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# SYNAPTIC INHIBITION OF THE M-CURRENT: SLOW EXCITATORY POST-SYNAPTIC POTENTIAL MECHANISM IN BULLFROG SYMPATHETIC NEURONES

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SUMMARY

1. Slow muscarinic excitatory post-synaptic currents (slow e.p.s.c.s) generated by preganglionic nerve stimuli were recorded in voltage-clamped bullfrog sympathetic neurones.

2. $I_M$ – an outward, voltage-dependent, $K^+$-current – was inhibited during the slow e.p.s.c., and membrane conductance was reduced in a voltage-dependent manner.

3. The slow e.p.s.c. was associated with reduced outward rectification in the steady-state current–voltage ($I/V$) curve at membrane potentials more positive than −60 mV, with no change in the shape of the non-rectifying part of the $I/V$ curve at more negative potential.

4. The amplitude of the slow e.p.s.c. was reduced by membrane hyperpolarization, to zero at membrane potentials equal to, or more negative than, −60 mV. The voltage sensitivity of the slow e.p.s.c. accorded with that of $I_M$.

5. It is concluded that the slow e.p.s.c. results from a selective inhibition of $I_M$.

## INTRODUCTION

Stimulation of cholinergic preganglionic fibres produces two excitatory post-synaptic potentials (e.p.s.p.s) in amphibian sympathetic neurones: a 'fast' e.p.s.p., mediated via nicotinic receptors; and a 'slow' e.p.s.p., mediated via atropine-sensitive muscarinic receptors (Nishi & Koketsu, 1968; Libet, Chichubu & Tosaka, 1968; Tosaka, Chichubu & Libet, 1968; Koketsu, 1969; Libet, 1970). The fast e.p.s.p. is analogous to the muscle end-plate potential and likewise results from the transient opening of cation-selective channels (Nishi & Koketsu, 1960; Blackman, Ginsborg & Ray, 1963; Kuba & Nishi, 1979: MacDermott, Connor, Dionne & Parsons, 1980). The slow e.p.s.p. is quite different: it has been attributed (in substantial part, at least) to the *closure* of $K^+$-channels (Weight & Votava, 1970; Kuba & Koketsu, 1976; but see Kobayashi & Libet, 1968, 1974, for a dissenting view).

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In the preceding paper (Adams, Brown & Constanti, 1982*b*) we reported a selective inhibition of the M-current ($I_M$) by exogenous muscarinic receptor agonists in bullfrog sympathetic neurones. $I_M$ is a time- and voltage-dependent $K^+$-current activated between −70 and 0 mV (Adams, Brown & Constanti, 1982*a*). The consequences of this inhibition – a sustained voltage-dependent inward (depolarizing) current, reduced input conductance and increased excitability – closely resemble the characteristic features of the slow e.p.s.p. We now report that $I_M$ is also inhibited by preganglionic stimulation and provide evidence that this underlies the slow e.p.s.p. This observation has been briefly reported in abstract form (Adams & Brown, 1980*b*).

## METHODS

Experiments were performed on voltage-clamped B neurones in isolated bullfrog lumbar sympathetic ganglia using the experimental methods described in the preceding two papers (Adams *et al.* 1982*a*, *b*), with the following modifications. (i) The $Ca^{2+}$ concentration was raised to 8 mM, to offset the high $Mg^{2+}$ concentration (10 mM). (ii) Preganglionic fibres were stimulated by drawing the descending sympathetic chain between the seventh and eighth or eighth and ninth ganglia (numbering of Ecker & Wiedersheim, 1896) into a suction electrode. This means that preganglionic B fibres should have been preferentially stimulated (Nishi, Soeda & Koketsu, 1965) so yielding a pure slow e.p.s.p. without contaminating slow i.p.s.p. (Libet *et al.*, 1968; Tosaka *et al.* 1968; Skok, 1973). In agreement, we did not detect a slow i.p.s.p. Slow excitatory post-synaptic currents (e.p.s.c.s) were evoked using repetitive or single preganglionic shocks as described in Results. When responses to single shocks were compared at different potentials , several minutes were allowed between each stimulus, since otherwise the slow e.p.s.c. tended to fade.

