion. Values of holding potential in clamp are shown on the records.

only phasic paravertebral neurones exhibit significant I_M, but muscarinic agonists increase excitability of all, including tonic neurones which normally show little accommodation during maintained depolarization.

Muscarinic blockade of resting and Ca-dependent K conductances has previously been described in other neurones (North, 1986), but not the depression of I_A. In the present experiments, all of these effects appear to be independent. Each of the conductances described is discrete. Two of the conductances are voltage-dependent (I_M and I_A), but have different kinetics and operate over different voltage ranges. Two are dependent on calcium influx during the action potential, but differ in time course; both are present in the third neurone subtype in the coeliac ganglion (Figure 1D).

Furthermore, the degree of blockade varied at different agonist concentrations. Resting conductance was decreased progressively as concentration was raised above 10 μM. I_M and $I_{Ca,K2}$ were almost completely blocked by both drugs at 10 μM. I_A and $I_{Ca,K1}$ were only partially reduced in amplitude and time course at 10–50 μM. The differential sensitivity of the two Ca, K conductances, despite presumably the same Ca^{2+} influx during one action potential, suggests that only some Ca channels are blocked by muscarinic agonists (see Belluzzi *et al.*, 1985).

Thus increased excitability of mammalian sympathetic ganglion cells induced by activation of muscarinic receptors may involve a number of distinct K^+ channels present in the membranes of different neurone sub-types. Whether or not the different mechanisms are linked to the same receptor (see North, 1986) remains to be clarified.

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