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MUSCARINIC INHIBITION OF M-CURRENT AND A POTASSIUM LEAK CONDUCTANCE IN NEURONES OF THE RAT BASOLATERAL AMYGDALA

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SUMMARY

1. Voltage-clamp recordings using a single microelectrode were obtained from pyramidal neurones of the basolateral amygdala (BLA) in slices of the rat ventral forebrain. Slow inward current relaxations during hyperpolarizing voltage steps from a holding potential of −40 mV were identified as the muscarinic-sensitive M-current (I_M), a time- and voltage-dependent potassium current previously identified in other neuronal cell types.

2. Activation of I_M was voltage dependent with a threshold of approximately −70 mV. At membrane potentials positive to this, the steady-state current–voltage (*I–V*) relationship showed substantial outward rectification, reflecting the time- and voltage-dependent opening of M-channels. The underlying conductance (g_M) also increased sharply with depolarization.

3. The reversal potential for I_M was −84 mV in medium containing 3·5 mM K^+. This was shifted positively by 27 mV when the external K^+ concentration was raised to 15 mM.

4. The time courses of M-current activation and deactivation were fitted by a single exponential. The time constant for I_M decay, measured at 24 °C, was strongly dependent on membrane potential, ranging from 330 ms at −40 mV to 12 ms at −100 mV.

5. Bath application of carbachol (0·5–40 μM) inhibited I_M, as evidenced by the reduction or elimination of the slow inward M-current relaxations evoked during hyperpolarizing steps from a holding potential of −40 mV. The outward rectification of the steady-state *I–V* relationship at membrane potentials positive to −70 mV was also largely eliminated. The inhibition of I_M by carbachol was dose dependent and antagonized by atropine.

6. Carbachol produced an inward current shift at a holding potential of −40 mV that was only partially attributable to inhibition of I_M. An inward current shift was also produced by carbachol at membrane potentials negative to −70 mV, where I_M is inactive. These effects were dose dependent and antagonized by atropine. They were attributed to the muscarinic inhibition of a voltage-insensitive potassium leak conductance (I_{Leak}).

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7. In most cells, carbachol reduced the slope of the instantaneous *I–V* relationship obtained from a holding potential of −70 mV so that it crossed the control *I–V* plot at the reversal potential for I_{Leak}. This was found to be −108 mV in 3·5 mM K^+ saline, shifting to −66 mV in 15 mM K^+ saline. However, in a few neurones, carbachol produced a parallel shift of the instantaneous *I–V* relationship and no reversal potential could be obtained, possibly due to the activation by carbachol of a non-specific cation conductance.

8. In unclamped BLA pyramidal neurones, carbachol produces a slow membrane depolarization accompanied by an increase in input resistance. It is concluded that these actions are mediated by muscarinic receptor activation, resulting in inhibition of both the M-current and a voltage-independent K^+ leak current.

INTRODUCTION

The basolateral nucleus of the amygdala (BLA) receives a substantial cholinergic innervation from the nucleus basalis and adjacent regions of the substantia inominata in the ventral forebrain (Carlsen, Zaborszky & Heimer, 1985; Hellendall, Godfrey, Ross, Armstrong & Price, 1986). Intracellular recordings obtained from pyramidal cells of the BLA using an *in vitro* forebrain slice preparation have revealed that stimulation of this cholinergic pathway produces a slow EPSP that is associated with a decrease in membrane conductance (Washburn & Moises, 1989, 1992). These effects appeared to be due to the activation of muscarinic receptors by synaptically released acetylcholine since they were mimicked by direct application of muscarinic agonists, were enhanced by inhibitors of acetylcholinesterase, and blocked by atropine but not by nicotinic cholinergic antagonists (Washburn & Moises, 1992). Similar membrane depolarizations in response to muscarinic agonists have also been observed in sympathetic neurones of the frog (Kuba & Koketsu, 1976) and rat (Brown & Constanti, 1980) as well as in pyramidal neurones of the mammalian hippocampus (Benardo & Prince, 1982; Cole & Nicoll, 1984) and cortex (McCormick & Prince, 1986, 1987).

In sympathetic neurones, production of the slow cholinergic depolarization and its underlying inward current was fully accounted for by inhibition of the M-current (I_M), a time- and voltage-dependent K^+ conductance that is blocked following activation of muscarinic cholinergic receptors (Brown & Adams, 1980; Adams, Brown & Constanti, 1982*a*, *b*). In hippocampal pyramidal neurones, muscarinic agonists also inhibit I_M, and this was originally postulated to be responsible for production of the slow cholinergic depolarization (Halliwell & Adams, 1982; Gahwiler & Brown, 1985). However, more recent studies have revealed that muscarinic agonists also inhibit a voltage-insensitive potassium leak conductance in hippocampal neurones (Madison, Lancaster & Nicoll, 1987; Benson, Blitzer & Landau, 1988). It is the inhibition of this resting current, rather than blockade of I_M, that is largely responsible for the inward current which underlies production of the membrane depolarization in these cells (Madison *et al.* 1987).

It is not known what membrane currents are responsible for production of the slow cholinergic depolarization in pyramidal neurones of the rat BLA. Previous recordings from unclamped BLA neurones have demonstrated that a muscarinic-induced depolarization was associated with an increase in input resistance, reversed at a

potential negative to −80 mV, was sensitive to changes in extracellular potassium concentration and was blocked by intracellular caesium (Washburn & Moises, 1992). These data suggest that the slow depolarization was due to inhibition of a potassium conductance. In the present study, a single-electrode voltage clamp was used to identify the M-current in pyramidal neurones of the BLA and determine its sensitivity to the cholinomimetic drug carbachol. The results indicate that activation of muscarinic receptors produces inhibition of both the M-current and a voltage-insensitive K^+ leak conductance in BLA pyramidal neurones and the reduction of both currents contributes to the generation of a prolonged membrane depolarization. Some of these results have appeared in abstract form (Moises & Womble, 1990; Womble & Moises, 1990, 1991).

METHODS

Preparation

Young adult male Sprague–Dawley rats (150–200 g; Charles River) were used. The animals were killed by decapitation, the brains rapidly removed and horizontal slices of the ventral forebrain containing the basolateral amygdala cut at 500 μm using a Vibroslice (World Precision Instruments). Slices were maintained in a holding chamber at room temperature submerged in artificial cerebrospinal fluid (ACSF) that was continuously bubbled with 95% O_2–5% CO_2. Composition of the ACSF was (mM): NaCl, 124; KCl, 3·5; $CaCl_2$, 3·0; $MgSO_4$, 1·5; NaH_2PO_4, 1·0; $NaHCO_3$, 26·2; glucose, 11·0. Individual slices were transferred to the recording chamber as needed and maintained under submersion between layers of nylon mesh, continuously superfused with oxygenated ACSF at a rate of 1·0–1·5 ml min^{-1}. All recordings were obtained at room temperature (23–25 °C). Drugs were dissolved in ACSF to their final concentration and applied to the slice by means of a multi-port valve system.

Recordings were routinely obtained in the presence of 1 μM tetrodotoxin (TTX) in order to block action potential generation and eliminate spontaneous synaptic activity. In various experiments, carbachol (carbamylcholine chloride, 0·5–40 μM), atropine sulphate (1 μM), caesium chloride (2 mM), cobalt chloride (2 mM), or cadmium chloride (200 μM) were added to the bathing medium. For preparation of calcium-free medium, $CaCl_2$ was eliminated, NaCl was reduced to 118·5 mM and $MgSO_4$ was raised to 10 mM. All drugs were obtained from Sigma Chemical Co.

Intracellular recording

In the horizontal brain slice preparation used here, the BLA consists of a roughly triangular area that can be identified by its relationship to surrounding anatomical landmarks, being bordered laterally by the external capsule, caudomedially by the lateral ventricle and rostromedially by the stria terminalis. Neurones within this region were impaled with a single microelectrode pulled from 1·2 mm o.d. thin-walled glass capillary tubing (WPI Instruments). Microelectrodes were filled with a solution of 2·7 M potassium chloride (KCl) and 0·4 M potassium acetate and had resistances of 30–100 MΩ. Initially, recordings were made using microelectrodes filled with 3 M KCl but these often showed highly unstable current-passing characteristics during voltage clamp (i.e. developed very large and rapid changes in electrode resistance). This problem was alleviated by the addition of a small amount of potassium acetate. In a few experiments, 100 mM cyclic adenosine 3′,5′-monophosphate (cyclic AMP) was also present in the solution filling the electrode.

