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Evidence for autoinhibition of stimulation-induced noradrenaline release from vasa deferentia of the guinea-pig and rat

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1 Both phenoxybenzamine and idazoxan increased the efflux of radioactivity elicited by a train of stimulation (4 pulses at 5 Hz) in vasa deferentia preincubated with [^{3}H]-noradrenaline. Phenoxybenzamine increased the release of radioactivity from vasa stimulated with a single pulse, whereas idazoxan did not.

2 The contractile response in both guinea-pig and rat vasa was biphasic: phenoxybenzamine enhanced the initial twitch component and reduced the second component in guinea-pig vasa stimulated with a single pulse or a train of pulses. Idazoxan enhanced both phases of the response of guinea-pig vasa stimulated with a train of pulses but did not affect the response to stimulation with a single pulse.

3 The effect of phenoxybenzamine in increasing the efflux of radioactivity produced by a single pulse of stimulation was abolished by cocaine, indicating that the increase in efflux was due to blockade of noradrenaline uptake.

4 Contractile responses of guinea-pig vasa stimulated with a single pulse in the presence of cocaine were unaltered by phenoxybenzamine, whereas with a train of stimulation the twitch component was enhanced and the second phase was reduced.

5 The effects of phenoxybenzamine or idazoxan on the efflux of radioactivity from rat vasa portions were qualitatively the same as were observed in whole vasa. The contractile response of the prostatic portion consisted of a rapid twitch with a single pulse of stimulation, but was biphasic with a train of stimulation; the response of the epididymal portion was biphasic with either a single pulse or a train of pulses.

6 These results suggest that there is no inhibitory feedback modulation of noradrenaline release with a single pulse of stimulation in guinea-pig and rat vasa deferentia whereas, with a train of stimulation, there is autoinhibition of noradrenaline release.

Introduction

There is considerable evidence for the concept that transmitter noradrenaline mediates an inhibitory feedback effect on its subsequent release by acting on prejunctional α-adrenoceptors (Starke, 1977; Westfall, 1977; Gillespie, 1980; Rand *et al.*, 1980; Langer, 1981). The effect of α-adrenoceptor antagonists in increasing the nerve stimulation-induced release of noradrenaline can be attributed to disruption of the autoinhibitory feedback loop by blockade of the prejunctional α-adrenoceptors. It follows that blockade of prejunctional α-adrenoceptors should not result in an increase in noradrenaline release elicited by a single pulse of stimulation since there is no noradrenaline in the biophase of the prejunctional α-adrenoceptors before that pulse has released transmitter, and such has been found to be the case in isolated atria (Rand *et al.*, 1973; 1975). However, Kalsner (1979a,b) showed that phenoxybenzamine (33 μM) increased the release of noradrenaline elicited by a single pulse from guinea-pig isolated vas deferens and construed this finding as evidence against the concept of autoinhibition of noradrenergic transmission. He rejected the possibility that blockade of noradrenaline uptake could explain his finding because pretreatment with cocaine (8.8 μM) and hydrocortisone (28 μM) did not prevent the effect of phenoxybenzamine.

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We decided to re-examine the question in isolated vasa deferentia from guinea-pigs and rats, using a lower concentration of phenoxybenzamine (3 μM) to reduce the degree of its neuronal uptake blocking effect, and a higher concentration of cocaine (30 μM) to obtain a relatively greater degree of neuronal uptake blockade with this agent. We also used the relatively specific α_2-adrenoceptor antagonist idazoxan (RX781094) (Doxey *et al.*, 1983), which has been shown by Marshall (1983) to have no effect on the release of noradrenaline from mouse vas deferens induced by a single pulse. Furthermore, since the contractions of the prostatic and epididymal portions are different with respect to the fast and slow components of the contractile response to a single pulse of stimulation (Anton *et al.*, 1977) and α-adrenoceptor antagonists affect the two components differently (Brown *et al.*, 1979), we decided to investigate whether there was any difference between the two portions in the effect of α-adrenoceptor antagonists on stimulation-induced release of noradrenaline.

