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# CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN GUINEA-PIG OLFACTORY CORTEX NEURONES *IN VITRO*

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SUMMARY

1. Guinea-pig olfactory cortex neurones *in vitro* (23–25 °C) were voltage clamped by means of a single-micro-electrode sample-and-hold technique.

2. Under current clamp at the resting potential ($\sim -80$ mV), brief depolarizing stimuli evoked trains of action potentials with little visible after-potential. However, in 90% of recorded cells held at membrane potentials between $-70$ and $-45$ mV, depolarizing current pulses evoked a slow after-hyperpolarization (a.h.p.) ($\sim 8$ mV) lasting several seconds and accompanied by an increase in input conductance.

3. The outward membrane current underlying the a.h.p. was revealed either by switching rapidly to voltage clamp at the end of a spike train ('hybrid' clamp) or by applying brief depolarizing commands from potentials between $-60$ to $-45$ mV. The tail current showed a distinct rising phase (time to peak $\sim 1$ s) and exponential decay ($\tau \sim 3$ s) and was suppressed by removal of external $Ca^{2+}$, or adding $Co^{2+}$ (1–2 mM), $Cd^{2+}$ (200 $\mu$M) or $Mg^{2+}$ (6 mM). The a.h.p. current reversal potential was $-96$ mV in 3 mM-$K^+$ medium.

4. Low concentrations (1–2 $\mu$M) of muscarine, carbachol, oxotremorine or the muscarinic ganglion stimulant, McN-A-343 (1–10 $\mu$M) reduced the a.h.p. current and leak conductance and induced a steady inward current, without affecting M-current ($I_M$) relaxations. $I_M$ inhibition generally required higher ($> 10$ $\mu$M) agonist concentrations, although oxotremorine remained ineffective at up to 50 $\mu$M.

5. The a.h.p. current was reduced by noradrenaline and tetraethylammonium (TEA), but not by apamin or tubocurarine. Apart from TEA, these agents had no effect on $I_M$.

6. Addition of tetrodotoxin (TTX, 1 $\mu$M) or removing external $Na^+$ depressed the a.h.p. current amplitude recorded under voltage clamp. The residual tail current could be further reduced by adding $Cd^{2+}$ or muscarinic agonists.

7. Repolarizing tail currents induced following positive voltage commands consisted mainly of $I_M$ and slow a.h.p. current with little evidence of a 'fast' $Ca^{2+}$-activated $K^+$ current ($I_C$).

8. It is concluded that the slow a.h.p. current that underlies the post-burst after-hyperpolarization of olfactory neurones, is a $Ca^{2+}$-dependent $K^+$ current distinct from $I_M$. It is suggested that the cholinergic modulation of this current

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(rather than $I_M$) may provide a more subtle control of cell excitability in cortical neurones.

## INTRODUCTION

Slow after-hyperpolarizations (a.h.p.s) following a single or train of action potentials have been described in a variety of mammalian central neurones (Kandel & Spencer, 1961; Schwartzkroin, 1975; Hotson & Prince, 1980; Llinás & Yarom, 1981). These long-duration a.h.p.s were attributed to the activation of a $Ca^{2+}$-dependent $K^+$ conductance and could be blocked by the removal of external $Ca^{2+}$, or the addition of external divalent cations which interfere with $Ca^{2+}$ entry (e.g. $Cd^{2+}$, $Mn^{2+}$ and $Co^{2+}$) (Hotson & Prince, 1980; Alger & Nicoll, 1980; Schwartzkroin & Stafstrom, 1980; Wong & Prince, 1981; Cole & Nicoll, 1984*a*; Adams & Lancaster, 1985; Lancaster & Adams, 1986).

Mammalian cortical neurones may also exhibit prolonged post-spike a.h.p.s (Connors, Gutnick & Prince, 1982; Stafstrom, Schwindt, Flatman & Crill, 1984) although no detailed account of their properties has yet been presented. We have previously reported that olfactory cortical neurones *in vitro* can generate a slow $Ca^{2+}$-dependent $K^+$ conductance that is reversibly reduced by muscarinic agonists (Constanti & Sim, 1985). In the present study, we have further examined the properties and pharmacology of this cortical a.h.p. (and the underlying slow outward membrane current) using a single micro-electrode voltage-clamp or 'hybrid'-clamp technique (Wilson & Goldner, 1975; Lancaster & Adams, 1984, 1986). Our results show that the a.h.p. current in olfactory cortical neurones is pharmacologically distinct from another muscarine-sensitive outward current, the M-current ($I_M$), present in these cells (Constanti & Galvan, 1983*b*) and is more sensitive to lower concentrations of muscarinic agonists. In addition, the a.h.p. current recorded in olfactory neurones exhibited some characteristics that were notably different from those of the slow $I_{a.h.p.}$ recently described in hippocampal pyramidal cells (Lancaster & Adams, 1986).

## METHODS

### *Preparation*

Albino guinea-pigs (250–350 g; either sex) were anaesthetized with ether, decapitated and their brains carefully removed. Rostro-caudal slices of olfactory cortex (500 $\mu$m thick) were then prepared using either a manual tissue chopper as previously described (Constanti & Sim, 1984) or an Oxford vibratome. The slices were pre-incubated in oxygenated Krebs solution at room temperature (23 °C) for 30 min before transferring to the recording chamber. The composition of the modified Krebs solution was (mM): NaCl, 118; KCl, 3; $CaCl_2$, 1·5; $NaHCO_3$, 25; $NaH_2PO_4 . 2H_2O$, 1·2; $MgCl_2 . 6H_2O$, 1; D-glucose, 11; (bubbled with 95% $O_2$:5% $CO_2$, pH 7·4). The slices were held between two nylon meshes (slice completely submerged) and superfused continuously at *ca.* 5 ml/min with oxygenated Krebs solution at 23–25 °C. In some experiments, the inflowing solution was pre-heated via a closed, steel heat-sink chamber to provide a constant temperature of 35 °C. Excess solution was removed by top suction.

For (nominal) $Ca^{2+}$-free Krebs solution, $CaCl_2$ was replaced with equimolar $MgCl_2$ (total $[Mg^{2+}]$ 2·5 mM). For low (25 mM)-$Na^+$ Krebs solution, NaCl was replaced by equimolar choline chloride, whereas for $Na^+$-free Krebs, the remaining $NaHCO_3$ was replaced by equimolar choline bicarbonate. Atropine (10 $\mu$M) was included to avoid any weak muscarinic agonist action of choline (Krnjević & Reinhardt, 1979). In some experiments, low (25 mM)-$Na^+$ Krebs solution was prepared by

replacing NaCl with equimolar *N*-methyl-D-glucamine and the pH adjusted to 7·4 by adding *ca.* 110 mM-HCl. Attempts to replace $Na^+$ with Tris (even at concentration as low as 25 mM) were found to be detrimental to the slices under our conditions.

All drugs (BDH Analar grade, or Sigma Ltd. prepared in Krebs solution) were applied in fixed concentrations to the superfusate (bath exchange time *ca.* 30 s). 4-m-Chlorophenylcarbamoyloxy-2-butynyltrimethyl ammonium, McN-A-343, was obtained from Research Biochemicals Industries, MA, U.S.A. Pirenzepine was a gift from Dr Karl Thomae GmbH, Biberach an der Riss, F.R.G.

*Electrical recording*

Intracellular micro-electrodes were filled with either 4 M-potassium acetate or 3 M-KCl (50–80 MΩ) and coupled to a single-micro-electrode current–voltage clamp pre-amplifier (Dagan 8100 or Axoclamp-2). The switching frequency used was 2–3 kHz (25% duty cycle with the Dagan or 30% with the Axoclamp) after adjustment of the capacity neutralization in 'switched current-clamp' mode. The limitations and pitfalls associated with the use of the sample-and-hold technique, in which the amplifier cycles between current-passing and voltage-recording modes have been discussed previously in some detail (Galvan & Adams, 1982; Halliwell & Adams, 1982; Adams, Brown & Constanti, 1982; Constanti & Galvan, 1983*a*; Johnston & Brown, 1983; Finkel & Redman, 1984).

Under voltage clamp, the phase and gain controls were usually adjusted to give a voltage-step settling time within 3 ms. With small negative voltage jumps ( $< 40$ mV), the membrane potential attained the command level with reasonable accuracy whereas the deviation with larger jumps could be up to 10% (depending on the holding potential). Any attempts to improve voltage control by further increasing the feed-back gain usually led to amplifier oscillation and invariable cell death. Deviations from the command potential were also recorded following positive voltage jumps, especially as more slow, outward current become activated. In experiments where tetrodotoxin (TTX) was omitted from the bathing medium, early transient inward currents representing uncontrolled $Na^+$ spikes were often apparent during the first 20–30 ms of the membrane current recorded following a small voltage step: several corresponding unclamped spikes then appeared at the onset of the voltage record. Thereafter, the clamp settled to the command potential, and slower graded outward currents developed. This problem of uncontrolled spikes could be minimized by clamping at relatively positive holding potentials ($V_h$, normally $-45$ or $-50$ mV) so that $Na^+$ spike generation was largely inactivated. The steady voltage level recorded at the soma following a step command was always used to construct current–voltage ($I$–$V$) curves. Sampled membrane currents (filtered at 200–300 Hz; low pass 48 dB/octave) and voltage were either photographed directly from the oscilloscope screen or recorded on a Gould Brush 2400 pen recorder (rise time $< 8$ ms for square-wave input). Membrane currents were not corrected for leakage or capacitative currents. Unless otherwise noted, each experimental sequence was repeated at least three times in different cells. The data presented below are based on stable recordings made from cortical neurones in *ca.* sixty slices. Neurones were encountered within the pyramidal cell layers II–IV of the olfactory cortex (O'Leary, 1937; Halliwell, 1982).

## RESULTS

### *Current-clamp recordings*

Recorded cells had stable resting potentials in the range $-91$ to $-72$ mV (mean $-82 \pm 0{\cdot}64$ mV (S.E. of mean), $n = 70$). Under resting conditions, a brief depolarizing current pulse (100–200 ms; 1–1·5 nA) evoked a train of action potentials with little visible a.h.p. However, as the cell was depolarized (by steady d.c. current injection) to potentials between $-70$ and $-45$ mV, a brief depolarizing pulse now evoked a single or train of spikes followed by a distinct, slow a.h.p., lasting several seconds.