## RESULTS

Fig. 1 shows representative potential changes following preganglionic stimulation. Two temporally separate events are seen: the initial fast excitatory post-synaptic potential with (normally) a superimposed spike; and the subsequent slow excitatory post-synaptic potential. The latter began after a latency of some 200–300 msec, peaked at about 2 sec and lasted some 10–20 sec. This accords with the initial description of the slow e.p.s.p. in these neurones by Tosaka *et al.* (1968) and Nishi & Koketsu (1968). However, and in contrast to these earlier reports, it should be noted that repetitive preganglionic stimulation was not necessary to elicit a slow e.p.s.p: as shown in Fig. 1*A* and *C*, a clear slow e.p.s.p. was observed after only a *single* orthodromic volley, although it could usually be augmented and prolonged by applying a short train of pulses at 1–10 Hz. The slow e.p.s.p. was not a negative spike-afterpotential since it was also observed under conditions when the initial fast e.p.s.p. did not generate a spike (Fig. 1*C*). The very prolonged late slow e.p.s.p. (Nishi & Koketsu, 1968; Jan, Jan & Kuffler, 1979) was not evident in our experiments, presumably because we did not stimulate the appropriate C-fibre inputs.

Fig. 2 shows the corresponding fast and slow inward currents in a voltage-clamped neurone (fast and slow e.p.s.c.) following a single preganglionic stimulus. The slow e.p.s.c. peaked after about 2 sec and corresponded to about 0·15 nA inward current at −30 mV. In this record it is preceded by a 0·7 sec hyperpolarizing command to −60 mV, to close the M-channels. As described earlier (Adams *et al.* 1982*a*), M-channel closure is signalled by the slow inward current relaxation. On repolarizing, the

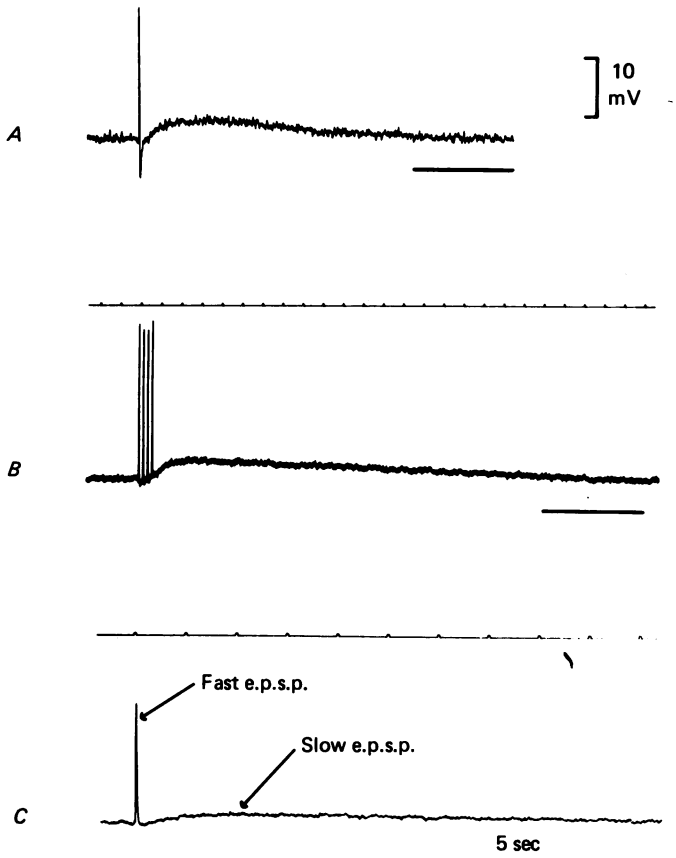


Fig. 1. Examples of excitatory potentials recorded from three bullfrog lumbar sympathetic neurones following preganglionic sympathetic chain stimulation. Single stimuli were applied in *A* and *C*, four stimuli at 5 Hz in *B*. The initial event consists of a fast e.p.s.p. with superimposed single spikes in *A* and *B* (the spikes being truncated by the recorder); in *C*, which is recorded at twice the speed, only the fast and slow e.p.s.p.s were generated, without a superimposed spike. Time traces, 1 sec.