Methods

Guinea-pigs (500–800g) and rats (300–500g) were killed by a blow to the head. Their vasa deferentia were dissected free from adjoining connective tissue and then desheathed. In some experiments, the rat vasa were divided into prostatic and epididymal portions. After dissection, noradrenaline stores were labelled by incubating the tissues for 60 min at 37°C in 0.84 μM (−)-[7,8-^{3}H]-noradrenaline hydrochloride (8 Ci mmol^{-1}). After incubation, each vas (or portion of vas) was attached to an isometric strain gauge and adjusted to provide a resting tension of 1 g force. The output was recorded on a Rikadenki chart recorder. The tissue was superfused with Krebs-Henseleit solution gassed with a 5% CO_2/95% O_2 mixture. The Krebs-Henseleit solution was maintained at 37°C and was delivered at a constant flow rate of 4 ml min^{-1} by an LKB peristaltic pump. Two platinum ring electrodes, 0.5 cm apart, were placed around the prostatic end for stimulating the intramural nerves. After 60 min of superfusion, a 'priming stimulation' consisting of a 30 s train of 2 ms pulses at 1 Hz was applied to assist in the removal of loosely bound radioactive compounds and superfusion was continued for a further 30 min.

Following the washout period, the tissues were stimulated 3 times at 30 min intervals with supramaximal monophasic square wave pulses of 2 ms duration using a Grass S88 stimulator. The stimuli delivered were monitored on a cathode ray oscilloscope. When cocaine was used, it was added to the superfusion fluid 15 min before the first period of stimulation and remained present throughout the experiment. When α-adrenoceptor antagonists were used, they were added to the superfusion fluid 25 min before and removed 5 min after the second period of stimulation.

The resting efflux of radioactivity was taken as the mean of the radioactivity in three 1 min samples collected before each period of stimulation. The stimulation-induced (S-I) efflux of radioactivity was determined by subtracting the mean resting efflux from the radioactivity present in each of five 1 min samples collected from the commencement of stimulation and summing the differences.

The amount of radioactivity present in the 1 min samples was measured in a Packard liquid scintillation counter and was expressed as disintegrations per min (d.p.m.). Corrections for counting efficiency were made by an automatic internal standard.

The Krebs-Henseleit solution was of the following composition (mmol l^{-1}): NaCl 118, KCl 4.7, $NaHCO_3$ 2.5, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-glucose 11.1, sodium edetate 0.067 and ascorbic acid 0.07.

The drugs used were: cocaine hydrochloride (MacFarlan Smith), phenoxybenzamine hydrochloride (Smith Kline & French) and idazoxan hydrochloride (RX781094) (Reckitt & Colman). Phenoxybenzamine was dissolved in a mixture (1:1) of propylene glycol and 0.2 μM acetic acid. Other drugs were dissolved in

Table 1 Efflux of radioactivity, before (resting efflux) and that induced by the first period of stimulation, from [^{3}H]-noradrenaline-labelled isolated superfused whole vasa deferentia of the guinea-pig and rat

	Stimulation (pulses)	n	*Resting efflux* (d.p.m. per min)	*Stimulation-induced efflux* (d.p.m.)
Guinea-pig	1	10	5111 ± 143	263 ± 16
	4	11	4732 ± 261	842 ± 102
Rat	1	4	6815 ± 246	224 ± 61
	4	4	6156 ± 277	489 ± 92

Data shows are means ± s.e.mean.

Table 2 Resting and stimulation-induced (S-I) effluxes of radioactivity in the second period in the absence (control) or presence of phenoxybenzamine or idazoxan

	Guinea-pig			*Rat*		
	Resting (%)	*S-I* (%)	n	*Resting* (%)	*S-I* (%)	n
Control						
1 pulse	82.3 ± 1.6	82.9 ± 4.4	10	81.2 ± 2.1	123 ± 21.3	4
4 pulses	81.0 ± 1.2	90.7 ± 4.1	11	95.2 ± 1.0	93.5 ± 14.1	4
Phenoxybenzamine (3 μM)						
1 pulse	109 ± 4.3*	263 ± 40.7 *	4	127.3 ± 9.8*	271 ± 38.4*	11
4 pulses	105 ± 4.5 *	360 ± 51*	6	118 ± 2.4*	395 ± 24*	4
Phenoxybenzamine (30 μM)						
1 pulse	306 ± 5.6*	700 ± 6.27*	6			
4 pulses	288.7 ± 11.1*	768.3 ± 153*	4			
Idazoxan (1 μM)						
1 pulse	88.2 ± 1.7	95.1 ± 7.5	12			
4 pulses	89.3 ± 2.1	252.1 ± 29.4*	10			

Results are expressed as percentages of the effluxes in the first period and means ± s.e.means are shown.
* $P < 0.05$ when compared with the corresponding controls.

distilled water. Stock solutions were subsequently diluted to give the required concentrations of drugs in Krebs-Henseleit solution.

