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EFFECTS OF DIVALENT CATIONS AND NORMORPHINE ON SPONTANEOUS EXCITATORY JUNCTION POTENTIALS IN THE MOUSE VAS DEFERENS

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1 Excitatory junction potentials (e.j.ps) occurring spontaneously or evoked by nerve stimulation were recorded intracellularly from smooth muscle cells of the mouse isolated vas deferens.

2 The amplitude of the evoked e.j.ps and the amplitude and frequency of spontaneous e.j.ps were measured before and during application of normorphine or solutions which might be expected to change the influx of calcium ions into the nerve terminals.

3 Spontaneous e.j.ps could be recorded even in solutions which contained tetrodotoxin (1 μM), no added calcium and EGTA (1 mM). A four fold increase in calcium concentration from 1.25 to 5 mM greatly increased the amplitude of the evoked e.j.ps but had no effect on the amplitude or frequency of the spontaneous e.j.ps.

4 Magnesium (12 mM) and cobalt (4 mM) both greatly reduced the evoked e.j.ps and also reduced the frequency of spontaneous e.j.ps.

5 Normorphine (2 μM) reduced the amplitude of the evoked e.j.p. by 70% but had no effect on the amplitude or frequency of spontaneous e.j.ps.

6 It is suggested that normorphine inhibits noradrenaline secretion from nerve varicosities by a mechanism different from that of magnesium and cobalt. One possibility is a block of action potential propagation along varicose fibres.

Introduction

Opiates inhibit the release of noradrenaline evoked by electrical stimulation of the postganglionic sympathetic fibres of the mouse vas deferens (Henderson, Hughes & Kosterlitz, 1972; Hughes, Kosterlitz & Leslie, 1975). This opiate action can be readily measured as a reduction in the amplitude of the excitatory junction potential (e.j.p.) evoked by nerve stimulation (Henderson & North, 1976). It has been suggested that opiates may depress such evoked release of noradrenaline by interfering directly with the ability of calcium to mediate stimulus secretion coupling (Bennet & Lavidis, 1980; Illes, Zieglgänsberger & Herz, 1980), perhaps by inhibiting voltage-dependent calcium currents or by preventing the association of intracellular calcium with the site which triggers noradrenaline release. Although there is little direct evidence for this (Milner, North & Vitek, 1981), the possibility is favoured by the lack of effects of opiates on spontaneous e.j.ps (Henderson, 1976; Bennett & Lavidis, 1980; Ito & Tajima, 1980) and analogy with the somatic neuromuscular junction at which spontaneous transmitter release differs from evoked release in being less sensitive to the extracellular calcium concentration. The present experiments sought to investigate more fully the effects and interactions of normorphine and calcium on the spontaneous e.j.p.

Methods

Vasa deferentia were removed from NMRI mice which had been killed by a blow to the head. The mesenteric sheath was removed and a 10–15 mm length of vas was pinned in a 1 ml tissue bath. The vas was perfused at 1.5 ml/min by a heated (37°C) solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, bubbled with 95% O_2/5% CO_2. Intracellular recordings were made

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from the smooth muscle with microelectrodes filled with 2.5 M KCl having d.c. resistances of 50–80 MΩ. Evoked e.j.ps were induced by stimulating intramural nerve fibres with platinum electrodes, using 1 ms duration pulse of sufficient intensity to give an e.j.p. of 20 mV (or 10 mV when the effects of high calcium were observed). E.j.ps were evoked at a frequency not exceeding 0.003 Hz. Spontaneous e.j.ps could be recorded in all impalements, but only those cells were used where the control frequency exceeded 0.25 Hz. Amplitudes and frequencies of spontaneous e.j.ps were measured from photographs of the oscilloscope recording taken over a 3–5 min period. The amplitude histograms of the spontaneous e.j.ps were markedly skewed, many potentials being difficult to distinguish from the recording noise. Because this noise varied among recordings, but never exceeded 1 mV, all spontaneous e.j.ps having amplitudes of less than 1 mV were discarded.