Only cells with resting potentials more negative than −55 mV and with overshooting action potentials greater than 70 mV in height were included in this study. Intracellular discontinuous current-clamp (DCC) and single-electrode voltage-clamp (SEVC) recordings were made using the Axoclamp 2A sample and hold amplifier (Axon Instruments), with a 30% duty cycle. The sampling frequency was approximately 3 kHz. Amplified signals were displayed on a storage oscilloscope while a separate oscilloscope was used to continuously monitor the headstage output. Capacitance compensation was set while in DCC mode. After switching to SEVC mode, the gain and phase controls were adjusted to obtain as square a voltage response as possible without inducing oscillations. Gain settings were typically 3–8 nA mV^{-1}. Care was taken to ensure that the voltage drop across the electrode dissipated completed during the interval between current injection and voltage sampling. With small negative voltage steps (< 40 mV), the clamp settling time was

$\leqslant 3$ ms, by which time the recorded voltage deflection was $> 95\%$ of the command voltage. The membrane potential actually obtained during a voltage step, rather than the designated command potential, was used for the construction of all current–voltage relationships. Records were discarded if the voltage response during the command pulse showed a sag in the membrane potential of more than 1–2 mV. This was never a problem in the present study since the hyperpolarizing voltage steps used here did not induce currents large enough to produce such a sag. However, loss of clamp control could occur during attempts to hold cells at potentials positive to -40 mV. At these levels, large, sustained outward currents were activated and therefore no attempts were made to analyse currents at potentials positive to -40 mV.

Reconstructions of pyramidal neurones in the rat BLA following intracellular labelling with Lucifer Yellow have revealed that these cells have a morphology that is very similar to other cortical and hippocampal pyramidal neurones (Washburn & Moises, 1992). Due to their extended processes, voltage clamping of pyramidal cells using a single microelectrode is hampered by the problem of voltage control decrement. However, analysis of hippocampal pyramidal neurones has revealed these cells to be electronically 'compact' with a equivalent electronic length of 0·9 at 35–37 °C (Brown, Fricke & Perkel, 1981; Johnston, 1981). At lower temperatures input resistance is increased, shortening the electronic length. Olfactory cortex neurones have an equivalent electrotonic length of only 0·6 at 23 °C (Constanti & Galvan, 1983*a*), implying that a steady-state voltage step will show only a small attenuation with distance, yielding greater voltage control of the dendrites. The lower temperature used in the present study (23–25 °C) thus helped to maximize the voltage control of BLA neurones during single-electrode voltage clamping. For more extensive discussions of the problems associated with this technique, see Halliwell & Adams (1982), Johnston & Brown (1983), and Adams & Galvan (1986).

Data analysis

Currents and voltages were filtered at 300 Hz and digitized on-line (333 Hz) using a TL-1 Interface and pClamp software (Axon Instruments) connected to an IBM AT computer. All data analysis was performed off-line using DAOS software (Laboratory Software Associates, Victoria, Australia) furnished with a cursor-based routine for exponential curve fitting using a least-squares procedure. Current traces were not corrected for leakage or capacitative transients. Steady-state current values were obtained from single exponential curves fitting to the current relaxation. Extrapolation of the fitted curve back to a point in time half-way through the preceding capacity transient provided an estimate for the instantaneous current response. These values were used for the construction of instantaneous and steady-state current–voltage relationships.

An extrapolation procedure was also used to analyse the repolarizing tail current. The initial portion of the tail was usually contaminated by the presence of a rapidly activating, transient outward current (see Fig. 1*A*), similar in appearance to the A-current (I_A) of other central neurones (Adams & Galvan, 1986; Storm, 1990). To exclude the contribution of this current and determine the instantaneous current level of the tail relaxation, single exponential curves were fitted to the tail current starting approximately 100 ms after the termination of the hyperpolarizing voltage step. The fitted curve was then extrapolated back to the preceding capacitive transient to provide a value for the instantaneous tail current response. Because of the presence of additional currents such as H-current and Ca^{2+}-activated K^+ currents which contributed to the tail following large hyperpolarizing voltage steps, care was taken to select only those cells with minimum contamination and tail current analysis was confined to only those records obtained with command potentials positive to approximately -70 mV.

Values given in the text are means with the standard error of the mean (S.E.M.) The S.E.M. was also included as error bars with those points on the graphs that refer to the means of three or more measurements. In some cases, the size of the point obscures the error bars.

RESULTS

M-current in BLA neurones

The results reported in this paper were generated from recordings of 123 BLA pyramidal neurones that displayed acceptable criteria for electrophysiological stability and health (see Methods). These neurones had a mean resting potential of

$-68{\cdot}6 \pm 0{\cdot}5$ mV ($n = 97$), which is similar to the value previously reported for pyramidal neurones in the rat BLA (Rainnie, Asprodini & Shinnick-Gallagher, 1991; Washburn & Moises, 1992). In normal ACSF, spontaneous synaptic activity within the slice often complicated the task of voltage clamping and thus all recordings were performed in the presence of 1 μM TTX.

M-current (I_M) has been identified in a wide variety of peripheral and central neurones as a sustained potassium conductance, activated by depolarization and inhibited by muscarinic cholinergic receptor activation (for reviews see Adams & Galvan, 1986; North, 1989; Storm, 1990). Figure 1*A* shows recordings obtained from a pyramidal BLA neurone subjected to a voltage-clamp protocol designed to test for the presence of an M-current. A series of 900 ms hyperpolarizing voltage steps were applied to the cell from a holding potential of -41 mV. Small hyperpolarizing steps (to command potentials positive to -70 mV) produced an initial instantaneous current response followed by a slow inward current relaxation that followed a single exponential time course. Overall, these relaxations averaged $77 \pm 7{\cdot}8$ pA ($n = 23$) in amplitude when measured during voltage steps to -55 mV from a holding potential of -41 mV. Stepping back to the holding potential yielded a smaller instantaneous current response (after tail current extrapolation; see Methods) than at the onset of the voltage step, indicating a conductance decrease during the step, followed by a slow outward tail current relaxation. With larger hyperpolarizing voltage steps, the inward current relaxations became smaller and faster, eventually reversing direction near -82 mV to become an outward relaxation (arrow), suggesting that the relaxations were due to a declining K^+ current. The close resemblance of these current relaxations to the M-current relaxations previously described in frog sympathetic ganglia (Brown & Adams, 1980), hippocampal pyramidal neurones (Halliwell & Adams, 1982) and olfactory cortical neurones (Constanti & Galvan, 1983*b*), coupled with the finding that the relaxations in BLA pyramidal neurones were eliminated by muscarinic receptor activation (see below), indicated that they were due to M-current deactivation.

The presence of several additional currents may be noted in the records shown in Fig. 1*A*. Voltage steps to command potentials negative to -80 mV activated the anomalous rectifier current (I_H or I_Q) previously described in hippocampal pyramidal neurones (Halliwell & Adams, 1982). Activation of I_H is manifested during the voltage step to -90 mV as a slowly developing inward current relaxation that follows the rapid decay of the reversed M-current. Repolarizing tail currents were seen upon termination of the hyperpolarizing voltage step (Fig. 1*A*). Following small voltage steps (command potentials positive to approximately -70 mV), the tail consisted of the rapidly decaying outward A-current (I_A) followed by a slow outward current relaxation produced by reactivation of I_M. Steps to more negative potentials were followed by slowly relaxing tail currents of increasing amplitude. Calcium-dependent K^+ currents appeared to contribute to these repolarizing currents since tail current amplitude was reduced by bath perfusion of 200 μM cadmium ($n = 4$), 2 mM cobalt ($n = 2$) or medium containing zero-calcium and 10 mM magnesium ($n = 2$), treatments that did not effect I_M, I_H or I_A. Mixed tail currents such as these were not used for analysis.