The radioisotope (−)-[7,8-^{3}H]-noradrenaline hydrochloride (specific activity 8 Ci mmol^{-1}) was obtained from the Radiochemical Centre, Amersham.

Means and standard errors are given throughout the paper and statistical analysis was by an unpaired, 2-tailed Student's *t* test. Analysis of variance was also performed on the data.

Results

Resting and stimulation-induced effluxes of radioactivity

The effluxes of radioactivity, from [^{3}H]-noradrenaline-labelled whole vasa deferentia from guinea-pigs and rats, occurring spontaneously and elicited by the first period of stimulation with 4 pulses at 5 Hz or with a single pulse are shown in Table 1. The resting effluxes

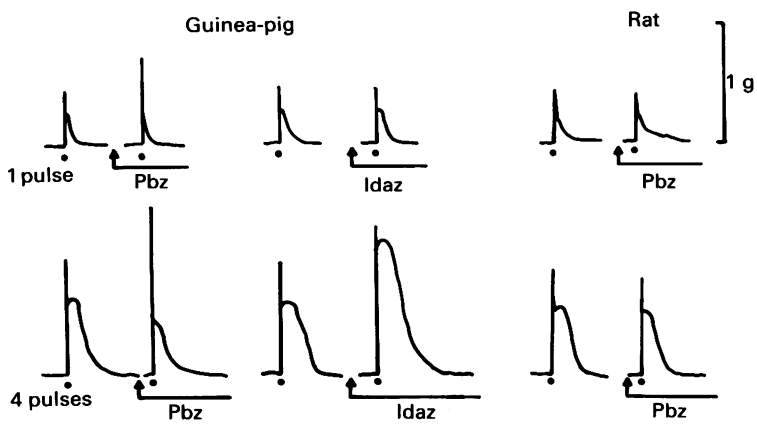

Figure 1 Isometric contractions of whole vasa deferentia from guinea-pig and rat in response to field stimulation with a single 2 ms pulse or 4.2 ms pulses at 5 Hz. Stimulation indicated by dots below records. The second of each pair of responses was elicited 25 min after the first after exposure for 25 min to either phenoxybenzamine (Pbz) 3 μM or idazoxan (Idaz) 1 μM.

of radioactivity were approximately 5000 d.p.m. per min for guinea-pig and 6500 d.p.m. per min for rat vasa. The effluxes were increased by about 250 d.p.m. by a single pulse of stimulation in guinea-pig and rat vasa, and by 830 d.p.m. and 490 d.p.m. in guinea-pig and rat vasa, respectively, by stimulation with a train of 4 pulses at 5 Hz.

The contractile responses to stimulation with a single pulse or trains of pulses were biphasic in both species. These consisted of an initial fast twitch component followed by a more prolonged secondary component, as shown in Figure 1.

Effects of phenoxybenzamine

Phenoxybenzamine (3 μM) slightly but significantly increased the resting effluxes of radioactivity from both guinea-pig and rat whole vasa deferentia to 25–50% above the corresponding control values (Table 2). A higher concentration of phenoxybenzamine (30 μM) produced about a 3 fold increase in the resting efflux of radioactivity from the guinea-pig vas deferens (Table 2).

Phenoxybenzamine (3 μM) increase the stimulation-induced effluxes of radioactivity elicited by a single pulse above the corresponding control value by about 3.6 fold in guinea-pig and 2.3 fold in rat vasa deferentia (Table 2). The higher concentration of phenoxybenzamine (30 μM) produced about an 8 fold increase in the stimulation-induced efflux of radioactivity elicited by a single pulse in guinea-pig vasa deferentia (Table 2).

Phenoxybenzamine (3 μM) increased the efflux of radioactivity elicited by 4 pulses of stimulation by 3.9 fold and 4.2 fold, respectively, in guinea-pig and rat vasa deferentia. The higher concentration of phenoxybenzamine (30 μM) produced about an 8 fold increase in the efflux of radioactivity elicited by 4 pulses of stimulation in guinea-pig vas deferens (Table 2).

In the guinea-pig, but not in the rat vas deferens, the initial rapid twitch component of the contractile responses to stimulation with 1 or 4 pulses was enhanced by phenoxybenzamine (3 μM); however, the second slow component of the response was diminished in vasa deferentia of both species, except with a single pulse in the rat vas (Figure 1).