Normorphine hydrochloride (Dr A.E. Jacobsen) was applied by changing the perfusing solution to one which differed only in its content of the drug. Solutions of changed ionic composition were made by adjusting the NaCl content to preserve the osmolarity. Calcium-free solutions contained ethylene glycol-bis (β-amino-ethyl ether)-N,N′-acetic acid (EGTA) (1 mM) and tetrodotoxin (1 μM). Cobalt containing solutions had a reduced (10 mM) $NaHCO_3$ content and adjusted NaCl concentration. Solutions of different ionic composition or those containing normorphine were applied for 8–10 min, during which time their effects reached a steady state.

Results

General observations

The resting potential of the smooth muscle cells was −50 to −70 mV, the value at impalement remaining constant throughout the period of recording from a single cell (up to 4 h). Spontaneous e.j.ps could be recorded from all cells (Figure 1), their frequency and amplitude being considerably greater in the more superficial muscle fibres than in the deeper cells. As has been described (Burnstock & Holman, 1962; Furness, 1970), the time course of the spontaneous e.j.ps was always much faster than that of an evoked e.j.p. of the same amplitude. The amplitude distribution of the spontaneous e.j.ps was heavily skewed toward those of low amplitude (Figure 2). Tetrodotoxin (1 μM) did not alter the amplitude histogram of the spontaneous e.j.p. (Figure 2); this concentration completely but reversibly abolished the evoked e.j.p.

Effect of changed ion concentrations

Spontaneous e.j.ps could still be recorded in solutions which contained no added calcium and EGTA (1 mM). However, this solution depolarized the muscle cells by 8–12 mV and it was therefore not possible to determine quantitatively the effects on amplitude and mean frequency. Reducing the calcium concentration from 2.5 to 1.25 mM caused no change or a slight fall (<5 mV) in membrane potential. The

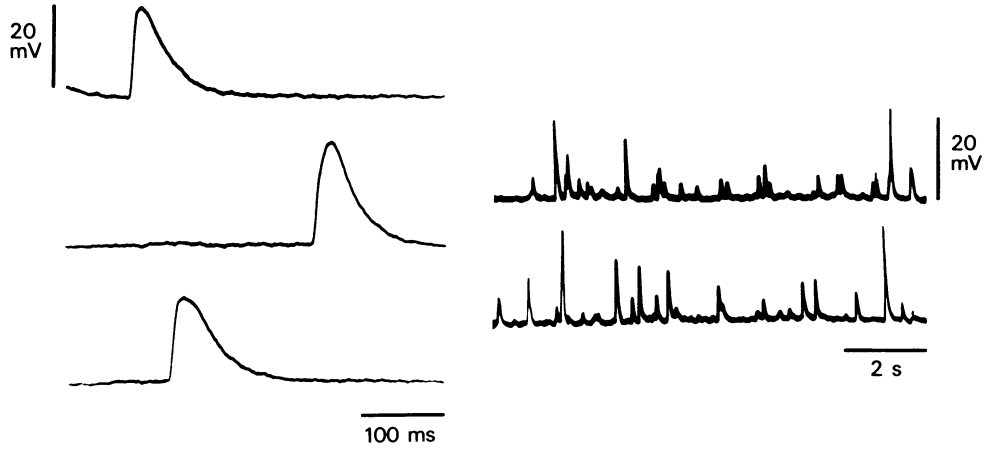

Figure 1 Intracellular recordings of spontaneous e.j.ps in a smooth muscle cell of the mouse vas deferens. Left and right panels show recordings from the same muscle cell on different time scales.

