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THE EFFECT OF STIMULUS INTENSITY ON α-ADRENOCEPTOR-MEDIATED FEEDBACK CONTROL OF NORADRENALINE RELEASE

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1 Mouse vas deferens stimulated transmurally (2.5 Hz, 2–32 V, 40–620 mA, for 45 s) responded with a twitch and a secondary contraction. Both responses were abolished by cinchocaine and were voltage-dependent.

2 In tissues previously incubated with [^{3}H]-(−)-noradrenaline, stimulation also produced an increase in tritium overflow from the tissue. Phentolamine increased tritium overflow by 19% at high stimulus intensities (30 V, 600 mA) and by 130% at low stimulus intensities (11 V, 200 mA).

3 It is concluded that α-adrenoceptor-mediated feedback control of noradrenaline release is more marked at low stimulus intensities and that this is compatible with a role for calcium ions in this control mechanism.

Introduction

Initial measurements of evoked tritium overflow from mouse vas deferens, previously incubated with [^{3}H]-(−)-noradrenaline showed that phentolamine (1×10^{-5} M) increased tritium overflow by only ≈ 20% (Harper & Hughes, unpublished observations). However, rises in noradrenaline output of 125% to 400% have been recorded during exposure to phentolamine at concentrations of 1×10^{-6} M and 1.8×10^{-4} M by Farnebo & Hamburger (1971) and by de Potter, Chubb, Put & de Schaepdryver (1971) respectively and we have now investigated this quantitative discrepancy between our results and those of these other workers.

Methods

Tuck No. 1 mice (28 to 35 g) were killed by cervical dislocation, one vas deferens removed, cleared of mesentery and placed in physiological saline (NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 25, glucose 11 mM; ascorbic acid (BDH) 57, disodium ethylenediaminetetra-acetic acid (BDH) 27 and β-oestradiol (Sigma) 3.7 μM; gassed with 5% CO_2 in O_2) at room temperature. Ligatures were attached to each end of the tissue which was incubated at 37°C for 10 min in 1.0 ml of the physiological saline. [^{3}H]-(−)-noradrenaline (10 μCi; specific activity 9.1 Ci/mmol; Radiochemical Centre, Amersham) was added and the incubation continued for 45 min. The tissue was removed from the bath, mounted between 1 mm diameter platinum wires held 5 mm apart and placed in a second tissue bath maintained at 37°C. The bath was drained and refilled with 1.0 ml of physiological saline every 2 min and the effluent was collected in liquid scintillation vials to which was added 10 ml of a liquid scintillation cocktail (naphthalene 100 g, 2,5-diphenyloxazole 7 g, 1,4-di-2-(5-phenyloxazolyl)-benzene 0.5 g, methanol 50 ml, glacial acetic acid 20 ml and dioxan to 1000 ml). The samples were counted for tritium and corrected for quenching by an external standard ratio method. The tissue was allowed to wash for 45 min and electrical stimulation was applied at 20 min intervals (2 ms pulse width, 2.5 Hz for 45 s starting 15 s after a change of bath fluid). Two intensities of stimulation were used, high intensity (30 V, 600 mA) and low intensity (11 V, 200 mA). Three periods of stimulation were applied and phentolamine mesylate (1×10^{-5} M; Ciba) was added to the bulk of the physiological saline as required and remained in contact with the tissue for 20 min before its effect on evoked overflow of tritium was determined. At the end of the experiment the tissue was combusted and the resulting samples were counted for tritium as above and corrected for recovery from the combustion process (94.3 ± 0.4%; mean ± s.e. mean; $n = 6$).

When contractile responses of the tissue were measured, incubation with [^{3}H]-(−)-noradrenaline was omitted and the ligature at the top of the tissue was connected to an isometric transducer (Dynamometer UF1; load 0.5 g) and the output displayed on

a Devices recorder (M2). Electrical stimulation was applied as above but at 6 min intervals and various voltages (2 to 32 V). In all experiments the voltage and current applied across the electrodes were monitored on a Solatron oscilloscope and all quantitative data refer to these measured values.

Results

Electrical stimulation of the mouse vas deferens resulted in a 'twitch' followed by a secondary contraction; the size of both responses was dependent on the applied voltage. At low voltages (2 to 20 V) the twitch predominated and both responses showed a peak with respect to the applied voltage. At high voltages (25 to 32 V) the twitch response was not seen and the secondary contraction increased in size above the peak seen at the lower voltages (Figure 1). Both responses were well maintained over a period of 2 h and were abolished by cinchocaine (Ciba) (2.6×10^{-5} M).

Electrical stimulation produced an increase in tritium overflow from the tissue compared with the resting release. Resting release was taken as the average tritium content of the two samples obtained immediately before electrical stimulation; usually 1 to 2×10^3 d/min per sample. For each period of stimulation the evoked overflow was calculated as the total tritium which appeared in two samples (that obtained during and that obtained immediately after electrical stimulation) in excess of the tritium expected from the resting release.

The first period of stimulation evoked a variable overflow compared with that occurring in later periods of stimulation and samples obtained during this time were disregarded. In the second stimulation period, a comparison between tissues stimulated at high intensity (30 V, 600 mA) and at low intensity (11 V, 200 mA) showed that evoked overflow of tritium was greater at the higher stimulus intensity (2542 ± 294 and 1797 ± 178 d/min respectively; mean ± s.e. mean; $n = 9$) but that the difference between these two values was only just statistically significant ($P < 0.05$ but > 0.02; Student's t test). In both cases, evoked overflow represented only a small fraction of the total tritium content of the tissue which fell within the range 4 to 13×10^5 d/min per tissue and was not significantly different in the two groups ($P > 0.6$).

