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THE CONTRACTILE EFFECTS OF 5-HYDROXYTRYPTAMINE ON THE RAT ISOLATED VAS DEFERENS

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1 5-Hydroxytryptamine (5-HT) (5.16–1291 μM) produced a phasic contraction followed later by rhythmic contractions in the rat vas deferens, primarily in the epididymal half. 5-HT (129 μM) produced no response in Ca^{2+}-free solution. Nifedipine (0.29 μM) or verapamil (2.04 μM) inhibited the initial phasic response to 5-HT, but inhibition of the rhythmic contractions required concentrations 5 fold (nifedipine) or 30 fold (verapamil) higher.

2 Methysergide (2.13 μM) abolished the phasic and reduced the frequency of the rhythmic contractions. Phentolamine (2.65 μM) did not affect the phasic response but reduced the amplitude of the rhythmic contractions. The combination of phentolamine (2.65 μM) and methysergide (2.13 μM) completely abolished the response to 5-HT (129 μM).

3 Desipramine (1.32 μM) had no effect on the phasic response to 5-HT (129 μM), but the rhythmic contractions were reduced in amplitude with no effect on their frequency.

4 In vasa deferentia removed from reserpine-treated or from guanethidine-denervated rats, both phasic and rhythmic components of the 5-HT (129 μM) contraction were augmented due to supersensitivity.

5 It is concluded that the phasic component of the 5-HT contraction is mediated by post-junctional 5-HT receptors, while the rhythmic component is mediated by the combination of post-junctional 5-HT receptors and noradrenaline released from neuronal stores. Assuming that nifedipine and verapamil are acting solely by inhibition of calcium channels, the phasic and rhythmic components of the 5-HT response may be mediated through separate Ca^{2+} channels. If this is correct, one channel might be a voltage-dependent channel and the other could be similar to, but distinct from the channel mediating the response to methoxamine.

Introduction

The rat and guinea-pig vas deferens are both contracted by 5-hydroxytryptamine (5-HT). There is an initial phasic response, followed by a sustained response with superimposed rhythmic contractions (Thoa & Maengwyn-Davies, 1968; Nishino, Irikura & Takayanagi, 1970; Ozawa & Katsuragi, 1974). The 5-HT content of the rat vas deferens is 543 ± 64 ng/g wet weight and it has been suggested that 5-HT may modulate neurotransmission in this tissue (Fuenmayor, Gomez, Campos & Romero, 1976). In contrast, Ambache & Zar (1971) and Ambache, Dunk, Verney & Zar (1973) have rejected the idea of involvement of 5-HT in motor transmission in the guinea-pig and rat vas deferens.

Cytochemical and biochemical studies have shown that under appropriate conditions, exogenous 5-HT is accumulated into the neuronal noradrenaline store in the vas deferens (Jaim-Etcheverry & Zieher, 1969; Thoa, Eccleston & Axelrod, 1969). It might therefore be expected that part of the contractile response with 5-HT would be mediated by release of endogenous noradrenaline. Nishino *et al.* (1970) found that the initial phasic contraction in the rat vas deferens was blocked by phentolamine, reserpine and imipramine. However, different results were obtained by Ozawa & Katsuragi (1974) using the guinea-pig vas deferens in which they reported that the initial phasic component was mediated by post-junctional 5-HT receptors, but that the sustained response involved a presynaptic action mediated through noradrenaline release.

We now describe the effects of calcium deprivation and of calcium channel inhibitors on responses to 5-HT in the rat intact vas deferens. In view of the conflicting results noted above, we have reinvestigated the contribution of direct and indirect actions to the two phases of the 5-HT contraction in the rat vas deferens. We have also extended these studies to the epididymal and prostatic halves of bisected vasa deferentia. A preliminary account of these results was given to the British Pharmacological Society (Hay & Wadsworth, 1981a).