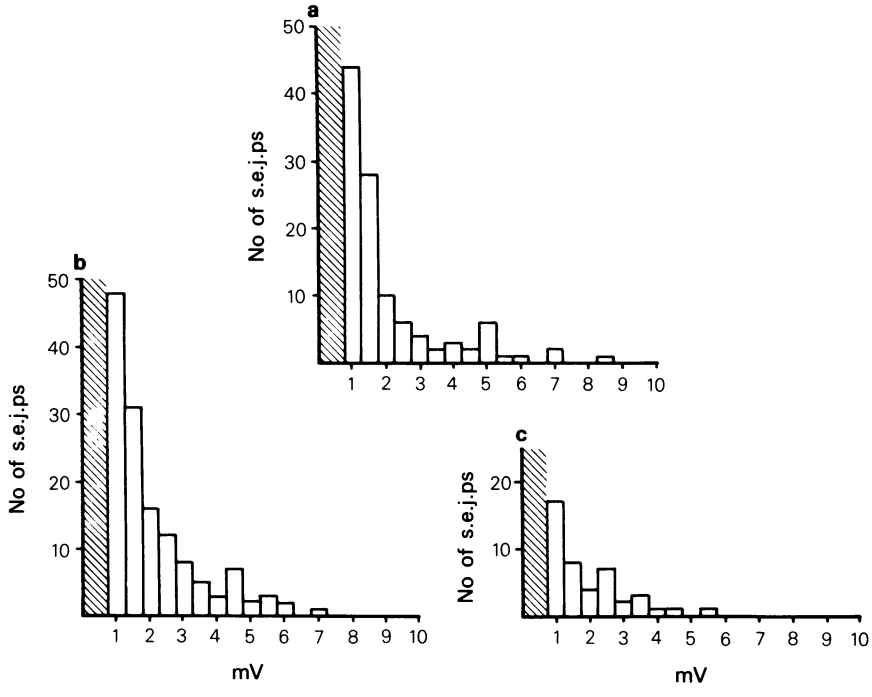

Figure 2 Persistence of spontaneous e.j.ps (s.e.j.ps) in one smooth muscule cell in a nominally calcium-free Krebs solution. (a) Amplitude histograms of spontaneous e.j.ps in a normal solution containing 2.5 mM Ca^{2+}. (b) In 2.5 mM Ca^{2+} and 1 μM tetrodotoxin. (c) In a solution containing no added Ca^{2+}, 1 mM EGTA and 1 μM tetrodotoxin. Each histogram represents the distribution of spontaneous e.j.p. amplitudes over a period of 5 min, starting after the drug had been present for 10 min.

Table 1 The effect of normorphine and divalent cations on the mean amplitude and frequency of spontaneous e.j.ps and the amplitude of evoked e.j.ps

	Relative change (%)		
	Spont s.e.j.ps		*e.j.ps*
Treatment	Frequency	Amplitude	Amplitude
Tetrodotoxin 1 μM	−4.6 ± 9.4 (4)	−4.3 ± 3.4 (4)	−100.0 (4)
Normorphine 2 μM	−2.8 ± 3.9 (5)	−4.2 ± 5.2 (5)	−72.6 ± 3.4*** (5)
Ca^{2+} 5 mM	+3.8 ± 8.5 (4)	−2.0 ± 6.3 (4)	+85.9 ± 10.3** (5)
Ca^{2+} 1.25 mM	−10.0 ± 5.7 (5)	−7.9 ± 6.9 (5)	−67.1 ± 4.2** (4)
Mg^{2+} 12 mM	−76.0 ± 6.8* (4)	−5.0 ± 6.3 (4)	−77.1 ± 4.6** (5)
Co^{2+} 4 mM	−68.0 ± 2.4** (4)	−6.2 ± 3.5− (4)	−100.0 (4)

Values are means ± s.e.mean of number of experiments indicated.
The mean amplitude of the spontaneous e.j.ps from 26 experiments on different preparations was 2.03 ± 0.08 mV, and the mean frequency was 0.63 ± 0.05 Hz (mean ± s.e.mean; $n = 26$).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

