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Effect of Cocaine on Tritium Overflow Evoked from Vasa Deferentia Previously Loaded with [^{3}H]Noradrenaline by Stimulation Using Different Types of Electrode

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Abstract—In mouse and guinea-pig vasa deferentia previously incubated with [^{3}H]noradrenaline, electrical stimulation applied through parallel electrodes (transmurally) increased overflow of tritium 2- to 5-fold above the resting value. Electrical stimulation applied using methods involving more substantial conduction of nerve impulses in neuronal elements in the tissues evoked a tritium overflow which was smaller (70%) than that evoked by transmural stimulation. Cinchocaine (25 μM), tetrodotoxin (0·5 μM) or the absence of calcium effectively abolished evoked overflow in both tissues whichever method of stimulation was used. In mouse vas deferens, cocaine (10 μM) did not alter overflow evoked by either transmural or axonal stimulation while 100 μM produced a reduction. In guinea-pig vas deferens, cocaine (10 μM) produced a statistically significant increase in evoked overflow of about 50% or more with both transmural and axonal stimulation. As in mouse vas deferens, 100 μM cocaine produced a reduction. It is concluded that the action of cocaine is independent of these methods of stimulation and that some difference in the arrangement of the noradrenergic nerves in the two species may account for the differential effect of cocaine observed.

In tissues such as ear artery, iris, atria and cortical brain slice, the application of drugs which block re-uptake of endogenous noradrenaline causes an increase in the noradrenaline which overflows from the tissue in response to electrical stimulation. In other tissues such as spleen, portal vein, colon and mouse vas deferens, blockade of noradrenaline re-uptake does not increase electrically evoked transmitter overflow (see El-Mas & Hughes (1990) for references). One factor which may contribute to this difference is the configuration of the electrode system used to apply electrical stimulation. For example, blockade of noradrenaline re-uptake in rabbit ear artery produced an increase in evoked noradrenaline overflow when electrical stimulation involved propagated nerve impulses but not when transmural stimulation was used (Rand et al 1988). We have previously reported that the noradrenaline re-uptake blocker, cocaine, does not increase evoked overflow of transmitter from mouse vas deferens when stimulation is applied transmurally (Hagan & Hughes 1981). This paper now reports a comparison of the effects of cocaine on overflow evoked by transmural stimulation and by propagated nerve impulses in this tissue and in guinea-pig vas deferens. An initial report of some of these results has previously been communicated to the British Pharmacological Society (El-Mas & Hughes 1991).

Materials and Methods

Mouse vas deferens

The methods used have been described in detail elsewhere (El-Mas & Hughes 1990); modifications are detailed below. In outline, mouse vasa deferentia were incubated for 40 min with 0·66 μM [^{3}H]noradrenaline. Only the epididymal half of the vas deferens was exposed to the incubation solution; the prostatic portion was held above the incubation fluid and protected from contact with the solution by being wrapped in moist cotton wool. This procedure ensured that only the epididymal part of the vas deferens became loaded with radiolabelled transmitter. The tissue was then mounted in a bath which was drained and refilled with modified Krebs solution (1·5 mL) every 2 min. After washing for 45 min, electrical stimulation (2·5 Hz, 2 ms, 90 s for 2 min every 20 min) was applied through parallel electrodes held 4 mm apart and running the length of the vas (long electrodes; 600 mA, 20–30 V) or through 3 mm diameter ring electrodes (3 mm apart) placed at the prostatic (unloaded) end of the vas (ring electrodes; 350 mA, 40–48 V). Two periods of stimulation were applied through long electrodes and thereafter the ring and long electrodes were used alternately. The effluent from the tissue bath was collected and counted for tritium; tritium overflow in each 2 min collection period was expressed in fractional terms by dividing by the tritium content of the tissue. At the end of the experiment the tissue was divided into 8 equal portions each of which was dissolved in 0·25 mL of OptiSolve (LKB). Acetic acid (4 M, 0·25 mL) was added to each tissue sample to prevent chemiluminescence and the tritium content measured by liquid scintillation counting. All tritium counts were corrected for quench using the spectral index of the external standard.