Effects of idazoxan

In guinea-pig whole vasa deferentia, the relatively specific α_2-adrenoceptor antagonist idazoxan (1 μM) had no significant effect on the resting efflux of radioactivity. The stimulation-induced efflux of radioactivity elicited by a train of 4 pulses was significantly increased to 1.4 fold above the corresponding control value (Table 2). However, in contrast to the finding with phenoxybenzamine, idazoxan did not significantly increase the efflux of radioactivity elicited by a single pulse. Analysis of variance showed no significant variance between control experiments at 1 pulse and those where idazoxan was present.

Idazoxan (1 μM) enhanced both phases of the contractile response of the guinea-pig vas deferens stimulated with a train of 4 pulses but did not appreciably affect the response to stimulation with a single pulse (Figure 1).

Effects of cocaine on the response to phenoxybenzamine

The blockade of neuronal re-uptake by phenoxybenzamine may explain the difference between the effects of this drug and idazoxan on the efflux of radioactivity elicited by a single pulse. Therefore, experiments were carried out to determine the effect of phenoxybenzamine (3 μM) in the presence of cocaine (30 μM), which blocks neuronal uptake.

The resting and stimulation-induced effluxes of radioactivity during the first period of stimulation in the presence of cocaine (30 μM) are given in Table 3. Exposure to cocaine increased the resting efflux of radioactivity from guinea-pig vas deferens by a mean

Table 3 Efflux of radioactivity, before (resting efflux) and that induced by the first period of stimulation, in the presence of cocaine (30 μM), from [^{3}H]-noradrenaline-labelled isolated superfused whole vasa deferentia of the guinea-pig and rat

Species	*Stimulation* (pulses)	n	*Resting efflux* (d.p.m. per min)	*Stimulation-induced efflux* (d.p.m.)
Guinea-pig	1	10	8515 ± 369	665 ± 110
	4	4	9696 ± 424	1493 ± 583
Rat	1	8	5479 ± 106	482 ± 64
	4	5	4806 ± 410	767 ± 83

Data shown are means ± s.e.mean.

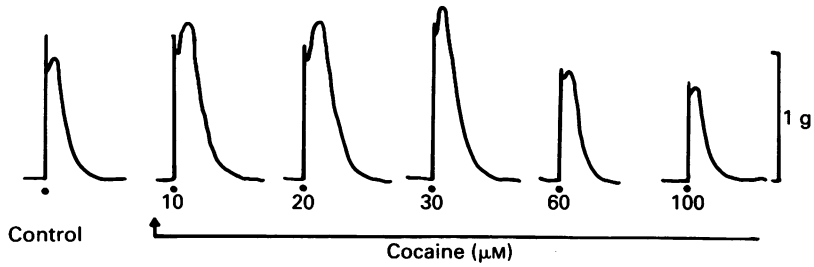

Figure 2 Contractile response of guinea-pig whole vas deferens elicited by a train of 4 2 ms pulses at 5 Hz (stimulation is indicated by the dots below the records) before (control) and after tissue had been exposed to each of the various concentrations of cocaine for 25 min.

Table 4 Effect of cocaine (30 μM) on the resting and stimulation induced (S-I) effluxes of radioactivity in the second period in the absence (control) or presence of phenoxybenzamine (3 μM)

	Guinea-pig			*Rat*		
	Resting (%)	*S-I* (%)	n	*Resting* (%)	*S-I* (%)	n
Control						
1 pulse	85.0 ± 2.38	111.6 ± 18.4	10	93.9 ± 3.48	94.6 ± 4.7	8
4 pulses	88.2 ± 4.8	74.3 ± 1.0	4	87.2 ± 3.4	92.2 ± 3.6	5
Phenoxybenzamine (3 μM)						
1 pulse	88.9 ± 1.96	88.0 ± 10.6	8	102.2 ± 2.8	111.4 ± 17.5	9
4 pulses	91.7 ± 4.6	272.4 ± 28.1*	10	99.0 ± 3.4	301.3 ± 22.6*	4

Results are expressed as percentages of the effluxes in the first period and means ± s.e.means are shown. Cocaine was present throughout the first and second periods.
* $P < 0.05$ when compared with the corresponding controls.

