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# Substance P inhibits the M-current in bullfrog sympathetic neurones

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Substance P (SP, 2.5–10 μM) was applied by rapid bath perfusion to bullfrog lumbar sympathetic neurones *in vitro*, voltage-clamped through a single micro-electrode. In unclamped cells, SP produced a depolarization accompanied by an increase in apparent input resistance. Under voltage-clamp a voltage-dependent inward current was induced by SP, during which the time-dependent relaxations induced by square voltage commands were inhibited. It is concluded that SP inhibits the M-current ($I_M$), a species of voltage-dependent $K^+$-current, and that $I_M$-inhibition was the primary cause of the inward current and membrane depolarization in the cells tested.

**Introduction** The M-current ($I_M$) is a time- and voltage-dependent $K^+$-current which, in bullfrog sympathetic neurones, is activated between − 70 mV and 0 mV, and contributes the major component of outward membrane current between rest potential and spike threshold (Brown & Adams, 1980; Adams, Brown & Constanti, 1982a). Inhibition of $I_M$ is the primary cause of the depolarizing action of muscarinic acetylcholine-receptor agonists and of the cholinergic slow excitatory postsynaptic potential in these cells (Adams, Brown & Constanti, 1982b; Adams & Brown, 1982).

$I_M$ is also inhibited by mammalian luteinizing hormone releasing hormone (LHRH) (Adams & Brown, 1980; see also Katayama & Nishi, 1982). This, or rather a closely-related peptide (Eiden & Eskay, 1980; Eiden, Loumaye, Sherwood & Eskay, 1982), is the transmitter responsible for the non-cholinergic late slow excitatory potential in bullfrog sympathetic neurones (Jan, Jan & Kuffler, 1979; 1980; Kuffler, 1980; Jan & Jan, 1982). Nishi, Katayama, Nakamura & Ushijima (1980) and Jan & Jan (1982) have reported that the depolarizing action of LHRH is closely imitated by substance P (SP), albeit acting via a different receptor. We have therefore tested whether SP, like LHRH, also inhibits $I_M$.

**Methods** Ninth and tenth lumbar sympathetic ganglia were isolated from bullfrogs (*Rana catesbiana*), briefly digested with 1% trypsin (Sigma type II) and maintained in flowing Ringer solution at ambient temperature (about 22°C). The composition of the Ringer solution was (mM): NaCl 115, KCl 2.5, $CaCl_2$ 2, $MgCl_2$ 10, and Tris-base 2.5 (buffered to pH 7.2 with HCl). Large B-cells were impaled with a single 3 M KCl-filled microelectrode and voltage-clamped using a Dagan 8100 sample-and-hold preamplifer, switched between current injection and voltage recording at 3 kHz with a 50% duty cycle. The recorded voltage usually attained $>90\%$ of the applied command; measured voltages refer to recorded voltage, not command voltage. Two samples of substance P were used, from Peninsula Biochemicals and Cambridge Research Biochemicals; their effects appeared identical. LHRH was obtained from Peninsula Biochemicals.

**Results** SP (2.5 to 10 μM) was applied to 16 neurones in ganglia from two bullfrogs. Twelve showed a clear response of the type described below, although the sensitivity varied; four showed no response at 10 μM. Two of the latter were also unresponsive to 10 μM LHRH (LHRH was not tested on the other two); these unresponsive cells also showed an unusually delayed response to muscarine.

Figure 1, showing the effects of SP on a single neurone, fully exemplifies the positive effects seen in all other cells. Under 'current-clamp' (Figure 1a), SP produced a reversible depolarization accompanied by an increased 'input resistance' (as measured by the peak voltage deflexion produced by constant hyperpolarizing current injections) and an increased tendency to anode-break discharges. This closely resembles the effect of muscarinic agonists (cf. Brown & Adams, 1980; Adams *et al.*, 1982b).

To test for M-current inhibition, the cell was voltage-clamped at a holding potential of − 30 mV so that a substantial fraction of M-channels were open, generating a steady outward current, and then subjected to square hyperpolarizing commands to − 56 mV (Figure 1b upper record). M-channels close during the hyperpolarization and the consequent