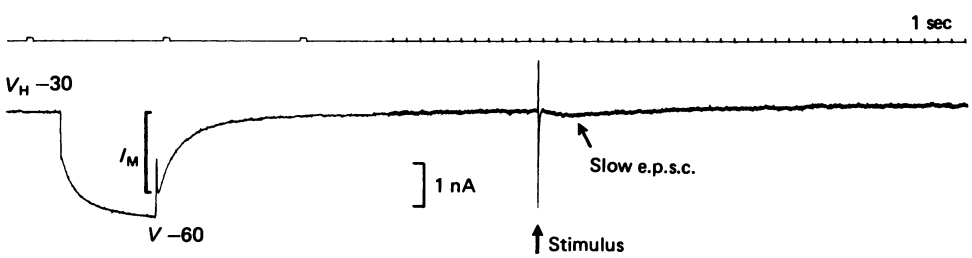


Fig. 2. Slow excitatory post-synaptic *current* (slow e.p.s.c.) evoked by a single preganglionic stimulus (at arrow) in a neurone voltage-clamped at −30 mV. The stimulus was preceded by a 0·7 sec hyperpolarizing voltage step to −60 mV, to reveal the amplitude of $I_M$ at −30 mV: this is given by the initial amplitude of the repolarizing outward relaxation (bracketed). Time is in seconds: note the recorder deceleration before applying the nerve stimulus.

M-channels re-open. The amplitude of the associated outward relaxation (bracketed) provides a measure of the steady outward M-current at −30 mV: this amounted to about 2 nA.

The effect of applying a voltage jump of this type *during* a slow e.p.s.c. is illustrated in Fig. 3. Here the slow e.p.s.c. was augmented (and prolonged) by repetitively

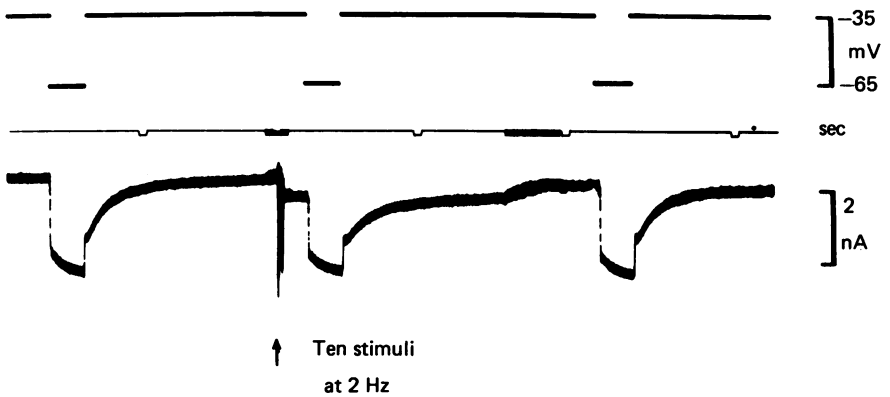


Fig. 3. Effect of repetitive preganglionic stimulation (ten shocks at 2 Hz) on steady current and on $I_M$ relaxations in a neurone voltage-clamped at −35 mV (two-electrode clamp). Note that the chart speed was slowed 100 times during the stimulus train and during recovery.

stimulating the preganglionic trunk. The amplitude of the repolarizing M-current tail is clearly reduced, from about 1·7 to 1·25 nA. This is almost precisely the magnitude of the peak inward current during the slow e.p.s.c. Two further points should be noted. (i) There is no change in the current level attained at the end of the hyperpolarizing command, i.e. there is no net inward current at −65 mV when the M-channels are shut. (ii) The instantaneous current step at the beginning of the voltage jump is reduced whereas that at the end is hardly changed. The former measures the membrane chord conductance at −35 mV, comprising the leak conductance plus the conductance of the open M-channels, whereas the latter measures the leak conductance at −65 mV, with very little M-conductance component. Hence, there is clearly a reduced chord conductance but only at a voltage when the M-conductance contributes to the chord conductance. These effects exactly imitate the effect of adding muscarine (see fig. 1 in Adams *et al.* 1982*b*), and indicate a selective reduction of $I_M$.