Phentolamine (1×10^{-5} M) applied after the second period of stimulation caused an increase in evoked overflow of tritium in the third period of stimulation with either high or low intensity stimulation (Figure 1). The increase (expressed as a percentage of the tritium overflow evoked by nerve stimulation before exposure of the tissue to phentolamine) was much

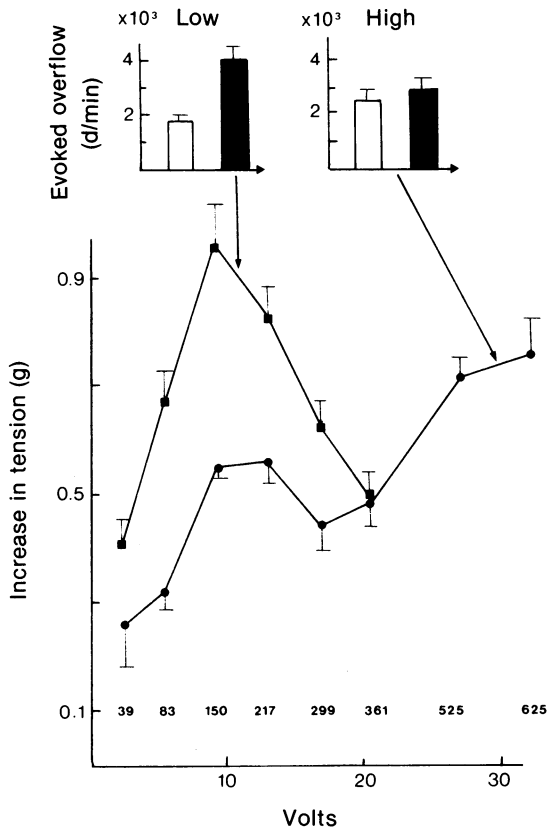

Figure 1 The effect of increasing voltage (abscissae) on the tension developed (g, mean ± s.e. mean, $n = 6$) during the twitch (■) and secondary contraction (●) by the mouse vas deferens in response to electrical stimulation (2.5 Hz, 2 ms pulse width for 45 s). The numbers across the lower part of the figure show the current passing across the electrodes (mA) at the corresponding voltages. The inset histograms show the overflow of tritium (mean ± s.e. mean; $n = 9$) from tissues previously incubated with [^{3}H]-(−)-noradrenaline evoked in response to low (11 V, 200 mA) and high (30 V, 600 mA) intensity electrical stimulation in the absence (open columns) and in the presence of phentolamine (1×10^{-5} M).

greater in tissues given low intensity stimulation ($130 \pm 12\%$; mean ± s.e. mean; $n = 9$) than in those given high intensity stimulation ($19 \pm 6\%$; mean ± s.e. mean; $n = 9$). In absolute terms the increases in evoked overflow produced by phentolamine during high and low intensity stimulation were $+ 419 \pm 194$ d/min and $+ 2329 \pm 274$ d/min respectively (mean ± s.e. mean; $n = 9$). In both cases the difference between the pairs of values was highly significant statistically ($P < 0.001$; Student's t test).

Discussion

Although high currents were passed across the electrodes, both the twitch response and the secondary contraction were mediated through neuronal elements as both were blocked by cinchocaine hydrochloride (2.6×10^{-5} M; Ciba) at a concentration that does not reduce the response to exogenous noradrenaline (Clark & Hughes, 1966). However, measurements of current flow after replacement of the tissue by an electrical insulator of similar dimensions showed that only about 0.2% of the applied current passed through the tissue, the remainder being shunted through the physiological saline.

Tritium overflow was increased by both high and low intensity stimulation the former producing a slightly greater tritium overflow than the latter. The potentiation of tritium overflow by phentolamine was 6.8 fold greater during low intensity stimulation than during high intensity stimulation when expressed in percentage terms and in absolute terms the effect was 5.5 fold greater. Thus, the influence of the α-adrenoceptor-mediated control mechanism on noradrenaline release is less marked at high stimulus intensities as used in our initial experiments and this explains the quantitative discrepancy between our initial results and those of other workers (Farnebo & Hamburger, 1971; de Potter *et al.*, 1971). This observation is compatible with a proposed role for calcium ions (see Westfall, 1977) in the α-adrenoceptor-mediated control mechanism. Katz & Kopin (1969) have shown that in field stimulated rat atria, high current stimulation induced a release of noradrenaline which was independent of the presence of calcium in the physiological saline. If a similar situation occurs in the mouse vas deferens and if the α-adrenoceptor-mediated control mechanism operates through an effect on available calcium, then blockade of the α-adrenoceptors would produce a much greater rise in noradrenaline output in response to low intensity stimulation than in response to high intensity stimulation where the release of noradrenaline is calcium independent.

References

CLARK, E.R. & HUGHES, I.E. (1966). Blocking action of some quaternary derivatives of 2-(2,6-xylyloxy)ethylamine on adrenergic nerves. *Br. J. Pharmac.*, **28**, 105–127.

FARNEBO, L-O. & HAMBURGER, B. (1971). Drug induced changes in the release of ^{3}H-noradrenaline from field stimulated rat iris. *Br. J. Pharmac.*, **43**, 97–106.

KATZ, R.I. & KOPIN I.J. (1969). Electrical field-stimulated release of norepinephrine-H^3 from rat atrium; effects of ions and drugs. *J. Pharmac. exp. Ther.*, **169**, 229–236.

de POTTER, W.P., CHUBB, I.W., PUT, A. & de SCHAEPDRYVER, A.F. (1971). Facilitation of the release of noradrenaline and dopamine-β-hydroxylase at low stimulation frequencies by α-blocking agents. *Archs int. Pharmacodyn. Ther.*, **193**, 191–197.

WESTFALL, T.C. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.*, **57**, 659–728.

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