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Methods

Vasa deferentia were removed from Wistar rats (235–460 g body weight), in some experiments bisected (Pennefather, Vardolov & Heath, 1974; Anton, Duncan & McGrath, 1977) and suspended under 0.5 g tension in Krebs-Henseleit solution at 36–38°C. Contractions were recorded isometrically. The Krebs-Henseleit solution contained Na^+ 144, K^+ 5.8, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, Cl^- 128.6 and glucose 11.1 mM and was bubbled with 95% O_2:5% CO_2. The nominally Ca^{2+}-free solution was made in the same way but omitting $CaCl_2$. In calcium-deprivation experiments, tissues were incubated for 30 min in Ca^{2+}-free Krebs-Henseleit solution before addition of 5-HT. In the continuing presence of 5-HT, increasing concentrations of $CaCl_2$ were then added cumulatively at 20 min intervals. The contact period for 5-HT was approximately 25 min, successive additions being made at intervals of approximately 60 min. Two control applications of 5-HT were usually made before the addition of antagonists; each concentration of antagonist was left in contact with the tissue for 20 min.

Some rats were pretreated with reserpine, using a schedule of either (a) 5 mg/kg i.p. at 48 h followed by 3 mg/kg i.p. at 24 h before use, or (b) 2.5 mg/kg i.p. 24 h before use. Another group of animals was injected with guanethidine (25 mg/kg i.p. daily for 14 days followed by 50 mg/kg i.p. daily for 2 days) in order to destroy adrenergic nerves (Heath, Evans, Gannon, Burnstock & James, 1972). This treatment reduces the response to single pulse transmural stimulation (150 V, 0.1 ms pulse width) to 9% of control (Hay & Wadsworth, 1982a) and the tissues are referred to in the text as denervated tissues.

With each addition of 5-HT or tyramine, the rhythmic contractions were quantified as follows. The mean amplitude was obtained by averaging the peak tension produced during the last 20 contractions or by those contractions occurring during the last 12.5 min of the drug contact period. Mean frequency was obtained by counting the number of contractions present in the last 12.5 min of the drug contact period.

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, tyramine HCl, reserpine (Sigma), methysergide hydrogen maleate, dihydroergotamine mesylate (Sandoz), verapamil HCl (Knoll), nifedipine (Bayer), phentolamine mesylate, guanethidine sulphate (Ciba), desipramine HCl (Geigy), methoxamine HCl (Wellcome), propranolol HCl (ICI), atropine sulphate (B.D.H.).

Results are expressed as mean ± s.e.mean. In the graphs, standard errors are shown by vertical bars, except where less than the height of the symbol. Statistical analysis of the data was made using Student's t test; the 0.05 level of probability was regarded as significant.

Results

5-HT (129 μM) usually produced a phasic contraction that reached a maximum after 24–60 s and then declined towards baseline. Rhythmic contractions, usually starting at the peak of this contraction, continued until wash-out (up to 3 h) (Figure 1). There was considereable variation in the sensitivity of different tissues and no correlation between the amplitude of the initial phasic and rhythmic responses in different tissues.

Throughout the concentration range 5.16–1291 μM, 5-HT produced contractions having phasic and rhythmic components. The concentration-response curves were shallow; in particular the increase in phasic response between 25.8 and 1291 μM was very slight (Figure 2). This may be due to desensitization (Miranda, 1976) since a second application of 5-HT (129 μM) gave a reduced phasic response (72 ± 9% of the first 5-HT contraction, $n = 17$, $P < 0.01$) although there was no difference in the rhythmic contractions (amplitude 117 ± 13%, $n = 13$, frequency 97 ± 7%, $n = 13$ of first 5-HT rhythmic response).

In the epididymal half, the initial phasic response was larger, and the rhythmic contractions were also larger and much more frequent than in the prostatic half (Figure 1, Table 1). In some cases, the prostatic half showed no response of any type. Tyramine (57.6 or 288 μM)-induced contractions also had larger phasic components and more frequent rhythmic contractions in the epididymal than in the prostatic half (Table 2). In some experiments vasa were trisected

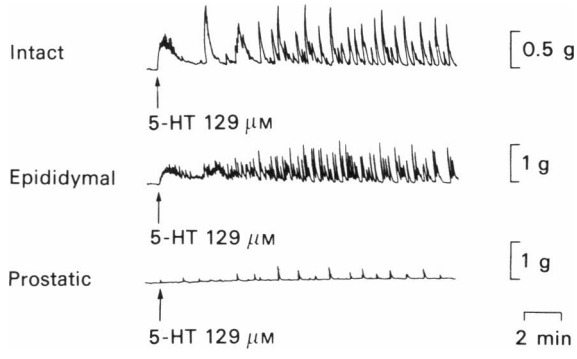

Figure 1 Responses to 5-hydroxytryptamine (5-HT 129 μM) in an intact and in epididymal and prostatic halves of a bisected vas deferens. The contraction occurred predominantly in the epididymal half.