Fig. 1 illustrates the various types of a.h.p. which could be observed in olfactory neurones held at $-60$ mV. A brief depolarizing current pulse (200 ms; $\sim 0{\cdot}8$ nA) evoked characteristic slow a.h.p.s in different cells varying in peak amplitude (4–12 mV) and duration (3–12 s) at $-60$ mV (Fig. 1*Aa* and *e*). In the present study,

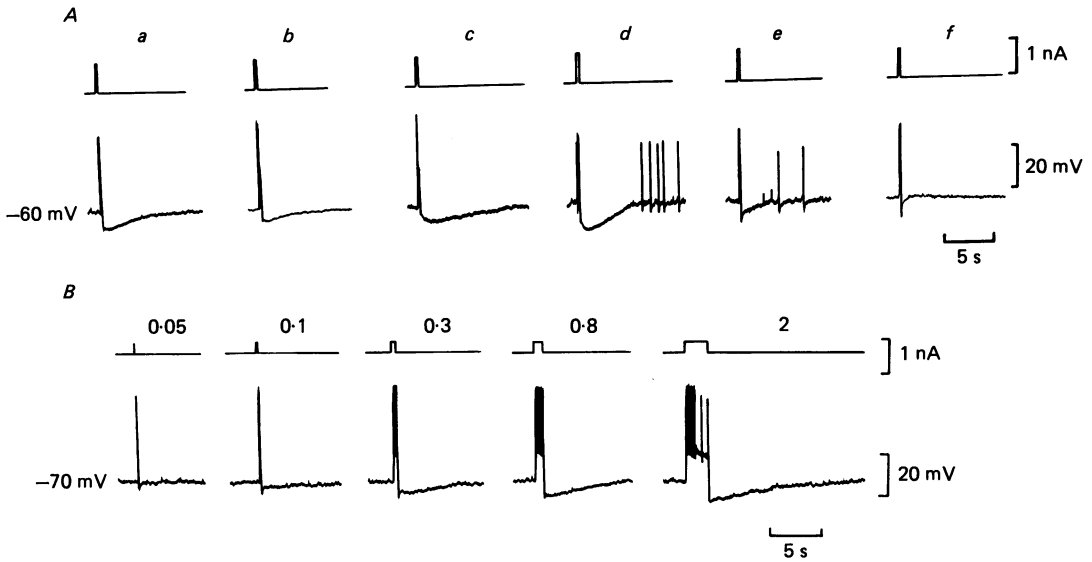


Fig. 1. Intracellular current-clamp records obtained from single olfactory cortical neurones at 25 °C. *A*, chart records *a–e* show some representative types of slow a.h.p. that could be evoked in different cells by injecting a brief depolarizing current pulse (200 ms, ~ 0·8 nA; upper traces, injected current; lower traces, membrane potential). In all cells, the resting potential was maintained at −60 mV by injecting steady depolarizing current. Note that the a.h.p. in *c* and *d* shows a typical slow rising phase. Record *f* represents a neurone in which no slow a.h.p. could be evoked. (All spikes are truncated by the chart recorder). *B*, different neurone; resting potential = −70 mV: effect of increasing current-pulse duration (indicated by numbers (s) above traces) on the amplitude and duration of the a.h.p. Note, that spike accommodation occurs during the longest (2 s) pulse.

these a.h.p. types were observed in *ca.* 90 % of cells recorded; the remainder displayed only a brief-duration a.h.p. (Fig. 1*Af*) even when their membrane potentials were depolarized by current injection. Unlike bull-frog sympathetic ganglion cells (Pennefather, Lancaster, Adams & Nicoll, 1985) the lack of long-duration a.h.p.s in some olfactory neurones was not merely a consequence of poor micro-electrode impalement, since these cells still showed 'healthy' spike amplitudes ( > 100 mV) and input conductances in the range of ~ 10 to 30 nS.

The character of the slow a.h.p. was dependent on both the amplitude and duration (Fig. 1*B*) of the injected current. With increasing current intensity, the peak amplitude of the evoked a.h.p. usually attained a maximum value, then showed some decline. On the other hand, increasing the duration of the injected current pulse increased the size and duration of the a.h.p. until the peak became occluded by the stimulus pulse and spike accommodation was revealed (Fig. 1*B*). The peak of the a.h.p. was associated with a fall in cell input resistance (up to 45 %, $n = 6$).

*Voltage-clamp recordings*

One way of revealing the underlying outward membrane current following a train of action potentials was to switch to voltage clamp at the end of a brief depolarizing current pulse ('hybrid clamp'; Pennefather *et al.* 1985; Lancaster & Adams, 1986). Fig. 2*A* shows a neurone held at −45 mV under current clamp, where a depolarizing

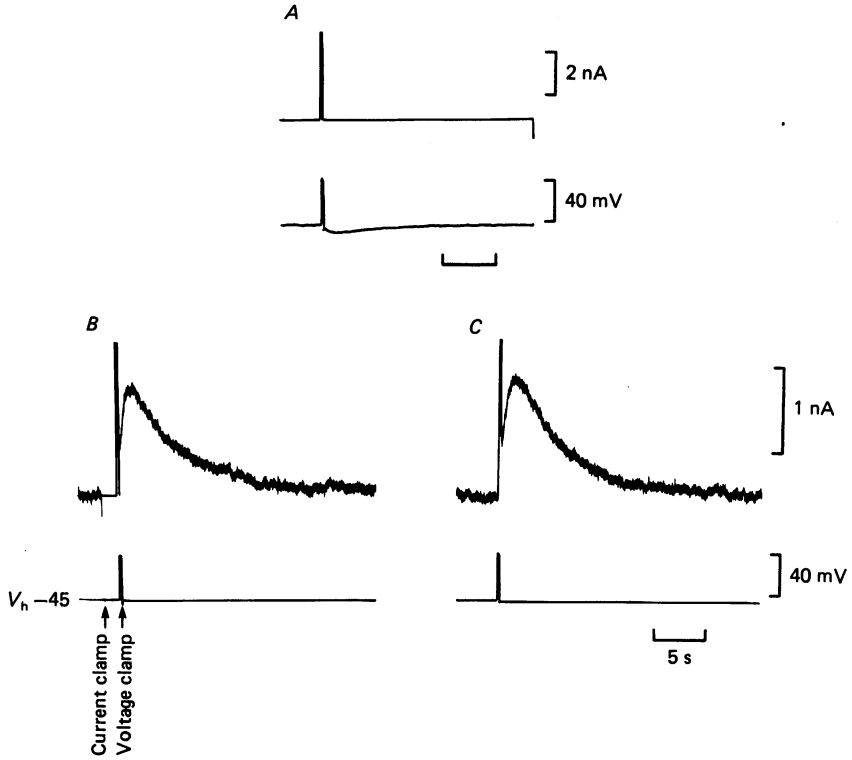


Fig. 2. Comparison of the a.h.p. current 'tails' evoked using either a 'hybrid'- or voltage-clamp protocol. *A*, typical slow a.h.p. evoked initially under current clamp in a neurone held at −45 mV by steady current injection (depolarizing pulse = 200 ms, 4 nA). *B*, the underlying outward a.h.p. tail current (upper trace) is revealed by switching to voltage clamp at −45 mV at the end of the current pulse. *C*, a similar slow outward tail is revealed under voltage-clamp following a +47 mV voltage jump (200 ms). Note that the tail currents show a distinct rising phase and similar decay time course. In this, and all subsequent Figures (unless otherwise indicated), TTX was absent from the bathing medium.

pulse evoked a slow a.h.p. The underlying slow outward tail current, showing a similar time course to the a.h.p., was recorded on switching rapidly to voltage clamp at −45 mV at the end of the current pulse (Fig. 2*B*). A similar slow outward tail current was evoked by applying a 200 ms depolarizing command under voltage clamp (in the absence of TTX) from a holding potential of −45 mV (Fig. 2*C*). The tail currents recorded showed a distinct rising phase with a peak after ~ 1 s (cf. Lancaster & Adams, 1986).

Fig. 3*A* shows the progressive activation of the slow outward tail current (a.h.p. current) following 100 ms positive jumps of increasing amplitude from a holding potential of −45 mV. The a.h.p. current recorded in this cell increased in size with increasing depolarization (up to a maximum of 1·4 nA) (Fig. 3*B*). In some cells, the amplitude of the a.h.p. current showed a decline at potentials between +5 and +40 mV, which could partly reflect a decrease in $Ca^{2+}$ influx as the $Ca^{2+}$ reversal potential ($E_{Ca}$) was approached (see below). However, no N-shaped inflexion in the activation curve was ever observed in this potential range, and deterioration of cell

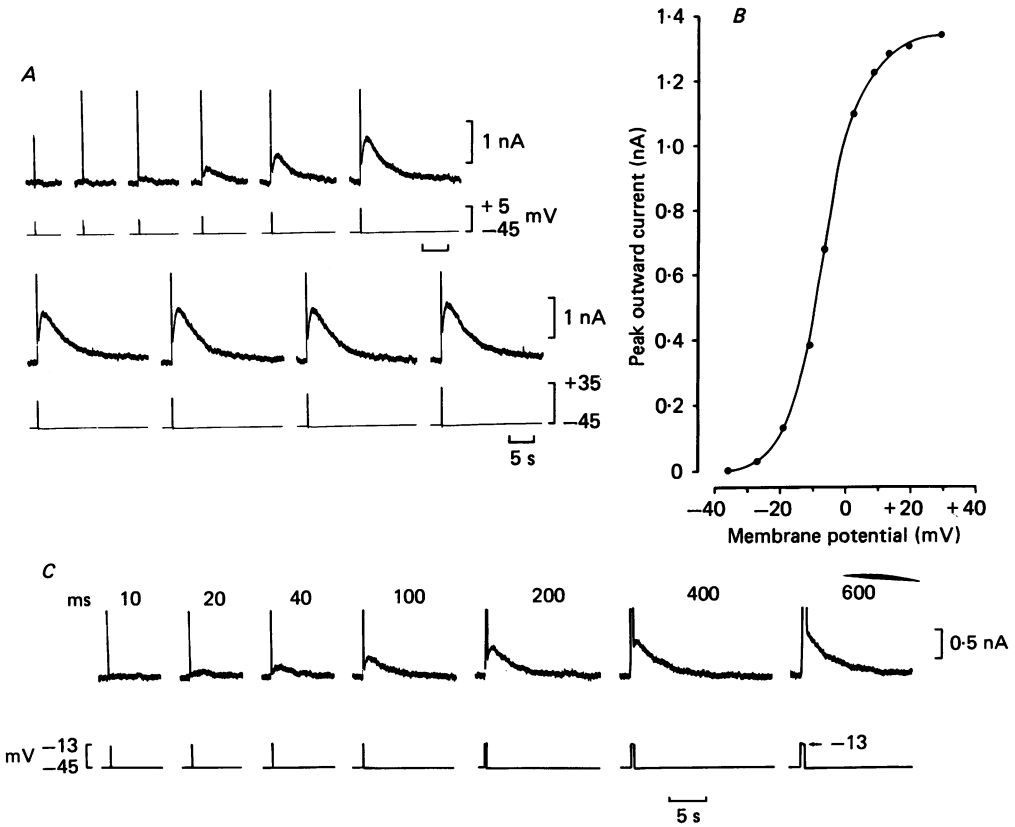


Fig. 3. *A*, continuous chart recording showing the progressive activation of the slow a.h.p. current in a neurone voltage clamped at −45 mV (upper traces, current; lower traces, voltage). 100 ms positive voltage jumps of increasing amplitude (from +9 to +77 mV) were applied at 30 s intervals. *B*, plot of peak amplitude of a.h.p. current against the membrane potential attained during the jump. *C*, (different cell) progressive activation of a.h.p. current using +32 mV voltage steps of increasing duration (10–600 ms). Note, the distinct rising phase of the tail current is lost when clamp command exceeds 400 ms.

membrane properties invariably resulted when voltage jumps to potentials more positive than +40 mV were applied.