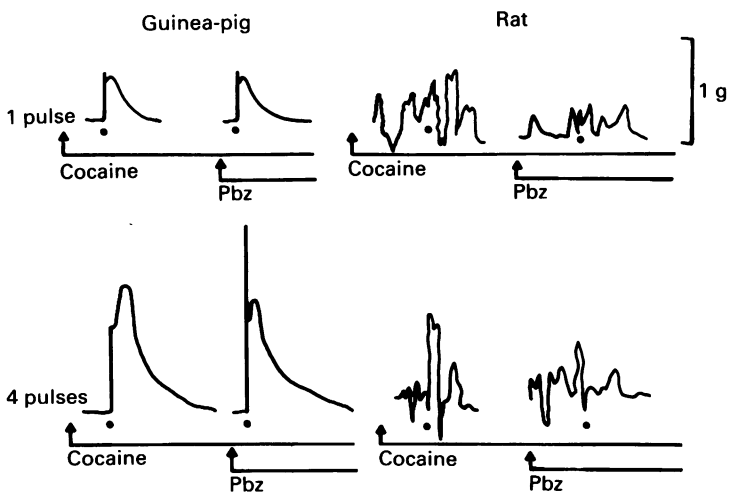

Figure 3 Effects of phenoxybenzamine (Pbz) 3 μM on contractile responses of whole vasa deferentia from guinea-pig and rat in the presence of cocaine (30 μM) elicited by a single 2 ms pulse and a train of 4 2 ms pulses at 5 Hz. Stimulation is indicated by the dots beneath the records. Cocaine was present throughout the experiments. Phenoxybenzamine was added 25 min before the portion of record shown. Note that cocaine induced irregular contractile activity in the rat vas deferens and this obliterated the responses to stimulation.

Table 5 Efflux of radioactivity, before (resting efflux) and that induced by the first period of stimulation from [^{3}H]-noradrenaline-labelled prostatic and epididymal portions of rat vasa deferentia

	Stimulation (pulses)	n	*Resting efflux* (d.p.m. per min)	*Stimulation-induced efflux* (d.p.m.)
Prostatic	1	3	4870 ± 213	160 ± 25
	4	3	4562 ± 155	279 ± 18
Epididymal	1	7	2822 ± 183	146 ± 19
	4	3	3447 ± 184	253 ± 6

Data shown are means ± s.e.mean.

of 84%, but decreased that from rat vas deferens by a mean of 21% (cf. Tables 1 and 3). The stimulation-induced effluxes were, however, increased in vasa deferentia from both species with both 1 and 4 pulses of stimulation by about 60% and 130%.

Cocaine in concentrations of 10, 20 and 30 μM increased the second slow component of the contractile response to stimulation in guinea-pig vasa deferentia without appreciably affecting the initial rapid twitch component; higher concentrations, 60 and 100 μM, reduced both phases of the response. Typical records from an experiment are shown in Figure 2. The above findings indicate that the local anaesthetic activity of cocaine is not appreciably manifested until the concentration exceeds 30 μM.

Phenoxybenzamine (3 μM) in the presence of cocaine (30 μM) slightly increased the resting release of radioactivity, but to a lesser extent than that occurring in the absence of cocaine, the overall mean increase being about 10% (Table 4, cf. Table 2).

The efflux of radioactivity elicited by a single pulse of stimulation was not significantly affected by phenoxybenzamine (3 μM) in the presence of cocaine (30 μM) (there was also no significant variance between control and drug experiments); but the effluxes elicited by 4 pulses were significantly increased under these conditions by 3.6 fold and 3.3 fold, respectively, in guinea-pig and rat vasa deferentia (Table 4). However, the effect of phenoxybenzamine (3 μM) in increasing the efflux elicited by 4 pulses was not as great in the presence as in the absence of cocaine (Table 4; cf. Table 2).

The contractile responses of guinea-pig vasa stimulated with a single pulse in the presence of

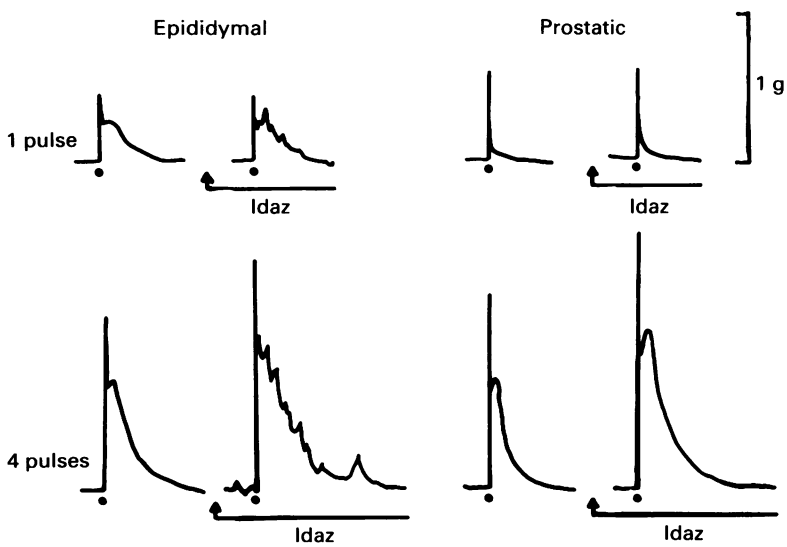

Figure 4 Effect of idazoxan (Idaz) 1 μM on contractile responses of epididymal and prostatic portions of rat vas deferens elicited by a single 2 ms pulse or 4 2 ms pulses at 5 Hz. Stimulation is indicated by the dots beneath the records. Idazoxan was present for 25 min before the second of each pair of responses was elicited.