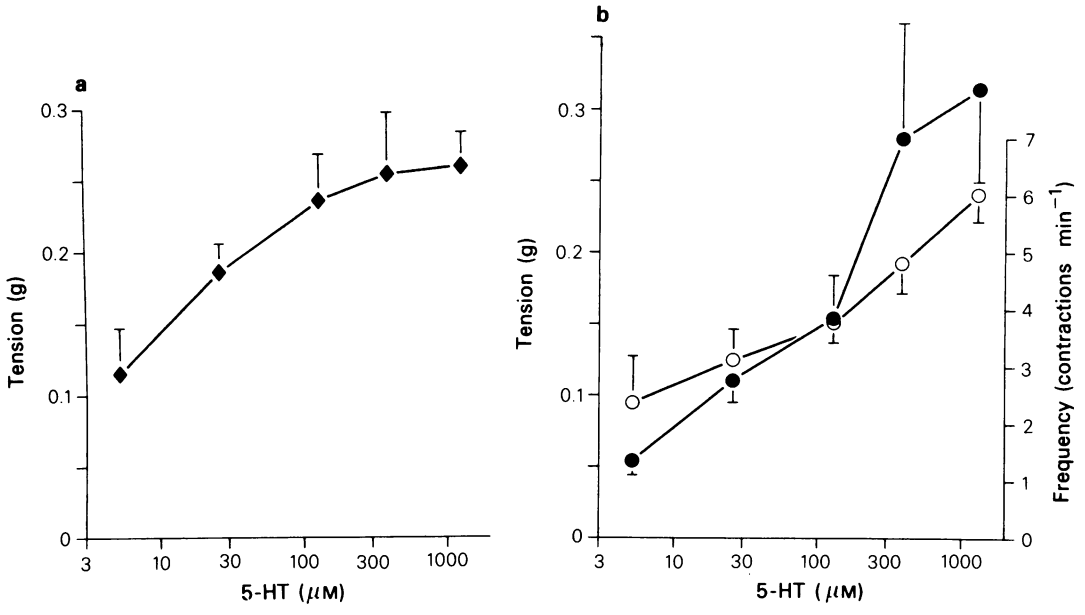

Figure 2 5-hydroxytryptamine (5-HT) concentration-response curves in intact vasa deferentia (a) initial phasic response (◆), (b) amplitude (●) and frequency (○) of rhythmic contractions; $n = 4$.

and the central third discarded. The prostatic third showed less response than the prostatic half both to 5-HT and to tyramine (especially the frequency of the rhythmic contractions). Such prostatic response as is present therefore seems to be largely due to contamination of the 'prostatic' half with 'epididymal' tissue.

Incubation for 30 min in nominally Ca^{2+}-free Krebs-Henseleit solution completely prevented both the phasic and rhythmic components of the 5-HT (129 μM) contraction. When $CaCl_2$ was subsequently added in the presence of 5-HT, very small rhythmic contractions appeared (at 0.1–0.5 mM Ca^{2+}). Increasing $[Ca^{2+}]$ from 0.5 to 1 mM caused a dramatic increase in frequency and a small increase in amplitude of the rhythmic contractions. The maximum frequency was achieved at 1.5 mM Ca^{2+} and the maximum amplitude at 4 mM Ca^{2+} (Figure 3a).