As inferred from current-clamp recordings (Fig. 1*B*), increasing the duration of the voltage jump resulted in a progressively stronger activation of the a.h.p. current (Fig. 3*C*). In this cell, a pulse of 20 ms was required before the a.h.p. current became apparent. However, as the duration of the voltage step increased, the distinct rising phase of the tail current was lost as it merged with the steady outward current developed during the clamp command.

*Dependence on external $K^+$ concentration*

Depolarizing voltage jumps to −20 mV from varying holding potentials between −50 and −110 mV evoked a series of slow outward tail currents under voltage clamp, that were clearly reversed to inward tails in Krebs medium containing 3 or 9 mM-$K^+$ (Fig. 4*A*). Plotting the peak amplitude of the a.h.p. current as a function of the

holding potential (Fig. 4*B*) indicated a reversal potential of −90 mV in 3 mM-$K^+$ medium in this cell. However, as the external $K^+$ concentration was raised to 9 mM (on adding 6 mM-KCl), the reversal potential was shifted by approximately 25 mV (Fig. 4*B*) (expected shift in $K^+$ reversal potential, $E_K$ = 28 mV, assuming intracellular $K^+$ concentration, $[K^+]_i$ = 130 mM; Harvey, Scholfield & Brown, 1974).

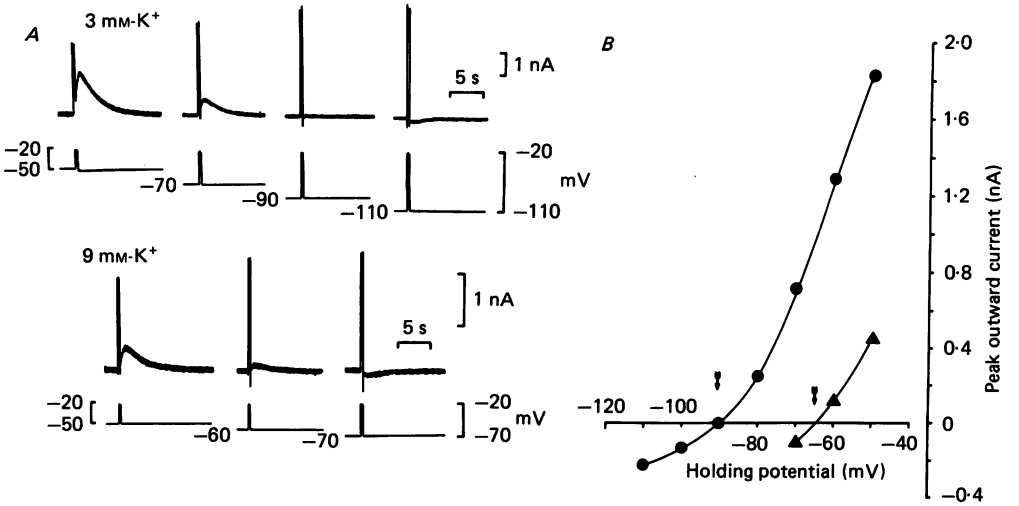


Fig. 4. The effect of raising external $K^+$ from 3 to 9 mM (by adding 6 mM-KCl) on the reversal potential of the slow a.h.p. current. *A*, slow a.h.p. currents were evoked in either a 3 or 9 mM-$K^+$ medium by applying 200 ms voltage steps to −20 mV while varying the holding potential from −50 to −110 mV. *B*, plot of peak amplitude of the a.h.p. current against holding potential, indicates a tail reversal potential of −90 mV (arrow) in 3 mM-$K^+$ (●) and −64·5 mV (arrow) in 9 mM-$K^+$ (▲). Note, the curvature of the *I–V* plots, particularly beyond the reversal potential.

The a.h.p. current evoked under voltage clamp or 'hybrid' clamp showed a similar reversal potential with a mean (±S.E. of mean) of −96±2·2 mV ($n$ = 6) in 3 mM-$K^+$ solution, which is close to the predicted $E_K$ of −95 mV. Interestingly, the time course of the decay of the tail current showed very little change between −50 and −110 mV (average $\tau$ = 3 s) (Fig. 4*A*) and was relatively consistent from cell to cell (mean $\tau$ in twelve cells at 25 °C = 3·3±0·29 s). The decay time constant was, however, rather sensitive to changes in temperature, becoming approximately four times shorter at 35 °C compared with its value at 25 °C (cf. Thompson, Masukawa & Prince (1985) in hippocampal neurones).

*Dependence on external $Ca^{2+}$ concentration, and effect of divalent cations*

Fig. 5 shows the effect of reducing external $[Ca^{2+}]$ on the slow outward tail current. On switching to nominal $Ca^{2+}$-free (2·5 mM-$Mg^{2+}$) Krebs medium, a slowly developing inward holding current ( ~ 0·2 nA) was observed under voltage clamp at −50 mV, along with a complete inhibition of the a.h.p. current evoked by brief positive jumps. This action was readily reversed on returning to normal Krebs (1·5 mM-$Ca^{2+}$) solution. A similar effect was observed after applying $Ca^{2+}$ channel blockers such as $Co^{2+}$

(1 mM), $Cd^{2+}$ (200 $\mu$M) or $Mg^{2+}$ (6 mM), suggesting that $Ca^{2+}$ entry was involved in the generation of the outward a.h.p. current.

These findings also argue against the possibility that a.h.p. generation was largely due to activation of an electrogenic pump mechanism as can occur in some other neurones (Kuno, Miyahara & Weakly, 1970; Jansen & Nicholls, 1973). The slow inward $I_M$ relaxations due to *de*-activation of an ongoing time- and voltage-dependent $K^+$ current (Constanti & Galvan, 1983*b*), monitored simultaneously during these experiments, remained unaffected by the removal of external $Ca^{2+}$ (Fig. 5*B*) or the addition of $Ca^{2+}$ channel blockers.

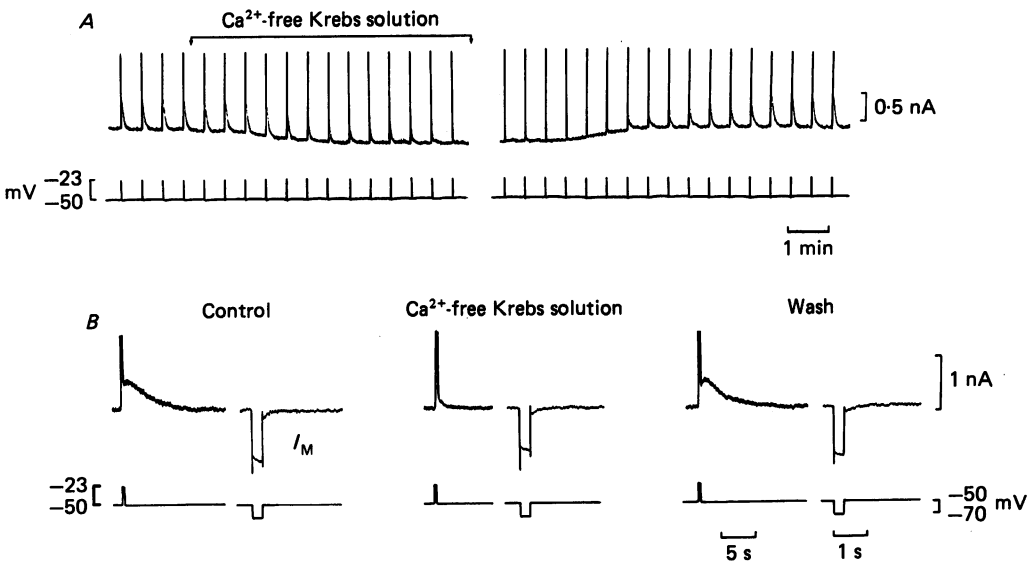


Fig. 5. Effect of exposure to a nominal $Ca^{2+}$-free (2·5 mM-$Mg^{2+}$) Krebs solution on the a.h.p. current. *A*, continuous chart recording showing a.h.p. tail currents evoked every 30 s by stepping from −50 to −23 mV for 250 ms. On switching to $Ca^{2+}$-free solution, a steady inward current (0·25 nA) is induced, along with a near complete inhibition of the a.h.p. current. The effect is rapidly reversed on returning to normal (1·5 mM-$Ca^{2+}$) Krebs solution. *B*, corresponding a.h.p. currents (displayed on a faster chart speed) obtained in control solution, after 6 min in $Ca^{2+}$-free Krebs solution, and after 10 min wash-out. Traces alongside show 'leak' currents evoked by 20 mV negative jumps (400 ms); the slow inward current relaxations reflect $I_M$ de-activation. Note, the 'leak' conductance is reversibly reduced by $Ca^{2+}$ removal.

The slow nature of the a.h.p. current may reflect a prolonged lifetime of internal $Ca^{2+}$ determined by $Ca^{2+}$ sequestration–extrusion mechanisms, rather than a long open time of a $Ca^{2+}$-dependent $K^+$ channel (Kuba, Morita & Nohmi, 1983). Although the slow-current decay time was apparently voltage independent (cf. also Lancaster & Adams, 1986) there could still be some voltage sensitivity of the underlying a.h.p. current mechanism. To test for this, we superimposed 10–20 mV negative voltage jumps (100–400 ms duration) near the peak of an evoked a.h.p. current and looked for any fast relaxations in membrane current on an expanded chart speed. In five cells examined ($V_h$ = −50 mV), we could not reveal any obvious relaxations in a.h.p. current, although the 'leak' conductance at the peak was clearly increased. The only relaxations observed were due to $I_M$ deactivation, but these were not notably different from those evoked in the absence of a background a.h.p. current.

The possibility that any a.h.p. current relaxations were faster than the resolution of our voltage clamp cannot, of course, be excluded.