Guinea-pig vas deferens

Vasa deferentia with hypogastric nerve attached were removed from male guinea-pigs, 250–437 g, as described by Hukovic (1961). The tissue was incubated in modified Krebs solution (mM: NaCl 118, KCl 5·4, $CaCl_2$ 2·5, $MgSO_4$ 0·57, KH_2PO_4 0·59, $NaHCO_3$ 25, glucose 11 also containing (μM)

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EDTA 27, ascorbic acid 57 (to retard oxidation of the radiolabelled noradrenaline) and 17-β-oestradiol 3·7 (to inhibit uptake$_2$); gassed with 5% CO_2–95% O_2) at 37°C for 45 min with 0·77 μM [^{3}H]noradrenaline. A tissue was then mounted on an electrode assembly in a tissue bath containing 4·5 mL of modified Krebs solution which was replaced with an equal volume every 2 min. The electrode assembly consisted of 2 parallel platinum wires 5 mm apart which ran the length of the tissue to provide transmural stimulation and a pair of hook electrodes over which the mesentery containing the hypogastric nerve was secured (to provide conducted axonal stimulation). As soon as the tissue was set up a brief initial period of stimulation was applied through the hook electrodes. Any tissue failing to show a contractile response was discarded. Thereafter stimulation was applied at 5 Hz, 1 ms for 90 s every 20 min (300 mA transmural; 100–180 mA axonal). The effluent from the tissue bath was counted for tritium and the results expressed as above, except that the tissue was not divided into portions and was dissolved in 2 mL of OptiSolve. Acetic acid (4 M, 1 mL) was added before scintillation counting.

Cocaine and other drugs were applied in the bulk of the physiological solution 14 min before a period of stimulation.

Drugs

Drugs used were; cinchocaine hydrochloride (Ciba, UK), cocaine hydrochloride (Boots, UK), ethylenediamine tetra-acetic acid (EDTA) (BDH, UK), 17-β-oestradiol (Sigma, UK), tetrodotoxin (Sigma, UK). [7,8-^{3}H]-(−)-Noradrenaline (15 Ci mmol^{-1})) was obtained from The Radiochemical Centre, Amersham, UK.

Statistical procedures

Where appropriate all results are expressed as mean ± s.e. Tests for statistical significance ($P < 0.05$) utilized Student's unpaired *t*-test or the Mann-Whitney rank test (Snedecor & Cochran 1980) as appropriate.

Results

Mouse vas deferens

The first period of stimulation (S1) through the long electrodes sometimes gave rise to an evoked overflow of tritium which was not representative of those in later periods and this data was routinely discarded. Immediately before the second period of stimulation (S2) through long electrodes, control tissues contained $6{\cdot}81 \pm 0{\cdot}99 \times 10^5$ disintegrations per min of tritium and resting overflow averaged 2772 ± 380 disintegrations per min (n = 8). Evoked overflow averaged 7353 ± 1269 disintegrations per min. The corresponding fractional resting and evoked overflows, those at the next period of stimulation (S3; through ring electrodes), those in subsequent periods (S4 (long) and S5 (ring) electrodes) and the ratio of these to the corresponding values obtained initially (i.e. S4/S2 and S5/S3) are shown in Table 1.

In a second group of tissues, tritium content immediately before the second period of stimulation (S2) was $7{\cdot}28 \pm 0{\cdot}73 \times 10^5$ disintegrations per min of tritium and resting overflow averaged 3274 ± 255 disintegrations per min (n = 8). Evoked overflow averaged 8482 ± 1028 disintegrations per min. None of these values differed significantly from the corresponding values obtained in the control group ($P > 0{\cdot}2$; Student's *t*-test). The fractional resting and evoked overflows before the next period of stimulation (S3; through the ring electrodes), those obtained on subsequent repetition of these two types of stimulation (S4 and S5) in the presence of cocaine (10 μM) and the ratio of these values to the corresponding values obtained initially (i.e. S4/S2 and S5/S3) are shown in Table 1. There is no statistically significant difference ($P > 0{\cdot}6$) between the ratios obtained in the presence of cocaine and those obtained in its absence, indicating that cocaine did not alter resting or evoked fractional overflow.