To study the effect of preganglionic stimulation on the current–voltage curve a series of voltage-commands like those in Fig. 3, but of varying amplitude, were applied before and during a series of repeated trains of preganglionic impulses. The trains were applied at intervals in such a way as to obtain an approximately constant inward current at −30 mV. The amplitude of the current excursions at the end of each voltage-command (1 sec duration) were plotted against command potential, giving the 'steady-state' current–voltage curves shown in Fig. 4. Again, the effect of preganglionic stimulation agrees closely with that of muscarine (cf. fig. 2 in Adams *et al.* 1982*b*): the outward rectification positive to −60 mV associated with opening

of M-channels is reduced, whereas the linear part of the $I/V$ curve at more negative potentials is unchanged. The change in the $I/V$ curve accords with that expected for a constant 53 % inhibition of $I_M$ throughout its voltage range (continuous lines).

The dotted line in Fig. 4 shows how the steady current induced by preganglionic stimulation, measured by the displacement of the $I/V$ curves, would be expected to

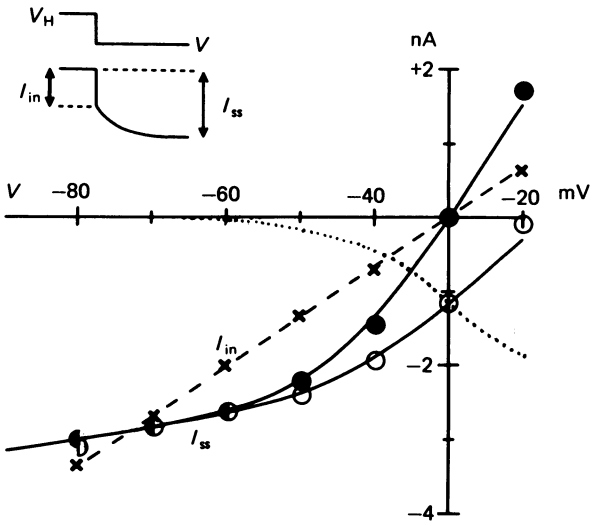


Fig. 4. Current–voltage curves constructed from an experiment like that illustrated in Fig. 3, but using a range of voltage commands before (●) and during (○) repeated trains of preganglionic stimulation. The trains produced a consistent inward current of about 1·2 nA at the holding potential of −30 mV. Circles show the current level attained at the end of the 1 sec voltage-commands ($I_{ss}$), plotted against command potential. Currents are set to zero at −30 mV before stimulation. The continuous curves are constructed on the assumption (see Adams *et al.* 1982*a*, *b*) that the total membrane current is the sum of a voltage-independent leak current ($I_L$) and a voltage-dependent M-current ($I_M$), given by

$$I_L = G_L(V - V_L);$$

$$I_M = G_M(V - V_M);$$

and

$$G_M = \bar{G}_M[1 + \exp 0{\cdot}1(-35 - V)]^{-1}$$

where $V$ = membrane potential, $V_L = -10$ mV, $V_M = -75$ mV (see below), $G_L = 15$ nS, and $\bar{G}_M = 75$ nS before stimulating the nerve and 35 nS during nerve stimulation. The crosses show the instantaneous $I/V$ curve before preganglionic stimulation ($I_{in}$), measured from the amplitudes of the instantaneous current steps accompanying each voltage command. This crosses the steady-state $I/V$ curve at the reversal potential for $I_M$ ($V_M = -75$ mV). The dotted line shows the apparent net inward current produced by preganglionic stimulation, i.e. the difference between the two continuous curves.

