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A hypothesis to explain the presynaptic effects of adrenoceptor antagonists

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1 The hypothesis of negative feedback regulation of transmitter release was examined in a range of tissues obtained from three species.

2 Tissues were transmurally stimulated with 100 pulses at 2 Hz with pulse durations from 50 μs to 5,000 μs, and the efflux of [^{3}H]-noradrenaline determined.

3 The stimulation-induced efflux of tritium increased with increasing pulse duration, but yohimbine, a prototypal α_2-antagonist had an effect which was consistently contrary to expectations for a negative feedback system. Enhancement of efflux by the antagonist, supposedly correlated directly with the extent of ongoing auto-inhibition, became smaller rather than larger as the stimulation-induced efflux rose with increases in pulse duration, with all other parameters of stimulation maintained constant. Similar findings were obtained in rat spleen with the haloalkylamine antagonist, phenoxybenzamine.

4 It is concluded that the presynaptic effects of adrenoceptor antagonists do not involve a negative feedback function nor do they relate, in any detectable way, to the extracellular concentration of transmitter.

5 The effects on stimulation-induced tritium efflux of yohimbine, phenoxybenzamine and enlargment of the pulse duration, in a variety of tissues, support the previously described hypothesis of a common action to enhance efflux. The antagonists increased efflux to approximately the same value between 50 and 1,000 μs pulse durations and that value was equivalent to that obtained in each given tissue with pulses of 1,000–2,000 μs in the absence of the antagonist.

6 Tetraethylammonium, an inhibitor of stimulation-induced potassium efflux from nerves had an effect on transmitter efflux in rat spleen essentially like that of the adrenoceptor antagonists. These findings provide further support for an alternative to the hypothesis of negative feedback. Yohimbine and other presynaptic antagonists may prolong the period of potassium efflux from nerve varicosities, and by this means prolong depolarization and the associated period of transmitter release, rather than act by disrupting an ongoing system sensing and responding to fluctuations in extracellular transmitter levels.

Introduction

The hypothesis that noradrenaline release from nerves is set by presynaptic inhibitory receptors responsive to the free synaptic concentration of previously released neurotransmitter is widely accepted (see review by Starke, 1981). Feedback regulation seemed to provide the explanation for the observed enhancement of stimulation-induced efflux by adrenoceptor antagonists and its inhibition by agonists. A body of work has appeared, however, which questions the validity of the presynaptic receptor hypothesis (e.g. Kalsner, 1979a,b; 1982a,b; 1983b,c; Kalsner & Chan, 1979; Drew, 1980; Holman & Surprenant, 1980; Fitzgerald *et al.*, 1981; Blakeley *et al.*, 1982).

Recently an alternative explanation was put forward to account for the effects of antagonists on stimulation-related efflux of transmitter (Kalsner, 1983a). Evidence was provided, from a study in guinea-pig atria, that yohimbine, a presumed prototypal presynaptic α_2-antagonist, prolongs the period of potassium efflux from nerve terminals during the action potential. In this way depolarization is prolonged and also the associated period of transmitter release, independently of the ambient concentration of free synaptic transmitter. The present study is a more extensive investigation into the applicability of the hypothesis to a range of cardiovascular and non-cardiovascular tissues taken from three species.