The initial phasic response to 5-HT (129 μM) was substantially inhibited by nifedipine (0.087–0.29 μM) (contractions reduced to 8±3% control, $n = 4$ by nifedipine 0.29 μM) or verapamil

Table 1 Components of the contraction produced by 5-hydroxytryptamine (5-HT, 129 μM) in rat vasa deferentia (mean ± s.e.mean)

	Phasic component Tension (g)	*Rhythmic component* Tension (g)	*Rhythmic component* Frequency (min^{-1})	n
Control				
Intact	0.22±0.02	0.37±0.02	3.48±0.18	80
Bisected: prostatic half	0.07±0.02	0.15±0.03	1.94±0.34	28
epididymal half	0.34±0.04	0.36±0.04	4.29±0.22	28
Trisected: prostatic third	0.02±0.01	0.08±0.05	0.46±0.27	4
epididymal third	0.69±0.11	0.25±0.03	5.50±0.38	4
Denervated				
Intact	0.58±0.11 (b)	0.56±0.14 (b)	4.78±0.86 (c)	10
Reserpinized				
Intact	0.50±0.14 (c)	0.74±0.11 (a)	5.07±0.52 (c)	6

(a) Significantly different from controls $P < 0.001$; (b) Significantly different from controls $P < 0.01$; (c) Significantly different from controls $P < 0.05$.
All data for prostatic half significantly different from epididymal half ($P < 0.001$).

Table 2 Components of the contraction produced by tyramine in rat vasa deferentia (mean ± s.e.mean)

	Phasic component Tension (g)	*Rhythmic component* Tension (g)	*Rhythmic component* Frequency (min^{-1})	n
Tyramine (57.6 μM)				
Control				
Intact	0.71 ± 0.11	0.50 ± 0.09	5.37 ± 0.77	6
Bisected: prostatic half	0.05 ± 0.02	0.34 ± 0.08	0.91 ± 0.32	8
epididymal half	0.24 ± 0.06	0.60 ± 0.15	2.73 ± 0.31	8
Trisected: prostatic third	0.04 ± 0.01	0.14 ± 0.09	0.18 ± 0.13	4
epididymal third	0.11 ± 0.06	0.19 ± 0.09	1.72 ± 0.59	4
After reserpinization	0 (a)	0.11 ± 0.08 (b)	0.31 ± 0.20 (a)	6
Tyramine (288 μM)				
Control				
Intact	1.01 ± 0.21	1.09 ± 0.36	5.70 ± 0.38	11
Bisected: prostatic half	0.29 ± 0.13	1.16 ± 0.23	2.36 ± 0.49	9
epididymal half	1.08 ± 0.11	0.82 ± 0.11	5.55 ± 0.30	9
Trisected: prostatic third	0.04 ± 0.02	1.12 ± 0.46	0.44 ± 0.22	4
epididymal third	0.29 ± 0.16	0.56 ± 0.21	1.96 ± 0.71	4
After reserpinization	0 (a)	0.53 ± 0.10 (d)	0.48 ± 0.14 (a)	4

Effect of reserpinization: (a) Significantly different from controls $P < 0.001$; (b) Significantly different from controls $P < 0.01$; (c) Significantly different from controls $P < 0.05$; (d) not significantly different from controls.

(2.04 μM) ($n = 2$), but the rhythmic response was more resistant. Since in untreated preparations, the rhythmic response to 5-HT (129 μM) increased in amplitude and decreased in frequency with time, the calcium channel inhibitors were added to one vas, the contralateral acting as control (Figure 3b, c). Nifedipine (0.29–0.58 μM) reduced the rhythmic response and nifedipine (1.44 μM) abolished it (Figure 3c). Verapamil (10.2–20.4 μM) reduced the amplitude but increased the frequency of the rhythmic contractions. These contractions of verapamil also increase the frequency of methoxamine-induced rhythmic contractions (Hay & Wadsworth, 1980), perhaps because of membrane depolarization (Haeusler, 1972). The rhythmic contractions were almost abolished by verapamil (102 μM) (Figure 3b).

Methysergide (2.13 μM) abolished the initial phasic contraction to 5-HT (129 μM), leaving in its place a small group of rhythmic contractions (Figure 4a). The later rhythmic contractions were reduced in frequency (by 58 ± 7%, $n = 13$, $P < 0.001$) and there was also a small, variable, but significant reduction in amplitude (of 21.9 ± 12.3%, $n = 13$, $P < 0.05$) (Figure 4a, Table 3). Methysergide (2.13 μM) had no effect on rhythmic contractions produced by methoxamine (8.1 μM) (amplitude + 3 ± 9%, frequency −17 ± 5%, $n = 6$) or tyramine (2.88 μM) ($n = 1$) showing that it was specific for 5-HT receptors.