*Pharmacology of a.h.p. current: muscarinic agonists*

The most outstanding property displayed by the slow outward tail current was its marked sensitivity to low concentrations of muscarinic agonists (Constanti & Sim, 1985). Muscarine itself and other agents such as carbachol, oxotremorine or the muscarinic ganglion stimulant McN-A-343 effectively reduced the a.h.p. current at

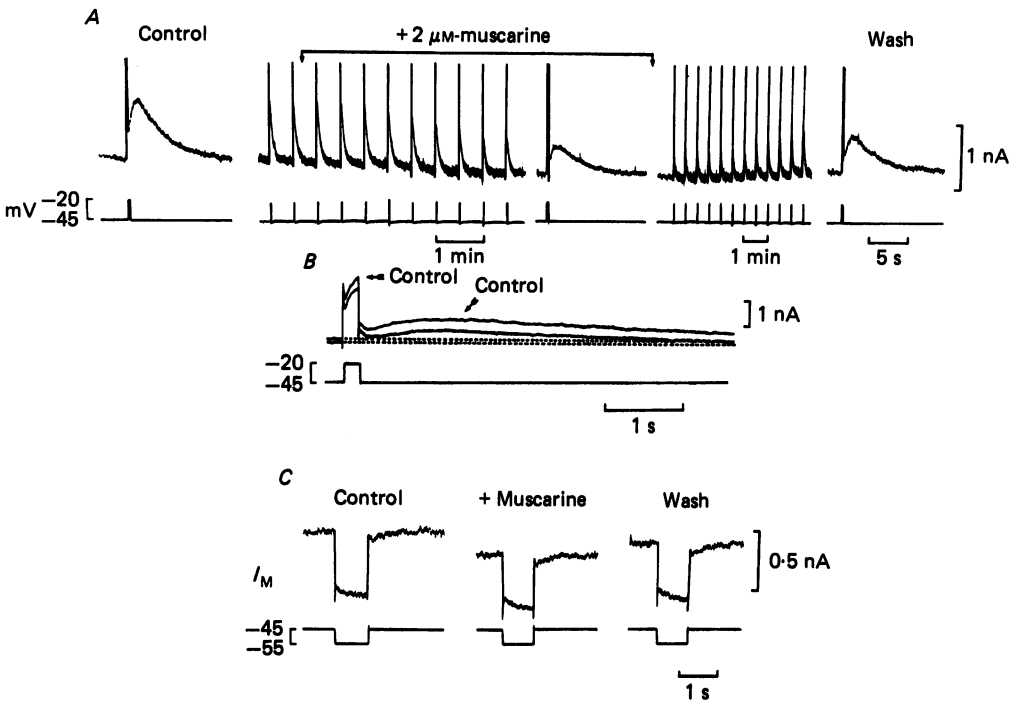


Fig. 6. Effect of a low concentration of (±) muscarine (2 μM) on the a.h.p. current and $I_M$ recorded in a neurone clamped at −45 mV. *A*, a 5 min application of muscarine reduced the peak amplitude at the a.h.p. current; a steady inward (depolarizing) current of 0·2 nA developed during exposure. Partial recovery is shown, 15 min after wash-out (note changes in chart speed). Steps were made to −20 mV for 200 ms every 30 s. Note, that the rising phase of the slow a.h.p. current is still apparent in muscarine. *B*, shows superimposed current traces (displayed at a lower gain and expanded time scale) taken in control solution and in muscarine. *C*, the 'leak' conductance is reduced by muscarine with no effect on the $I_M$ relaxation evoked by a −10 mV (400 ms) voltage jump.

1–10 μM concentrations. On bath application of 2 μM-muscarine (Fig. 6), a steady inward current developed ( ~ 0·25 nA) along with a clear reduction (48 %) of the a.h.p. current evoked by jumping to −20 mV. This effect was readily reversed on wash-out (Fig. 6). Even at this relatively low concentration of muscarine, the 'leak' conductance was clearly reduced by *ca.* 12 % (range 10–21 %; $n = 8$), but with no apparent effect on the amplitude of $I_M$ relaxations (Fig. 6*C*). A similar effect was observed with other muscarinic agonists such as carbachol (1–10 μM), oxotremorine

(0·5–10 μM) or McN-A-343 (1–10 μM), again, with no apparent effect on $I_M$ at these concentrations.

*Noradrenaline*

Madison & Nicoll (1982), showed that noradrenaline, acting on β-adrenoceptors, inhibited the slow $Ca^{2+}$-dependent a.h.p. in hippocampal neurones in a reversible manner; a corresponding reduction of the slow hippocampal $I_{a.h.p.}$ was also reported

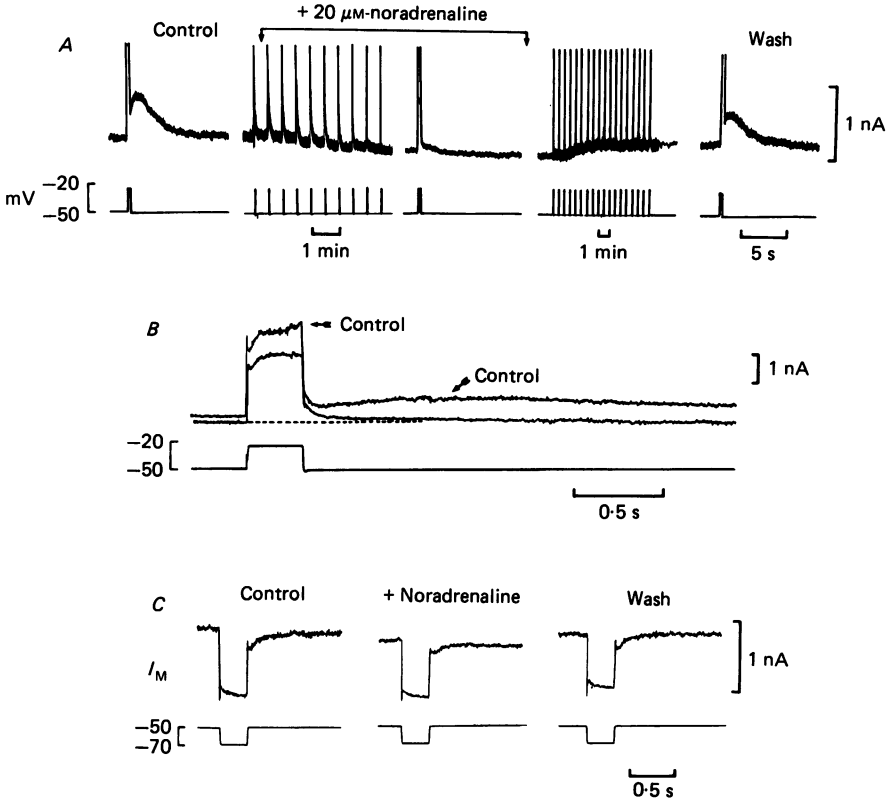


Fig. 7. Effect of noradrenaline on the a.h.p. current and $I_M$ recorded in a neurone clamped at −50 mV. *A*, addition of noradrenaline (20 μM) effectively inhibited the a.h.p. current and induced a steady inward current (0·18 nA). Partial reversal is shown after 25 min wash-out. *B*, superimposed fast-speed records taken in control solution and noradrenaline. *C*, 'leak' conductance is reduced by noradrenaline without affecting $I_M$ (voltage jump = −20 mV, 300 ms).

by Lancaster & Adams (1986). We found a comparable inhibitory effect of noradrenaline (1–50 μM) on the cortical slow outward tail current recorded under voltage clamp (average reduction 55 %, at 2 μM; $n = 4$). An example is shown in Fig. 7*A* and *B*. In the presence of 20 μM-noradrenaline, the outward tail was effectively reduced by 86 % and a steady inward current of ~ 0·13 nA developed at −50 mV (Fig. 7*A*). The 'leak' conductance of the membrane was also reduced (15 %); however, as seen with low concentrations of muscarinic agonists, $I_M$ remained unaffected (Fig. 7*C*).

*Tetraethylammonium*

In the presence of the $K^+$ channel blocker, tetraethylammonium (TEA) (1 mM), a steady inward current was induced ( ~ 0·58 nA) and the evoked a.h.p. current following a brief depolarizing jump from −50 mV was clearly inhibited (Fig. 8). However, concomitant with its inhibition of the a.h.p. current, TEA also reduced $I_M$ in all six cells tested (28 ± 4·4 %), thereby contributing in part, to the steady inward current developed. This suggests a rather non-selective effect of this agent towards these currents.

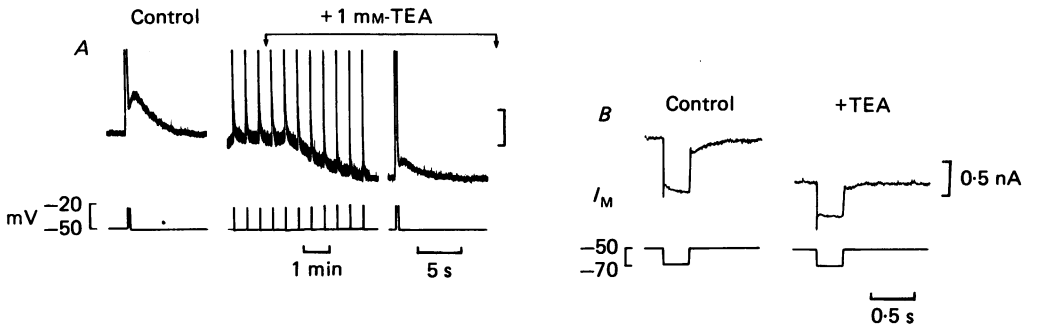


Fig. 8. Effect of TEA on a.h.p. current and $I_M$ recorded under voltage clamp at −50 mV. *A*, addition of TEA (1 mM) induced a steady inward current (0·6 nA) and a clear inhibition of the a.h.p. current evoked by a +30 mV (200 ms) voltage jump. *B*, both the 'leak' conductance and $I_M$ relaxation were reduced by this concentration of TEA (voltage jump = −20 mV, 300 ms).

*Tubocurarine and apamin*

The slow a.h.p. and a.h.p. current in bull-frog sympathetic ganglion cells can be inhibited by tubocurarine (Nohmi & Kuba, 1984; Tokimasa, 1984) or the bee-venom toxin, apamin (Pennefather *et al.* 1985). However, neither tubocurarine (10–500 μM; $n = 3$) nor apamin (1 μM; $n = 3$) had any effect on the a.h.p. current or underlying cell excitability in olfactory neurones. These agents cannot, therefore, be regarded as selective blockers of slow $Ca^{2+}$-dependent $K^+$ conductances in all nerve cells.

*Comparison of muscarinic sensitivity of a.h.p. current and $I_M$*

Constanti & Galvan (1983*b*) described the muscarinic sensitivity of $I_M$ in olfactory neurones, that was similar to that shown by the $I_M$ in bull-frog ganglia (Adams *et al.* 1982) and hippocampal neurones (Halliwell & Adams, 1982). Since the a.h.p. current could be activated within the same potential range as $I_M$ (more positive than −60 mV) and was also affected by muscarine, it was important to establish whether these two currents could be distinguished in terms of their muscarinic sensitivity.