Methods

Tissue preparation

Rats, guinea-pigs and rabbits of either sex were killed by cervical dislocation or decapitation and the appropriate tissues immediately removed and placed in oxygenated (95% O_2 plus 5% CO_2) Krebs-Henseleit (Krebs) solution at pH 7.4 (composition (mM): NaCl 115.3, KCl 4.6, $CaCl_2$ 2.3, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, KH_2PO_4 1.1, glucose 7.8 and disodium edetate 0.03). The left atria of rabbits was cut into four longitudinal strips and that of the rat into two strips. The two vasa deferentia were desheathed and left intact; the ureters were cut into distal and proximal halves and the two distal portions considered as one matched pair and the two proximal another; capsular strips of the rat spleen were used, with two adjoining strips (1.7 cm long) taken from each spleen. In experiments with phenoxybenzamine and tetraethylammonium (TEA) one single strip (1.0 cm long) was taken from each spleen. The two central ear arteries of the rabbit were opened longitudinally and each cut into two equal segments. All tissues were trimmed of adherent fat and connective tissue. The tissues were incubated for 60 min in 4.0 ml of continuously oxygenated Krebs solution containing (−)-[7,8-^{3}H]-noradrenaline (10 μCi ml^{-1}, 7.6–10.0 $\times$ 10^{-7} M) at 37°C. Following incubation, the tissues were briefly washed with fresh Krebs solution and then mounted under 1 g tension (for support), between platinum electrodes in a superfusion apparatus. The preparations were continuously superfused with warmed (37°C) and oxygenated Krebs solution by a Harvard peristaltic pump which maintained a constant flow rate of 5 ml min^{-1}. The Krebs superfusion solution routinely contained cocaine (8.8 $\times$ 10^{-6} M) and normetanephrine (1 $\times$ 10^{-5} M) to block neuronal and extraneuronal uptake.

Drugs and radiochemicals

The drugs used and their sources were : phenoxybenzamine hydrochloride (SKF), cocaine hydrochloride (BDH Ltd), (±)-normetanephrine hydrochloride (Calbiochem), tetraethylammonium bromide (Aldrich) and yohimbine hydrochloride (Nutritional Biochemicals). The radioisotope (−)-[7,8-^{3}H]-noradrenaline hydrochloride (specific activity 10–13 Ci mmol^{-1}) was obtained from the Radiochemical Centre, Amersham. It was diluted to a stock concentration of 100 μCi ml^{-1} in (−)-ascorbic acid (50 μg ml^{-1}) and stored in 5 ml aliquots at 4°C under nitrogen gas. To obtain a final concentration of 10 μCi ml^{-1} (7.6–10.0 $\times$ 10^{-7} M) in the incubation medium, 0.4 ml of the stock was added to 3.6 ml of Krebs solution.

Protocols

The efflux of [^{3}H]-noradrenaline from the preparations was determined by counting 1.0 ml aliquots of the 15.0 ml superfusate collected in vials by fraction collectors which rotated every 3 min. The aliquots were transferred to vials containing 10 ml of Aqueous Counting Scintillant (Amersham) and counted in a Beckman LS-230 counter with automatic external standardization to determine efficiency. Basal efflux is expressed as disintegrations per minute (d.p.m.) and determined from the total radioactivity detected in the 3 min sample collected immediately before each stimulation. Transmural stimulation was always begun at the onset of a 3 min collection period. Stimulation-induced efflux was calculated as the difference between basal efflux and total d.p.m. in the 3 min samples collected during and immediately after stimulation.

After a 90 min equilibration period, including one initial stimulation (10 pulses, 2 Hz) at 30 min following suspension, each pair of tissues was stimulated transmurally with a train of 100 monophasic pulses at 2 Hz and supramaximal voltage. The pulse durations utilized were 50, 100, 200, 500, 1,000, 2,000 and 5,000 μs with 9 min between each stimulation. One of each pair of tissues was exposed to yohimbine (3 $\times$ 10^{-6} M), phenoxybenzamine (3 $\times$ 10^{-6} M) or tetraethylammonium (3 $\times$ 10^{-3} M) for 30 min, followed without washout, by the identical stimulation cycle done simultaneously in both the control and treated preparations. No tissue was used for more than one stimulation cycle. Mean data are presented with their standard errors and Student's t test was used for comparisons between means; a P value of 0.05 or less was considered significant. When intra-tissue comparisons were made a paired analysis was used, otherwise the unpaired t test was used.