Phentolamine (2.65 μM) had no significant effect on the initial phasic response to 5-HT (129 μM) (−19 ± 11%, $n = 7$) but reduced the amplitude of the rhythmic contractions with no significant effect on their frequency (amplitude −31 ± 13%, $n = 10$, $P < 0.05$, frequency −13 ± 8%, $n = 10$) (Figure 4b, Table 3). After phentolamine (2.65 μM), rhythmic responses to methoxamine (8.1 μM) were abolished or negligible ($n = 20$). Dihydroergotamine (1.47 μM) also substantially decreased the amplitude of the rhythmic contractions to 5-HT (129 μM) and slightly reduced their frequency (Table 3). The rhythmic part of the response to 5-HT, although markedly reduced by either methysergide or phentolamine alone, could be abolished only by giving both in combination (Figure 4a, b). In bisected vasa deferentia, as in the intact preparation, methysergide inhibited the phasic response and the frequency of the rhythmic contractions, while phentolamine inhibited the amplitude of the rhythmic contractions.

Desipramine (1.32 μM) abolished the first phase of the response to tyramine (288 μM), also markedly reducing the amplitude but not the frequency of the subsequent rhythmic contractions ($n = 1$) (Figure 5a). Desipramine (1.32 μM) reduced the amplitude of the rhythmic contractions to 5-HT (129 μM) with no effect on their frequency, nor on the initial phasic response (Figure 4c, Table 3). Rhythmic contractions induced by methoxamine (8.1 μM) were not affected by desipramine (1.32 μM) ($n = 2$).

In vasa deferentia from reserpine-treated animals the tyramine (57.6 or 288 μM) response had a negligible phasic component and a substantially reduced rhythmic component (Table 2, Figure 5b) and similar results were obtained in denervated vasa deferentia, showing that these treatments were effective in depleting nearly all the releasable noradrenaline. However, the 5-HT contraction was not reduced: both the

