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INTRACELLULAR OBSERVATIONS ON THE EFFECTS OF MUSCARINIC AGONISTS ON RAT SYMPATHETIC NEURONES

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1 Responses of single neurones in isolated superior cervical ganglia of the rat to muscarinic agonists were recorded with intracellular microelectrodes.

2 (±)-Muscarine (1 to 10 μM) and methylfurmethide (1 to 3 μM) produced reversible membrane depolarizations (≤15 mV) accompanied by a fall in input conductance and an increased tendency toward repetitive spike discharges. The spike configuration was unchanged.

3 Analysis of steady-state current/voltage curves revealed the most consistent muscarinic effect to be a large reduction (~50% at 10 μM muscarine) in input slope conductance around rest potential. This conductance decrease diminished as the membrane was hyperpolarized, and the normal increase in slope conductance with membrane depolarization was depressed. The current/voltage curves in the presence and absence of agonist did not usually intersect; in a few tests, the curves intersected at between −65 and −88 mV (i.e. 9 to 28 mV hyperpolarized to rest potential).

4 Divalent cations (10 mM $[Ca^{2+}]$ or $[Mg^{2+}]$) showed a small muscarine-like effect on the current/voltage and slope conductance/voltage curves, but did not affect the action of muscarine itself.

5 Tetraethylammonium (TEA, 5 mM) also had a small muscarine-like effect, and depressed or reversed the action of muscarine. However, TEA differed from muscarine in blocking orthodromic transmission and prolonging direct spike repolarization.

6 It is concluded that the primary effect of muscarinic agonists is to alter the rectifying properties of the cell within the potential range −80 to −40 mV.

Introduction

There is general agreement that muscarinic depolarization of sympathetic neurones is accompanied by a fall in cell input conductance, and that the primary ionic conductance so affected is that of K^+ (e.g. frog: Weight & Votava, 1970; Kuba & Koketsu, 1974; 1976a and b; rabbit: Kobayashi & Libet, 1970; rat: Suzuki & Volle, 1978). The ^{86}Rb-flux tests in the preceding paper (Brown, Fatherazi, Garthwaite & White, 1980) accord with this interpretation. However, there is some uncertainty regarding the properties of this conductance change and whether other mechanisms than a fall in K^+-conductance might also be involved. Thus, the relatively small conductance decrease originally described by Weight & Votava (1970) appears insufficient to account for the depolarization in simple ohmic terms. Further, Kuba & Koketsu (1976a) noted a substantial voltage-sensitivity in the depolarization, to the extent that the latter rarely showed inversion on hyperpolarizing the cell. They suggested that (i) muscarinic agonists might affect the rectifying properties of the cell and that (ii) there may be additional inward currents involving Na^+ and/or Ca^{2+} in the synaptically-mediated slow e.p.s.p.

One problem which might affect the interpretation of previous experiments is that the agonists used, acetylcholine (either as the natural transmitter or as added agonist), methacholine and bethanechol, also have nicotinic effects; the degree to which these effects were suppressed by the nicotinic antagonists used is not entirely clear. In the present experiments, we have examined further the effects of two 'specific' muscarinic agonists ((±)-muscarine and methylfurmethide; see Brown *et al.*, 1980) on rat sympathetic neurones.

Methods

Superior cervical ganglia were isolated from Wistar rats (about 200 g weight), anaesthetized with urethane; the ganglia were desheathed and maintained in a bath of flowing Krebs solution at a temperature of 29 to 30°C. The design of the recording bath was

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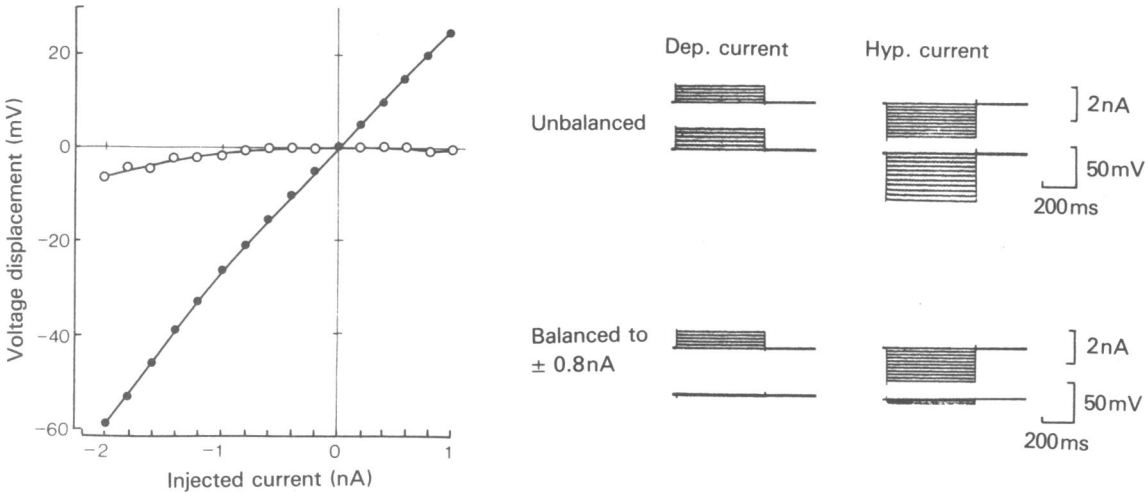

Figure 1 An example of microelectode characteristics recorded at the end of an experiment after withdrawing the electrode from a cell. The graph shows the current voltage characteristics in the unbalanced state (●) and when the voltage deflection produced by ±0.8 nA of current was balanced out (○). Representative current and voltage recordings are illustrated on the right.

essentially that used by Scholfield (1978) with some small modifications. A Peltier temperature-controlling block with water-cooled heat sink was used to provide manual temperature control of the bath fluid (measured by means of a pre-calibrated electronic thermometer with a Cu/Con thermocouple junction). Drugs flowed into the bath at the same rate through separate flowlines, all enclosed within a heated water jacket. The perfusion rate was adjusted so that the bath was filled within 10 to 20 s. The drug solutions in their reservoirs were bubbled continuously with 95% O_2 and 5% CO_2 (pH ~ 7.4); in some experiments, 95% O_2 and 5% CO_2 was gently bubbled directly into the bath chamber without disturbing the recording.

Superficial neurones were impaled with a single glass microelectrode filled with 4 M potassium acetate (neutralized to pH 7 with glacial acetic acid). The electrodes were prepared from prefibered glass (Clark Electro-medical GC 100F-6) using a Livingstone-type puller on a weak pull setting. This yielded electrodes with tip resistances of 30 to 60 MΩ that were capable of passing up to ±1 nA of current (tested before and after impalement) without appreciable rectification (see Figure 1); when necessary, corrections were made for electrode rectification at higher injecting currents. Our preferred method of impalement was by brief electrical oscillation of the electrode tip, under foot pedal control. Membrane potential was recorded with respect to ground via a high input-impedance probe with current injection facility: the electrode impedance and capacitance were nullified by an active bridge circuit (Colburn & Schwartz, 1968). Voltage recordings were monitored on a storage oscilloscope and Bryans d.c. chart recorder and stored for analysis on magnetic tape (Racal Thermionic Store-4 FM tape recorder; band-width 0 to 1.25 kHz at 3.75 inches/s tape speed). Neurones were activated orthodromically through a pair of Pt stimulating electrodes on the preganglionic nerve trunk. Cells with maintained resting potentials > -50 mV and spikes > 70 mV were selected for study; the average duration of impalement of cells used was 2 to 4 h.

(±)-Muscarine was obtained from Sigma. All other chemicals were reagent grade. Methylfurmethide was a gift from Dr A. Ungar.

Results

Muscarinic agonists

(±)-Muscarine (1 to 10 μM) and methylfurmethide (Me-Fur 1 to 3 μM) were selected as representative muscarinic agonists for testing on the basis of their high potency and relatively rapid reversal on washing (see Brown *et al.*, 1980). Their effects are illustrated in Figures 2 and 3 and are summarized in Table 1.