Results

Stimulation-induced efflux

Guinea-pig vas deferens and ureter preparations, rabbit left atria and ear artery strip and rat atrial and spleen strips, mounted and stimulated in separate experiments with 100 pulses at 2 Hz, showed overflows of ^{3}H-transmitter, in the absence of yohimbine, which increased progressively with increasing pulse duration from 50 to 5,000 μs (Table 1). Values obtained previously in guinea-pig atria (Kalsner, 1983a) are shown for comparative purposes. The correlation between increasing pulse duration and increasing efflux was evident even over the limited range of 50 to 1,000 μs, which is of special interest here (*vide infra*); the correlation coefficient (r) values

Table 1 The effect of yohimbine[a] on stimulation-induced efflux of [^{3}H]-noradrenaline in rat, guinea-pig and rabbit tissues

Animal/ tissue	*Expt. group*	*No.*	*Transmitter efflux* ($\times 10^3$ d.p.m.) *at pulse duration* (μs) 50	100	200	500	1,000	2,000	5,000
Guinea-pig									
Atria[b]	C	7	9.8± 0.6	12.2± 0.7	19.1± 2.4	19.2±2.4	25.7±2.5	41.0±4.7	
	Y	7	39.7± 6.4*	47.1± 5.7*	53.7±10.6*	47.4±8.7*	47.3±7.7*	64.0±8.7*	
Vas deferens	C	6	16.4± 1.5	18.2± 2.2	21.7± 2.5	26.5±2.8	35.9±4.0	63.1±7.0	146.0±17.1
	Y	6	53.7±11.0*	49.3± 9.0*	51.3± 7.9*	43.9±4.2*	47.7±6.1*	64.4±9.9	144.4±25.8
Ureter	C	6	7.8± 1.3	8.5± 1.0	9.5± 1.1	17.6±3.9	27.1±6.0	43.9±8.3	104.6±10.6
	Y	6	23.2± 1.2*	17.6± 1.5*	15.8± 1.6*	23.4±3.4	33.1±5.7	54.2±9.2	132.0±18.2
Rabbit									
Atria	C	5	9.4± 2.9	13.3± 2.9	16.4± 3.8	17.8±4.4	22.4±4.8	31.3±6.8	52.8± 3.0
	Y	5	27.3± 6.4*	32.1± 4.9*	32.4± 5.1*	28.8±4.2*	29.5±3.4	36.9±6.4	65.7±14.7
Ear artery	C	6	4.6± 0.7	5.3± 0.6	5.8± 0.7	9.2±0.9	13.5±1.0	19.1±1.2	34.2± 2.8
	Y	6	9.6± 1.3*	10.3± 1.1*	10.9± 1.0*	12.1±0.8*	14.5±1.3	17.7±1.2	27.0± 1.7
Rat									
Atria	C	6	31.0± 1.1	32.2± 1.1	29.0± 1.9	31.7±1.8	40.9±2.4	60.8±4.6	126.7± 8.5
	Y	6	69.6± 9.6*	65.1±10.4*	53.8± 8.7*	47.4±6.2*	48.9±4.5	60.7±3.9	110.5± 7.9
Spleen	C	5	10.9± 1.7	14.5± 1.6	17.5± 2.2	23.6±3.0	33.2±3.6	51.1±5.0	109.9± 8.8
	Y	5	40.7± 5.6*	48.8± 7.4*	47.3± 6.6*	43.3±5.1*	45.3±5.0*	59.6±5.3*	108.8± 8.4

[a]Yohimbine hydrochloride (3×10^{-6} M) was administered 30 min before the onset of stimulation to one of each set of tissues and maintained throughout the stimulation cycle (50–5,000 μs durations). [b]Guinea-pig atria data obtained from Kalsner (1983a), for comparative purposes. Asterisks indicate mean efflux values in treated tissue group significantly different from that of matched control (untreated) group ($P < 0.05$) at the specified pulse duration. Efflux values in control tissues: 100 vs 500 or 50 vs 500 pulse durations, $P < 0.05$ in all cases except rat atria (NS); 500 vs 1,000, 1,000 vs 2,000 and 2,000 vs 5,000 pulse durations, $P < 0.05$. Efflux values in yohimbine treated tissues 100 vs 500 or 50 vs 500 pulse durations (NS); 500 vs 1,000 pulse durations (NS) except for guinea-pig ureter ($P < 0.005$) and rabbit ear artery ($P < 0.05$); 1,000 vs 2,000 pulse durations, $P < 0.05$ except for rabbit atria (NS); 2,000 vs 5,000 pulse durations, $P < 0.05$ in all cases. Number of values at 5,000 μs pulse duration was 3 and 4 in untreated and yohimbine treated rabbit left atria, respectively; 5 in untreated ear artery and 4 in untreated and in treated guinea-pig ureter. Number of values at all other pulse durations correspond to column of indicated values.