Figure 1*B* shows the current–voltage (*I–V*) relationships for both the instantaneous

(I_{In}; ○) and steady-state (I_{SS}; ●) currents obtained from the current traces shown in Fig. 1*A*. Measurement of I_{SS} at command potentials negative to −80 mV was complicated by activation of I_H. However, we were able to overcome this contamination to some extent by taking advantage of the very rapid decay of the M-

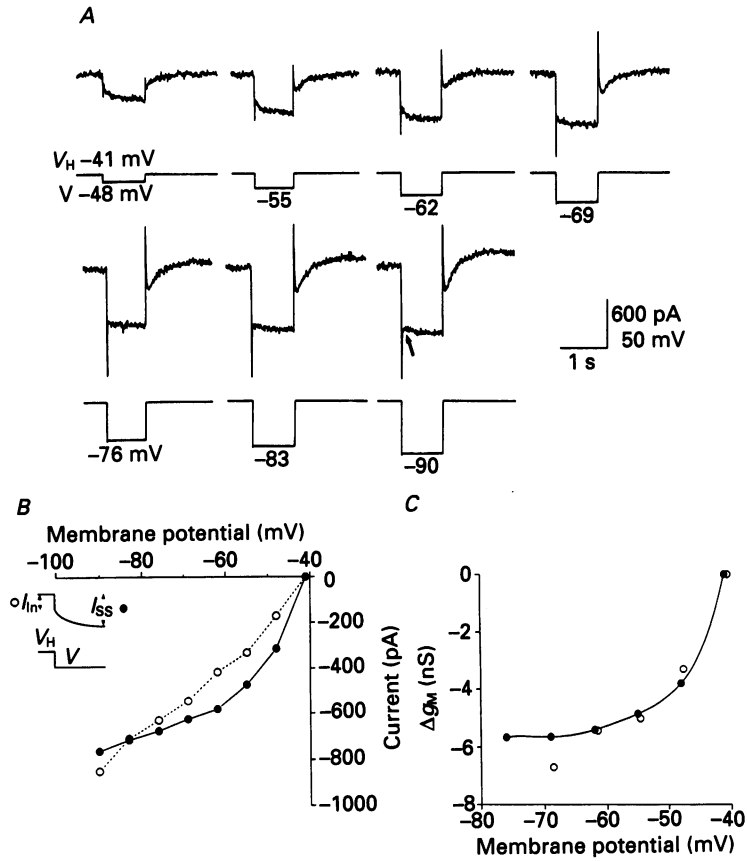

Fig. 1. Voltage dependence of the M-current. *A*, current relaxations reflecting the deactivation of I_M during a series of increasing hyperpolarizing voltage commands from a holding potential of −41 mV. At the most negative command potential (−90 mV), I_M has reversed direction to become a rapidly decaying outward relaxation (arrow), followed by a slow inward current relaxation produced by activation of the H-current. *B*, current–voltage relationship obtained from the records in *A*, showing the amplitude of the instantaneous current responses (I_{In}, ○) and the steady-state current levels (I_{SS}, ●), plotted against step command potential. *C*, change in M-conductance (Δg_M) during and after the hyperpolarizing voltage step calculated from the amplitudes of the slow current relaxations during the command step (●) and upon returning to the holding potential (○) as described in the text.

current relaxations at these command potentials. Thus, the I_M steady-state current level was estimated from single exponential curves fitted to approximately the first 150 ms after the onset of the voltage step. In addition, we also examined the kinetics of I_M in five BLA neurones which had no H-current. These cells yielded similar results, suggesting that contamination by I_H did not seriously distort our findings.

The *I–V* relationships in Fig. 1*B* demonstrate that M-channel activity is voltage- and time-dependent. The pronounced outward rectification of the steady-state curve at membrane potentials between −70 and −40 mV is consistent with the voltage-dependent nature of M-channel opening. In contrast, the instantaneous current response is approximately linear over the entire range of voltages. This implies that the rectification of the steady-state curve resulted not from rectification in individual M-channels, but from changes in the total number of channels open at each potential. The separation between the I_{In} and I_{SS} curves reflects the time dependency of channel closing during the 900 ms hyperpolarizing voltage step, while the cross-over point of the two curves yielded an estimate for the M-current reversal potential.

Reversal potential for I_M

The relatively negative reversal potential obtained from the cross-over point of the steady-state and instantaneous *I–V* relationships depicted in Fig. 1*B* indicated that the current relaxations resulted from the deactivation of a conductance that was probably carried by K^+ ions, similar to the M-current of other neurones (Brown & Adams, 1980; Adams *et al.* 1982*a*; Halliwell & Adams, 1982). To examine the ionic nature of the relaxations in BLA pyramidal neurones, reversal potentials were determined for the current relaxations evoked by hyperpolarizing commands from a holding potential of −40 mV, first in normal medium and then after switching to ACSF containing an elevated K^+ concentration. In some of these experiments, 2 mM caesium was included in the bathing saline to eliminate contamination by I_H (Halliwell & Adams, 1982; Moises & Womble, 1990). Reversal potentials did not differ significantly in the presence or absence of caesium.

In the cell shown in Fig. 2, the inward current relaxations reversed direction at a potential of −84 mV when recordings were obtained in control medium containing 3·5 mM extracellular K^+. Raising the external K^+ concentration to 15 mM shifted the reversal potential to −56 mV. Overall, the reversal potential of the inward current relaxations recorded in normal medium was $-83{\cdot}8 \pm 1{\cdot}0$ mV ($n = 20$). Although this value may slightly underestimate the true reversal potential due to contamination by I_H, it is consistent with the reversal potentials reported for I_M in rat and frog sympathetic neurones (Constanti & Brown, 1981; Adams *et al.* 1982*a*) and hippocampal pyramidal neurones (Halliwell & Adams, 1982). In five BLA neurones, elevation of the extracellular $[K^+]$ to 15 mM shifted the reversal potential of the inward current relaxation by 27 mV, to $-56{\cdot}6 \pm 2{\cdot}7$ mV. Although this is somewhat less than the predicted shift of 37 mV for a pure potassium conductance, these data indicate that the M-current of BLA pyramidal neurones is largely, but perhaps not exclusively, carried by potassium ions.

Conductance changes

A measure of M-channel closings and openings could be obtained from the amplitudes of the M-current relaxations recorded during and immediately following hyperpolarizing commands from a depolarized holding potential such as those shown in Fig. 1*A*. The change in steady-state M-conductance (Δg_M) was determined by dividing these current amplitudes by the driving force (Brown & Adams, 1980; Adams *et al.* 1982*a*). Thus, for M-current deactivation at the command potential *V*,

$-\Delta g_M(V) = -\Delta I_M(V)/(V - V_M)$, where $\Delta I_M(V)$ is the amplitude of the M-current relaxation and V_M is the M-current reversal potential. Conversely, the change in M-conductance during reactivation of I_M upon returning to the holding potential V_H is given by $+\Delta g_M(V_H) = +\Delta I_M(V_H)/(V_H - V_M)$, where $\Delta I_M(V_H)$ is the amplitude of the M-current tail relaxation.

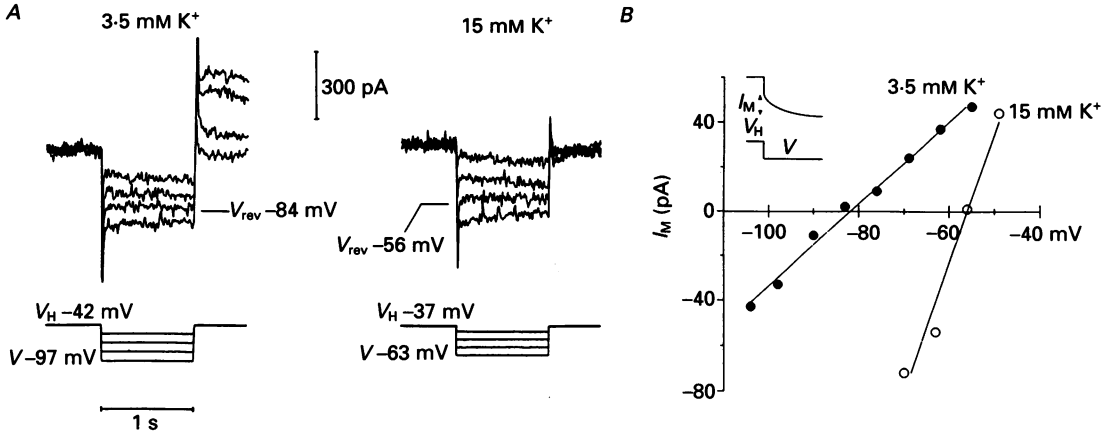

Fig. 2. Effect of extracellular K^+ concentration on the reversal potential for I_M. *A*, current traces elicited by a series of step hyperpolarizations from the indicated holding potentials while recording in ACSF containing 3·5 mM or 15 mM K^+. Resting potentials were −63 and −45 mV, respectively. The reversal potential (V_{rev}) was shifted to a more positive potential in ACSF containing elevated extracellular K^+. Caesium (2 mM) was included in the bathing saline to block the H-current. *B*, plot of M-current amplitude, calculated as the difference between the instantaneous and steady-state current responses, against step command potential. The reversal potential for I_M was the voltage level at which there was no net current, −84 mV in normal ACSF (●) and −56 mV in ACSF containing 15 mM K^+ (○).