Bath-application of muscarine produced a depolarization of 4 to 6 mV at 1 μM and 7.5 to 15 mV at 10

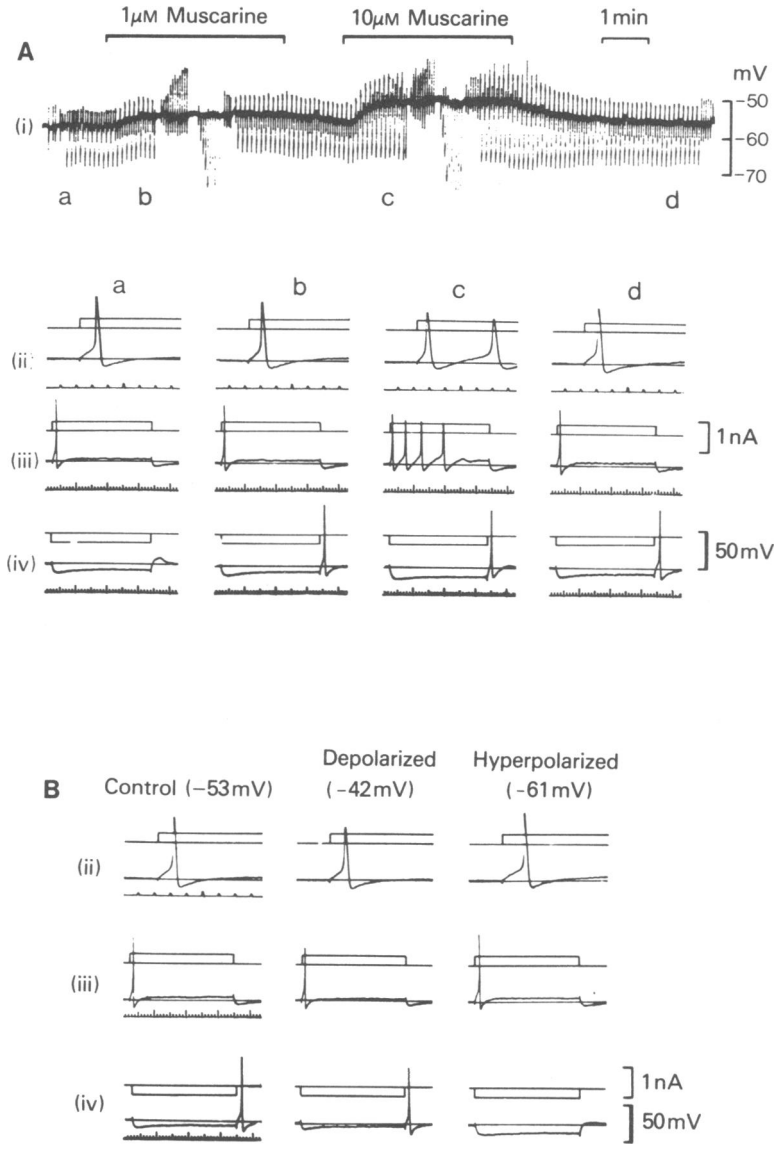

Figure 2(A) Responses of a single ganglion cell to 1 and 10 μM muscarine, separated by a brief perfusion with normal Krebs solution. The upper trace (i) is a continuous d.c. record of membrane potential (calibrated to zero potential after withdrawal of the microelectrode). Downward deflections indicate electrotonic potentials produced by constant hyperpolarizing current pulses (~300 ms duration, −0.3 nA, 0.17 Hz). (Upward blips are severely attenuated spikes induced by depolarizing current pulses.) The records in rows (ii), (iii) and (iv) are oscilloscope records of the responses to the depolarizing [(ii) and (iii)] and hyperpolarizing [(iv)] current injections corresponding to times (a), (b), (c) and (d) on the continuous chart record; in each record the upper beam shows current, the middle beam voltage and lower beam the time-base in 10 ms divisions. The responses to depolarizing current pulses are shown at two different sweep speeds. (B) Effects of changing the membrane potential of the same cell by injecting continuous current on the responses to depolarizing [(ii) and (iii)] and hyperpolarizing [(iv)] current pulses (0.3 nA). The membrane potential registered on passing constant depolarizing current was confirmed from the change in peak amplitude of the anode-break spike (assuming full removal of Na^+-inactivation during the preceding hyperpolarization).

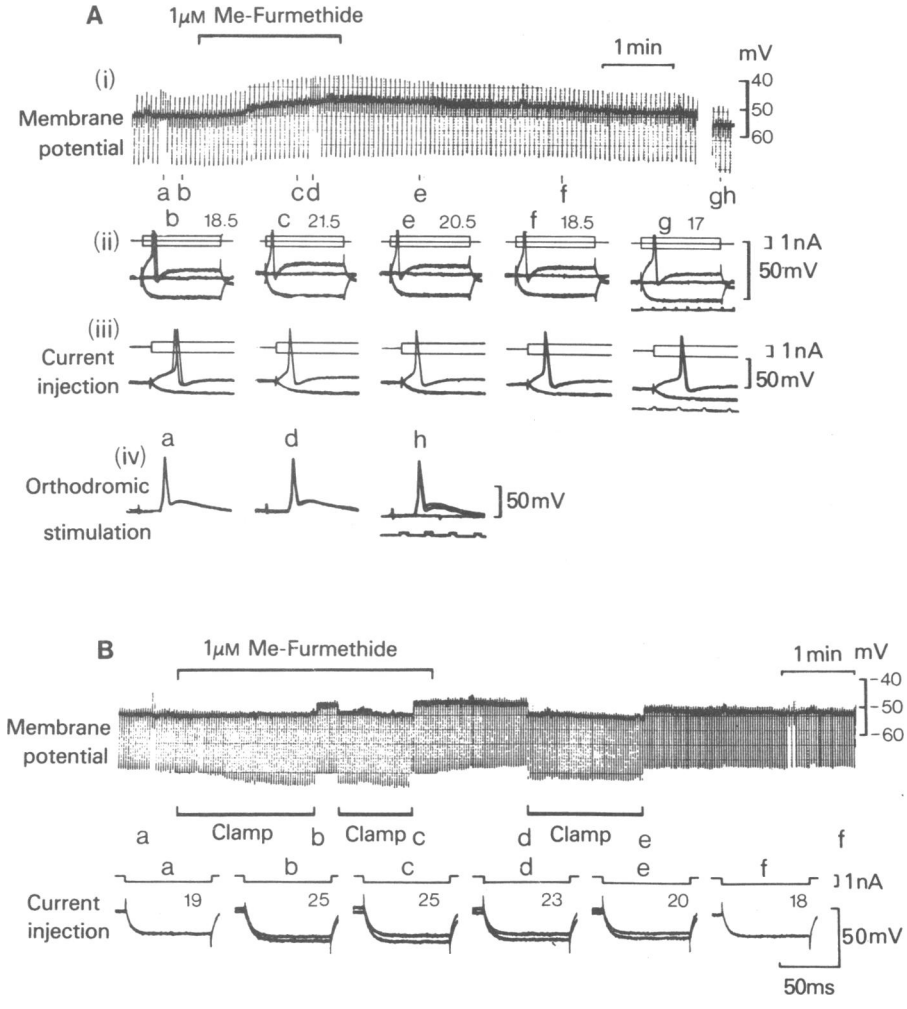

Figure 3 Two responses to 1 μM methylfurmethide recorded in the same cell (A) under conditions where the membrane was allowed depolarize and (B) when the membrane potential was maintained at the pre-drug level by passing hyperpolarizing current under manual control ('clamp'). The 'clamp' was intermittently released to reveal the amount of drug-induced depolarization. Upper traces in each case are continuous d.c. chart records as in Figure 1. Lower records in (A) show: (ii) and (iii), responses to alternating depolarizing and hyperpolarizing current pulses (±0.5 nA, 70 ms, 0.5 Hz), recorded at two different gains and sweep speeds; and (iv) responses to supramaximal orthodromic stimuli. Time trace shows 10 ms intervals. An interval of 6 min elapsed between (f) and (gh) in (A). In (B) only responses to hyperpolarizing pulses (−0.5 nA) were recorded; records (b), (c), (d) and (e) show superimposed electrotonic potentials in the presence and absence of the 'clamp'. The letters above the records refer to the corresponding time on the continuous chart record and the numbers give the amplitude of hyperpolarizing electrotonic potentials (mV). Two or three superimposed oscilloscope traces are shown on each record.