Table 2 The effects of pulse duration changes, yohimbine[a], phenoxybenzamine[a] (Pbz), and tetraethylammonium (TEA)[a] on per pulse efflux of [^{3}H]-noradrenaline

Animal	Tissue	Experimental group	No. of values	Per pulse ^{3}H-transmitter efflux: 50 μs duration (× 10^2 d.p.m.)	1,000 μs duration	Efflux ratio (1,000 μs/50 μs)
Guinea-pig	Atria	Control	7	0.98 ± 0.06	2.57 ± 0.25	2.60 ± 0.36
		Yohimbine	7	3.97 ± 0.64	4.73 ± 0.77	1.36 ± 0.37*
	Vas deferens	Control	6	1.64 ± 0.15	3.59 ± 0.40	2.20 ± 0.12
		Yohimbine	6	5.38 ± 1.10	4.77 ± 0.61	1.00 ± 0.11*
	Ureter	Control	6	0.78 ± 0.13	2.71 ± 0.60	3.62 ± 0.65
		Yohimbine	6	2.32 ± 0.12	3.31 ± 0.57	1.43 ± 0.25*
Rabbit	Atria	Control	5	0.94 ± 0.29	2.24 ± 0.48	3.54 ± 1.10
		Yohimbine	5	2.73 ± 0.64	2.95 ± 0.34	1.38 ± 0.28*
	Ear artery	Control	6	0.46 ± 0.07	1.35 ± 0.10	3.21 ± 0.33
		Yohimbine	6	0.96 ± 0.13	1.45 ± 0.13	1.67 ± 0.22*
Rat	Atria	Control	6	3.10 ± 0.11	4.09 ± 0.24	1.32 ± 0.06
		Yohimbine	6	6.96 ± 0.96	4.89 ± 0.45	0.75 ± 0.10*
	Spleen	Control	5	1.10 ± 0.17	3.32 ± 0.36	3.26 ± 0.32
		Yohimbine	5	4.07 ± 0.56	4.53 ± 0.50	1.17 ± 0.07*
		Control	4	0.58 ± 0.07	1.27 ± 0.09	2.08 ± 0.07
		TEA	4	6.98 ± 1.33	9.10 ± 1.66	1.33 ± 0.06*
		Control	4	0.64 ± 0.08	1.44 ± 0.07	2.37 ± 0.23
		Pbz	4	4.75 ± 0.82	5.20 ± 0.85	1.10 ± 0.01*

Asterisks indicate mean efflux ratios of yohimbine treated group significantly different from that of matched control group ($P < 0.05$). [a]Yohimbine hydrochloride (3×10^{-6} M), tetraethylammonium bromide (3×10^{-3} M) phenoxybenzamine hydrochloride (3×10^{-6} M) were administered 30 min before stimulation to one of each set of tissues, as indicated. Per pulse efflux values in controls 50 vs 1,000 μs, $P < 0.01$ in all tissues. Per pulse efflux values in yohimbine-treated tissues 50 vs 1,000 μs, $P < 0.02$ in rabbit ear artery, all others NS.

were 0.98, 0.99 and 0.99 in guinea-pig atria, vas deferens and ureter, respectively ($P < 0.0025$); 0.92 and 0.99 in rabbit atria and ear artery ($P < 0.025$); 0.88 and 0.99 in rat atria and spleen ($P < 0.05$). The magnitude and steepness of the increase in transmitter efflux with elongation of the pulse duration varied from tissue to tissue, but the efflux with pulses of 1,000 μs duration, for example, was consistently greater than with 500 μs pulses in all the tissues studied, as it was between 500 and 100 μs (except for rat atria; Table 1). As shown in Table 2, the mean per pulse overflow at 1,000 μs was, in all tissues but one, between 2 and 3 times that at 50 μs. These findings are essentially similar to those described previously for guinea-pig atria (Table 2 and Kalsner, 1983a).