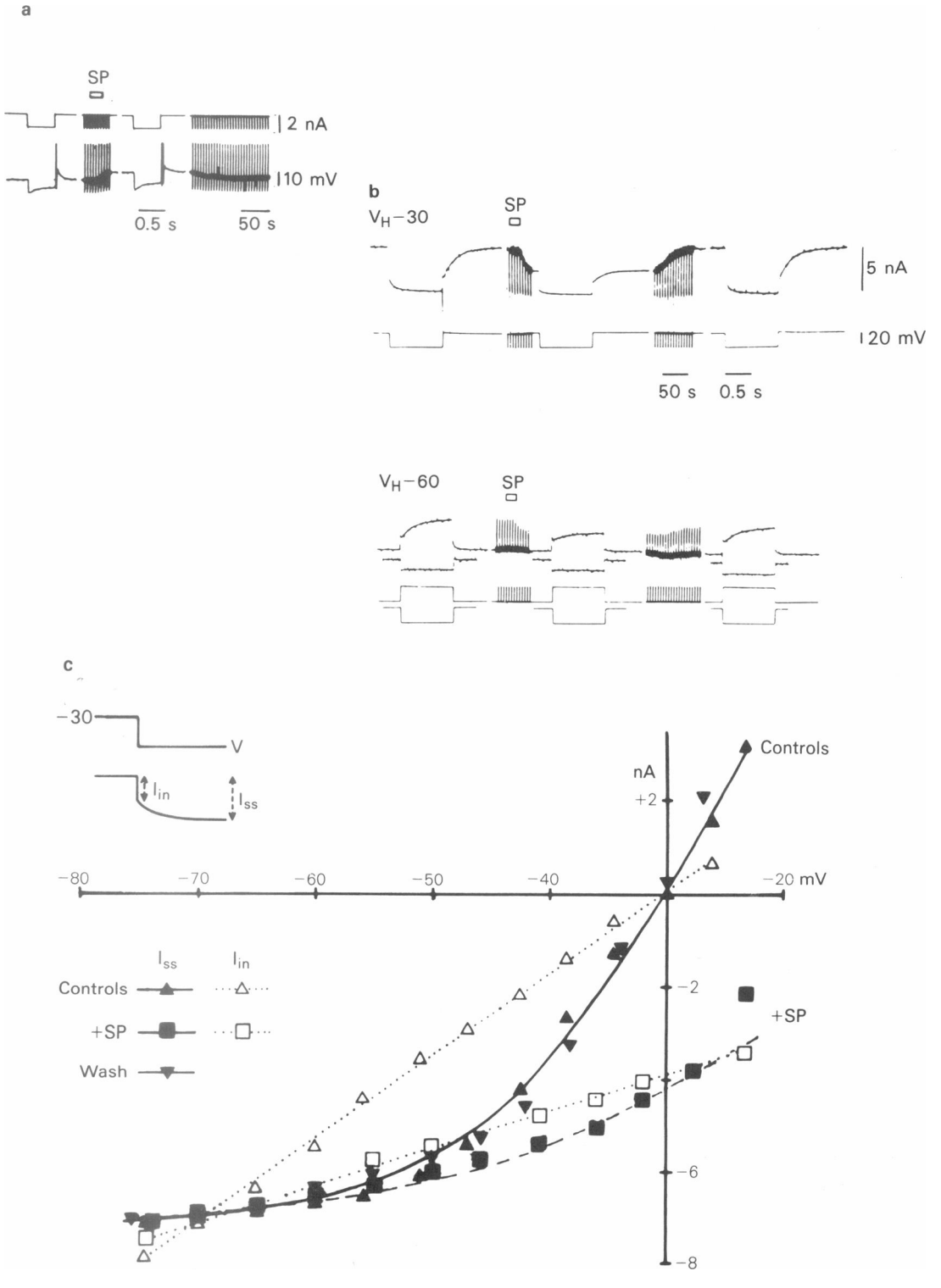
a
SP
2 nA
10 mV
0.5 s
50 s
b
$V_H$−30
SP
5 nA
20 mV
50 s
0.5 s
$V_H$−60
SP
c
−30
V
$I_{in}$
$I_{ss}$
Controls
nA
+2
−80
−70
−60
−50
−40
−20 mV
$I_{ss}$
$I_{in}$
Controls
+SP
Wash
−2
+SP
−6
−8

**Figure 1** Effects of substance P (SP) 10 $\mu$M and luteinizing hormone releasing hormone (LHRH) 10 $\mu$M recorded in a single bullfrog lumber sympathetic neurone (estimated diameter 38 $\mu$m). In each record current is displayed on the upper channel and membrane potential on the lower channel; the recorder speed was intermittently accelerated 100 × to display current and voltage transients. (**a**) Response to SP, applied for 20s recorded under 'current-clamp' at a resting potential of −37 mV. (**b**) Responses to similar (20s) applications of SP recorded under voltage-clamp at holding potentials of −30 mV (upper records) and −60 mV (lower records: current and voltage baselines are offset for hyperpolarizing voltage commands). (**c**) Current/voltage curves obtained before (▲, △) and during (■, □) sustained SP-perfusion, and after washing out the SP (▲, △), measured from the current excursions generated by a series of increasing 1s voltage commands from a holding potential of −30 mV. Solid symbols ($I_{ss}$) show 'steady-state' currents, measured as the displacement of the current at the end of each voltage command from the initial holding current; open symbols ($I_{in}$) show the initial 'instantaneous' current excursions accompanying the voltage command-steps. (The current-clamp records in (**a**) were obtained after the voltage-clamp tests; the low resting potential in (**a**) therefore reflects some cell deterioration, but the effect of SP depicted in (**a**) replicates that seen in other cells).