μM; Me-Fur produced a depolarization of up to 7 mV at 3 μM. The depolarization was usually accompanied by an increase in the voltage deflection produced by constant-current hyperpolarizing pulses (70 to 500 ms duration, of sufficient strength to give a peak voltage excursion of 10 to 20 mV from rest potential), indicating a *reduced* input conductance, of up to 50%. This was not the result of the membrane depolarization *per se* (as might occur if the cells showed inward rectification), since (i) the passage of sustained depolarizing

current through the microelectrode in the absence of agonist *increased* the apparent input conductance measured by the brief hyperpolarizing current pulses (Figure 2B), and (ii) the reduction in input conductance became larger when the membrane depolarization was prevented by 'manual voltage-clamping' (Figure 3B).

Action potentials At the concentrations used, neither muscarine nor Me-Fur modified the action potentials generated by orthodromic (preganglionic nerve) stimulation or by current-injection in a manner other than that expected from the reduced membrane potential (compare, for example, records (ii) in Figure 2A and (ii) in Figure 2B). In particular, both the falling phase and the after-hyperpolarization were unaffected (cf. amphibian cells: Kuba & Koketsu, 1976b). The agonists themselves did not usually generate action potentials; more frequently, they caused the appearance of repetitive spiking during the injection of depolarizing current pulses, in place of the normal single spikes (compare columns (c) and (a) in Figure 2A). In the absence of spikes, oscillations of the membrane potential were often seen (e.g. record (c, iii) in Figure 2A). Again, these effects could not be replicated by injecting sustained depolarizing currents (Figure 2B) however large the superimposed depolarizing current pulses. Muscarinic agonists also increased the tendency for 'anode-break' spiking on cessation of brief hyperpolarizing current pulses, and occasionally induced multiple anode-break spikes. In one cell, sustained firing at ~8 Hz persisted for several seconds after a depolarizing pulse.

Current voltage (I/V) curves Curves were constructed by measuring the 'steady-state' voltage attained during injection of long (70 to 500 ms) current pulses delivered at 1 to 3 s intervals). Since the ganglia were not treated with tetrodotoxin, points could not be obtained beyond a few mV in the depolarizing direction due to spike generation; however, curves were normally extended to −100 mV in the hyperpolarizing direction. Some typical I/V plots are illustrated in Figures 4 and 5.

The primary effect of muscarine and Me-Fur at both low and high concentrations was to increase the slope of the I/V curve in the region of most pronounced rectification, that is, between rest potential and membrane potentials 10 to 20 mV hyperpolarized to rest. In some instances, either with the higher muscarine concentrations (10 μM: Figure 4) or shortly after the first application of muscarine, the slope at more hyperpolarized levels was also increased, but this was not always seen.

Slope conductance Plots of slope conductance (G_s, measured from tangents drawn to the I/V curves at each point) against membrane potential (V) indicated an approximately exponential increase in slope conductance in the range −80 to −50 mV (e.g., Figure 4Bb). Addition of a muscarinic agonist shifted this curve by some 5 to 10 mV in the depolarizing direction without greatly altering its slope, though often introducing a 'dip' at −65 to −60 mV. At high agonist concentrations, the voltage-insensitive conductance at membrane potentials more negative than −80 mV was also reduced. Passage of sustained

Table 1 Summary of muscarinic effects on membrane potential and input conductance of rat sympathetic neurones at 29 to 30°C

Agonist	*Concn*	ΔE_m	$\frac{\Delta G_i}{G_i}$	$\frac{\Delta G_s^{\circ}}{G_s^{\circ}}$	$\frac{\Delta G_s^{min}}{G_s^{min}}$	ΔI°
		(mV)	(%)	(%)	(%)	(nA)
Muscarine	1 μM	+5.1 ±0.9 (4)	−12.3 ±11.7 (4)	−22.3 ±22.4 (4)	−1 ±18.3 (4)	+0.19 ±0.08 (4)
	10 μM	+11.2 ±2.9 (15)	−36.6 ±11.6 (15)	−53.7 ±13.8 (9)	−15.9 ±20.0 (8)	+0.31 ±0.19 (9)
Me-Fur	1 μM	+4.3 ±0.9 (5)	−11.6 ±13.4 (5)	−43, −47	−17, −5	+0.10, +0.15
	3 μM	+7, +4	−18, 0	−46, −19	−25, −4	+0.14, +0.12

Numbers are means ± s.d. (number of applications). ΔE_m = change in resting potential (+ = depolarization); $\Delta G_i/G_i$ = % change in input conductance measured using constant-current pulses; $\Delta G_s^{\circ}/G_s^{\circ}$ = % change in input slope conductance measured at the pre-drug resting potential, determined from current-voltage curves; $\Delta G_s^{min}/G_s^{min}$ = % change in minimum slope conductance at hyperpolarized levels of membrane potential; ΔI° = net inward current measured from the lateral displacement of the current/voltage curves at the pre-drug resting potential. E_m ranged from −40 to −73 mV, G_i from 22 to 126 nS, G_s° from 32 to 96 nS and G_s^{min} from 11 to 51 nS.

depolarizing current produced variable and generally small shifts in the G_s/V relationship, which did not resemble the effects induced by muscarinic agonists.

Subtraction of the slope conductance in the presence of agonist from that at rest, yielded a measure of the reduction in G_s produced by the agonist (interrupted curves in Figure 4Bb). This was clearly voltage-dependent, with a peak slope corresponding to an e fold reduction for 4 to 7 mV depolarization. The maximum decrease in slope conductance, at or around rest-potential, amounted to some 50 to 60% of the resting slope conductance. (Since the I/V curves could not be pursued far in the depolarizing direction, greater reductions might obtain at more depolarized levels though, in many cases, the reduction had reached a peak below rest potential.)

Agonist-induced currents The net current induced by the agonist can be calculated from the lateral shift of the I/V curve with respect to control (see Appendix). As expected from the voltage-dependence of G_s, the inward current diminished with membrane hyperpolarization from a value of between 0.1 and 0.8 nA at rest potential to a steady value of <0.05 nA at $V \leq -80$ mV (Figure 4Bd). On a few occasions, with high agonist concentrations, or at early times during a first agonist application (see below), the agonist-induced current became *outward* at hyperpolarized values: this is illustrated for 10 μM muscarine in Figure 4Bd. Inversion of agonist-currents on hyperpolarization, at membrane potentials between -65 and -85 mV, was observed on 6/19 occasions with muscarine and 2/6 with Me-Fur.

Prolonged agonist applications The effects of muscarine and Me-Fur usually peaked after 1.5 to 2 min application. During more prolonged applications, both the depolarization and the shift in the I/V curves declined somewhat; the inversion of the agonist current on hyperpolarization seemed particularly transient. On washing, partial recovery of the membrane potential and conductance change occurred quite