Yohimbine treatment

In order to prevent complications with long pulse durations, due to transmitter depletion, individual tissues were subjected to only one stimulation cycle (pulse duration 50 to 5,000 μs), and matching tissues, or tissue segments, taken from the same preparations were used to study the effects of antagonists. Yohimbine, a presumed specific α_2-presynaptic receptor antagonist, was used at a concentration determined previously to produce a marked or maximal increase in ^{3}H-transmitter overflow, namely 3×10^{-6} M (Kalsner, 1983a,b). The presynaptic antagonist increased the overflow of tritium during field stimulation in each of the tissues studied at pulse durations between 50 and 500 μs and in some tissues also at 1,000 μs (Table 1). This was not the case at the longest pulse duration of 5,000 μs and, with the exception of rat spleen, and the previously reported guinea-pig atria, the effect of yohimbine was not statistically significant at 2,000 μs.

An examination of the absolute amounts of ^{3}H-transmitter leaving the tissues during field stimulation, in the presence of yohimbine, showed that in most cases an approximately equivalent amount of tritium was released with pulse durations in the range of 50 to 1,000 μs. In those few tissues where a progressive increase in efflux with lengthening pulse duration (50 to 1,000 μs) was still evident (e.g. ear artery), it was considerably reduced in amount compared with control tissues not exposed to yohimbine (Tables 1 and 2). The loss of the positive relationship between pulse duration and ^{3}H-efflux, recorded in control tissues is evident from the finding that the stimulation-induced efflux at a pulse duration of 1,000 μs was only marginally greater than that at

50 μs in all yohimbine-treated tissues (Table 2). This interpretation was verified by the generally poor total correlation between pulse duration (50–1,000 μs) and efflux in the majority of the yohimbine-treated tissues. The correlation coefficients (r) were 0.79 (NS), 0.60 (NS) and 0.85 ($P<0.05$) in guinea pig atria, vas deferens, and ureter; 0.16 (NS) and 0.99 ($P<0.05$) in rabbit atria and ear artery and 0.77 (NS) and 0.01 (NS) in rat atria and spleen.

Patterns of efflux enhancement

Increases in ^{3}H-transmitter efflux induced by yohimbine during field stimulation, relative to matched controls, were most pronounced during stimulation with pulses of short duration than with those of long duration.

The declining percentage magnification of efflux by yohimbine with incremental enlargement of the pulse duration is consequently the result of rising efflux values in tissues not treated with the presynaptic antagonist and relatively unvarying efflux values in the yohimbine-treated tissues, at pulse durations of 50 to 1,000 μs (Table 2): this is obvious from Figure 1, where the efflux values obtained in yohimbine-treated tissues are presented as percentages of the values recorded at 50, 100, 200, 500, 1,000, 2,000 and 5,000 μs in matching control tissues, taken from the same animals. Correlation coefficients (r) between increasing pulse duration (50–2000 μs) and decreasing percentage increment in ^{3}H-efflux ranged between 0.81 and 0.97 ($P<0.05$) in all tissues except guinea-pig ureter, where significance was not quite reached ($r=0.53$, $P>0.05$). With longer pulse durations (2,000 and 5,000 μs) absolute efflux (d.p.m.) climbs similarly in both yohimbine and untreated tissues, indicating a yohimbine-insensitive mechanism in transmitter release. Described another way, tritium efflux (d.p.m.), during stimulation in the presence of yohimbine ranged from 225%–374% of comparable values in matched tissues, not treated with the antagonist, at