In twenty neurones tested, we consistently found that relatively larger concentrations ( > 10 μM) of muscarinic agonists were required to produce any notable inhibition of the $I_M$ relaxation ( > 10 %) than to inhibit the a.h.p. current. In addition, oxotremorine (and McN-A-343) which effectively reduced the a.h.p. current at 1–2 μM concentrations, had little effect on $I_M$, even when applied at 30 μM (or above) in cells previously shown to respond to muscarine or carbachol (Fig. 9).

This approximately tenfold difference in sensitivity to muscarinic agonists displayed by the a.h.p. current and $I_M$ suggested possible differences in the underlying muscarinic receptor types and/or intracellular transduction mechanisms involved. Muscarinic receptors may currently be subdivided into $M_1$ and $M_2$ types, exhibiting high or low affinity respectively for the antagonist pirenzepine (Hammer & Giachetti, 1982). In some tissues (e.g. heart), the $M_2$ sites may be regulated

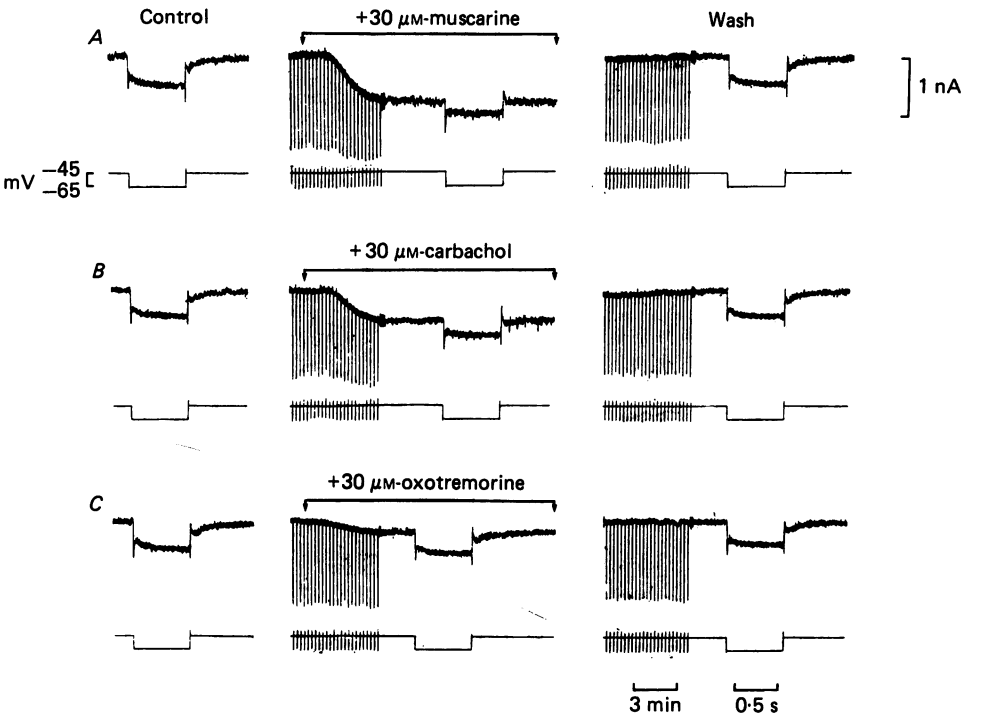


Fig. 9. Comparison of the effects of muscarinic agonists on $I_M$ relaxations recorded in a single neurone voltage clamped at −45 mV. In each row, records show initially the control $I_M$ relaxation evoked by a −20 mV (650 ms) voltage jump on a fast chart speed. The speed was slowed during drug applications and speeded up after 5 min drug exposure. *A* and *B*, application of muscarine or carbachol (30 μM) produced an inward shift in holding current, reduction in 'leak' conductance and clear inhibition of $I_M$, whereas in *C*, 30 μM-oxotremorine induced a smaller inward current, and reduction in 'leak' but had no effect on the $I_M$ relaxation. Each drug application was followed by a 30 min wash-out period.

allosterically be gallamine (Stockton, Birdsall, Burgen & Hulme, 1983). In the present study, we found that the inhibitory action of low concentrations of muscarinic agonists (e.g. 2 μM-muscarine) on the a.h.p. current remained unaffected in the presence of 50–300 nM-pirenzepine ($n = 3$ cells) ($K_D$ at $M_1$ sites is *ca.* 20 nM, cf. Hammer, Berrie, Birdsall, Burgen & Hulme, 1980) but was antagonized by 20 μM-gallamine (Fig. 10). Higher concentrations of pirenzepine ( > 300 nM) however, blocked the action of muscarine.

Interestingly, our preliminary experiments revealed that inhibition of $I_M$ by muscarine or carbachol (30–50 μM) was *also* insensitive to low concentrations of pirenzepine but was blocked by gallamine (Constanti & Sim, 1987), suggesting that muscarinic inhibition of the cortical a.h.p. current and $I_M$ is mediated by $M_2$-type receptors (not necessarily identical).

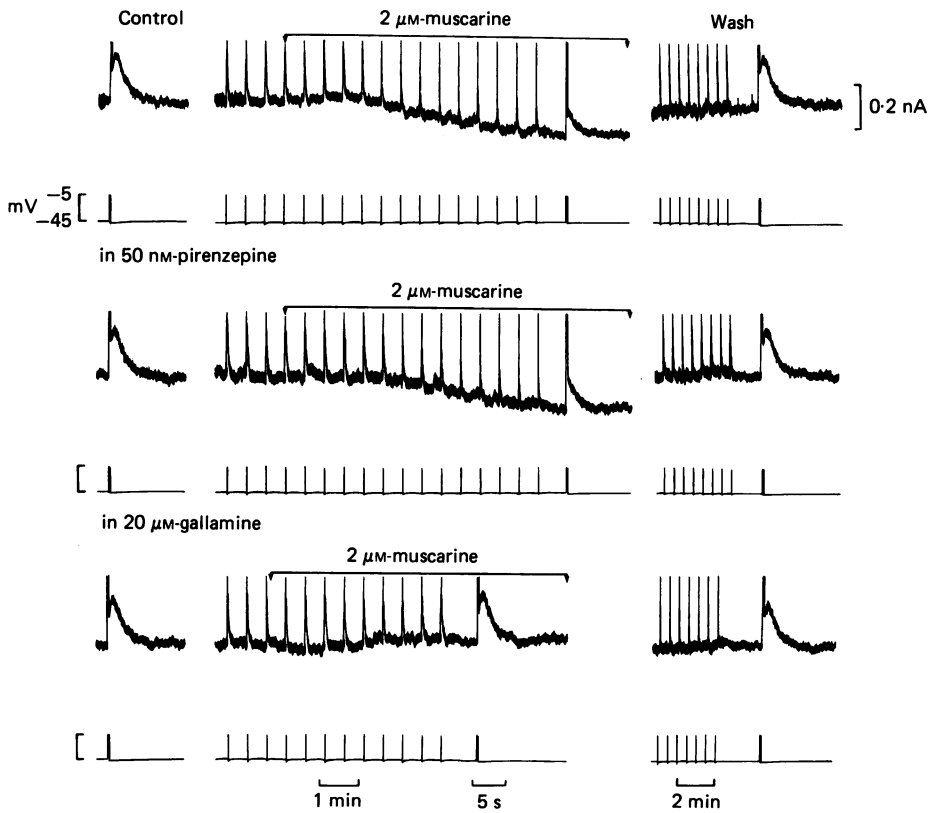


Fig. 10. Pirenzepine does not prevent the inhibition of the a.h.p. current by muscarine. Continuous record of clamp currents measured in a single neurone clamped at −45 mV. Upper row shows inhibition of a.h.p. current by 2 μM-muscarine recorded in normal Krebs solution. +40 mV (200 ms) voltage jumps were applied every 30 s (note changes in chart speed). Middle row: the effect of muscarine is still apparent in 50 nM-pirenzepine but is abolished in 20 μM-gallamine (lower row). Each muscarine application was followed by a 10 min wash-out period. Pirenzepine was allowed to equilibrate for 20 min and gallamine for 10 min before testing muscarine.

*An unexpected effect of TTX*

In our early 'hybrid'-clamp experiments, we noted that on adding TTX (1 μM) to the bathing medium, the a.h.p. tail current was reduced *before* there was a clear inhibition of direct $Na^+$ spike generation. In TTX, even large and prolonged depolarizing current pulses failed to restore the a.h.p. to its previous amplitude. Under voltage clamp at −45 to −50 mV, application of TTX regularly evoked a steady inward current of up to 0·1 nA ($n = 8$) along with a gradually developing inhibition (62 %) of the slow outward tail current (Fig. 11*A*). The 'activation' curve of the a.h.p. current was clearly depressed by TTX (Fig. 11*B*) in an apparently 'non-competitive' manner. The distinct rising phase was largely lost; however, some residual slow tail current always remained. The 'leak' conductance was also reduced ($< 10$ %) without affecting $I_M$.

We could never obtain a recovery of the a.h.p. current following removal of TTX,

even after 1 h of washing, although under current clamp, spike generation had partially recovered. A similar inhibition of the slow a.h.p. current by TTX was recorded in guinea-pig hippocampal CA1 neurones under voltage clamp (A. Constanti & J. A. Sim, unpublished observation).