Table 6 Resting and stimulation induced (S-I) effluxes of radioactivity in the second period in the absence (control) or presence of phenoxybenzamine or idazoxan in rat vasa deferentia portions

	Epididymal portion			*Prostatic portion*		
	Resting (%)	*S-I* (%)	n	*Resting* (%)	*S-I* (%)	n
Control						
1 pulse	89.9 ± 2.0	93.1 ± 7.2	7	94.2 ± 0.5	108 ± 10.3	3
4 pulses	91.9 ± 1.3	87.6 ± 3.3	3	94.2 ± 0.6	87.7 ± 1.5	3
Phenoxybenzamine (3 μM)						
1 pulse	123.8 ± 14.5*	310 ± 69.9*	4	123.5 ± 14.9*	330 ± 96.0*	4
4 pulses	97.7 ± 3.4	318.6 ± 37.1*	3	103.6 ± 6.0*	411 ± 62.7*	4
Idazoxan (1 μM)						
1 pulse	88.7 ± 1.9	107.6 ± 24	8	90.4 ± 2	92.7 ± 10.8	3
4 pulses	86.5 ± 2.4	215 ± 2.1*	4	86.3 ± 2.7	232.5 ± 6.4*	4

Results are expressed as percentages of the effluxes in the first period and means ± s.e.means are shown.
* $P < 0.05$ when compared with the corresponding controls.

cocaine were unaltered by phenoxybenzamine, whereas with a train of 4 pulses of stimulation, the twitch component was enhanced and the slow phase was reduced (Figure 3). Rat vasa deferentia contracted spontaneously when cocaine was added, making measurement of stimulation-induced contractions impossible, as shown in Figure 3.

Comparison of epididymal and prostatic portions of rat vas deferens

The resting efflux of radioactivity from the prostatic portion of the vas deferens was consistently greater than that from the epididymal portion, the mean values being 4716 d.p.m. (s.e.mean = 136, $n = 6$) and 3134 d.p.m. (s.e.mean = 126, $n = 10$), respectively. The stimulation-induced efflux of radioactivity was similar for both portions and greater for 4 pulses of stimulation than for a single pulse (Table 5). The sums of the various effluxes from the 2 portions were in reasonable accord with the corresponding effluxes from the whole vas deferens (cf. Table 1).

The contractile response of the prostatic portion consisted of a single, rapid twitch with 1 pulse of stimulation and was biphasic with 4 pulses. The contractile response of the epididymal portion was biphasic with both 1 and 4 pulses of stimulation (Figure 4).

Despite the differences in contractile responses, the effects of phenoxybenzamine or idazoxan on the efflux of radioactivity from the two portions were qualitatively the same as those observed in whole vasa deferentia, that is, phenoxybenzamine (3 μM) increased the resting and stimulation-induced effluxes of radioactivity elicited by 1 or 4 pulses. However, idazoxan (1 μM) had no significant effect on resting efflux or efflux elicited by a single pulse (there was also no significant variance between control and drug experiments), but increased efflux elicited by 4 pulses at 5 Hz. The results are summarized in Table 6.

Idazoxan did not affect the height of the contractile response of prostatic and epididymal portions stimulated with single pulses; however, with trains of stimulation there was an increase in both the twitch and slow component in both portions of the tissue, as shown in Figure 4. In the epididymal portion, irregularities were induced in the slow component of the response by idazoxan.

Discussion

The efflux of radioactivity from [^{3}H]-noradrenaline-labelled vasa deferentia provides an index of the release of noradrenaline from transmitter stores (Langer, 1970; Langer & Vogt, 1971). When the sympathetic nerves are stimulated, the compounds carrying the tritium marker are noradrenaline and metabolites formed by the action of monoamine oxidase and catechol-*O*-methyltransferase. In the presence of phenoxybenzamine, the stimulation-induced efflux of radioactivity is increased, and noradrenaline itself accounts for almost all of the radioactivity: this is because phenoxybenzamine blocks both neuronal and extraneuronal uptake of noradrenaline and hence impedes the access of noradrenaline released exocytotically by nerve impulses to the intracellularly located sites of the metabolizing enzymes.