At the end of the experiment each tissue was cut into 8 equal lengths and the distribution of tritium in these portions expressed as a percentage of the tissue total tritium content (Fig. 1).

More than 95% of the tritium content was found to be contained in the epididymal 5/8ths of the tissue and there was no statistically significant difference ($P > 0{\cdot}2$; Student's *t*-test) between the distribution of tritium in the 8 portions of the control and cocaine treated tissues.

Application of cinchocaine (25 μM; single experiment) or tetrodotoxin (0·5 μM; single experiment) in place of cocaine,

Table 1. Fractional overflow of tritium evoked by stimulation through long (transmural) or ring electrodes in control mouse vas deferens previously incubated with [^{3}H]noradrenaline and in tissues exposed to cocaine during S4 and S5. Values are mean ± s.e.m., n = 8.

		Control tissues (fractional overflow ($\times 10^{-3}$))		Cocaine-treated (after S3) (fractional overflow ($\times 10^{-3}$))	
Type of stimulation		Resting	Evoked	Resting	Evoked
Transmural	S2	4·19 ± 0·22	10·88 ± 0·83	4·59 ± 0·21	11·87 ± 1·06
Ring	S3	3·76 ± 0·28	6·17 ± 0·95	3·98 ± 0·24	5·54 ± 0·60
				Cocaine (10 μM)	
Transmural	S4	3·58 ± 0·29	10·12 ± 0·70	3·93 ± 0·29	10·61 ± 0·60
Ring	S5	3·28 ± 0·24	5·75 ± 1·00	3·49 ± 0·25	5·35 ± 0·94
Ratio	S4/S2	0·84 ± 0·03	0·94 ± 0·07	0·86 ± 0·03	0·91 ± 0·09
Ratio	S5/S3	0·91 ± 0·05	0·93 ± 0·07	0·89 ± 0·05	0·96 ± 0·11

None of the mean values from the control group are significantly different from corresponding values in the cocaine-treated group.

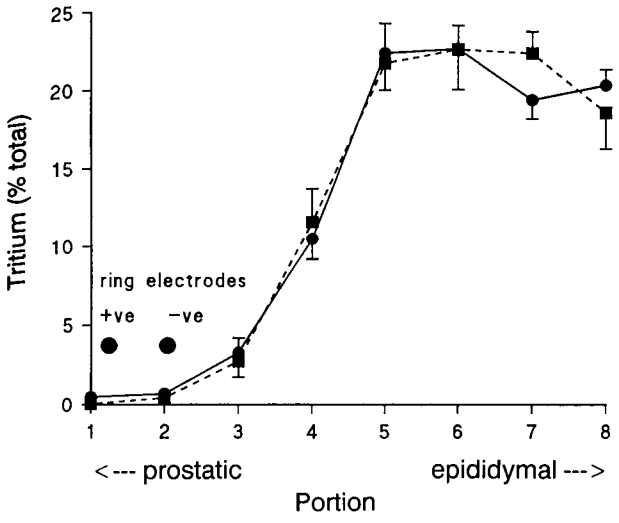

FIG. 1. Distribution of tritium (% of total tritium content; mean ± s.e.m.; n = 8) in the equal sequential portions taken from the prostatic to the epididymal end of mouse vas deferens previously incubated with [^{3}H]noradrenaline and then subjected to the experimental procedure outlined above. There is no statistically significant difference ($P > 0.4$, Student's t-test) between corresponding portions of the control (●) and cocaine-treated (10 μM, ■) tissues. Note the position of the ring electrodes relative to the distribution of tritium.

or exposure to calcium-free Krebs' solution (2 experiments) reduced the S4/S2 and S5/S3 ratios to less than 0·06 indicating that these treatments effectively abolished evoked overflow. Cocaine (100 μM) also substantially reduced these ratios giving values of 0·31 and 0·21, respectively.