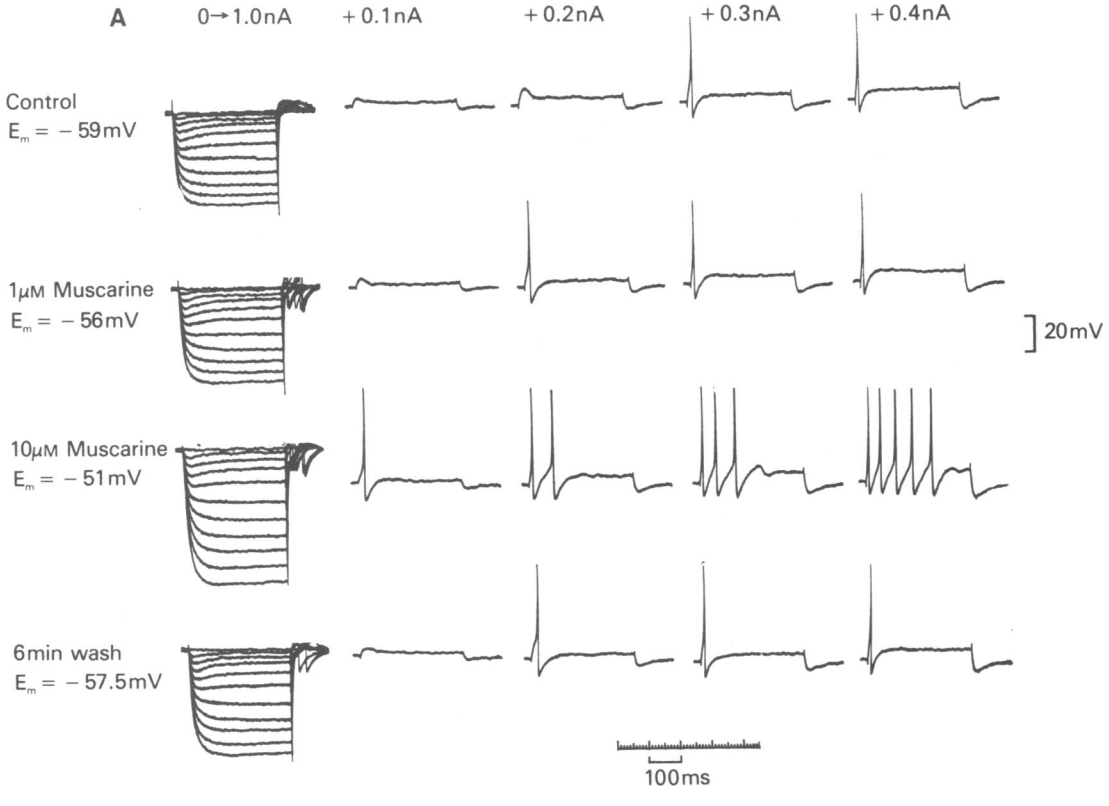

Figure 4A See legend opposite

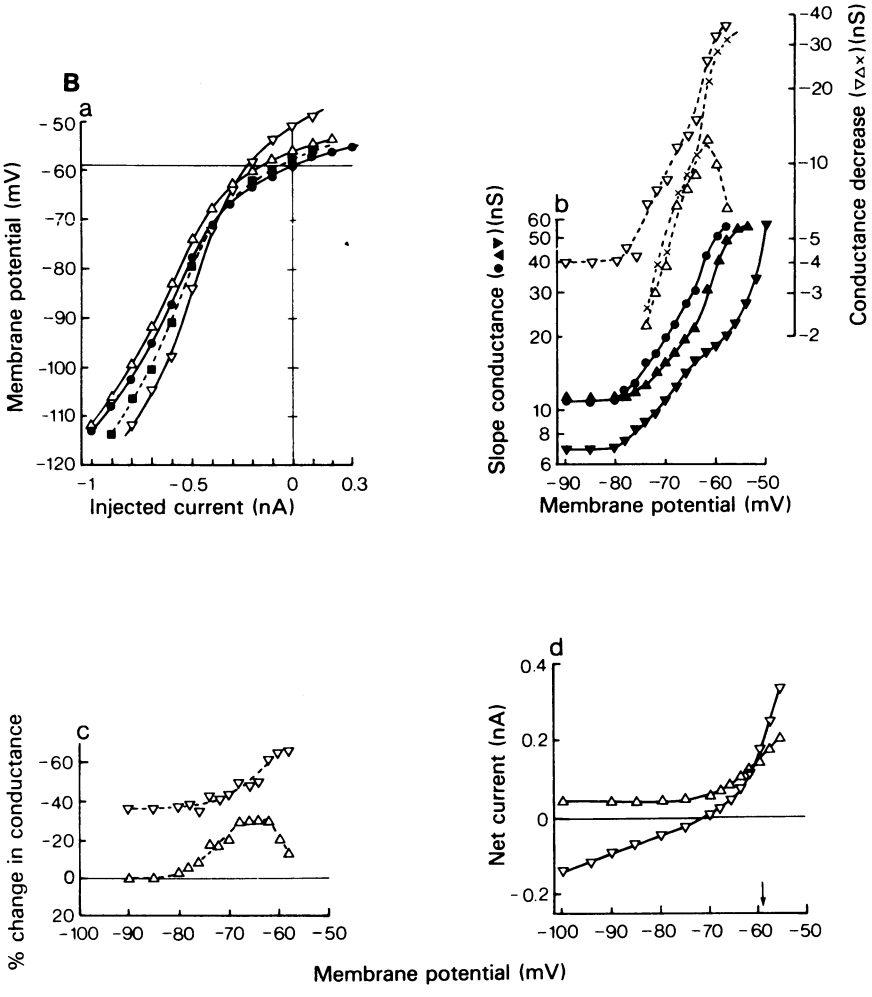

Figure 4 Current-voltage (I/V) relationships determined during the responses illustrated in Figure 2. (A) Records of voltage deflections produced by sequential hyperpolarizing current pulses from 0 to −1 nA in 0.1 nA steps (left column) and depolarizing pulses from +0.1 to +0.4 nA (right). (B) Curves constructed from steady-state voltage deflections illustrated in (A) (measured after 300 ms) as follows: (a) I/V curves (i) before (●), (ii) between 1 and 2.5 min after adding 1 μM muscarine (△), (iii) between 1.5 and 3 min after a subsequent addition of 10 μM muscarine (▽), and (iv) 5 min after washing out the muscarine (■). (b) Relation between slope-conductance and membrane potential before (●) and during 1 μM (▲) or 10 μM (▼) muscarine. Slope conductance was determined from tangents drawn to the curves at each point in (a) and expressed as nS on a logarithmic ordinate scale. The open symbols above (△,▽) show the corresponding conductance decreases obtained after subtraction from the control measurements. The crosses (× ... ×) show the conductance decrease produced by 10 μM muscarine after further subtracting the voltage-insensitive component of the conductance decrease recorded at membrane potentials more negative than −80 mV; note that the slope of the residual (voltage-sensitive) component is the same as that observed at 1 μM muscarine (△) but is displaced 2 mV to the left and extends to a higher peak value. (c) Relative decrease in slope conductance (%) induced by 1 μM (△) or 10 μM (▽) muscarine as a function of membrane potential. (d) Net current (nA) at different membrane potentials determined from the lateral displacement (relative to control) of the current-voltage curves shown in (a) (inward current +ve) induced by 1 μM (△) or 10 μM muscarine (▽). (All curves fitted by eye).

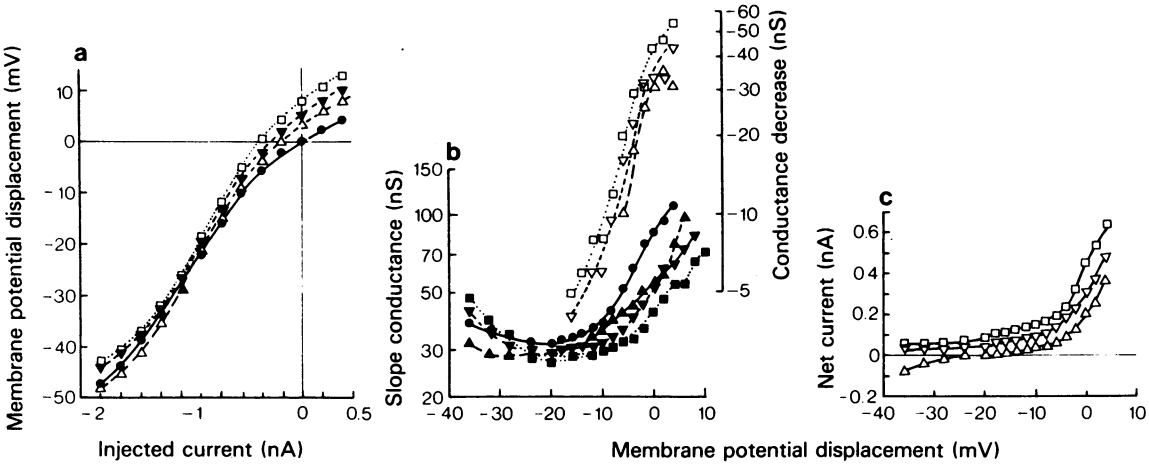

Figure 5 Effects of cumulatively increasing concentrations of muscarine (1 μM; ▲,△; 3 μM: ▼,▽; 10 μM: ■,□). (a) Current-voltage curves (cf. Figure 4B(a)) measured at the peak depolarization produced by each concentration of muscarine. (b) Voltage-dependence of the slope-conductance (cf. Figure 4B(b)). Open symbols here show the conductance decrease produced by muscarine, measured by subtraction from control conductances. (Note that the slope of the curves remains constant with incrementing dose, but that a lateral shift of 2 and 4 mV occurs in the hyperpolarizing direction as the concentration is raised from 1 to 3 μM and 3 to 10 μM respectively.) (c) Net currents induced by muscarine, measured from the lateral displacement of the I/V curves in (a). The net current reversed at a membrane potential about 25 mV negative to rest after (1 μM) application of muscarine, but not after subsequent, higher doses. (The membrane potentials in this experiment were designated relative to that before adding muscarine, because of some uncertainty regarding the absolute membrane potential. This was probably about −60 mV.)

rapidly (within 1 to 3 min; see top record in Figure 2A), but full restoration of the resting membrane potential and conductance required 10 to 15 min washing. A second application of muscarine within 2 min of washing regenerated a depolarization and further reduced input conductance. Thus, although there was some 'fade' in response, partial recovery was rapid and a cumulative or 'semi-cumulative' dose regimen could be used in the manner described previously (Brown *et al.*, 1980).