The concept that noradrenaline, released as the sympathetic neurotransmitter, normally exerts an

inhibitory effect on its subsequent release by a feedback action on prejunctional α-adrenoceptors is well established (for references see Introduction). It follows from the concept that blockade of the prejunctional α-adrenoceptors involved in the autoinhibitory feedback loop should result in an increase in the release of transmitter noradrenaline evoked by a train of nerve impulses, and hence in an increased efflux from the tissue. It would also follow that the noradrenaline released by a single nerve impulse should not be increased by blockade of prejunctional α-adrenoceptors since no noradrenaline has yet been released to act on these receptors. (It is of course possible that spontaneously released noradrenaline could be exerting a tonic effect on prejunctional α-adrenoceptors, and if this were so it would vitiate the deduction about the expected effect of α-adrenoceptors on the release of noradrenaline evoked by a single pulse of stimulation; however, as it transpires, it is not necessary to evoke such a tonic effect.)

Given the hypothesis of autoinhibition of noradrenergic transmission, observations on the effect of blockade of prejunctional α-adrenoceptors provide a useful test of the validity of the hypothesis. Kalsner (1979a) showed that a high concentration of phenoxybenzamine (33 μM) produced a 3 fold increase in the efflux of noradrenaline from guinea-pig vas deferens evoked by a single pulse of stimulation. In similar experiments, we found that a lower concentration of phenoxybenzamine (3 μM) produced 3.6 fold and 2.3 fold increases in noradrenaline effluxes from guinea-pig and rat vasa deferentia, respectively, and a higher concentration of phenoxybenzamine (30 μM) produced an 8 fold increase in the release from the guinea-pig vas deferens. Accordingly, as Kalsner (1979a,b) has already pointed out, it appears that the hypothesis of autoinhibition of noradrenergic transmission is not sustained by these findings, and that another explanation should be sought to account for the increase in stimulation-induced release of noradrenaline produced by phenoxybenzamine. There is, in fact, another explanation which satisfactorily accounts for the effect of phenoxybenzamine in such a way that the findings do not challenge the hypothesis of autoinhibition of noradrenergic transmission.

There is abundant evidence that phenoxybenzamine not only blocks α-adrenoceptors but also inhibits noradrenaline uptake in sympathetically innervated tissues, including the vas deferens (Iversen, 1965; Iversen & Langer, 1969). It is only slightly less potent than cocaine as a blocker of noradrenaline uptake and has an apparent K_i value for this action of 7.4–11.3 μM in rat vas deferens (Iversen & Langer, 1969). Phenoxybenzamine (30 or 33 μM) would therefore cause a very substantial (almost complete) blockade of noradrenaline uptake; the blockade would be less, but nevertheless considerable, with 3 μM phenoxybenzamine. Kalsner (1979a) rejected the possibility that blockade of noradrenaline uptake by phenoxybenzamine accounted for the increase in the efflux evoked by a single pulse of stimulation on the grounds that the effect of phenoxybenzamine (33 μM) was the same in the absence and in the presence of cocaine (8.8 μM) and hydrocortisone (28 μM), which were used with the intention of blocking both neuronal and extraneuronal uptake of noradrenaline. However, since 33 μM phenoxybenzamine is producing almost complete blockade of neuronal uptake, the addition of 8.8 μM cocaine is of little consequence and would not be expected to contribute appreciably. The same is probably true for blockade of extraneuronal uptake by phenoxybenzamine and hydrocortisone.

In contrast, when the more modest concentration of 3 μM phenoxybenzamine is used, pretreatment with cocaine (30 μM) abolishes the effect of phenoxybenzamine in increasing the noradrenaline efflux evoked by a single pulse of stimulation. Cocaine 30 μM was selected because it would produce an almost complete blockade of neuronal uptake of noradrenaline without any appreciable manifestation of blockade of nerve conduction. Thus the effect of 3 μM phenoxybenzamine in increasing noradrenaline release evoked by a single pulse of stimulation can be explained entirely by blockade of neuronal uptake.