Guinea-pig vas deferens

The first period of stimulation (S1) through transmural electrodes gave an evoked overflow of tritium which was sometimes discrepant from those in later periods and this data was routinely discarded. Immediately before the second period of stimulation through hook electrodes (S2; axonal) control tissues contained $2{\cdot}16 \pm 0{\cdot}21 \times 10^6$ d min^{-1} of tritium and resting overflow averaged 7184 ± 611 d min^{-1} (n = 5). Evoked overflow averaged $30\,536 \pm 6161$ d min^{-1}. The fractional resting and evoked overflows at this and the next period of stimulation (S3; transmural electrodes), those obtained on subsequent repetition of these two types of stimulation (S4 (axonal) and S5 (transmural)) and the ratio of these to the corresponding initial values (i.e. S4/S2 and S5/S3) are shown in Table 2.

In a second group of tissues tritium content immediately before the second period of stimulation (S2) was $1{\cdot}98 \pm 0{\cdot}17 \times 10^6$ d min^{-1} and resting overflow averaged 6494 ± 243 d min^{-1} (n = 5). Evoked overflow averaged 25620 ± 4731 d min^{-1}. None of these values are significantly different from the corresponding values in the first group of tissues ($P > 0{\cdot}3$; Student's t-test). The fractional resting and evoked overflows at this and the next period of stimulation (S3; transmural), those in subsequent periods (S4 (hook) and S5 (transmural)) in the presence of cocaine (10 μM), and the ratio of these values to those obtained initially (i.e. S4/S2 and S5/S3) are shown in Table 2. In comparison with control tissues, exposure to cocaine produced a significant increase in fractional evoked overflow with both types of stimulation but did not affect fractional resting overflow.

Application of cinchocaine (25 μM; single experiment) or tetrodotoxin (0·5 μM; single experiment), or exposure to calcium-free Krebs solution (2 experiments) gave S4/S2 and S5/S3 ratios of less than 0·05 indicating that these treatments effectively abolished evoked overflow. Cocaine (100 μM) gave S4/S2 and S5/S3 ratios for fractional evoked overflow of 0·23 and 0·17, respectively, indicating that with both types of stimulation-evoked overflow were substantially reduced by this concentration of cocaine.

Three experiments were conducted in which the Krebs solution used with mouse vas deferens was substituted for that normally used. In each of these experiments cocaine (10 μM) produced an increase in overflow evoked by transmural or by axonal stimulation yielding S4/S2 and S5/S3 ratios of $1{\cdot}53 \pm 0{\cdot}23$ and $1{\cdot}49 \pm 0{\cdot}31$, respectively (n = 3).

Discussion

Tritium overflow evoked by electrical stimulation of a tissue previously incubated with tritiated noradrenaline represents the difference between the tritium released and that taken back up into the cells. About 60% of the tritium is present as noradrenaline in mouse vas deferens under control conditions and this increases to about 70% in the presence of cocaine (El-Mas & Hughes 1990). No attempt has been made in these experiments to separate the tritium into different

Table 2. Fractional overflow of tritium evoked by stimulation through hook (axonal) or parallel (transmural) electrodes in control guinea-pig vas deferens previously incubated with [^{3}H]noradrenaline and in tissues exposed to cocaine during S4 and S5. Values are mean ± s.e.m., n = 5.

Type of stimulation		Control tissues (Fractional overflow ($\times 10^{-3}$)) Resting	Control tissues (Fractional overflow ($\times 10^{-3}$)) Evoked	Cocaine-treated (after S3) (Fractional overflow ($\times 10^{-3}$)) Resting	Cocaine-treated (after S3) (Fractional overflow ($\times 10^{-3}$)) Evoked
Axonal	S2	3·33 ± 0·28	14·16 ± 2·91	3·27 ± 0·11	13·09 ± 2·47
Transmural	S3	3·10 ± 0·14	21·31 ± 3·45	3·01 ± 0·12	20·21 ± 2·40
				Cocaine (10 μM)	
Axonal	S4	2·91 ± 0·12	13·11 ± 1·70	2·84 ± 0·13	20·93 ± 2·41*
Transmural	S5	2·78 ± 0·14	19·55 ± 2·30	2·81 ± 0·09	35·91 ± 3·23†
Ratio	S4/S2	0·88 ± 0·09	0·92 ± 0·13	0·87 ± 0·11	1·63 ± 0·16‡
Ratio	S5/S3	0·90 ± 0·10	0·91 ± 0·11	0·91 ± 0·12	1·76 ± 0·18‡