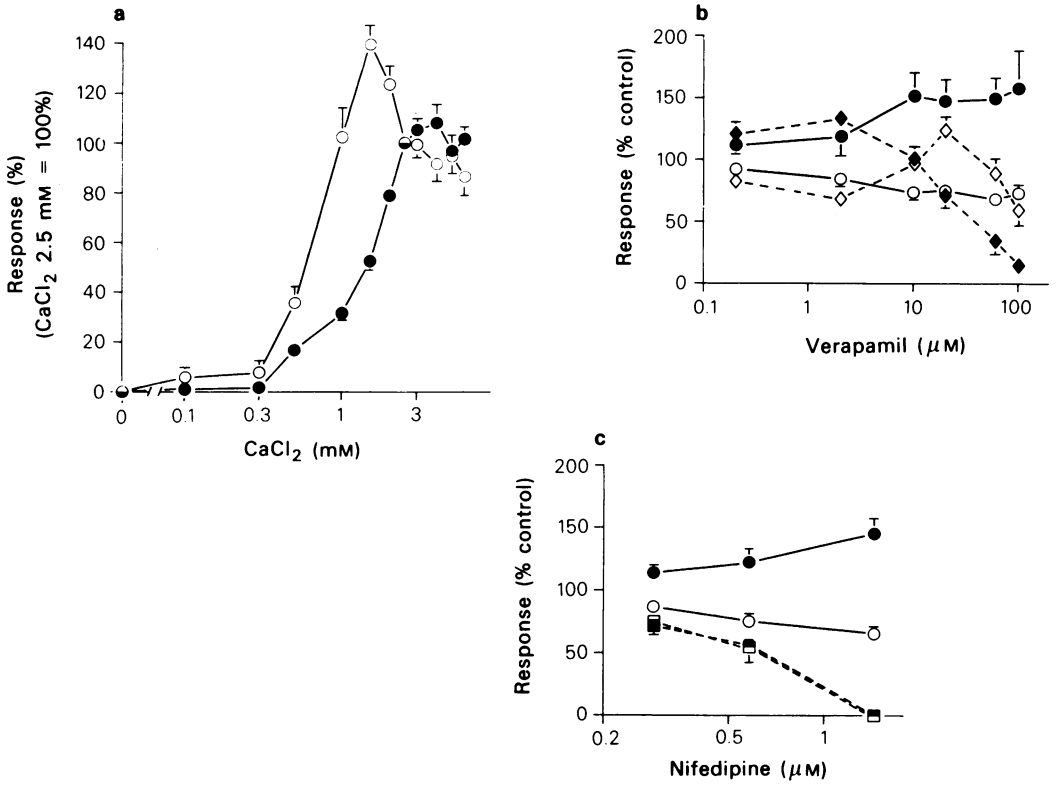

Figure 3 (a) Effect of extracellular calcium concentration on the amplitude (●) and frequency (○) of rhythmic contractions produced by 5-hydroxytryptamine (5-HT 129 μM) in intact vasa deferentia, $n = 8$. (b) Effect of verapamil on rhythmic contractions to 5-HT (129 μM) in intact vasa deferentia: amplitude (◆) and frequency (◇) in the presence of various concentrations of verapamil; amplitude (●) and frequency (○) of contralateral control preparations, $n = 4-6$. (c) Effect of nifedipine on rhythmic responses to 5-HT (129 μM) in intact vasa deferentia: amplitude (■) and frequency (□) in the presence of various concentrations of nifedipine; amplitude (●) and frequency (○) of contralateral control preparations, $n = 5-9$. In both (b) and (c) the amplitude and frequency of the rhythmic contractions is expressed as a percentage of the contractions before addition of nifedipine or verapamil. In untreated tissues (●, ○) there was an increase in amplitude and decrease in frequency during the experiment.

phasic response and the rhythmic contractions were larger than the controls (Figure 4d and Table 1). This increased sensitivity is probably caused by denervation supersensitivity, which results in non-specific potentiation of the effect of noradrenaline, acetylcholine, angiotension, K^+ and Ba^{2+} (Kasuya, Goto, Hashimoto, Watanabe, Munakata & Watanabe, 1969).

Neither atropine (1.44 μM) nor propranolol (3.38 μM) had any effects on either component of the 5-HT (129 μM) contraction ($n = 1-4$).

Discussion

The epididymal half of the rat vas deferens gave a larger response to 5-HT than did the prostatic half, and in this respect 5-HT resembles noradrenaline (Kasuya & Suzuki, 1979) and tyramine (Vardolov & Pennefather, 1976; Table 2), while KCl produces a greater response in the prostatic half (Hay & Wadsworth, 1982a). Although 5-HT, noradrenaline and tyramine all produce rhythmic contractions while KCl does not, there must be additional factors to account for this specificity, since $BaCl_2$ produces rhythmic contractions that are equally prominent in both halves (Hay & Wadsworth, unpublished observations).

We conclude that the phasic component of the 5-HT contraction is mediated via postsynaptic 5-HT receptors, since it is abolished by methysergide, but not blocked by α-adrenoceptor antagonists, by block of $Uptake_1$, or by denervation or reserpinization. The rhythmic component is mediated by a combined effect on 5-HT receptors (mainly affecting the frequency) and noradrenaline release (mainly affecting amp-