change as the membrane holding potential was adjusted: it should decrease with hyperpolarization, reaching zero at about −70 mV. Fig. 5 shows an experimental test of this prediction, using single preganglionic shocks. The slow e.p.s.c. became negligible at membrane potentials of −50 mV and greater. The records on the right show current relaxations produced by a sequence of voltage-commands from −30 mV to the voltage indicated. The relaxations associated with M-channel closure

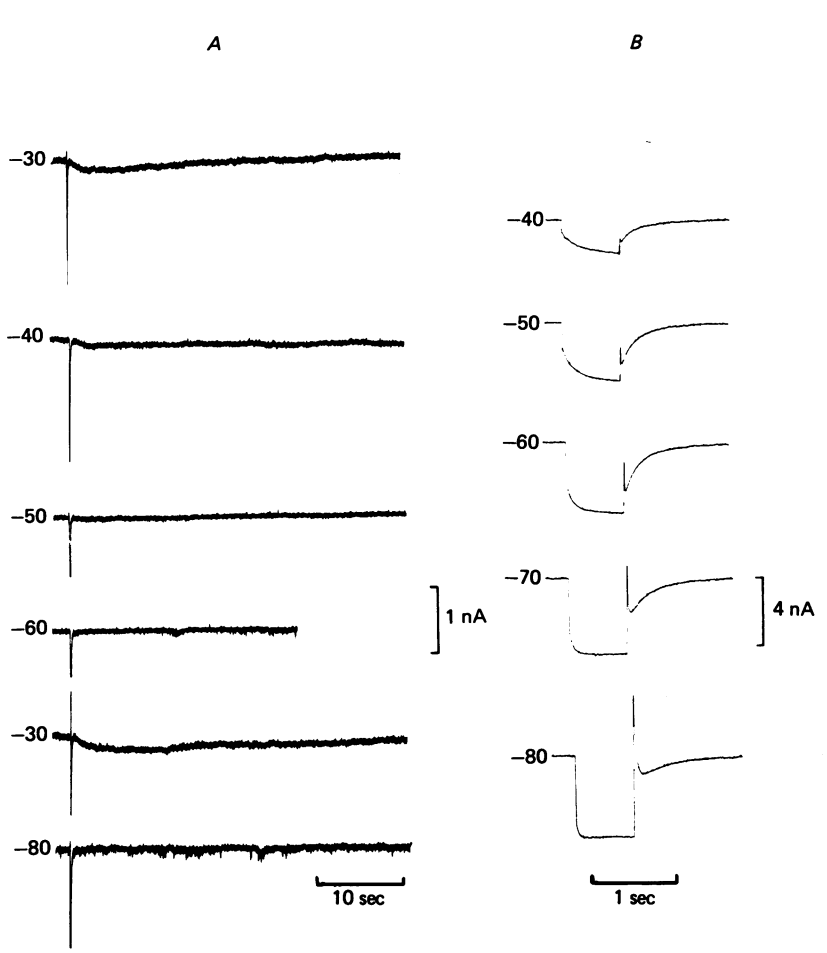


Fig. 5. *A*, slow e.p.s.c.s following single preganglionic shocks recorded at different holding potentials, in the order indicated. The initial currents are fast e.p.s.c.s; the small base line deflexions at −80 mV are miniature fast e.p.s.c.s. The amplitudes of the fast e.p.s.c.s are variably truncated (off-scale).

*B*, currents generated by a series of voltage steps to the command potentials indicated from a holding potential of −30 mV (cf. Fig. 2). Single micro-electrode voltage clamp. The upward current 'spikes' are A-currents (see Adams *et al.* 1982*a*).

remained inward down to −80 mV, indicating a reversal potential for $I_M$ beyond this: hence, the absence of a slow e.p.s.c. at −60 and −80 mV was not due to the absence of a $K^+$-current driving force, but to the fact that the M-channels were already shut.

Fig. 6 summarizes the voltage dependence of the slow e.p.s.c. in three experiments (including that illustrated in Fig. 5). The observed voltage dependence accords quite well with that expected for selective $I_M$ inhibition (continuous line).