A comparable effect to TTX was observed on partial or total replacement of external $Na^+$ by choline (+10 $\mu$M-atropine). However, in contrast with TTX, the effect of $Na^+$ substitution on the a.h.p. current was readily reversed after a 15 min

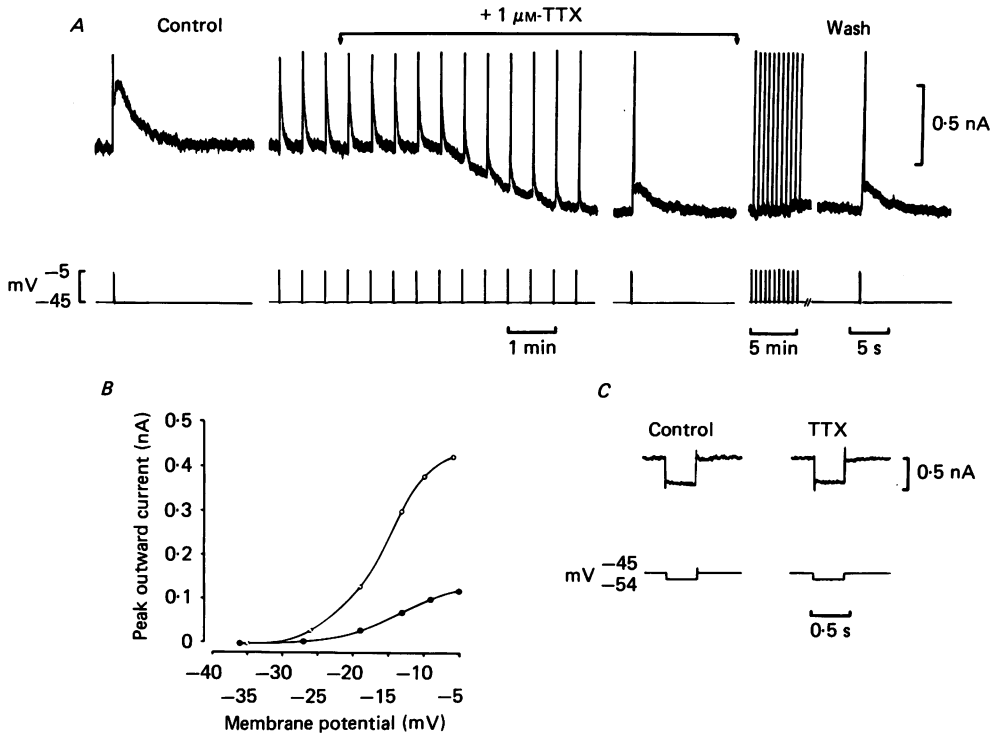


Fig. 11. Effect of TTX on the slow a.h.p. current. *A*, a.h.p. current evoked by a +40 mV (100 ms) voltage jump from a holding potential of −45 mV. Application of TTX induced an inward current of 0·4 nA and reduced the a.h.p. current amplitude by 64 %. The effect of TTX was not reversible, even after a 25 min wash-out. *B*, plot of the peak amplitude of the a.h.p. current as a function of the membrane potential attained during the evoking voltage jump in normal Krebs solution (○) and in the presence of TTX (●). Note, the effect of TTX appeared 'non-competitive'. *C*, 'leak' conductance recorded simultaneously, was slightly reduced in TTX (voltage jump = −10 mV, 400 ms).

wash in normal Krebs solution. The residual tail current, recorded in the presence of TTX or in $Na^+$-free solution, was still dependent on $Ca^{2+}$ entry since it was further reduced on removal of external $Ca^{2+}$ ions or addition of $Cd^{2+}$ (200 $\mu$M), with a further development of steady inward current.

Fig. 12 shows an experiment where longer ( ~ 1 s) voltage jumps were applied in order to evoke the a.h.p. current from a holding potential of −50 mV. Small positive commands evoked slow outward relaxations (contaminated by some early transient inward current) followed by repolarizing tail currents that decayed with two distinct components. With the largest positive jump,

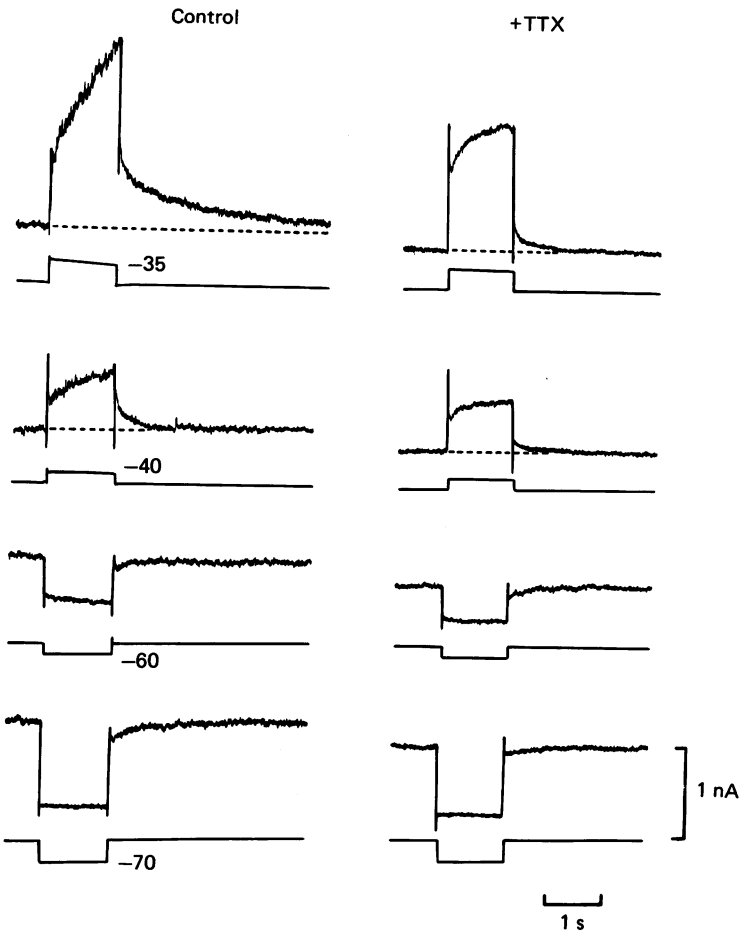


Fig. 12. Effect of TTX on clamp currents evoked by 1·1 s voltage commands from a holding potential of −50 mV. Records on the left-hand were obtained in normal Krebs solution in response to positive or negative voltage jumps to the potentials indicated by numbers alongside traces. Right-hand records show corresponding currents recorded after 5 min in 1 μM-TTX. Both the 'creeping' outward current developed during positive voltage commands and the ensuing slow outward tail current are clearly reduced by TTX; a steady inward current of 0·25 nA was developed along with a 25% reduction in 'leak' conductance. No recovery from TTX was observed after 30 min wash-out (not shown).

activation of the a.h.p. current produced a slow outward current 'creep' during the command pulse, and a prominent slowly decaying tail current thereafter. Both the 'creep' and the slow tail current were clearly reduced in amplitude in the presence of TTX.

One possible interpretation of these results is that generation of a substantial component of the a.h.p. current is dependent on $Na^+$ entry through TTX-sensitive channels. Some alternative possibilities are presented in the Discussion.

*Is there another $Ca^{2+}$-dependent $K^+$ current?*

Brown & Griffith (1983) and Lancaster & Adams (1986) concluded that a 'fast', $Ca^{2+}$-activated $K^+$ current ($I_C$), distinct from $I_{a.h.p.}$, was present in voltage-clamped

hippocampal neurones. $I_C$ displayed time- and voltage-dependence, was insensitive to muscarine, but was reduced by $Ca^{2+}$ removal, adding $Cd^{2+}$ or by TEA. It was activated at potentials positive to −45 mV (i.e. within the same voltage range as $I_M$) and decayed with a similar single-exponential time course at the end of a depolarizing voltage command. In view of these findings, we investigated whether a similar $I_C$ current existed in olfactory neurones.

Fig. 13 shows a cell that was subjected to 1 s depolarizing voltage commands from a holding potential at −45 mV. A +20 mV jump evoked a typical slow outward current relaxation which was followed by an outward tail current on repolarization

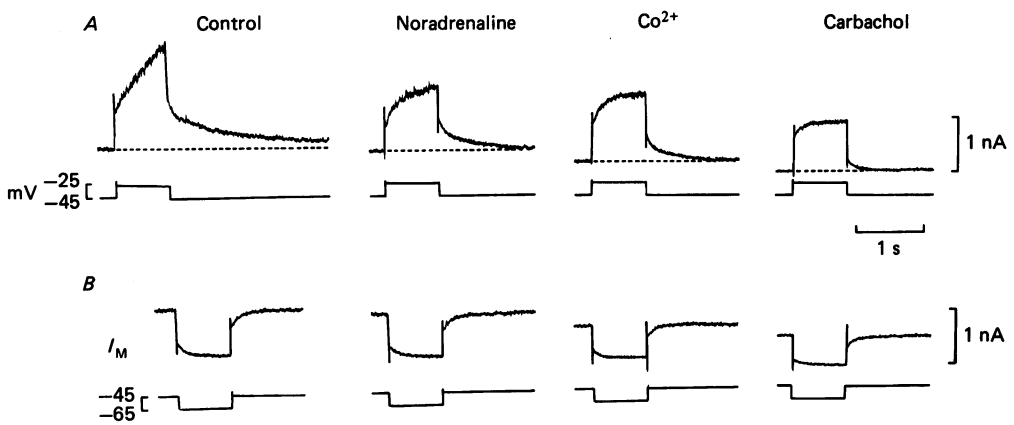


Fig. 13. *A*, clamp currents recorded from a cell clamped at −45 mV following 800 ms voltage steps to −25 mV. Traces read from left to right. Record on far left was obtained in control Krebs solution; subsequent records were obtained after cumulative exposures to noradrenaline (50 $\mu$M, 10 min), $Co^{2+}$ (2 mM, 5 min) and finally carbachol (50 $\mu$M, 5 min). Noradrenaline–$Co^{2+}$ abolishes the 'creeping' outward current relaxation activated by membrane depolarization and the *slowly* decaying component of tail-current. The residual outward relaxation and *faster* decaying tail ($I_M$?) are then clearly reduced by carbachol, *B*, corresponding $I_M$ relaxations produced by negative jumps from −45 to −65 mV. Note, progressive development of steady inward current and reduction in 'leak' conductance on adding noradrenaline, $Co^{2+}$ and carbachol.

(Fig. 13*A*). If a fast $Ca^{2+}$-activated $K^+$ current ($I_C$) was present, then the tail current might be expected to consist of at least three components: namely, M-, a.h.p.- and C-currents. Application of noradrenaline (50 $\mu$M) reduced the outward current developed during the depolarizing jump, and a large proportion of the slow outward a.h.p. tail current without affecting $I_M$ (Fig. 13*B*; see also Fig. 7). On addition of $Co^{2+}$ (2 mM), (no intermediate wash), the outward current evoked during the command was little affected, and there was no notable effect on the *fast* component of the tail current, although the residual slower component was further reduced. This suggests that the faster-decaying tail current was not dependent on $Ca^{2+}$ entry. Indeed, on further addition of 50 $\mu$M-carbachol, the outward current evoked during the jump, and the residual tail current amplitude were clearly reduced relative to the 'control' in noradrenaline–$Co^{2+}$ solution, implying that the residual tail consisted mainly of $I_M$. Similar results were obtained in three other neurones using this

protocol, and in one neurone where noradrenaline was first applied to eliminate the a.h.p. component followed by muscarine then $Co^{2+}$. Our experiments therefore suggest that the main components of the repolarizing current in olfactory cortical neurones consist of $I_M$ and a.h.p. current, with little if any, detectable $I_C$.