Unlike the increase in efflux of noradrenaline produced by phenoxybenzamine with a single pulse of stimulation in vasa deferentia, phenoxybenzamine does not enhance the release of noradrenaline from guinea-pig atria elicited by a single pulse (Rand *et al.*, 1973; 1975). The difference between the two tissues possibly lies in differences in the widths of their neuroeffector clefts. The narrower the neuroeffector cleft, the more important is neuronal uptake in the regulation of transmitter concentration in the biophase of the cleft. The vas deferens has narrow neuroeffector clefts and the highest noradrenaline content of any tissue (Dixon & Gosling, 1972; Furness & Iwayama, 1972; Furness, 1974): it is likely, therefore, that it would be more sensitive to blockade of uptake than the atrium which has a wider neuroeffector cleft.

The recently developed α-adrenoceptor antagonist idazoxan has been shown to have a selective action on prejunctional α-adrenoceptors in various tissues, including the vas deferens (Doxey *et al.*, 1983). In a concentration of 1 μM, idazoxan did not increase the efflux of noradrenaline from the vas deferens evoked by a single pulse of stimulation. This concentration of idazoxan has no neuronal uptake blocking activity, at least in rat atria, and did not increase the efflux of noradrenaline from the atria evoked by a single pulse of stimulation (unpublished observations). Furthermore, Marshall (1983) has shown that neither idazoxan nor yohimbine affected the noradrenaline

efflux from mouse vas deferens evoked by a single pulse of stimulation.

When vasa deferentia were stimulated with 4 pulses at 5 Hz, idazoxan (1 μM) increased the efflux of noradrenaline, as did phenoxybenzamine (3 μM) in the presence as well as in the absence of cocaine (30 μM). In the presence of cocaine, the phenoxybenzamine-induced increase in noradrenaline efflux evoked by 4 pulses was slightly less than that produced in the absence of cocaine, suggesting that a small portion of the increase in efflux was due to blockade of noradrenaline re-uptake, but the greater part was due to disruption of the autoinhibitory feedback loop resulting from blockade of prejunctional α-adrenoceptors.

The actions of the α-adrenoceptor antagonists on the efflux of noradrenaline from the epididymal and prostatic portions followed the same pattern as for whole vas deferens. Phenoxybenzamine increased the noradrenaline effluxes elicited by either a single pulse or a train of pulses, whereas idazoxan increased only the efflux of noradrenaline elicited by a train of pulses. The two components of the contractile responses of the vas deferens contribute to different extents in the two portions, so the two portions respond differently to various drugs (Brown *et al.*, 1979; McDonald & McGrath, 1980). The ability to block either component preferentially strongly suggests that there is non-noradrenergic transmission in the vas deferens as well as noradrenergic transmission. Even though the noradrenergic twitch component predominates at the prostatic end of the tissue, efflux of noradrenaline from both portions was affected in a similar manner. The only difference in noradrenaline effluxes was that the prostatic end had a higher resting efflux; this difference is probably due to the greater noradrenaline content, which has been found to be 14.5 $\mu g\,g^{-1}$ in the prostatic portion and 7.0 $\mu g\,g^{-1}$ in the epididymal portion (Zieher & Jaim-Etcheverry, 1971).

The enhancement by idazoxan of both phases of the contractile responses stimulated with a train of pulses is consistent with it possessing only antagonistic activity on α_2-adrenoceptors. The initial twitch component of the response has been considered to be non-noradrenergic whereas the second slow component is noradrenergic and due to activation of postjunctional α_2-adrenoceptors. Enhancement of both components by idazoxan could be explained in terms of disruption of a noradrenaline-mediated inhibition of the release of both transmitters involving α_2-adrenoceptors.

In conclusion, the effect of phenoxybenzamine in increasing the release of noradrenaline elicited by a single pulse of stimulation in guinea-pig and rat vasa deferentia does not involve α_2-adrenoceptors (as the specific α_2-adrenoceptor antagonist idazoxan did not increase efflux), but is due to block of uptake of noradrenaline, since phenoxybenzamine did not increase noradrenaline release when cocaine was present. The discrepancy between these results and those of Kalsner (1979a) lies in the concentrations of the drugs used and in the choice of α-adrenoceptor antagonists. With trains of stimulation, a functional negative feedback system exists in the vas deferens, as revealed by an increase in the stimulation-induced release of noradrenaline produced by idazoxan and phenoxybenzamine in the presence of cocaine. Although the prostatic and epididymal portions differ in their postjunctional responses and possibly in their innervation, the regulation of noradrenaline release appears to involve the same mechanisms throughout the length of the tissue. The non-noradrenergic component of the response also seems to be subject to an inhibitory effect on transmitter release by an action of noradrenaline on prejunctional α_2-adrenoceptors.

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