## DISCUSSION

The present experiments indicate a single mechanism for the slow e.p.s.c.: a selective inhibition of that voltage-dependent $K^+$-current we have previously termed

the M-current (Adams *et al.* 1982*a*). Evidence for this may be summarized as follows. (i) $I_M$ relaxations associated with the voltage-dependent opening and closing of M-channels are reduced during the slow e.p.s.c. (ii) Membrane chord conductance is reduced at voltages where the M-conductance ($G_M$) is substantial but not where $G_M$ is negligible. (iii) The slow e.p.s.c. shows the same dependence on membrane potential

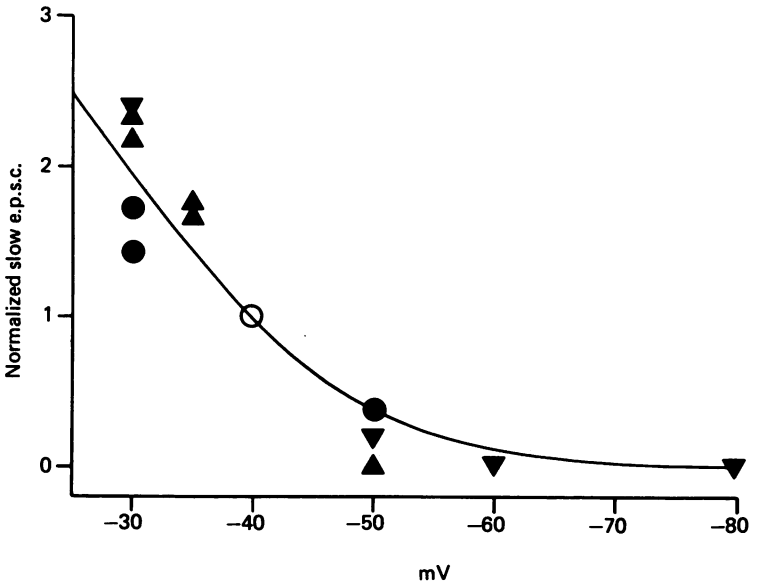


Fig. 6. Relative amplitude of the slow e.p.s.c. recorded at different holding potentials, normalized about that at −40 mV. Each symbol (●, ▲, ▼) represents a different neurone clamped with a single micro-electrode. The continuous curve shows the relationship between slow e.p.s.c. amplitude and membrane potential ($V$) if the slow e.p.s.c. resulted entirely from $I_M$ inhibition, assuming that

$$I_M = G_M\,(V-(-90)) \text{ and } G_M = \bar{G}_M\,[1-\exp 0{\cdot}1(-35-V)] \quad \text{(see Fig. 4).}$$

as does $I_M$. Under our experimental conditions the slow e.p.s.c. generated at −30 mV by a single preganglionic stimulus corresponded to the closure of some 5–10% of the open M-channels; with repeated trains of stimuli up to 50% of the channels could be closed. The equivalent circuit model of Adams *et al.* (1982*a*) suggests that, from a rest potential of between −50 and −55 mV, slow depolarizations of up to 5 mV would be expected from this amount of M-channel closure.

Thus, we confirm Weight & Votava's (1970) conclusion that the amphibian slow e.p.s.p. results from a reduced $K^+$-conductance, but with the refinement of defining the species of $K^+$-conductance affected. Selective depression of $I_M$ explains the peculiar voltage sensitivity of the slow e.p.s.p. (an *increase* with membrane depolarization) initially reported by Kobayshi & Libet (1968: their fig. 3) and by Nishi, Soeda & Koketsu (1969). However, deviations from the predicted behaviour shown in Fig. 6 have also been reported. *Firstly*, Nishi *et al.* (1969) and Kuba & Koketsu (1976) reported a further increase in the amplitude of the slow e.p.s.p. in a proportion of neurones (labelled 'type 1') during membrane hyperpolarization beyond −70 mV, which they ascribed to an additional inward current unrelated to the fall in $G_K$. This we have not observed: the slow e.p.s.c. recorded