Concentration-sensitivity Figure 5 shows I/V relationships obtained with cumulatively-increasing concentrations of muscarine. The major effect of increasing concentration was to shift the G_s/V curves further in the depolarizing direction; thus, the conductance-decreases showed the same voltage-sensitivity in terms of slope, but became larger at any given membrane potential. The net currents (Figure 5c) likewise became larger while retaining their voltage-sensitivity. After the first application of muscarine (1 μM), the current inverted at about −80 mV, but this was not duplicated at the higher concentrations and probably reflects the transient inversion effect of initial applications mentioned earlier.

Effect of divalent cations

The shift of the G_s/V relationship produced by muscarine rather resembled that expected for an increase in the divalent cation concentration (see Muller & Finkelstein, 1972). Hence, it seemed worth testing whether the action of muscarine was imitated by divalent cations.

Figure 6 shows I/V and G_s/V plots from one such experiment in which $[Ca^{2+}]_o$ was first raised from 2.52 to 10 mM and then reduced to 1.26 mM. (Lower concentrations were impracticable due to increasing cell 'leakiness'.) Raising $[Ca^{2+}]_o$ did exert a small 'muscarine-like' depolarizing shift in the I/V and G_s/V relationships, which was quantitatively replicated by raising $[Mg^{2+}]_o$ from 1.2 to 10 mM; reducing $[Ca^{2+}]_o$ had the opposite effect. It may also be noted in Figure 6A that the I/V curves intersected at a membrane potential comparable to the inversion potential for muscarine in this cell (interrupted lines in

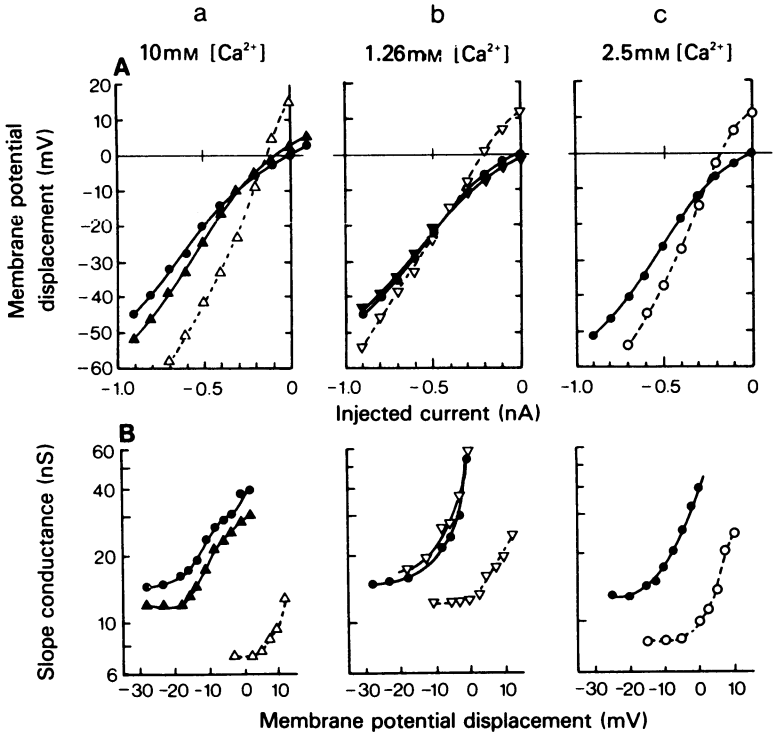

Figure 6 Effects of changing $[Ca^{2+}]_o$ on current/voltage curves (A) and slope-conductance/voltage curves (B) obtained in a single cell. (See Figure 4.) Filled symbols: curves in the absence of muscarine; open symbols, curves in the presence of muscarine. (●,○) 2.5 mM $[Ca^{2+}]$; (▲,△) 10 mM $[Ca^{2+}]$; (▼,▽) 1.26 mM $[Ca^{2+}]$. Thus, in (a) a current/voltage curve was first obtained in 2.5 mM $[Ca^{2+}]$ (●), then in 10 mM $[Ca^{2+}]$ (▲) and then muscarine added, still in 10 mM $[Ca^{2+}]$ (△). Likewise in (b) the first curve (●) was in 2.5 mM $[Ca^{2+}]$, then in 1.26 mM $[Ca^{2+}]$ (▼) and finally in muscarine +1.26 mM $[Ca^{2+}]$ (▽). (c) Simply shows curves in 2.5 mM $[Ca^{2+}]$ before (●) and during (○) muscarine perfusion.

Figure 6), suggesting that the Ca^{2+}-induced and muscarine-induced conductance changes involved the same ionic species.

In other respects however, the action of divalent cations was quite different from that of muscarine. Thus, raised $[Ca^{2+}]_o$ reduced the excitability of the neurone to depolarizing current injections, even though the cell was slightly depolarized; the current threshold for spike generation was elevated, and only single spikes were generated (Figure 7). In contrast, in the presence of muscarine, depolarizing pulses produced repetitive spikes. (Indeed, in the cell of Figure 7, a single pulse initiated a sustained spike discharge lasting several seconds at the peak of the muscarine depolarization.)

Raising or lowering the $[Ca^{2+}]$ concentration did not clearly alter the action of muscarine. Thus, both the depolarization and the shift in the I/V and G_s/V curves produced by muscarine in 10 mM $[Ca^{2+}]_o$ were essentially additive to the effect of Ca^{2+} itself (Figure 6Aa) and comparable to that seen in control solution (Figure 6Ac). Furthermore, in the presence of muscarine, depolarizing pulses still induced repetitive spikes in both high (10 mM) and low (1.26 mM) $[Ca^{2+}]$ media (Figure 7). (The nature of these discharges changed somewhat with changing $[Ca^{2+}]_o$. At 10 mM, the spike discharges were sustained, but at a relatively low frequency, whereas at 1.26 mM, the discharge was initially rapid but ceased after a few spikes. These changes in repetitive activity closely resemble those described in frog motoneurones on altering $[Ca^{2+}]_o$ in the absence of drugs, compare present Figure 7 with Figure 8 of Barrett & Barrett (1976), and hence may be attributed to the effect of