## DISCUSSION

### *The slow a.h.p. current is distinct from $I_M$*

In the present study, we have described some properties of a muscarine-sensitive $Ca^{2+}$-dependent $K^+$ current in olfactory cortical neurones that underlies the slow post-burst a.h.p. Although this current was activated within the same voltage range as $I_M$ (i.e. at potentials positive to $-60$ mV; Constanti & Galvan, 1983*b*) and showed a similar reversal potential (around $-96$ mV in 3 mM-$K^+$ medium), the slow a.h.p. current was pharmacologically distinct. In keeping with other reported $Ca^{2+}$-activated $K^+$ currents (Meech, 1978) the a.h.p. current (but not $I_M$) was sensitive to $Ca^{2+}$ removal or to the presence of $Ca^{2+}$ current blockers. In addition, it was clearly reduced by noradrenaline or *low* concentrations of muscarinic agonists. Interestingly, we found that oxotremorine and McN-A-343 were potent inhibitors of the a.h.p. current, but had negligible effects on $I_M$ even at concentrations exceeding 30 $\mu$M. The specific $M_1$-antagonist pirenzepine (cf. Hammer & Giachettti, 1982) did not affect the action of muscarinic agonists on the a.h.p. current (or on $I_M$) at concentrations below 300 nM, although higher concentrations had a blocking effect. In contrast, gallamine, a proposed 'cardioselective' $M_2$ antagonist (see Birdsall & Hulme, 1983) clearly prevented the muscarinic inhibition of the a.h.p. current at 20 $\mu$M (cf. opposite result reported in hippocampal neurones by Cole & Nicoll, 1984*a*). From our observations, we tentatively suggest that muscarinic inhibition of the cortical a.h.p. current and perhaps also $I_M$ may be linked to $M_2$-receptor mechanisms (Hammer *et al.* 1980). The biochemical transduction mechanisms that mediate these responses, however, have not yet been clarified.

Gil & Wolfe (1985) recently reported that oxotremorine and McN-A-343 inhibited adenylate cyclase activity in rat brain, an action now typically associated with $M_2$-receptor activation (Hammer & Giachetti, 1982; Birdsall & Hulme, 1983). However, it has been shown that a *rise* in intracellular cyclic nucleotides (cyclic AMP or cyclic GMP) can modulate the slow a.h.p. in hippocampal neurones (Madison & Nicoll, 1982; Cole & Nicoll, 1984*b*) as can activation of protein kinase C by phorbol esters (Baraban, Snyder & Alger, 1985; Malenka, Madison, Andrade & Nicoll, 1986).

### *Comparison with hippocampal slow a.h.p. current (hippocampal $I_{a.h.p.}$)*

Our slow a.h.p. current bore some obvious resemblance to $I_{a.h.p.}$ recorded in hippocampal neurones (Lancaster & Adams, 1986). The two currents were similar in terms of their slow decay time course ($\tau \sim 3$ s at 25 °C), apparent voltage independence and sensitivity to noradrenaline (Madison & Nicoll, 1982, 1986; Lancaster & Adams, 1986). However, Lancaster & Adams (1986) found a rather variable effect of TEA on the hippocampal $I_{a.h.p.}$ (from negligible to 50 % inhibition) with little effect on the holding current at around $-70$ mV. A similar TEA insensitivity was reported for the slow $Ca^{2+}$-dependent $K^+$ conductances in bull-frog ganglia (Pennefather *et al.* 1985; Tokimasa, 1985) and in guinea-pig myenteric neurones (Tokimasa, Hasuo,

Akasu & North, 1985). In contrast, 1 mM-TEA reduced the olfactory slow a.h.p. current and 'leak' conductance, and induced a steady inward current at holding potentials around $-50$ mV, in all cells studies ($n = 6$).

The olfactory a.h.p. current developed with a distinct delay when *brief* depolarizing pulses were used either under 'hybrid' or voltage clamp (in the absence of TTX; see below). In contrast, recording from guinea-pig hippocampal CA1 neurones *in vitro* showed that a rising phase of the hippocampal $I_{a.h.p.}$ was still apparent, even in the presence of TTX (A. Constanti & J. A. Sim, unpublished observation; cf. Lancaster & Adams, 1986). It is possible that some of the reported differences between the a.h.p. current and hippocampal $I_{a.h.p.}$ stem from a difference in experimental conditions.

The mechanism responsible for the latency in a.h.p. generation is unknown. One attractive possibility is that $Ca^{2+}$ entry induced by depolarization (via voltage-activated $Ca^{2+}$ channels: Constanti, Galvan, Franz & Sim, 1985) promotes further $Ca^{2+}$ release from intracellular reservoirs for activation of the a.h.p. conductance as suggested for bull-frog neurones (Kuba, 1980; Kuba *et al.* 1983). Alternatively the delay could reflect a spatial separation of $Ca^{2+}$ entry and a.h.p. generation sites. Our present data do not provide a distinction between these possibilities.

*Sensitivity to TTX*

The most unexpected property of the slow a.h.p. current was its apparent sensitivity to TTX (or to removal of $Na^+$ from the bathing medium) implying that a.h.p. generation was somehow related to neuronal $Na^+$ entry. In TTX, the a.h.p. tail current amplitude was reduced by 62%, largely lost its distinct rising phase and showed no recovery on wash-out, even though $Na^+$ spike generation had partly recovered.

It could be argued that the inhibition of the a.h.p. was merely due to suppression of the spike burst, and consequent reduction in $Ca^{2+}$ entry. Although this must largely apply under current clamp and also when using the 'hybrid'-clamp method, it is worth emphasizing that in TTX, the a.h.p. could not be restored to its 'control' amplitude by using large and prolonged depolarizing stimuli.

Under voltage clamp at around $-50$ mV, multiple $Na^+$ spiking was largely suppressed; occasionally, one or two uncontrolled spikes were evident on the voltage record. However, the a.h.p. current amplitude was clearly reliant on the *duration* and amplitude of the command step and not the early breakthrough of uncontrolled spikes. The TTX effect always developed in a gradual rather than an all-or-none manner expected from a sudden loss of $Na^+$ spike generation. This would suggest that $Na^+$ entry under voltage clamp may not be via the fast $Na^+$ channels that mediate the action potential, but via more persistently activated channels such as those underlying the persistent $Na^+$ conductance ($I_{NaP}$) reported in other mammalian central neurones (Connors *et al.* 1982; Stafstrom, Schwindt, Chubb & Crill, 1985; French & Gage, 1985). We have also detected a similar TTX-sensitive persistent inward current in voltage-clamped olfactory neurones (A. Constanti & J. A. Sim, unpublished obeservation).

$Na^+$-activated $K^+$ currents have previously been reported in avian neurones (Bader, Bernheim & Bertrand, 1985) and mammalian cardiac cells (Kameyama, Kakei, Sato, Shibusaki, Matsuda & Irisawa, 1984); however, these were considered as being distinct from any $Ca^{2+}$-activated $K^+$ currents. Our slow a.h.p. tail current

behaved like a typical $Ca^{2+}$-dependent current in being abolished by removal of external $Ca^{2+}$ or addition of $Cd^{2+}$, yet only a portion of the current was sensitive to TTX or $Na^+$ removal. Perhaps both intracellular $Ca^{2+}$ and $Na^+$ act synergistically to evoke the a.h.p., or a rise in internal $Na^+$ could be releasing $Ca^{2+}$ from internal stores. Some $Ca^{2+}$ could also be entering through TTX-blockable fast $Na^+$ channels, as shown in squid axon (Baker, Hodgkin & Ridgway, 1971); however, this process would still have been expected to occur in the absence of external $Na^+$.

A possibility that we cannot easily exclude is that the apparent $Na^+$ dependence of the a.h.p. current resulted from a poor space-clamp control of the cortical neurones with their associated dendrites, particularly if the $I_{NaP}$ and *some* a.h.p. generation sites were dendritically located (cf. Llinás & Yarom, 1981). Electrotonic spread of a small voltage step at the soma to more distal regions might then be facilitated by activation of $I_{NaP}$ and therefore be prevented by adding TTX or removing $Na^+$. Clearly, a more detailed examination of these possibilities is required, perhaps using dissociated cortical neurones and direct patch-clamp recording of single-channel activities.

*Apparent absence of $I_C$*

Our present results suggest that following positive voltage commands, the repolarizing tail currents consisted mainly of $I_M$ and slow a.h.p. current with little detectable fast $Ca^{2+}$-sensitive component ($I_C$; Lancaster & Adams, 1986) or the more slower $Ca^{2+}$-dependent component (also designated $I_C$) reported by Brown & Griffith (1983) in hippocampal neurones. If another $Ca^{2+}$-activated current did exist in our cells, then its kinetics may have been beyond the resolution of our voltage-clamp method.

In TTX, the hippocampal $I_{a.h.p.}$ current would be largely attenuated, and therefore difficult to detect with long (1–2 s) clamp commands; the recorded $I_C$ tail of Brown & Griffith (1983) could, therefore have contained some residual hippocampal $I_{a.h.p.}$ component. Furthermore, the kinetic properties of their $I_C$ were not always clearly distinguishable from those of $I_M$, despite the presence of muscarine or carbachol (20–50 $\mu$M) in the bathing medium. The 'residual' hippocampal $I_{a.h.p.}$ tail was more clearly distinguishable in the records of Lancaster & Adams (1986), however, the probable contribution of $I_M$ to their tail currents was not always fully taken into account. In our view, the possible effect of divalent cations, e.g. $Cd^{2+}$ or removal of external $Ca^{2+}$ on $I_M$ relaxations in hippocampal neurones needs to be carefully re-examined.

*Significance of a.h.p. current*

The importance of slow $Ca^{2+}$-activated $K^+$ conductances in governing neuronal repetitive discharge is now well recognized (Baldiserra & Gustafsson, 1974; Partridge, 1982). In hippocampal neurones, it has been suggested that both $I_{a.h.p.}$ and $I_M$ contribute to the accommodation of cell firing in response to prolonged depolarizing stimuli (Madison & Nicoll, 1984). It is likely that the slow a.h.p. current and M-current serve a similar role in olfactory cortical neurones, although we feel that the former may have a more dominant influence. In neurones where the slow a.h.p. current was lacking, the accommodation of action potential discharge was absent,

yet under voltage clamp, the $I_M$ relaxations were still very prominent. Since the inhibition of the a.h.p. current was invariably associated with a development of inward current and reduction in leak conductance, it is possible that a 'background' a.h.p. current component could be contributing to the 'resting' cell permeability at relatively positive membrane potentials. We have no information however, on whether the a.h.p. current *per se* was subject to inactivation. Direct evidence that the cortical a.h.p. current can be inhibited by synaptically-released acetylcholine, as it can in the hippocampus (Cole & Nicoll, 1984*a*), is presently lacking. However, we propose that the modulation of this current, rather than $I_M$, could provide a more refined mechanism for the cholinergic control of cell excitability in cortical neurones.

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## REFERENCES

ADAMS, P. R., BROWN, D. A. & CONSTANTI, A. (1982). M-currents and other potassium currents in bullfrog sympathetic neurones. *Journal of Physiology* **330**, 537–572.

ADAMS, P. R. & LANCASTER, B. (1985). Components of Ca-activated K current in rat hippocampal neurones *in vitro*. *Journal of Physiology* **362**, 23*P*.