Both measures of the change in membrane conductance were equivalent over the voltage range of −40 to −70 mV, as shown in Fig. 1*C*. The $\Delta g_M(V)$ plot (●) indicated a marked decrease in membrane conductance between the level seen at the holding potential of −41 mV and that measured during the hyperpolarizing voltage step, reflecting the voltage-dependent closing of M-channels during the step. The curve levelled off at a potential of −70 mV, indicating that essentially all M-channels were deactivated by a step to this membrane potential. Similarly, the $\Delta g_M(V_H)$ plot (○) represents the increase in membrane conductance due to the voltage-dependent opening of M-channels upon returning to the holding potential. The activation of I_M at membrane potentials positive to −40 mV was not examined since depolarization beyond this potential evoked large outward currents which quickly obscured the much smaller I_M.

Total steady-state M-conductance (g_M) was obtained by subtracting the plateau value of the $\Delta g_M(V)$ conductance present at −70 mV from the $\Delta g_M(V)$ measured at each voltage level up to −40 mV (Constanti & Brown, 1981; Adams *et al.* 1982*a*). The estimates obtained in this manner from twelve experiments are plotted against membrane potential in Fig. 3*A*. M-channels were completely closed at membrane

potentials of −70 mV and below, but positive to this M-conductance increased sharply with membrane depolarization, reaching a level of 5·0±0·8 nS ($n = 12$) at −40 mV. This behaviour is qualitatively very similar to that shown by M-currents in rat and frog sympathetic neurones (Constanti & Brown, 1981; Adams *et al.* 1982*a*), which also exhibit a strongly voltage-dependent M-conductance.

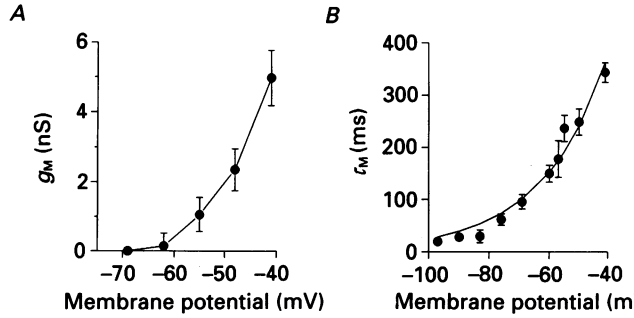

Fig. 3. *A*, mean voltage dependence of the steady-state M-conductance (g_M). Points are the means of results derived from twelve experiments of the type illustrated in Fig. 1. *B*, mean voltage dependence of decay time constants (τ_M) for M-current relaxations. Each point represents the mean of three to forty-one determinations. The curve was drawn according to $\tau(V) = \tau(0)\exp(AV)$, with $\tau(0) = 2360$ ms and $A = 0{\cdot}046$ mV^{-1}.

Time course of I_M inactivation

The decay of the current relaxations seen during a hyperpolarizing voltage step followed a single exponential time course which accelerated as the command potential was made more negative. Thus, measurements were made of the rates of decay during a series of hyperpolarizing voltage steps. At very negative command potentials (negative to −80 mV), the decay rate was determined from the first 150 ms of the current response to reduce contamination by I_H. In addition, to determine the time course of decay at potentials close to the reversal potential for I_M, measurements were made in ACSF containing 15 mM $[K^+]_o$. While raising the extracellular $[K^+]$ shifted the reversal potential to a less negative potential, it did not appear to alter the kinetics of decay since the time constants obtained in high K^+ saline agreed with those obtained in normal medium at those command potentials where both could be measured accurately.

The time constants for M-current decay are plotted against membrane potential in Fig. 3*B*. The time constant (τ_M) varied strongly with voltage, being slowest at approximately −40 mV where it was 330 ms and accelerated with hyperpolarization to 12 ms near −100 mV. The decline in τ_M could be fitted by $\tau(V) = \tau(0)\exp(AV)$, where $\tau(V)$ is the rate of M-current decay at the command voltage, $\tau(0)$ is the rate at 0 mV and A is a slope factor (Constanti & Galvan, 1983*a*). The fitted curve shown in Fig. 3*B* yielded values of 2360 ms and 0·046 mV^{-1} for $\tau(0)$ and A, respectively. In contrast, the rate of I_M activation during the repolarizing tail current did not vary with changes in the amplitude of the preceding voltage step (for small hyperpolarizations) (Fig. 1*A*). Time constant measurements for these relaxations were used to estimate τ_M at the holding potential of −40 mV. A similar strong voltage

dependency of the rate of M-current decay has also been reported in rat and frog sympathetic neurones (Constanti & Brown, 1981; Adams *et al.* 1982*a*).

Muscarinic inhibition of I_M

Muscarinic agonists have been shown to produce a slow membrane depolarization in BLA pyramidal neurones (Washburn & Moises, 1992). To examine the ionic

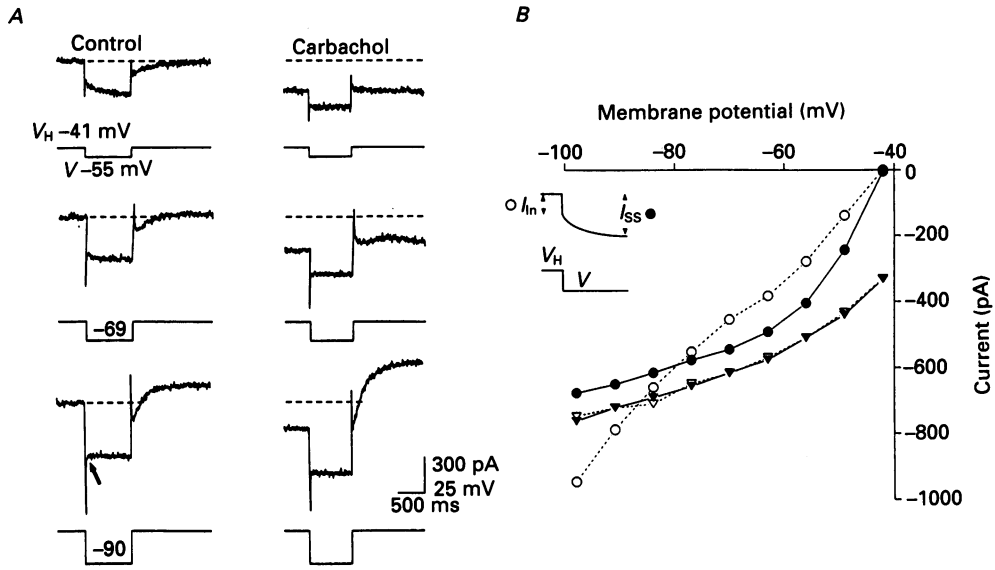

Fig. 4. Effect of carbachol on I_M. *A*, M-current relaxations evoked by hyperpolarizing voltage steps from a holding potential of −41 mV, before and during bath application of carbachol (40 μM). In control medium, I_M reversed direction and decayed rapidly (arrow) during the voltage step to −90 mV. In the presence of carbachol, the holding current showed an inward shift of 294 pA and M-current relaxations were eliminated. Dashed lines indicate control baseline current levels recorded at the holding potential. *B*, current–voltage relationships determined from the cell illustrated in *A*, showing control instantaneous (I_{In}, ○) and steady-state (I_{SS}, ●) current responses and instantaneous (▽) and steady-state (▼) responses in the presence of carbachol, plotted against step command potential. Carbachol reduced the outward rectification in the steady-state *I–V* relationship observed between command potentials of −40 and −70 mV, and produced an inward current shift at all membrane potentials.

mechanisms underlying this depolarization, we tested carbachol for its ability to modulate potassium conductances in the BLA. In current-clamp recordings, bath applications of 10–40 μM carbachol depolarized BLA neurones an average of 6·5 ± 1·0 mV (n = 13) from their normal resting potential of approximately −70 mV. In voltage clamp, this effect was reflected by a mean reduction of 53 ± 9 pA (n = 15) in the baseline current level recorded at a holding potential of −70 mV. It is this inward current shift that underlies the membrane depolarization seen in current-clamp records.