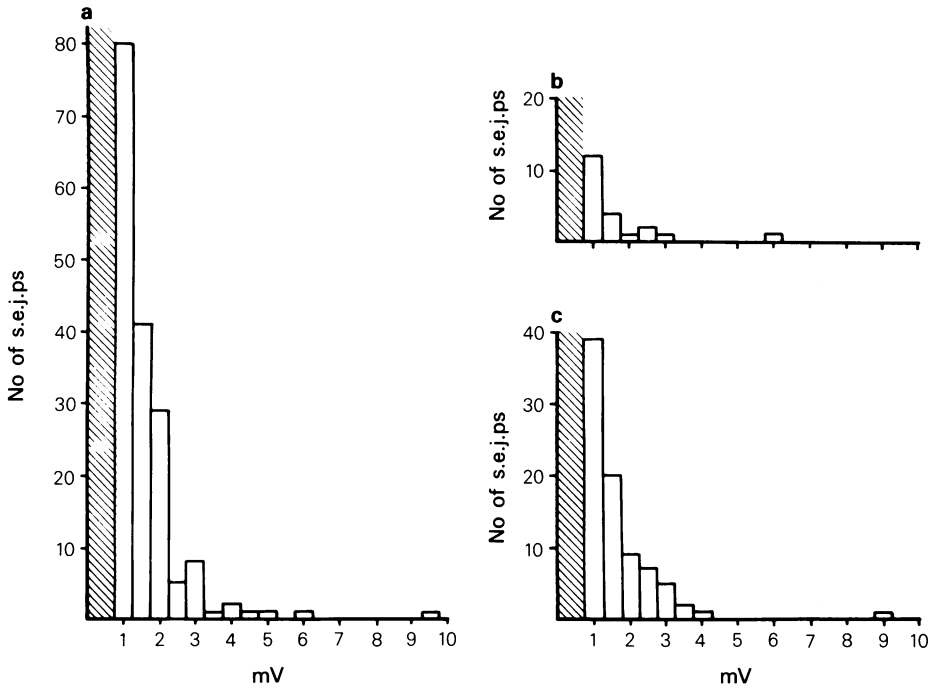

Figure 3 The inhibition by magnesium of the amplitude and frequency of spontaneous e.j.ps (s.e.j.ps) from one smooth muscle cell. (a) Amplitude histograms of spontaneous e.j.ps in a normal Krebs solution containing 1.2 mM Mg^{2+}; (b) After an increase of the Mg^{2+} concentration to 12 mM. (c) Partial recovery after returning to normal Krebs solution containing 1.2 mM Mg^{2+}. Each histogram represents the distribution of spontaneous e.j.p. amplitudes over a period of 5 min, after the new magnesium concentration had been present for 10 min.

evoked e.j.p. was reduced in amplitude to 30% of its control value (Table 1) but there was no change in the amplitude or frequency of spontaneous e.j.ps. Increasing the calcium concentration from 2.5 to 5 mM caused no change or a slight increase (<5 mV) in membrane potential. The evoked e.j.p. was increased in amplitude to approximately double its control value (Table 1) but the amplitude and frequency of spontaneous e.j.ps were not changed. TTX (1 μM) was present in those experiments in which calcium concentrations were reduced.

Magnesium chloride (12 mM) and cobalt chloride (4 mM) did not affect the resting potential of the smooth muscle cells, but each markedly depressed the amplitude of the evoked e.j.p. (Table 1). A presynaptic action in depressing transmitter release similar to that occurring at the somatic neuromuscular junction (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Weakly, 1973) was suggested by the finding that neither magnesium nor cobalt depressed the amplitude of the spontaneous e.j.p. However, both magnesium (Figure 3) and cobalt greatly reduced the frequency of the spontaneous e.j.ps (Table 1).

Raising the potassium concentration from 5.7 to 12 mM depolarized the smooth muscle cells by 8–12 mV, so that comparisons of spontaneous e.j.p. amplitude and frequency with those in control solutions were of limited value.

Effects of normorphine

Normorphine (2 μM) had no effect on the amplitude or frequency of spontaneous e.j.ps (Figure 4, Table 1). This concentration reduced the amplitude of the evoked e.j.p. to about 30% of its control. Normorphine also had no effect on the amplitude or frequency of the spontaneous e.j.p. in solutions which contained 12 mM potassium.