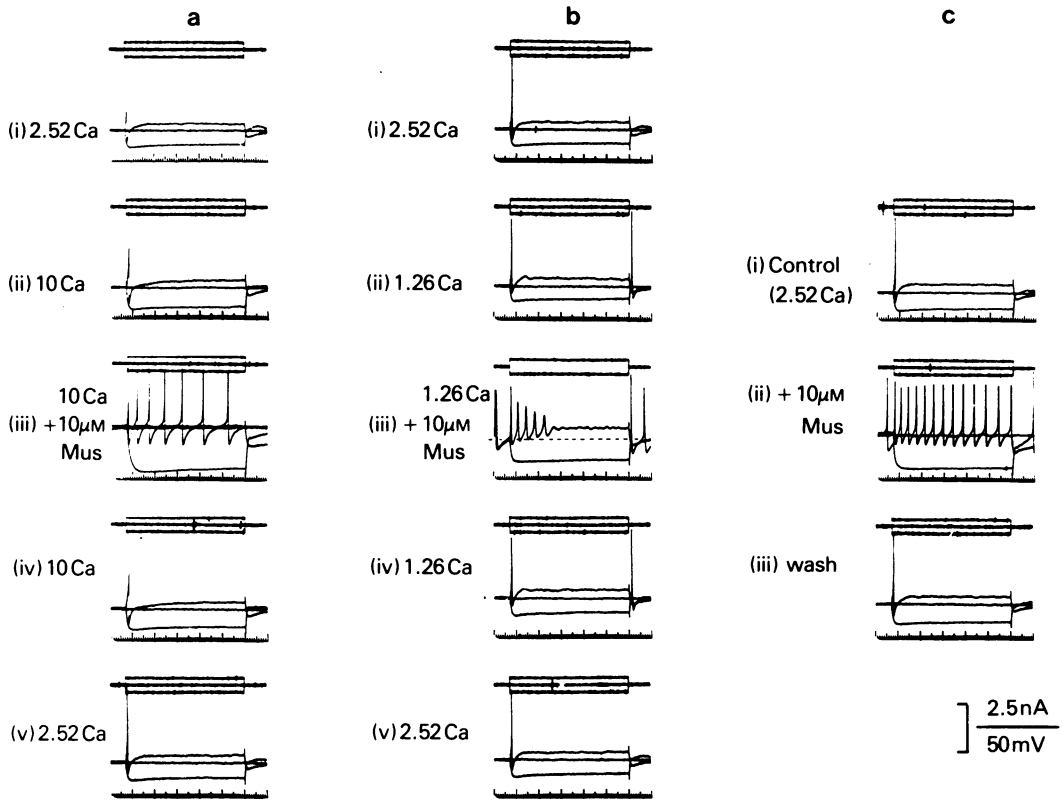

Figure 7 Oscilloscope records of the experiment depicted in Figure 6 showing cell responses to alternating depolarizing and hyperpolarizing current pulses (±0·4 nA; 530 ms) displayed as in Figures 2 and 3. Upper beams: current (2.5 nA calibration bar); middle beams, volta (50 mV calibration); lower beams, time (each large division = 100 ms).

Ca^{2+} itself rather than to any specific interaction with muscarine.)

Effect of temperature

The depolarization and conductance changes produced by muscarine were not radically altered on raising the normal operating temperature of 29 to 30°C up to 38.5°C or on reducing the temperature to 26°C (Table 2). Raising the temperature (i) increased the resting input conductance and hyperpolarized the cell by a few mV; (ii) increased the net inward current generated by muscarine; and (iii) reduced the firing frequency of the cell when challenged by depolarizing pulses in the presence of muscarine (Figure 8). The latter may have resulted from the prolongation of the spike after-hyperpolarization at the higher temperature; this after-hyperpolarization probably involves a Ca^{2+}-activated K^+ current (McAfee & Yarowsky, 1979), and its effect on firing frequency accords with previous observations on motoneurones (Barrett & Barrett, 1976) (compare also with the effect of raising $[Ca^{2+}]_o$ described above: Figure 7).

Effect of tetraethylammonium (TEA)

Kuba & Koketsu (1976b) suggested that muscarinic agonists impair delayed rectification. If so, then TEA might be expected to show some muscarine-like activity. Figures 9 to 11 show a comparison of 5 mM TEA and 10 μM muscarine on the same neurone.

TEA alone produced a small (≤4 mV) depolarization, accompanied by a ≤10% fall in input conductance (Figures 9 and 10; see also McAfee & Yar-

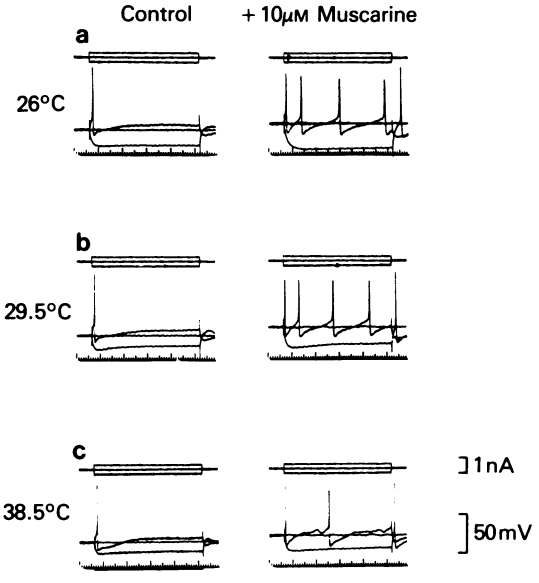

Figure 8 Oscilloscope records of the effects of muscarine recorded at three different temperatures in the same cell. Current pulses were ±0.3 nA: 450 ms; large time marks = 100 ms.

owsky, 1979). I/V curves showed a reduced slope conductance at rest potential and inversion of the net current at −80 to −90 mV (Figure 11). These effects did indeed resemble those seen with low doses of muscarine. However, TEA also showed several striking differences from muscarine: (i) it blocked orthodromic transmission (reversibly), presumably by blocking nicotinic receptors (Acheson & Pereira, 1946; Riker, 1965); (ii) it greatly prolonged spike repolarization, as previously described in frog neurones (Riker, 1964); and (iii) it did not induce repetitive spikes (compare records (b) in Figure 10A and B).

Interestingly, TEA blocked the effect of subsequently-added muscarine (Figure 9c) and reversed the effect of previously-added muscarine (Figure 9d); i.e., TEA added in the presence of muscarine hyperpolarized the cell and increased input conductance. On the other hand, muscarine did not prevent the effect of TEA on the action potential (compare record (c) in Figure 10A with record (b) in Figure 10B).

Discussion

The principal action of muscarinic agonists discerned in these experiments was to alter the normal steady-state rectifying properties of the rat ganglion cell membrane, so that the increase in conductance which normally occurs between −80 mV and rest potential was depressed. As a result of the reduced input conductance at rest potential, a net inward current of 0.1 to 0.8 nA was generated, depolarizing the cell by some 5 to 15 mV.

The shift in the steady-state I/V curve produced by muscarine and Me-Fur accords with the effect of acetylcholine (in the presence of curare) on frog ganglion cells described by Kuba & Koketsu (1974, 1976b). The latter authors attributed this to a suppression of delayed rectification; however, we have shown that the component of rectification in the rat which is apparently depressed by muscarine clearly differs from the conventional TEA-sensitive delayed rectifier since, unlike TEA, muscarinic agonists did not broaden the spike. The small 'muscarine-like' effect of TEA on the rat cells, and the subsequent blockade of muscarine, are probably attributable to a quite separate side effect of this notoriously 'unspecific' drug. In support of this contention, muscarine did not prevent the effect of TEA on the spike. Kuba & Koketsu (1977) found that TEA potentiated the muscarinic depolarization produced by acetylcholine on frog neurones: this we did not see, and can offer no

Table 2 Effect of changing temperature on the response of a single ganglion cell to 10 μM muscarine

Temperature	ΔE_m	ΔI°	G_i	ΔG_i	$\frac{\Delta G_i}{G_i}$	G°	ΔG°	$\frac{\Delta G^{\circ}}{G^{\circ}}$
(°C)	(mv)	(nA)	(nS)	(nS)	(%)	(nS)	(nS)	(%)
26	+7	+0.13	14.3	−5.2	−36	37.0	−25.4	−69
29	+9	+0.19	18.8	−7.0	−37	34.5	−18.9	−55
38.5	+8	+0.25	21.4	−6.0	−28	48.7	−30	−62

See footnote to Table 1 for symbols.