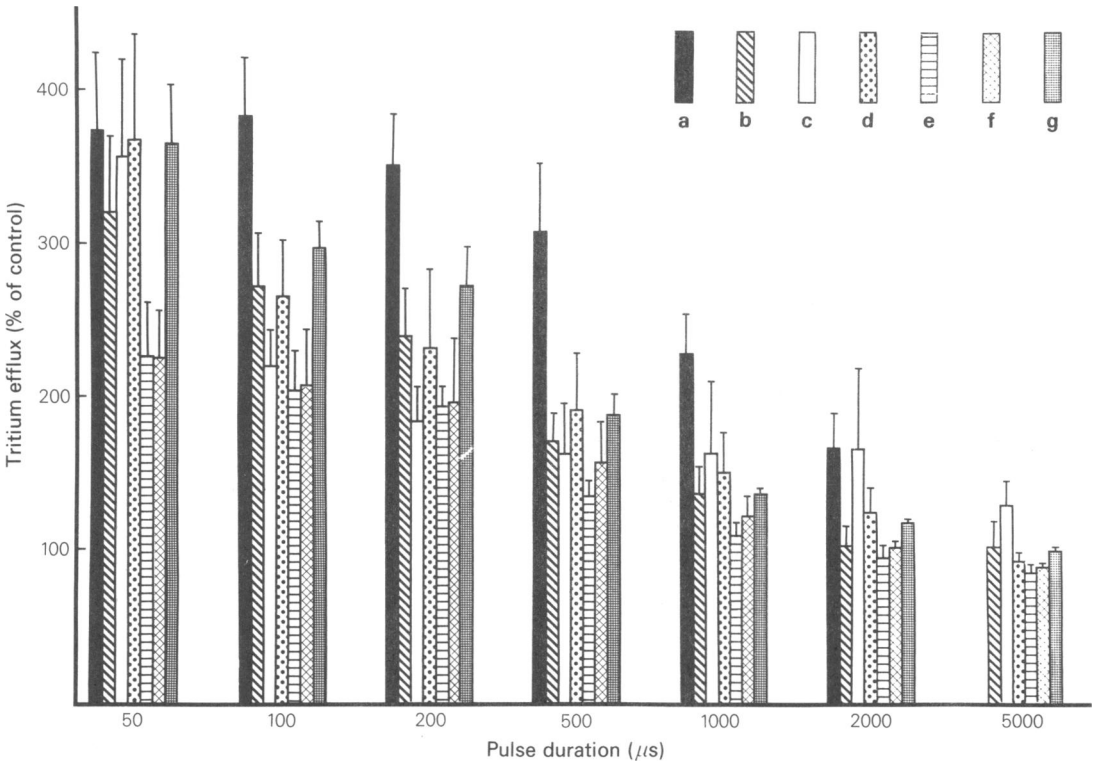

Figure 1 The relationship between pulse duration and stimulation-induced efflux with 100 pulses in 7 tissues. Columns shown with standard errors (vertical bars) represent stimulation-induced efflux in yohimbine-treated groups as % of efflux in matched control groups. Percentage values obtained by comparison of individually determined efflux values for paired yohimbine-treated and control tissues taken from the same animals. Mean efflux data are shown in Table 1. Symbols represent: guinea-pig atria (**a**), vas deferens (**b**) and ureter (**c**); rabbit atria (**d**) and central ear atery (**e**); rat atria (**f**) and spleen (**g**).

the 50 μs pulse duration, but decreased to between 95%–166% of control values when the pulses were of 2,000 μs duration, and to between 84% and 128% of control values at 5,000 μs duration in the test tissues represented in Figure 1.

Low calcium and efflux

The extracellular calcium was reduced to near zero by omission of $CaCl_2$ from the Krebs superfusion medium, beginning 30 min before the onset of stimulation, in one of the test tissues, namely guinea-pig ureter, to assess the linkage between stimulation-induced tritium efflux and neurosecretion. The lack of extracellular calcium depressed almost entirely (greater than 95%) the efflux of tritium in response to field stimulation in both untreated (2) and yohimbine-treated (2) ureters, regardless of the pulse duration employed, over the broad range of 50–5,000 μs, confirming the involvement of neurosecretory events. Even at the longest pulse durations of 2,000 and 5,000 μs, efflux values in untreated and antagonist-treated tissues were reduced to less than 2% of the values obtained under standard calcium conditions. This observation is in keeping with the previously described calcium dependency of ^{3}H-efflux in guinea-pig atria (Kalsner, 1983a).