Carbachol also inhibited the M-current. This effect can be discerned in several ways from the records shown in Fig. 4*A*. First, bath application of 40 μM carbachol eliminated the current relaxations induced by applying hyperpolarizing steps from

a holding potential of −41 mV, leaving only flat, ohmic-type current responses. This result indicates that in the presence of carbachol, all M-channels were closed before the voltage step was applied. Second, carbachol eliminated the repolarizing tail currents that were previously produced by reactivation of the M-current upon

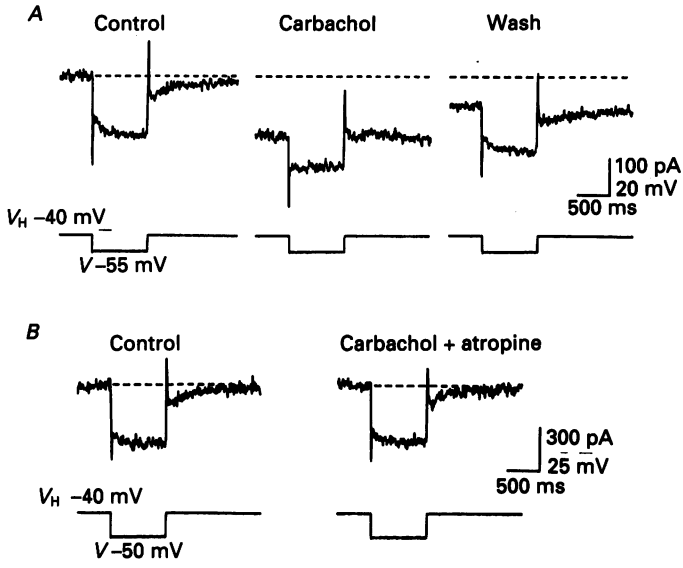

Fig. 5. Muscarinic block of I_M. Dashed lines indicate control baseline current levels at a holding potential of −40 mV. *A*, a hyperpolarizing voltage step to −55 mV evoked a slow inward M-current relaxation (Control). Bath application of 40 μM carbachol eliminated the inward current relaxation and produced a 116 pA inward shift in holding current. These effects were partially reversed after washing for 30 min with ACSF containing 1 μM atropine. *B*, in a different cell, inclusion of atropine (1 μM) in the bathing medium blocks the inhibitory actions of carbachol (40 μM) on both I_M and the holding current.

returning to the holding potential. (Note that the tail currents following the voltage step to −90 mV were contaminated by the presence of additional currents.) Finally, the instantaneous current responses at the onset and termination of the hyperpolarizing voltage step were equal in size in the presence of carbachol. This suggests that no ion channels were turned off or on by the voltage step and thus current flow occurred only through membrane leak channels. The reduction of the initial instantaneous current response indicated that membrane conductance at the holding potential was also reduced by the agonist. Chord conductance measurements were obtained by dividing I_{In} recorded at the onset of the hyperpolarizing step by the amplitude of the voltage step. Carbachol reduced membrane conductance at −40 mV from 21·6 ± 2·6 nS ($n = 11$) to 14·2 ± 2·6 nS ($n = 7$).

In Fig. 4*B*, we have plotted the amplitudes of the instantaneous (open symbols) and steady-state (filled symbols) current responses obtained from the records shown in Fig. 4*A* against the step command potential. The curve of the control steady-state current responses (○) shows a strong outward rectification between clamp potentials of −70 and −40 mV, reflecting the voltage-dependent opening of M-channels. In the presence of carbachol, I_M was blocked and this steady-state rectification was greatly

reduced (▼). Although this reduction was largest at depolarized membrane potentials, implying that carbachol was a more effective blocker of I_M at these potentials, it seems likely that the apparent voltage sensitivity of carbachol's action can be attributed to the voltage dependency of opening for the M-channel itself (see

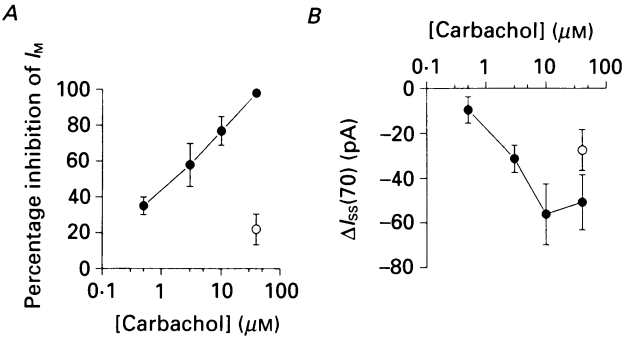

Fig. 6. *A*, concentration–response curve for the inhibitory action of carbachol on the M-current. The concentration of carbachol is plotted against the percentage inhibition of I_M (●), determined from measurements of M-current amplitude during hyperpolarizing steps to −55 mV from a holding potential of −40 mV. The inhibitory effect of 40 μM-carbachol on I_M was almost totally blocked by 1 μM atropine (○). Each point represents the mean of four to nine measurements. *B*, concentration–response relationship for inhibition of the K^+ leak conductance by carbachol. The magnitude of the inward current shift (in pA) recorded at a holding potential of −70 mV ($\Delta I_{ss}(70)$) is plotted against carbachol concentration (●). The inhibitory action of 40 μM carbachol on the leak conductance was antagonized by 1 μM atropine (○). Each point represents the mean of three to ten measurements.

Adams *et al.* 1982*b*). Thus, within the voltage range of −70 to −40 mV, larger numbers of M-channels would initially be open at more depolarized membrane potentials and therefore available for inhibitory modulation. The majority of these would subsequently be closed by carbachol, producing a larger net decrease in membrane current compared to that obtained at a less depolarized membrane potential where fewer M-channels were initially open. Similar results were obtained in six other neurones.

The inhibitory effects of carbachol on the M-current were reversible upon washing (Fig. 5*A*) and were blocked in the presence of 1 μM atropine (Fig. 5*B*), indicating their mediation via muscarinic cholinergic receptors. Interestingly, prolonged washing or administration of ACSF containing atropine were rarely successful in restoring the carbachol-induced inward shift in holding current back to the control level (Fig. 5*A*). Benson *et al.* (1988) reported in rat hippocampal pyramidal neurones that carbachol produced a similar inward shift that was not reversed by atropine and suggested that this might be attributed to long-term receptor desensitization. In contrast, Madison *et al.* (1987) found the inward shift to be reversible in hippocampal pyramidal neurones.

Figure 6*A* shows the dose–response relationship for inhibition of the M-current by carbachol, determined in twenty-four cells using a clamp protocol like that illustrated in Fig. 5*A*. Carbachol reduced M-current amplitude in a dose-dependent

manner (●), with an IC_{50} of approximately 2 μM. At a concentration of 40 μM, carbachol completely eliminated the slow inward current relaxations (Fig. 5*A*), indicating that there was no carbachol-insensitive portion to the M-current. The inhibitory effect of even the highest concentration of carbachol tested (40 μM) was blocked by 1 μM atropine (Fig. 6*A*, ○).