Discussion

Spontanous release of noradrenaline appears to be qualitatively analogous to spontaneous release of acetylcholine at the neuromuscular junction (Hubbard, 1961; Hubbard, Jones & Landau, 1968) in several respects. First, both occur, but at much reduced frequency, in the complete absence of extracellular calcium ions. Second, the frequency of

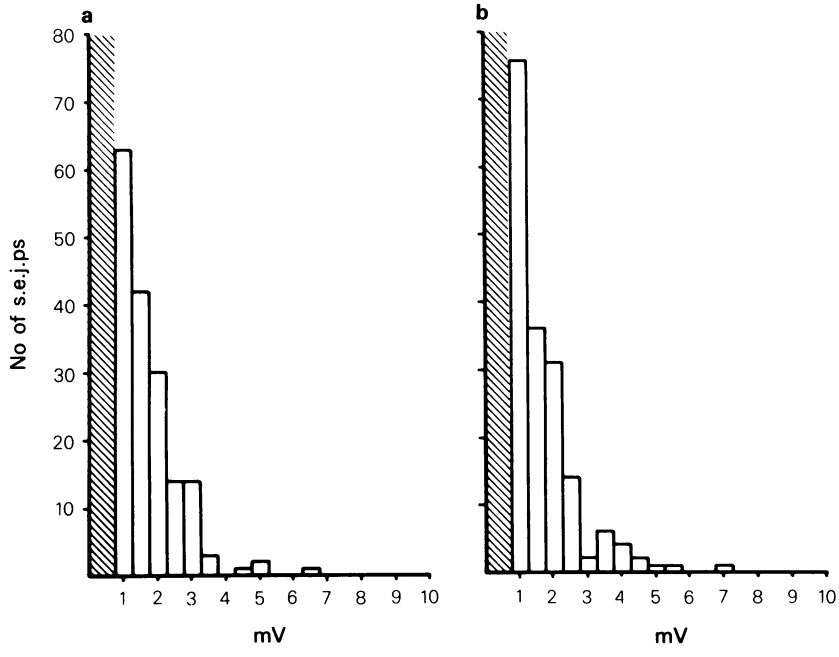

Figure 4 The lack of effect of normorphine on the amplitude and frequency of the spontaneous e.j.ps (s.e.p.js) from one smooth muscle cell. (a) Amplitude histograms of spontaneous e.j.ps in a normal Krebs solution. (b) In the presence of 2 μM normorphine. Each histogram represents the distribution of spontaneous e.j.p. amplitudes over a period of 5 min, starting after the drug had been present for 10 min.

release at both sites is depressed by extracellular magnesium ions. Third, four fold changes in extracellular calcium concentration, while greatly affecting evoked transmitter release, have relatively little or no effect on spontaneous release. Magnesium ions block calcium entry into the nerve terminal (Kharasch, Mellow & Silinsky, 1980) and cobalt appears to have a similar action (Hagiwara & Byerly, 1981). This implies that a significant proportion of spontaneous noradrenaline release is the consequence of calcium entry into the nerve terminal.

Normorphine has no effect on the spontaneous e.j.p. in a concentration which almost completely abolished the evoked e.j.p. Two basic sites of normorphine action might be considered. First, normorphine may hyperpolarize and/or increase the conductance of the nerve fibres; this could either simply prevent their excitation or block action potential propagation at varicosities and branch points. Such opiate actions occur in nerve fibres of the myenteric plexus (North & Tonini, 1977; Morita & North, 1981). Second, normorphine may affect depolarization-secretion coupling. It could do this either by changing the voltage dependence of the calcium activation process, without substantially changing resting calcium entry, or by impairing the ability of calcium ions to activate an intracellular release site. The latter mechanism has the corollary that such a site is different for evoked and spontaneous release, certain evidence for which exists (Kharasch *et al.*, 1981). It also has the merit of agreeing with direct (Guerrero-Munoz, Cerrata, Guerrero & Way, 1978) and indirect (Tokimasa, Morita & North, 1981) evidence that opiates inhibit the binding of calcium ions to an intracellular site.

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