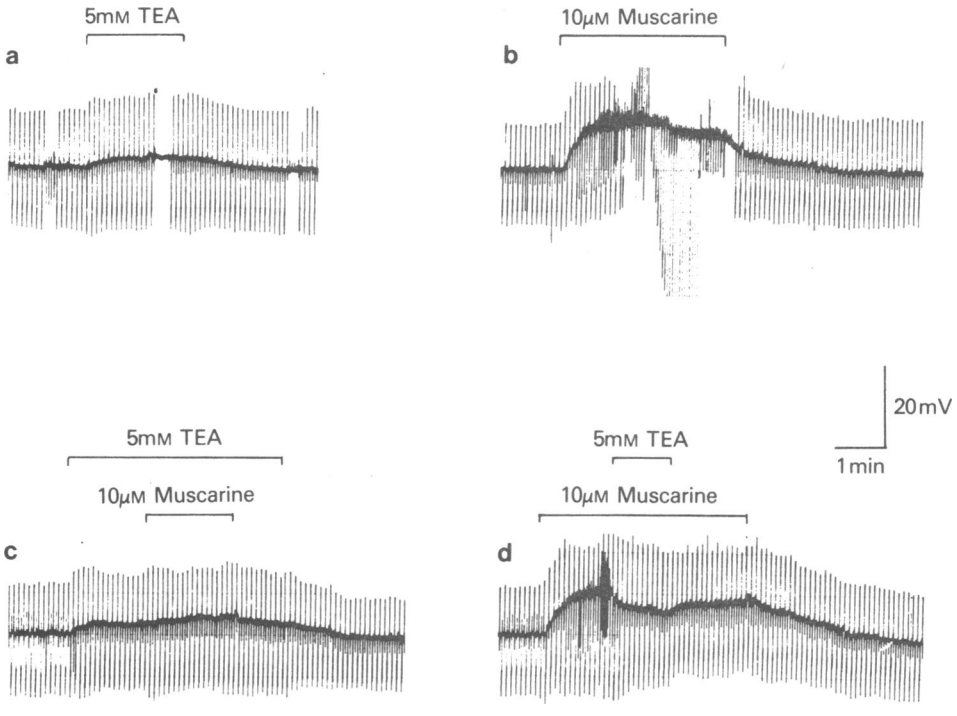

Figure 9 d.c. chart-records of the effects of tetraethylammonium (TEA) and muscarine, alone and in combination, on the membrane potential (thick line) and input conductance of a single neurone (downward deflections are peak voltage responses to −0.3 nA hyperpolarizing current pulses, 450 ms duration). Drug applications were made in the order and for the periods indicated, at about 15 to 20 min intervals.

explanation for the apparent difference between frog and rat cells.

In some instances, muscarine also reduced the non-rectifying component of membrane conductance observed at more negative membrane potentials; in such cases, the inward current sometimes reversed to an outward current at membrane potentials between −65 and −85 mV. This recalls the original observation of Weight & Votava (1970) that the muscarinic slow e.p.s.p. in frog neurones reversed at or near E_K: Kuba & Koketsu (1976a) also noted current reversal in a minority of neurones. In our experiments, such reversals were restricted to high agonist concentrations or to the first application, and were not a necessary accompaniment to the depolarization; hence we regard a fall in the steady-state (voltage-independent) conductance of the cell, and consequent current reversal, as secondary to the change in voltage-dependent conductance.

Kuba & Koketsu (1976a) also reported an occasional component of increasing inward current as the frog cell was hyperpolarized in the presence of acetylcholine, which they attributed to an increase in g_{Na} and/or g_{Ca}. This we did not observe, and suspect that it might have resulted from a small component of unblocked nicotinic receptor activation.

Elevation of external divalent cation concentration superficially mimicked the action of a low concentration of muscarine. However, as pointed out by Muller & Finkelstein (1972), an effect of this type may have several explanations: e.g., a change in the ionic conductance of the channels, in the total number of channels available, in the energy difference between open and closed channel states, or in surface-charge density (the most probable change induced by divalent cations). It seems unlikely that muscarinic agonists and divalent cations have a common point of action at this latter level, or that the effect of muscarine is mediated by a change in divalent cation gradient, since the actions of Ca^{2+} and muscarine were essentially additive and non-interactive.

In conclusion: the primary effect of muscarinic agonists was to modify the rectifying properties of rat ganglion cells. Recent experiments on bullfrog ganglia

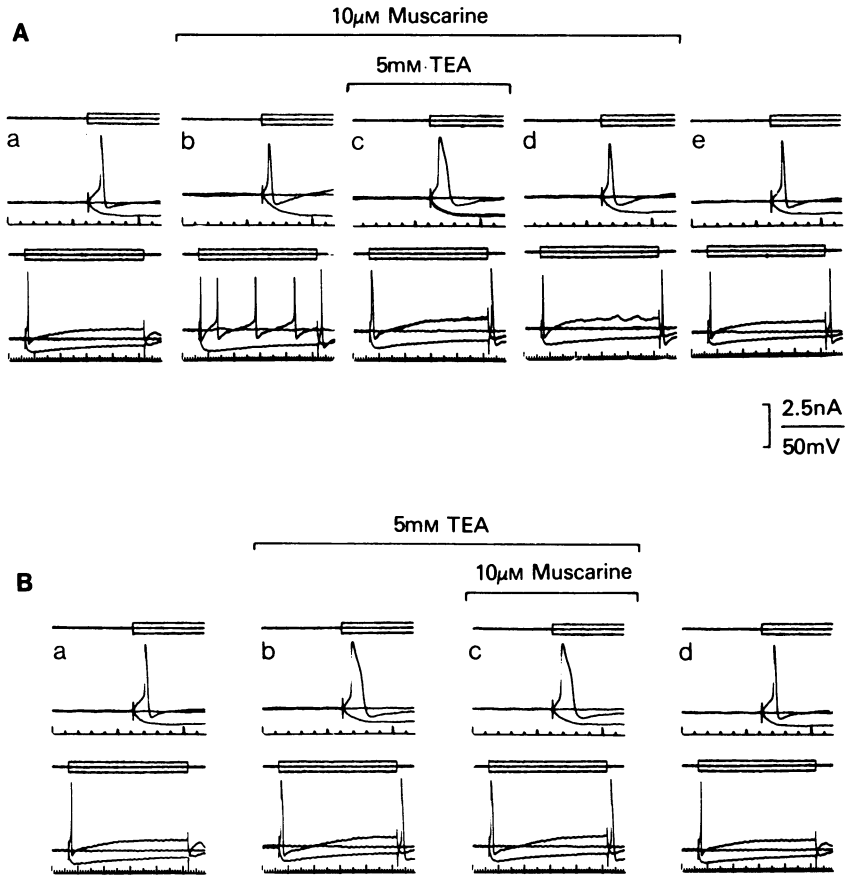

Figure 10 Oscilloscope records of the responses to alternate depolarizing and hyperpolarizing current pulses (±0.3 nA; 450 ms) recorded during responses (d) and (c) in Figure 9 (A and B respectively). In each, responses are displayed at two different sweep speeds to show changes in spike configuration and spike discharge rates. (Note the membrane potential oscillations in A(d) but not in A(c) and B(c).)

(Brown & Adams, 1980) have revealed a voltage-sensitive K^+-current operating within ±15 to 20 mV of rest potential, quite distinct from the conventional delayed rectifier, as the specific target for muscarinic agonists. Our observed shifts in the I/V curves are compatible with the operation of a similar current in rat ganglia. The striking repetitive spike discharges induced by muscarine suggest that this current, or its mammalian equivalent, may act as a braking control on spike frequency. The function of muscarinic transmission in both ganglia and the central nervous system (see Krnjević, Pumain & Renaud, 1971; Dingledine, Dodd & Kelly, 1977) may therefore be to remove this brake and to accelerate spike discharge rates.