Effect of carbachol on M-current kinetics

One possible mechanism by which muscarinic receptor activation might reduce the M-current is by shifting the voltage sensitivity curve for M-current conductance (Fig. 3*A*) to a more depolarized level. If this were the case, it should be possible to overcome the drug-induced blockade and achieve a normal level of M-current simply by displacing the membrane potential to a more depolarized level. Unfortunately, we were unable to directly assess this idea because at membrane potentials positive to −40 mV, I_M was obscured by the activation of additional currents. However, we could obtain indirect evidence relating to this possibility by assessing the effect of carbachol on the voltage sensitivity of M-channel kinetics. One manifestation of this voltage sensitivity is the fact that the rate of M-current decay is accelerated with membrane hyperpolarization (Fig. 3*B*). If carbachol induced a positive shift in this measure of voltage sensitivity, it should be manifested as an acceleration in the time course of M-current decay during a hyperpolarizing voltage step. To test this, decay rate measurements were obtained in six neurones on the residual I_M after partial inhibition of the M-current using a low concentration of carbachol. M-current amplitude, measured during a voltage step to −55 mV from a holding potential of −40 mV, was reduced by 0·5 μM carbachol by 36%, from 78±11 to 50±7 pA. However, the time constant of I_M decay was unaffected (164±23 ms *versus* 147±33 ms in the presence of carbachol). Similarly, the rate of M-current reactivation during the repolarizing tail current was also unchanged (331±26 ms *versus* 321±39 ms). Therefore, in rat BLA pyramidal neurones, as in bullfrog sympathetic neurones (Adams *et al.* 1982*b*), muscarinic receptor activation does not appear to affect the time courses of M-current activation or deactivation.

Muscarinic inhibition of a voltage-insensitive K^+ leak conductance

Studies in hippocampal pyramidal neurones have shown that muscarinic receptor activation not only blocks the M-current but also a voltage-independent K^+ leak conductance (I_{Leak}) (Madison *et al.* 1987; Benson *et al.* 1988). It is the blockade of this conductance that appears to be responsible for the inward shift in the steady-state current level in these cells at membrane potentials where I_M is inactive. A similar muscarinic-sensitive leak current appeared to also be present in BLA pyramidal neurones, since an inward current shift was produced by carbachol at membrane potentials negative to −70 mV, where M-current was not active. For example, in Fig. 4*A*, the control voltage steps to −69 or −90 mV resulted in the closure of all M-channels, as evidenced by the ohmic nature of the steady-state current response. Nevertheless, administration of carbachol produced an additional inward shift of the steady-state current level recorded at both of these command potentials. This inward current shift was also reflected in the steady-state *I–V* relationship for this cell (Fig. 4*B*). At membrane potentials negative to −70 mV, the control steady-state *I–V* plot

(●) was linear, indicating the presence of a voltage-insensitive leak conductance. In the presence of carbachol (▼), this portion of the steady-state I–V curve was shifted downward in parallel with the control curve, suggesting the inhibition of this non-rectifying conductance. Chord conductance measurements, obtained from the I_{In}

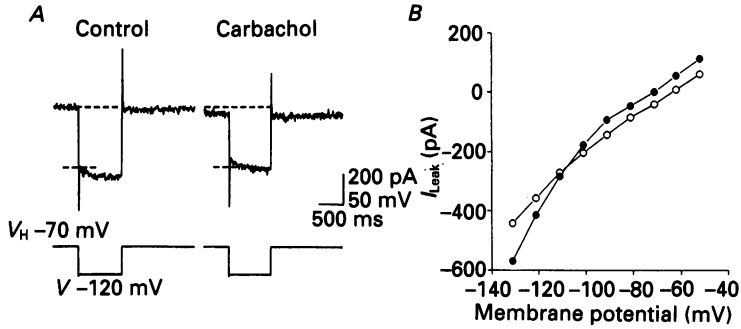

Fig. 7. Carbachol inhibits a K^+ leak conductance. *A*, clamp currents recorded from a cell held at −70 mV, where I_M is inactive. In control saline, a hyperpolarizing voltage step to −120 mV activated the slowly developing inward H-current. Application of carbachol (40 μM) induced an inward shift in the holding current at the V_H of −70 mV but an outward shift of the instantaneous current response recorded at the onset of the hyperpolarizing step to −120 mV. I_H was not affected by carbachol. Dashed lines indicate control levels for the holding and instantaneous current. *B*, current–voltage relationship for the cell in *A*, obtained by plotting the amplitude of the instantaneous current response (I_{Leak}) against step command potential, before (●) and during (○) application of carbachol (40 μM).

amplitude recorded at the termination of the hyperpolarizing voltage step divided by the amplitude of the voltage step, showed that carbachol reduced membrane conductance by 4·4 nS at a membrane potential of −76 mV, from 11·0 ± 1·8 nS (n = 10) to 6·6 ± 1·8 nS (n = 6). Thus, carbachol appeared to inhibit both the M-current and a voltage-insensitive leak current.

The dose–response relationship for the inhibitory action of carbachol on the potassium leak conductance was examined by plotting the net change in steady holding current recorded at a holding potential of −70 mV ($\Delta I_{SS}(70)$) as a function of carbachol concentration (Fig. 6*B*, ●). The inhibition of I_{Leak} by carbachol was concentration dependent, with the maximal effect observed at a concentration of 10 μM. By expressing the reductions in holding current produced at lower concentrations of agonist as a percentage of the maximal response obtained at 10 μM, we were able to estimate an IC_{50} of 2 μM. Atropine (1 μM, ○) antagonized the reductions in leak conductance produced by the highest concentration of carbachol tested (40 μM), indicating mediation of this effect via activation of muscarinic cholinergic receptors.

Leak current reversal potential

To examine the ionic properties of the leak conductance in BLA pyramidal neurones, we first obtained an estimate of its reversal potential from measurements of the instantaneous current responses evoked by voltage steps from a holding

potential where I_M was inactive, before and in the presence of carbachol. The results from one such experiment are shown in Fig. 7*A*. A 900 ms hyperpolarizing command to −120 mV from a holding potential of −70 mV activated I_H, identified as the slowly developing inward current relaxation during the voltage step. Administration of carbachol (40 μM) produced an inward shift in the holding current at −70 mV. However, the instantaneous current response recorded at the onset of the voltage step to −120 mV showed an *outward* shift in the presence of carbachol, indicating that the reversal potential for I_{Leak} was between −70 and −120 mV. Confirmation of this was provided by comparing the *I–V* relationships, shown in Fig. 7*B*, for the instantaneous current responses recorded in this cell after stepping to command potentials of −50 to −130 mV from a holding potential of −70 mV, before (●) and during (○) application of 40 μM carbachol. The slope of the *I–V* curve was reduced by carbachol over the range of −90 to −140 mV, reflecting a decrease in membrane conductance. The cross-over point for the two curves provided an estimate of the reversal potential for the carbachol-sensitive I_{Leak}, which was −108 mV in this cell. In this manner, a mean reversal potential for I_{Leak} of −107·5 ± 2·7 mV (range −120 to −97 mV) was obtained in eleven BLA neurones recorded in normal ACSF containing 3·5 mM $[K^+]_o$. Raising the extracellular K^+ concentration to 15 mM shifted the reversal potential to −66·3 ± 4·3 mV ($n = 3$). This 41 mV positive shift in the reversal potential agrees well with the 37 mV shift predicted by the Nernst equation for a pure K^+ conductance, indicating that the leak current of BLA pyramidal neurones is a potassium conductance. A similar muscarinic-sensitive K^+ leak conductance with a reversal potential of −92 mV in 5 mM K^+ (equivalent to −101 mV in 3·5 mM K^+) has been reported in hippocampal pyramidal neurones (Benson *et al.* 1988).

However, in a few BLA neurones (5 of 19), carbachol acted to *increase* membrane conductance at potentials negative to −70 mV. In these cells, plots of I_{In} before and during carbachol application did not cross over the range of tested potentials (−50 to −140 mV) and no reversal potential could be obtained. A similar response has been reported for a small subset of hippocampal pyramidal neurones (Benson *et al.* 1988).