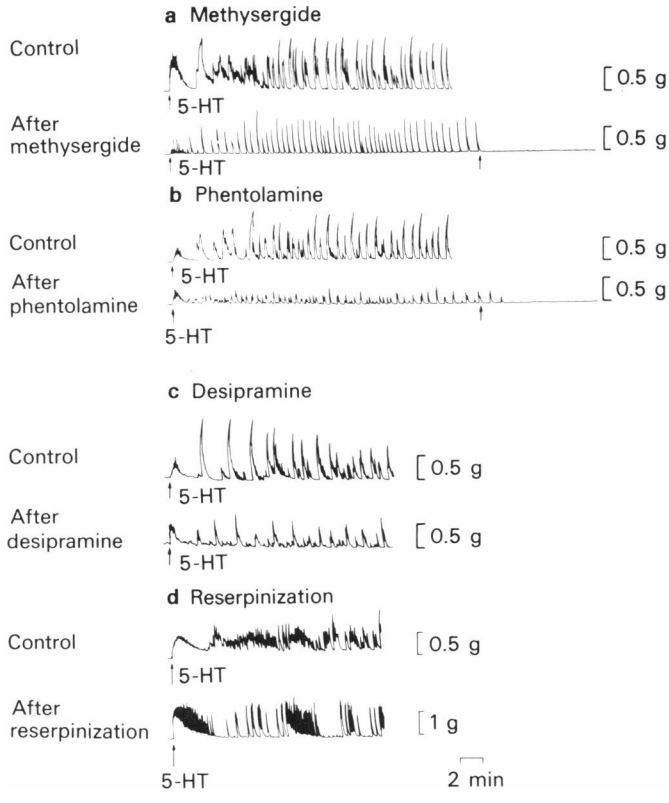

Figure 4 Effects of methysergide, phentolamine, desipramine and reserpinization on responses to 5-hydroxytryptamine (5-HT 129 μM) in intact vasa deferentia. In each section the upper panel shows the control response while the lower panel shows the response (a,b,c) 20 min after addition of the antagonist or (d) in a tissue taken from a reserpinized rat. (a) The phasic response was abolished by methysergide (2.13 μM) and the remaining rhythmic response was abolished by subsequent addition of phentolamine (2.65 μM, at arrow). (b) Phentolamine (2.65 μM) had no effect on the phasic response but reduced the amplitude of the rhythmic response which was abolished by subsequent addition of methysergide (2.13 μM, at arrow). (c) Desipramine (1.32 μM) produced a small reduction in the force of the rhythmic contractions. (d) Both the phasic and the rhythmic components of the 5-HT response were present in the reserpinized vas deferens.

litude). Thus the frequency of the rhythmic contractions was reduced by methysergide, and their amplitude reduced by procedures that prevent the action of noradrenaline (phentolamine, dihydroergotamine) or that prevent release of noradrenaline in response to agents entering the neurone via $Uptake_1$(desipramine). Although desipramine has substantial α-adrenoceptor antagonist action (Barnett, Symchowicz & Taber, 1968), in our experiments it antagonized tyramine but not methoxamine. Reserpine and denervation did not reduce the rhythmic contractions but we believe this is because these procedures induce supersensitivity to 5-HT (Kasuya *et al.*, 1969; Wakade, Kanwar & Gulati, 1970) which cancels out the loss of the noradrenaline component. In our experiments, the 5-HT contraction had no cholinergic or β-adrenoceptor component. Ozawa & Katsuragi (1974) concluded that the first phase of the 5-HT contraction in the guinea-pig vas deferens was mediated by 5-HT receptors and the second phase by noradrenaline release. Our conclusions in the rat vas deferens are in agreement with theirs, but in addition we have found that 5-HT receptors make a contribution to the rhythmic response. We have not been able to repeat the results of Nishino *et al.* (1970) who reported that reserpinization and α-adrenoceptor antagonists, but not a 5-HT antagonist, blocked the first phase of the response to 5-HT (77.4 μM) in the rat vas deferens. The 5-HT contraction has both 5-HT and noradrenaline release components in vascular smooth muscle (Starke & Weitzell, 1978; Marin & Sanchez, 1980) and in the rat anococcygeus (Oriowo, 1981). The action on 5-HT receptors is generally apparent with lower concentrations than those required to release noradrenaline. It is possible that this is also true in the rat vas deferens, since the initial phasic response (entirely involving 5-HT receptors) is maximal at 129 μM, whereas the amplitude of the

Table 3 Effects of methysergide, phentolamine, dihydroergotamine and desipramine on components of the contraction produced by 5-hydroxytryptamine (5-HT 129 μM) in rat intact vasa deferentia (mean ± s.e.mean)

	Phasic component	*Rhythmic component*	
	Tension (g)	Tension (g)	Frequency (min^{-1})
Control	0.40 ± 0.08	0.49 ± 0.07	3.89 ± 0.58
Methysergide (2.13 μM)	0.04 ± 0.01	0.41 ± 0.09	1.67 ± 0.45
n	7(a)	13(c)	13(a)
Control	0.25 ± 0.04	0.40 ± 0.04	5.02 ± 0.60
Phentolamine (2.65 μM)	0.20 ± 0.05	0.26 ± 0.05	4.30 ± 0.59
n	7(d)	10(c)	10(d)
Control		0.48 ± 0.10	2.13 ± 0.45
Dihydroergotamine (1.47 μM)	(e)	0.23 ± 0.15	1.60 ± 0.44
n		3(d)	3(c)
Control	0.29 ± 0.11	0.40 ± 0.06	4.48 ± 0.58
Desipramine (1.32 μM)	0.26 ± 0.12	0.21 ± 0.05	4.53 ± 0.42
n	3(d)	3(d)	3(d)