*$P < 0{\cdot}05$; † $P < 0{\cdot}01$ (Student's t-test) in comparison with control values. ‡ $P < 0{\cdot}05$ (Mann-Whitney rank test) in comparison with control ratios.

molecular species and it must be remembered throughout this work that changes in tritium overflow may not accurately reflect changes in synaptic noradrenaline concentrations (Marshall 1983).

The observation that cinchocaine, tetrodotoxin or omission of calcium from the bathing solution effectively abolished electrically evoked overflow of tritium suggests that in both tissues, both methods of stimulation produced an evoked overflow by activation of neuronal elements. In both mouse and guinea-pig vas deferens initial tissue content of tritium, the resting and the evoked overflow in the control group and in the group to be treated with cocaine (10 μM), were not significantly different allowing valid comparisons between appropriate groups. In both tissues resting overflow reduced slightly as the experiment progressed even when data was expressed in fractional terms, the appropriate ratios being consistently below unity. This may well reflect the progressive decline in a more loosely bound fraction which is small in relation to that more firmly bound in the vesicles. Comparison of control tissues and those treated with cocaine shows that cocaine did not alter resting overflow in a statistically significant manner in either tissue.

When expressed in fractional terms, evoked tritium overflow fell very slightly as the experiment progressed, although the appropriate ratios did not differ significantly from unity ($P > 0{\cdot}3$; Student's t-test).

In mouse vas deferens, consistent with our previous finding (El-Mas & Hughes 1990), application of cocaine did not change fractional evoked overflow when the tissue was stimulated transmurally. Neither did it change fractional evoked overflow when stimulation was applied by ring electrodes placed at the prostatic end of the tissue. Since the method of loading the tissue with tritiated noradrenaline has been shown to result in a very high proportion of the tritium in the tissue being located away from these electrodes, the evoked release must involve conduction of impulses from the excited area around the ring electrodes to the tritium-containing areas further up the vas. The possibility that these conducted impulses may not reach all loaded varicosities either because of damage to axonal elements in the tissue or because of failure of conduction at fine axonal branching points may account for the smaller fractional overflow (70%) evoked by this type of stimulation than by transmural stimulation. The latter would be expected to excite all neuronal elements at the level of the varicosities, even those not functionally connected to other conducting elements. Alternatively, the intensity of stimulation through the ring electrodes may not achieve a maximal activation of all neuronal elements.

In guinea-pig vas deferens, isolation of the hypogastric nerve is much easier than in the mouse and stimulation could therefore be applied transmurally or directly to the hypogastric nerve. As in the mouse vas deferens, these conducted impulses released less tritium than did transmural stimulation, and in control tissues fractional resting and evoked overflow fell slightly as the experiment progressed, although the latter did not reach statistical significance ($P > 0{\cdot}3$; Student's t-test). In contrast to the results in mouse vas deferens, however, cocaine (10 μM) markedly and significantly increase evoked tritium overflow both when stimulation was applied transmurally and when stimulation was applied using impulses conducted through the hypogastric nerve. This effect was still seen when experiments were conducted in the Mg-free Krebs solution normally used for mouse vas deferens. The effect is unlikely to be due to a difference in sensitivity to cocaine as a higher concentration reduced evoked overflow in both tissues, probably due to a local anaesthetic action known to be evident at this higher concentration (McCulloch et al 1985). It is clear therefore that in neither of these tissues is the effect of cocaine dependent on whether transmural or axonal stimulation is applied. The data do show, however, that blockade of noradrenaline re-uptake affects tritium overflow differently in the different species. Since we have shown previously that the lack of effect of cocaine in mouse vas deferens is still observed under a variety of experimental conditions it would appear that some difference in the arrangement or organization of the innervation in the two species must account for the observed effects.

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