Inhibition of both I_M and I_{Leak} contribute to the inward current shift

In addition to blocking the M-current and the K^+ leak current, carbachol has also been shown in hippocampal pyramidal neurones to inhibit a slow Ca^{2+}-activated K^+ current (I_{AHP}) that underlies the late after-hyperpolarization following a series of action potentials (Madison *et al.* 1987). A similar muscarinic-sensitive current has been identified in BLA pyramidal neurones (Womble & Moises, 1990) and thus it is possible that inhibition of this current might contribute to the inward current shift produced here by carbachol. This was probably not the case, however, since application of carbachol (10–40 μM) produced an inward current shift of 53 ± 9 pA ($n = 15$) in cells voltage-clamped at a holding potential of −70 mV, which is below the usual activation range for Ca^{2+} currents. To test the role of I_{AHP} more directly, neurones were impaled with microelectrodes containing 100 mM cyclic AMP, a treatment that blocks I_{AHP} in hippocampal neurones (Madison *et al.* 1987; Benson *et al.* 1988). This procedure eliminated I_{AHP} in BLA pyramidal neurones ($n = 2$), but

did not prevent the carbachol-induced inward current shift at holding potentials of −40 or −70 mV (data not shown). Thus, inhibition of I_{AHP} does not appear to contribute to the carbachol-induced inward current shift.

Although it can be assumed that muscarinic inhibition of I_{Leak} was responsible for the inward current shift at potentials negative to the activation range of I_M, the

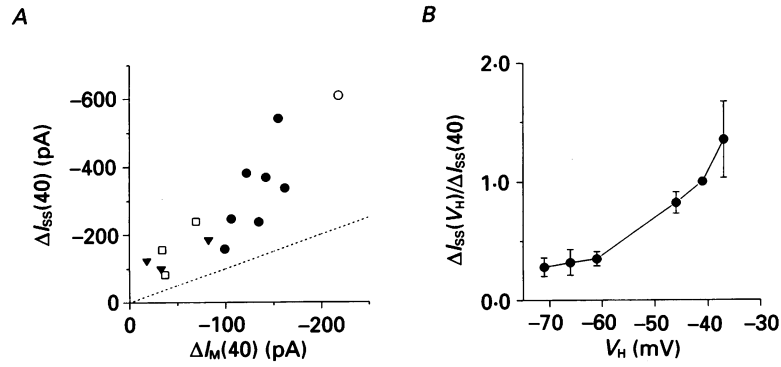

Fig. 8. Inhibition of both I_M and I_{Leak} contribute to the inward current shift produced by carbachol. *A*, the relationship between the magnitude of the carbachol-induced inward current shift and the change in M-current amplitude is illustrated by plotting the reduction in steady-state current at a holding potential of −40 mV ($\Delta I_{SS}(40)$) against the reduction in M-current amplitude recorded at the same potential ($\Delta I_M(40)$). Total M-current amplitude at −40 mV was estimated from the amplitude of the repolarizing tail current measured upon returning to the holding potential after a hyperpolarizing voltage step to −65 mV (see text). Different degrees of I_M inhibition and inward current shifts were produced by varying the carbachol concentration (in μM): ▼, 0·5; □, 3; ○, 10; ●, 40. The dashed line shows the 1:1 relationship expected if the inward current shift produced by carbachol was entirely due to inhibition of I_M. *B*, amplitude of the inward current shift produced by carbachol (10–40 μM) as a function of membrane potential. Plotted against the holding potential (V_H) is the magnitude of the inward current shift at various holding potentials ($\Delta I_{SS}(V_H)$), normalized to the current shift seen in the same cell at a holding potential of −40 mV ($\Delta I_{SS}(40)$). Each point represents the mean of three to twenty-three measurements.

question still remained whether inhibition of the M-current alone might account for the inward shift in holding current seen at more depolarized membrane potentials. To determine this, we systematically compared the magnitude of the inward current shift and the degree of M-current inhibition produced by carbachol in neurones voltage-clamped at a fixed holding potential. The graph in Fig. 8*A* summarizes the results from fourteen experiments in which we compared the carbachol-induced change in steady-state current recorded at a holding potential of −40 mV ($\Delta I_{SS}(40)$) against the decrease in M-current amplitude recorded at the same holding potential ($\Delta I_M(40)$). Total M-current amplitude at the holding potential of −40 mV was estimated from the size of the tail current following termination of a hyperpolarizing voltage step to −65 mV. Since nearly all M-channels were closed by steps to this clamp potential (Fig. 3*A*), the amplitude of the repolarizing tail on returning to the holding potential provided a measure of the total amount of sustained M-current active at this holding potential. The dashed line in the graph shows the expected

relationship if the inward current shift produced by carbachol was entirely due to inhibition of I_M. In all cases, the magnitude of the inward current shift exceeded the reduction in total M-current amplitude, regardless of agonist concentration. Since inhibition of I_M alone was not sufficient to fully account for the inward shift in steady-state current produced by carbachol at a membrane potential of −40 mV, we conclude that inhibition of I_{Leak} also contributed to the reduction in holding current at this potential.

The graph in Fig. 8*B* summarizes the results from a different series of experiments in twenty-three cells in which we examined how the inward current shift produced by carbachol (10–40 μM) varied as a function of holding potential (V_H). The carbachol-induced change in steady-state current at each holding potential ($\Delta I_{SS}(V_H)$) was normalized to the change in current level that was produced in the same cell at a holding potential of −40 mV ($\Delta I_{SS}(40)$). If, as in bullfrog sympathetic neurones (Adams *et al.* 1982*b*), inhibition of I_M alone accounted for the carbachol-induced inward current shift at all membrane potentials, this relationship would follow the activation curve for I_M, declining with membrane hyperpolarization to a value of 0 at a V_H of −70 mV, the threshold for I_M activation. Although the curve in Fig. 8*B* did show a voltage-dependent decline as the holding potential was made progressively more negative, it did not intersect with the abscissa but rather levelled off to a value of approximately 0·3 at a potential of −70 mV. Since all M-channels are closed at this membrane potential, this finding supports the idea that the substantial inward current shift produced by carbachol at a holding potential of −70 mV is due solely to inhibition of I_{Leak}.

The relative contributions that inhibition of I_M and I_{Leak} make to the total inward current shift at each membrane potential can also be obtained from the graph shown in Fig. 8*B*. Since inhibition of I_M does not contribute to the inward current shift at a holding potential of −70 mV, the remaining component of 0·3 must be due to inhibition of the leak current. This predicts that, at a holding potential of −40 mV, approximately 30% of the total inward current shift produced by carbachol was due to inhibition of I_{Leak}, while 70% resulted from block of the M-current. This estimation was confirmed by direct measurement. At a holding potential of −40 mV, carbachol (10–40 μM) produced an average inward current shift of 249 ± 44 pA (n = 14). However, at the same holding potential, the total sustained M-current was only 175 ± 19 pA (n = 12), or 70% of the total current shift. This value for total I_M was obtained from the amplitudes of the repolarizing tail currents produced upon returning to the holding potential following hyperpolarizing voltage steps to −65 mV, as in Fig. 8*A*. Thus, even complete inhibition of I_M could account for only a portion of the total inward current shift seen at a holding potential of −40 mV, with the remainder due to inhibition of the leak conductance. At more negative potentials, the relative contribution of the leak current increased, until at potentials negative to −70 mV inhibition of I_{Leak} accounted for 100% of the inward current shift produced by carbachol.

DISCUSSION

The M-current is a persistent, voltage- and time-dependent K^+ conductance, so named because of its sensitivity to muscarinic agonists (Brown & Adams, 1980). It is found in a wide variety of cells, including frog sympathetic neurones (Brown & Adams, 1980; Adams *et al.* 1982*a*, *b*), rat sympathetic neurones (Constanti & Brown, 1981) and many mammalian central neurones (hippocampal pyramidal cells: Halliwell & Adams, 1982; spinal cord neurones: Nowak & MacDonald, 1983; olfactory cortex: Constanti & Galvan, 1983*b*; cingulate cortex: McCormick & Prince, 1986; human neocortex: Halliwell, 1986). The M-currents found in these neurones all share the common characteristics of inhibition by muscarinic agonists, voltage sensitivity and slow rates of decay and activation.

The M-current described here in BLA pyramidal neurones exhibited similar characteristics. It was activated by membrane depolarization with a threshold of −70 mV, the normal resting potential for BLA pyramidal neurones. The M-conductance curve (g_M) showed a voltage-dependent increase with depolarization over the range of −70 to −40 mV. A maximum level for the steady-state M-conductance was not obtained in the BLA neurones recorded here, but at −40 mV it is only one-third of that reported for rat sympathetic neurones (Constanti & Brown, 1981). However, comparisons of relative cell surface areas suggests that the density of M-channels is similar for both cell types. The linear relationship of the instantaneous *I–V* curve in BLA neurones implies that individual M-channels do not exhibit rectification. Instead, the steep increase in g_M seen with membrane depolarization was due to the different proportions of channels open at each membrane potential, as is the case in rat and frog sympathetic neurones (Constanti & Brown, 1981; Adams *et al.* 1982*a*). Finally, the M-current recorded in BLA pyramidal neurones had a relatively negative reversal potential (−84 mV) which was shifted in a positive direction after elevation of the extracellular K^+ concentration, indicating that I_M is predominantly a K^+ conductance.