ALGER, B. E. & NICOLL, R. A. (1980). Epileptiform burst after-hyperpolarization: calcium-dependent potassium potential in hippocampal CA1 pyramidal cells. *Science* **210**, 1122–1124.

BADER, C. R., BERNHEIM, L. & BERTRAND, D. (1985). Sodium-activated potassium current in cultured avian neurones. *Nature* **317**, 540–542.

BAKER, P. F., HODGKIN, A. L. & RIDGWAY, E. B. (1971). Depolarization and calcium entry in squid giant axons. *Journal of Physiology* **218**, 709–755.

BALDISERRA, F. & GUSTAFSSON, B. (1974). Firing behaviour of a neurone model based on the afterhyperpolarization conductance time course and algebraical summation. Adaptation and steady state firing. *Acta physiologica scandinavica* **92**, 27–47.

BARABAN, J. M., SNYDER, S. H. & ALGER, B. E. (1985). Protein kinase C regulates ionic conductance in hippocampal pyramidal neurones: electrophysiological effects of phorbol esters. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 2538–2542.

BIRDSALL, N. J. M. & HULME, E. C. (1983). Muscarinic receptor subclasses. *Trends in Pharmacological Sciences* **51**, 459–462.

BROWN, D. A. & GRIFFITH, W. (1983). Calcium-activated outward current in voltage-clamped hippocampal neurones of the guinea-pig. *Journal of Physiology* **337**, 287–301.

COLE, A. E. & NICOLL, R. A. (1984*a*). Characterization of a slow cholinergic post-synaptic potential recorded *in vitro* from rat hippocampal pyramidal cells. *Journal of Physiology* **352**, 173–188.

COLE, A. E. & NICOLL, R. A. (1984*b*). The pharmacology of cholinergic excitatory responses in hippocampal pyramidal cells. *Brain Research* **305**, 283–290.

CONNORS, B. W., GUTNICK, M. J. & PRINCE, D. A. (1982). Electrophysiological properties of neocortical neurons *in vitro*. *Journal of Neurophysiology* **48**, 1302–1320.

CONSTANTI, A. & GALVAN, M. (1983*a*). Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones. *Journal of Physiology* **335**, 153–178.

CONSTANTI, A. & GALVAN, M. (1983*b*). M-current in voltage-clamped olfactory cortex neurones. *Neuroscience Letters* **39**, 65–70.

CONSTANTI, A., GALVAN, M., FRANZ, P. & SIM, J. A. (1985). Calcium-dependent inward currents in voltage-clamped guinea-pig olfactory cortex neurones. *Pflügers Archiv* **404**, 259–265.

CONSTANTI, A. & SIM, J. A. (1984). A different approach for preparing guinea-pig olfactory cortex slices. *Journal of Physiology* **357**, 117*P*.

CONSTANTI, A. & SIM, J. A. (1985). A slow, muscarine-sensitive Ca-dependent K current in guinea-pig olfactory cortex neurones *in vitro*. *Journal of Physiology* **365**, 47*P*.

CONSTANTI, A. & SIM, J. A. (1987). Muscarinic receptors mediating depression of the M-current in guinea-pig olfactory cortex neurones may be of the $M_2$-subtype. *British Journal of Pharmacology* **90**, 3–5.

FINKEL, A. S. & REDMAN, S. (1984). Theory and operation of a single micro-electrode voltage clamp. *Journal of Neuroscience Methods* **11**, 101–127.

FRENCH, C. R. & GAGE, P. W. (1985). A threshold sodium current in pyramidal cells in rat hippocampus. *Neuroscience Letters* **56**, 289–293.

GALVAN, M. & ADAMS, P. R. (1982). Control of calcium current in rat sympathetic neurons by norepinephrine. *Brain Research* **244**, 135–144.

GIL, D. W. & WOLFE, B. B. (1985). Pirenzepine distinguishes between muscarinic receptor-mediated phosphoinositide breakdown and inhibition of adenylate cyclase. *Journal of Pharmacology and Experimental Therapeutics* **232**, 608–616.

HALLIWELL, J. V. (1982). Neuronal interactions underlying electrical activity evoked from the guinea-pig piriform cortex *in vitro*: an anatomical and electrophysiological study. PhD Thesis, London University.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

HAMMER, R., BERRIE, E. P., BIRDSALL, N. J. M., BURGEN, A. S. V. & HULME, E. C. (1980). Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* **283**, 90–92.

HAMMER, R. & GIACHETTI, A. (1982). Muscarinic receptor subtypes: $M_1$ and $M_2$: Biochemical and functional characterisation. *Life Sciences* **31**, 2991–2998.

HARVEY, J. A., SCHOLFIELD, C. N. & BROWN, D. A. (1974). Evoked surface-positive potentials in isolated mammalian olfactory cortex. *Brain Research* **76**, 235–245.

HOTSON, J. R. & PRINCE, D. A. (1980). A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurones. *Journal of Neurophysiology* **43**, 409–419.

JANSEN, J. K. & NICHOLLS, J. G. (1973). Conductance changes, an electrogenic pump and the hyperpolarization of leech neurones following impulses. *Journal of Physiology* **229**, 635–655.

JOHNSTON, D. & BROWN, T. H. (1983). Interpretation of voltage clamp measurements in hippocampal neurons. *Journal of Neurophysiology* **50**, 464–486.

KAMEYAMA, M., KAKEI, M., SATO, R., SHIBASAKI, T., MATSUDA, H. & IRISAWA, H. (1984). Intracellular $Na^+$ activates a $K^+$-channel in mammalian cardiac cells. *Nature* **309**, 354–356.

KANDEL, E. R. & SPENCER, W. A. (1961). Electrophysiology of hippocampal neurons. II. After potentials and repetitive firing. *Journal of Neurophysiology* **24**, 243–259.

KRNJEVIĆ, K. & REINHARDT, W. (1979). Choline excites cortical neurons. *Science* **206**, 1321–1323.

KUBA, K. (1980). Release of calcium ions linked to the activation of potassium conductance in a caffeine-treated sympathetic neurone. *Journal of Physiology* **298**, 251–269.

KUBA, K., MORITA, K. & NOHMI, M. (1983). Origin of calcium ions involved in the generation of a slow after hyperpolarization in bullfrog sympathetic neurones. *Pflügers Archiv* **399**, 194–202.

KUNO, M., MIYAHARA, J. J. & WEAKLY, J. N. (1970). Post-tetanic hyperpolarization produced by an electrogenic pump in dorsal spino-cerebellar tract neurones of the cat. *Journal of Physiology* **210**, 839–855.

LANCASTER, B. & ADAMS, P. R. (1984). Single electrode voltage clamp of the slow AHP current in rat hippocampal pyramidal cells. *Neuroscience Abstracts* **10**, 872.

LANCASTER, B. & ADAMS, P. R. (1986). Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. *Journal of Neurophysiology* **55**, 1268–1282.

LLINÁS, R. & YAROM, Y. (1981). Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. *Journal of Physiology* **315**, 549–567.

MADISON, D. V. & NICOLL, R. A. (1982). Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature* **299**, 636–638.

MADISON, D. V. & NICOLL, R. A. (1984). Control of the repetitive discharge of rat CA1 pyramidal neurones *in vitro*. *Journal of Physiology* **354**, 319–331.

MADISON, D. V. & NICOLL, R. A. (1986). Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones *in vitro*. *Journal of Physiology* **372**, 221–244.

MALENKA, R. C., MADISON, D. V., ANDRADE, R. & NICOLL, R. A. (1986). Phorbol esters mimic some cholinergic actions in hippocampal pyramidal neurons. *Journal of Neuroscience* **6**, 475–480.

MEECH, R. W. (1978). Calcium dependent potassium activation in nervous tissues. *Annual Review of Biophysics and Bioengineering* **7**, 1–18.

NOHMI, M. & KUBA, K. (1984). (+) Tubocurarine blocks the $Ca^{++}$ dependent $K^+$ channel of the bullfrog sympathetic ganglion cell. *Brain Research* **301**, 146–148.

O'LEARY, J. L. (1937). Structure of the primary olfactory cortex of the mouse. *Journal of Comparative Neurology* **67**, 1–31.

PARTRIDGE, L. D. (1982). A mechanism for the control of low-frequency repetitive firing. *Cellular and Molecular Neurobiology* **2**, 33–45.

PENNEFATHER, P., LANCASTER, B., ADAMS, P. R. & NICOLL, R. A. (1985). Two-distinct Ca-dependent K currents in bullfrog sympathetic ganglion cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **82**, 3040–3044.

SCHWARTZKROIN, P. A. (1975). Characteristics of CA1 neurons recorded intracellularly in the hippocampal *in vitro* slice preparation. *Brain Research* **85**, 423–436.

SCHWARTZKROIN, P. A. & STAFSTROM, C. E. (1980). Effects of EGTA on the calcium-activated afterhyperpolarization in hippocampal $CA_3$ pyramidal cells. *Science* **210**, 1125–1127.

STAFSTROM, C. E., SCHWINDT, P. C., CHUBB, M. C. & CRILL, W. E. (1985). Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in vitro*. *Journal of Neurophysiology* **53**, 153–170.

STAFSTROM, C. E., SCHWINDT, P. C., FLATMAN, J. A. & CRILL, W. E. (1984). Properties of subthreshold response and action potential recorded in layer V neurons from cat sensorimotor cortex *in vitro*. *Journal of Neurophysiology* **52**, 244–263.

STOCKTON, J. M., BIRDSALL, N. J. M., BURGEN, A. S. V. & HULME, E. C. (1983). Modification of the binding properties of muscarinic receptors by gallamine. *Molecular Pharmacology* **23**, 551–557.

THOMPSON, S. M., MASUKAWA, L. M. & PRINCE, D. A. (1985). Temperature dependence of intrinsic membrane properties and synaptic potentials in hippocampus. *Journal of Neuroscience* **5**, 817–824.

TOKIMASA, T. (1984). Calcium-dependent hyperpolarization in bullfrog sympathetic neurones. *Neuroscience* **12**, 929–932.

TOKIMASA, T. (1985). Intracellular $Ca^{2+}$ ions inactivate $K^+$ current in bullfrog sympathetic neurons. *Brain Research* **337**, 386–391.

TOKIMASA, T., HASUO, H., AKASU, T. & NORTH, R. A. (1985). Hyperpolarizing after-current in myenteric neurons of guinea-pig ileum. *Neuroscience Abstracts* **11**, 1182.

WILSON, W. & GOLDNER, M. M. (1975). Voltage clamping with a single micro-electrode. *Journal of Neurobiology* **6**, 411–422.

WONG, R. K. S. & PRINCE, D. A. (1981). Afterpotential generation in hippocampal pyramidal cells. *Journal of Neurophysiology* **45**, 86–97.