(a) Significantly different from control $P < 0.001$ (paired test); (b) significantly different from control $P < 0.01$ (paired test); (c) significantly different from control $P < 0.05$ (paired test); (d) not significantly different from control (paired test); (e) the effects of dihydroergotamine were investigated on the rhythmic component only.

rhythmic contractions (predominantly involving released noradrenaline) increases in a dose-related manner up to the highest concentration used (1291 μM).

Both components of the 5-HT contraction were dependent on extracellular Ca^{2+} but the calcium channel inhibitors preferentially inhibited the phasic response. This may indicate that Ca^{2+} required for

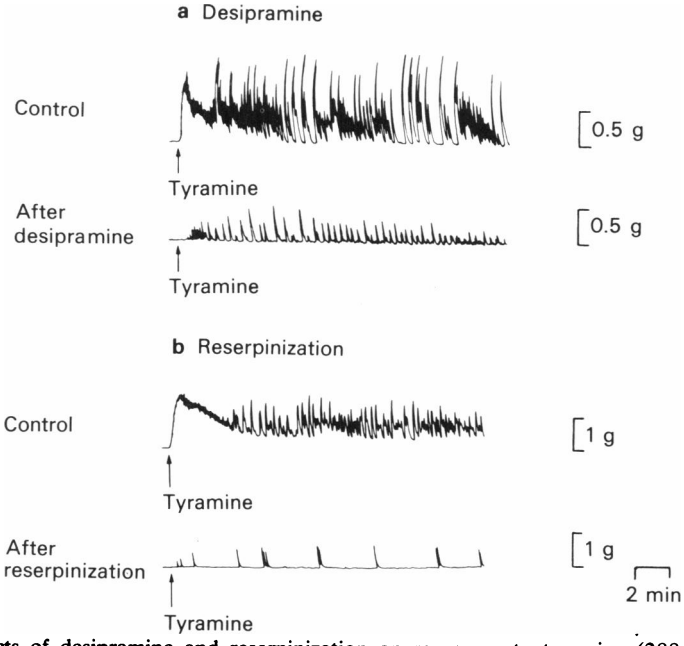

Figure 5 The effects of desipramine and reserpinization on responses to tyramine (288 μM) in intact vasa deferentia. In both sections, the upper panel shows the control response, while the lower panel shows the response (a) 20 min after the addition of desipramine or (b) in a tissue taken from a reserpinized rat. (a) Desipramine (1.32 μM) abolished the phasic and markedly reduced the amplitude of the rhythmic response to tyramine. (b) Both components of the tyramine response were almost abolished by reserpinization.

activation of the two components of the contraction is entering through separate types of Ca^{2+} channel. The phasic response was inhibited by nifedipine and verapamil in concentrations similar to those that inhibit KCl contractions (Hay & Wadsworth, 1982b). We conclude that this part of the response is caused by Ca^{2+} entering through voltage-dependent Ca^{2+} channels (Bolton, 1979), which then inactivate. The rhythmic contractions are less sensitive to calcium channel inhibitors and in this respect are similar to rhythmic contractions produced by methoxamine (Hay & Wadsworth, 1981b). However, there appear to be slight differences between the Ca^{2+} channels mediating the rhythmic contractions induced by 5-HT and those induced by methoxamine. Although verapamil has equal potency, nifedipine causes 50% inhibition of 5-HT-induced rhythmic contractions at 0.53 μM, but 50% inhibition of methoxamine-induced rhythmic contractions requires 2.42 μM (Hay & Wadsworth, 1981b). It is therefore possible that postsynaptic 5-HT receptors and adrenoceptors are linked to separate populations of Ca^{2+} channels, but further studies are necessary to substantiate this.

The rat vas deferens contains appreciable quantities of 5-HT, probably in mast cells (Fuenmayor *et al.*, 1976), although there is no evidence that this has any physiological function. However, if released in locally high concentration, this would exert a powerful contractile effect via both direct and indirect actions possibly involving a specific population of Ca^{2+} channels.

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