The M-current is unusual because it is a sustained, voltage-sensitive current that is activated by even small depolarizations from the normal resting potential of the cell. Activation of the M-current would tend to resist depolarizing influences and thus play an important role in stabilizing the cell at its resting potential. Although activation of I_M is too slow for it to contribute to membrane repolarization during a single action potential, the M-current does help to regulate the neuronal firing rate in hippocampal pyramidal neurones by contributing to both the accommodation response (Madison & Nicoll, 1984) and production of the medium after-hyperpolarization (Constanti & Sim, 1987; Storm, 1989).

Previous studies have demonstrated inhibition of the M-current by cholinergic muscarinic agonists in several neuronal types (sympathetic neurones: Constanti & Brown, 1981; Adams *et al.* 1982*b*; hippocampal pyramidal neurones: Halliwell & Adams, 1982; cingulate cortical neurones: McCormick & Prince, 1986). In hippocampal pyramidal neurones, M-current inhibition has also been produced by synaptically released acetylcholine following stimulation of cholinergic afferents in the slice (Madison *et al.* 1987). In the present study, we showed that bath application of carbachol inhibited the M-current and decreased membrane conductance in BLA

pyramidal neurones. The inhibitory effects of carbachol on I_M were not associated with a change in the time courses of partially inhibited currents, suggesting that the voltage sensitivities of I_M activation and decay were unchanged. Although carbachol appeared to block I_M in a voltage-dependent manner with greater inhibition occurring at more depolarized potentials, the lack of effect on the voltage sensitivity of I_M suggested that carbachol acted independently of voltage to simply reduce the number of open M-channels at each membrane potential. The inhibition of the M-current and the decrease in membrane conductance induced by carbachol were atropine sensitive, confirming that inhibition of this conductance resulted from the activation of muscarinic receptors. The effect of carbachol was also dose dependent, with complete blockade at a concentration of 40 μM. The IC_{50} was 2 μM, similar to the half-maximal concentration of 5 μM reported for M-current inhibition by carbachol in hippocampal pyramidal neurones (Madison *et al.* 1987).

Several lines of evidence indicated that in addition to inhibiting the M-current in BLA pyramidal neurones, carbachol also blocked a non-rectifying K^+ leak conductance. First, at depolarized membrane potentials, carbachol induced an inward current shift that was larger in magnitude than the accompanying loss of M-current. Second, carbachol produced an inward current shift at membrane potentials negative to -70 mV, where I_M was inactive. Muscarinic inhibition of I_{AHP}, a Ca^{2+}-activated K^+ current, was ruled out as contributing to these actions of carbachol since they were not prevented when I_{AHP} was blocked by recording with microelectrodes containing cyclic AMP. Third, carbachol reduced the initial instantaneous current response and this action showed a reversal potential of -108 mV, considerably more negative than the reversal potential derived for I_M. This very negative reversal potential for I_{Leak} and the finding that the reversal potential was shifted positively in a Nernstian manner by elevation of the extracellular K^+ concentration demonstrated that the leak current is carried by K^+ ions. Finally, at membrane potentials negative to the activation threshold for the M-current, the steady state I–V relationship was uniformly reduced by carbachol in a voltage-independent manner. The reduction of I_{Leak} by carbachol was concentration dependent, with an IC_{50} of 2 μM, and was blocked by atropine, indicating that it was mediated by muscarinic receptor activation. These results all point to the inhibition by carbachol of a voltage-insensitive K^+ leak conductance in BLA neurones, similar to the block seen in hippocampal pyramidal neurones (Madison *et al.* 1987; Benson *et al.* 1988).

Muscarinic agonists have been shown to produce a prolonged membrane depolarization in several neuronal cell types, including sympathetic neurones (Kuba & Koketsu, 1976; Brown & Constanti, 1980) and pyramidal neurones of the hippocampus (Benardo & Prince, 1982; Cole & Nicoll, 1984), cerebral cortex (McCormick & Prince, 1986, 1987) and BLA (Washburn & Moises, 1992). In rat and frog sympathetic neurones, inhibition of I_M alone was found to fully account for the inward current shift that underlies production of this depolarization (Constanti & Brown, 1981; Adams *et al.* 1982*b*). In these cells, the magnitude of the inward current shift produced at depolarized potentials by muscarinic agonists equalled the amount of M-current which was blocked at that membrane potential. Moreover, no inward current shift was seen at membrane potentials negative to the activation threshold

for I_M. Thus, a muscarinic-sensitive K^+ leak current was absent in these cells. In early studies with hippocampal pyramidal cells, it was also concluded that inhibition of I_M alone could fully account for production of the membrane depolarization (Halliwell & Adams, 1982) or the slow cholinergic EPSP (Gahwiler & Brown, 1985). However, later studies demonstrated that muscarinic agonists also blocked a voltage-insensitive K^+ leak conductance in these cells (Madison *et al.* 1987; Benson *et al.* 1988) and this conductance was more sensitive to muscarinic inhibition than was I_M (Madison *et al.* 1987). Thus, stimulation of cholinergic pathways elicited a slow EPSP even in the absence of any noticeable M-current inhibition, leading to the conclusion that inhibition of a K^+ leak current, rather than inhibition of I_M, was responsible for production of the slow EPSP (Madison *et al.* 1987).

We have found in BLA pyramidal neurones that muscarinic receptor activation blocks both the M-current and a voltage-insensitive K^+ leak conductance. At depolarized membrane potentials, inhibition of both currents by carbachol contributed to the slow membrane depolarization and its underlying inward current shift. However, at membrane potentials negative to the activation threshold for I_M, the inward current shift was due solely to inhibition of the leak current. In an unclamped BLA neurone, with a resting potential of -70 mV, it seems likely that production of the slow EPSP in response to cholinergic synaptic stimulation would be largely due to inhibition of I_{Leak}, since M-current is inactive at this membrane potential. But, at less negative membrane potentials, the magnitude of the depolarizing response would be enhanced by muscarinic inhibition of the M-current.

Although it was clear that carbachol inhibited a K^+ leak conductance in BLA pyramidal neurones, this action alone could not fully explain our results. In the majority of cells, the action of carbachol produced a reversal potential estimate for I_{Leak} of -108 mV in 3·5 mM K^+ ACSF. However, this is well below the theoretical value of -97 mV for the potassium reversal potential (E_K), calculated assuming an internal K^+ concentration of 165 mM (Benson *et al.* 1988), and below the measured reversal potentials of -96 and -97 mV, respectively, for I_{AHP} and the hyperpolarization-activated inward rectifier current (I_{IR}), two other K^+ currents in BLA pyramidal neurones (M. D. Womble & H. C. Moises, unpublished observations). In addition, carbachol *increased* membrane conductance at negative membrane potentials in a few BLA neurones, yielding no clear reversal potential. These findings follow a pattern similar to that seen in hippocampal pyramidal neurones where the results were attributed to a dual action by carbachol: inhibition of a K^+ leak conductance in the cell soma, coupled with activation of a non-specific cation conductance in the dendrites (Benson *et al.* 1988). Modelling by these authors suggested a reversal potential for the dendritic conductance of -50 mV, indicating a mixed Na^+–K^+ current. The presence of similar anomalous *I–V* relationships in some BLA pyramidal neurones, coupled with the very negative reversal potential for I_{Leak}, suggests that a similar conductance may also be present in amygdaloid neurones.

The present results indicate that muscarinic receptor activation acts to inhibit both the M-current and a K^+ leak current in BLA pyramidal neurones. In unclamped neurones these effects are manifested by an increase in input resistance and a sustained depolarization, thus bringing the membrane potential closer to the

threshold level for spike generation. This cholinergically induced depolarization would in turn greatly enhance neuronal excitability, resulting in increases in the rate and duration of action potential firing and in the production of spontaneous action potentials.

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