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Appendix

In this paper, we have used the lateral shift in the I/V curve as a means of calculating the net current induced by muscarinic drugs (see Figures 4 and 5). By using a simple electrical circuit representation of a cell (Figure 12; cf. Ginsborg, 1967) it can readily be shown that this subtraction method yields the extra current induced to flow across the membrane by agonist, irrespective of whether the cell input conductance is

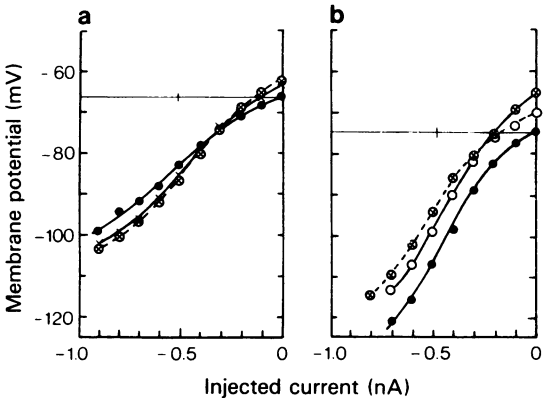

Figure 11 Current/voltage curves constructed during the responses shown in Figure 9 in the absence of drug (●), in 10 μM muscarine (○), in 5 mM tetraethylammonium (TEA) (×) and in the presence of both TEA and muscarine (⊗). The sequences were: (a) control, TEA, TEA + muscarine (record (c) in Figure 9); (b) control, muscarine, muscarine + TEA ((d) in Figure 9). Note that the muscarine and control curves in (a) crossed at the same absolute membrane potential as the [muscarine + TEA] and TEA curves in (b).

decreased (as in our experiments) or increased (as is more conventional).

1. *Conductance increase mechanism*

Consider a cell with membrane potential E and input conductance G, showing ohmic behaviour in response to applied transmembrane current ('current-clamp' conditions). At rest, the steady-state current-voltage relation (cell capacitance omitted) will be described by

$$V = E + (I/G) \qquad A1$$

where V is the recorded membrane potential and I the applied current (Figure 12b). In the presence of an agonist that *increases* membrane conductance by g (switch S closed), the total input conductance increases to (G + g) and the membrane potential will shift by an amount ΔV, determined by the electromotive driving force (e − E) (e is the agonist equilibrium potential). Thus in the active state, the current-voltage relation will be

$$V = [E + \Delta V] + \left[\frac{I}{G + g}\right] \qquad A2$$

where

$$\Delta V = (e - E)\left[\frac{g}{G + g}\right] \text{(Ginsborg, 1967)} \qquad A3$$

Taking an arbitrary level V′ on the membrane potential axis, this will correspond to a transmembrane current I_1 in the resting state and I_2 in the presence of agonist; the difference $I_2 - I_1$ is the extra current that would have to be applied across the membrane in order to maintain the membrane potential at V′ in the presence of agonist. This extra current can be shown to be equivalent to the net current (I_{net}) induced by agonist at the membrane potential V′. From eqns A1 and A2,

$$I_1 = (V' - E)G$$

$$I_2 = [V' - (E + \Delta V)](G + g).$$

Substituting for ΔV from eqn A3, and rearranging gives:

$$(I_2 - I_1) = I_{net} = g(V' - e). \qquad A4$$

2. *Conductance decrease mechanism*

For an agonist that decreases membrane conductance by g, the resting state of the cell may now be represented in the electrical circuit by leaving switch S closed (Figure 12a). The recorded resting membrane potential is then

$$V_{rest} = \left[\frac{EG + eg}{G + g}\right] \qquad A5$$

In the presence of agonist, S is opened and the recorded potential is

$$V_{active} = E$$

The potential change induced by agonist will be given by:

$$(V_{active} - V_{rest}) = \Delta V = \left[\frac{(E - e)g}{G + g}\right]$$

$$= \left[\frac{(V_{active} - e)g}{G + g}\right] \qquad A6$$

It will be noted that, unlike the conductance increase mechanism (cf. eqn A3) the expression for ΔV involves a term of membrane potential at the height of the response (V_{active}) and not the true resting membrane potential V_{rest}. To obtain ΔV in terms of the latter, eqn A5 is rearranged thus:

$$E = \frac{V_{rest}(G + g) - eg}{G} = V_{active}$$

Now substituting for V_{active} in eqn A6 gives

$$\Delta V = (V_{rest} - e)\frac{g}{G} \qquad A7$$

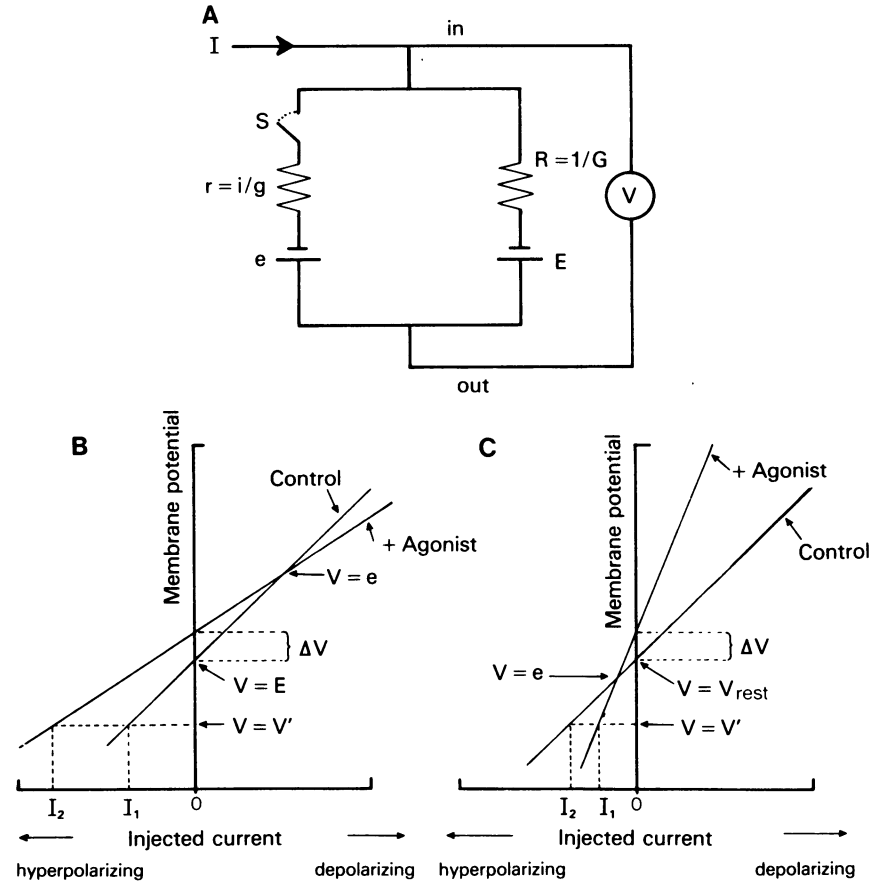

Figure 12 (a) Equivalent electrical circuit for a cell membrane showing ohmic behaviour (capacitance omitted). Right hand branch 1/G, E, represents resting conductance and membrane potential respectively; left hand branch represents the chemosensitive membrane (resistance 1/g and equilibrium potential e) connected in parallel. Additional of agonist corresponds to closure of switch S. Injection of current I can be made from an external d.c. source. The battery e is arbitarily shown with the negative terminal facing inward. (b) Simplified steady-state current/voltage relationships for a cell membrane at 'rest' (control) and in the presence of an agonist that increases membrane conductance and depolarizes the cell by ΔV. The curves intersect at the agonist equilibrium potential e. (c) Current/voltage relationships for membrane at 'rest' and in the presence of agonist that decreases conductance and depolarizes by ΔV. In both (a) and (b) the net agonist-induced current at the membrane potential V' is given by the difference $I_2 - I_1$ (see text).

The 'resting' current-voltage relation (Figure 12c) will be

$$V = V_{rest} + \left[\frac{I}{G + g}\right]$$

and in the 'active' state,

$$V = V_{rest} + \Delta V + (I/G)$$

At the arbitrary potential V', the corresponding currents I_2 and I_1 at rest and with agonist present respectively, will now be given by

$$I_2 = (V' - V_{rest})(G + g)$$

and

$$I_1 = (V' - V_{rest} - \Delta V)G$$

As before, the net current will be:

$$(I_2 - I_1) = (V' - V_{rest})g + \Delta V.G$$

Substituting for ΔV from eqn A7 and rearranging again gives equation A4:

$$I_{net} = g(V' - e)$$

Note

One interesting point emerges on comparing eqns A3 and A7: the potential change following a conductance decrease is a linear function of the conductance change, in contrast to the usual hyperbolic function for a conductance increase (cf. Ginsborg, 1967). Thus, if the total conductance change were directly proportional to the number of receptors activated, there would be 'spare receptors' for the depolarization produced by a conductance increase but not for the depolarization resulting from a conductance decrease.

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