The smooth curves in (**c**) are drawn assuming two parallel components of resting membrane conductance, a constant leak conductance $G_L$ with a reversal potential of −10 mV, and a voltage-dependent M-conductance $G_M$ following the formulation:

$$G_M = G_M \left\{1 + \exp\left[0.1\,(-35 - V)\right]\right\}^{-1}$$

where $G_M$ is the maximum value of $G_M$ when all M-channels are open and V is the membrane potential (see Adams *et al.*, 1982a). Total membrane current is then given by

$$I = I_L + I_M = G_L\,(V + 10) + G_M\,(V - V_{rev})$$

were $V_{rev}$ is the reversal potential for $I_M$. $V_{rev}$ is given by the potential (−70 mV) at which the 'instantaneous' I/V curve (dotted line) intersects the steady-state curve; in other words, where the $I_M$ closure-relaxation reversed from inward to outward. Conductances used for computation were: $G_L$ = 20 nS (estimated from a line drawn through the last three points negative to −60 mV); and $G_M$ = 250 nS before SP and 78 nS during SP. (Note: potentials are recorded potentials. There was a small drift, of about + 2.5 mV, in the recorded holding potential in SP solution.)

time-dependent decline in the outward M-current is manifested by a slow inward relaxation (see Adams *et al.*, 1982a). On repolarizing, the M-channels reopen, giving rise to a slow outward relaxation. Assuming that most of the M-channels had closed by the end of the hyperpolarization, the initial amplitude of the repolarizing outward relaxation gives an approximate measure of the contribution of $I_M$ to the steady membrane current at the holding potential ($= I_M(V_H)$). When 10 $\mu$M SP was added to the perfusion fluid, a large inward current developed at the holding potential, and $I_M(V_H)$ declined by some 70%. In contrast, there was little change in the absolute current level attained at the end of each hyperpolarizing command, implying that SP was ineffective at a potential where the M-channels were shut. This was confirmed by resetting the holding potential to −60 mV (lower record of Figure 1b). The M-current is now deactivated, but was intermittently reactivated by depolarizing commands, appearing as an outward relaxation. This outward relaxation was reduced by SP, showing that SP still inhibited the M-current. However, there was no change in steady holding current while the M-current was deactivated; nor was the leak conductance changed, as measured from the current excursions produced by hyperpolarizing voltage steps.

Figure 1c shows the effect of SP on the instantaneous and steady-state current/voltage (I/V) curves. The instantaneous I/V curves (dotted lines) generated from the holding potential of −30 mV were approximately linear and measure instantaneous current flow through the leak channels and through those M-channels open at −30 mV. SP reduced the slope of the curves from 175 nS to 78 nS: assuming a constant leak conductance of 20 nS (see above), this may be attributed to a fall in the total conductance of the M-channels ($G_M$), from 155 to 48 nS. The most obvious effect of SP on the steady-state I/V curves was a dramatic reduction in the very pronounced outward rectification observed at potentials positive to −70 mV. The latter results from the increasing contribution of outward $I_M$ to the total membrane current. The effect of SP on this outward rectification could be accounted for simply by assuming that SP reduced $G_M$ by a constant 69% at all potentials, without changing its voltage-sensitivity (see legend to Figure 1).

The effects of SP in this, and 4 other cells, were qualitatively replicated by LHRH; in these tests SP was appreciably the more potent.

**Discussion** These experiments show that SP, like LHRH, can inhibit the M-current ($I_M$) in bullfrog sympathetic ganglia. In this sample of cells $I_M$-inhibition alone appeared sufficient to account for the inward current produced by SP between −60 and −30 mV, and hence for the depolarization of these cells. Subsequent tests (S.W. Jones, unpublished observations) have revealed, in a fraction of cells, an additional and variable component of inward current, accompanied by a conductance increase, on

hyperpolarizing the cell beyond −60 mV (i.e. outside the M-current range). This accords with observations on LHRH by Sejnowski (1982) and by Katayama & Nishi (1982). Notwithstanding, $L_M$-inhibition was the most striking and prevalent feature of the responses to SP. If, as indicated by the experiments of Jan & Jan (1982), SP and LHRH act via different receptors, then at least five distinct types of agonist are capable of inducing M-channel closure in bullfrog sympathetic neurones: muscarinic agonists, LHRH, SP, uridine nucleotides and adenosine nucleotides, and $Ba^{2+}$ ions (see Adams & Brown, 1980; Brown, Constanti & Adams, 1981; Adams *et al.*, 1982; Akasu, Hirai & Koketsu, 1982). This suggests a complex linkage between receptor and channel closure: the nature of this linkage remains unknown.

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