under voltage clamp fell to zero at membrane potentials > −60 mV, like the behaviour of the slow e.p.s.p. in Kuba & Koketsu's 'type 3' cell (Fig. 6: cf. fig. 3 in Kuba & Koketsu, 1976). In the preceding paper (Adams *et al.* 1982*b*), we reported a rare inward current at potentials positive to −70 mV following brief application of a muscarinic agonist, but this declined at more depolarized levels. Since the normal resting potential of these cells is between −50 and −55 mV, such a current is unlikely to contribute significantly to the 'normal' slow e.p.s.p.; and in our experiments there was a good quantitative match between the amplitude of the slow e.p.s.c. and the amount of $I_M$ inhibition (see Figs. 3 and 4). A *second* deviation from simple $I_M$ inhibition is the reported *reversal* of the slow e.p.s.p. at membrane potentials beyond $E_K$ (Weight & Votava, 1970). Since $E_K$ in normal Ringer solution is between −80 and −90 mV, M-channels are shut at this voltage and no current flow can be generated by their closure. However, Kobayashi & Libet (1974) and Kuba & Koketsu (1976) were unable to confirm slow e.p.s.p. reversal and suggested that this might have resulted from contamination by the slow i.p.s.p.

This mechanism of e.p.s.p. generation is an interesting example of a type of transmitter action in which the transmitter does not open new ionic channels but intead modifies a pre-existing channel normally controlled by membrane voltage. Other examples of such a mechanism include the control of cardiac currents by autonomic nerves (see Noble, 1979; Giles & Shibata, 1981) and the synaptic inhibition of voltage-sensitive inward and outward currents in certain invertebrate neurones (Wilson & Wachtel, 1978; Klein & Kandel, 1980).

The present experiments show that this concept can now be extended to the vertebrate nervous system. In fact, $I_M$ inhibition may be quite a widespread mechanism for synaptic excitation. Thus, cholinergic agonists also inhibit M-currents in mammalian sympathetic neurones (Constanti & Brown, 1981) and in mammalian hippocampal neurones (Adams, Brown & Halliwell, 1981), suggesting that a comparable mechanism underlies cholinergic synaptic excitation at these loci. M-currents are also sensitive to some peptides (LHRH in bullfrog sympathetic neurones: Adams & Brown (1980*a*); angiotensin rat sympathetic neurones: Constanti & Brown (1981); substance P in cultured spinal neurones, R. L. MacDonald, unpublished observations), so $I_M$ inhibition may also explain some forms of slow peptidergic synaptic excitation.

As pointed out in the preceding paper (Adams *et al.* 1982*b*), the essential result of $I_M$ inhibition is not necessarily the direct generation of cell discharge by an excitatory post-synaptic potential, but rather the facilitation of the recipient neurone's response to other forms of synaptic or non-synaptic excitation or, using a commonly applied vernacular for synaptic transmission, a 'gain control' rather than a 'switch'. One can envisage how a concurrent activation of a 'slow', $I_M$ inhibiting input could facilitate and sustain a neurone's response to another 'fast' input, and how significant this might be in the hippocampus or cerebral cortex. It is rather more difficult to understand its role in frog sympathetic ganglia, which have a one-to-one input–output relationship, and in which both 'slow' and 'fast' transmitter are one and the same substance released from the same preganglionic fibre. Since the slow e.p.s.c. increases with repetitive stimuli, one possibility is that it provides a mechanism for safeguarding transmission in neurones in which $I_M$ would normally exert a strong limitation on sustained repetitive discharge. We know of no substantive evidence that this is so in frog ganglia; however, in mammalian ganglia atropine depresses the delayed facilitation observed between 1 and 20 sec after a conditioning train of preganglionic shocks (Libet, 1964; Brimble & Wallis, 1974) and reduces the compound action potential amplitude during repetitive stimulation (Libet, 1974), just in the manner

expected if the maintenance of excitability during and after stimulus trains *was* dependent upon muscarinic inhibition of $I_M$.

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