Phenoxybenzamine and efflux

To determine if the observed effects of yohimbine on stimulation-induced efflux are peculiar to that compound, other experiments were done with rat spleen, using phenoxybenzamine (3×10^{-6} M) as the presynaptic antagonist. As shown in Table 3, the haloalkylamine adrenoceptor antagonist increased stimulation-induced efflux similarly to yohimbine. Although the absolute magnitude of the increase in efflux appeared greater with phenoxybenzamine, the largest effect of the antagonist (relative to matched control values) was, as with yohimbine, clearly at the shorter pulse durations. In absolute terms (d.p.m.) the efflux, in the presence of phenoxybenzamine, appeared to be approximately the same over the broad range between 50 and 2,000 μs. This is in contrast to the established pattern of rising efflux values in control tissues not treated with an adrenoceptor antagonist.

Tetraethylammonium and efflux

In still other experiments, rat spleen strips were treated with tetraethylammonium (TEA), the prototype inhibitor of potassium efflux (Hille, 1977). In confirmation of a previous report with guinea-pig atria, the quaternary ion (3×10^{-3} M) increased

Table 3 The effect of tetraethylammonium[a] (TEA) and phenoxybenzamine[b] (Pbz) on ^{3}H-transmitter efflux in rat spleen

Group	No. of values	Stimulation-induced d.p.m. at pulse duration (μs) 50	100	200	500	1,000	2,000	5,000
(a) Control	4	6.4 ± 0.8	8.2 ± 0.5	9.6 ± 0.6	11.4 ± 0.3	14.4 ± 0.7	23.3 ± 0.9	54.8 ± 2.3
Pbz	4	47.5 ± 8.2*	64.1 ± 9.0*	69.1 ± 8.4*	61.1 ± 10.1*	52.5 ± 9.2*	52.0 ± 8.5*	71.6 ± 7.1
% of control[c]		764 ± 99	772 ± 75	719 ± 66	539 ± 94	370 ± 66	225 ± 37	131 ± 13
(b) Control	4	5.8 ± 0.7	7.1 ± 0.8	9.1 ± 0.5	10.5 ± 0.7	12.7 ± 0.9	19.1 ± 2.0	44.4 ± 3.9
TEA	4	69.8 ± 13.3*	98.2 ± 19.6*	102.3 ± 21.3*	99.0 ± 21.1*	91.0 ± 16.6*	79.5 ± 12.4*	77.7 ± 9.2*
% of control[c]		1221 ± 228	1378 ± 203	1113 ± 197	918 ± 144	708 ± 97	411 ± 25	174 ± 10

[a] Tetraethylammonium bromide (3×10^{-3} M) was administered 30 min before the onset of stimulation in one of each set of spleen strips.
[b] Phenoxybenzamine hydrochloride (3×10^{-6} M) was administered 30 min before the onset of stimulation in one of each set of spleen strips.
[c] Percentages obtained by individual comparison of each treated tissue as % of untreated matched control. Asterisks indicate mean stimulation-induced efflux values in treated group at given pulse duration, significantly different from that of corresponding untreated group.

stimulation-induced efflux in spleen to a greater extent than did yohimbine (Kalsner, 1983a), and somewhat more than did phenoxybenzamine. In absolute terms, the efflux (d.p.m.) approached a constant value over the entire range of pulse durations. The absolute efflux of tritium in the presence of tetraethylammonium was only 33% greater at 1,000 μs than at 50 μs compared with the much larger difference in matched control tissues (Table 2). In terms of percentage increase the quaternary ion, like yohimbine and the haloalkylamine, had its most pronounced effect in rat spleen at the shortest pulse durations. The size of the enhancement of efflux by TEA declined from 1,221% to 411% of control values between pulse durations of 50 and 2,000 μs. As was observed with yohimbine and phenoxybenzamine, TEA had only a modest effect on efflux at the longest pulse duration (Tables 1 and 3).

Discussion

In the present experiments the presynaptic actions of yohimbine, considered widely as the standard presynaptic α_2-adrenoceptor antagonist, were examined in a variety of tissues. The test conditions utilized were designed specifically to increase the amount of transmitter liberated at individual varicosities during a programmed period of stimulation. This was achieved by changing the duration of the individual pulse. It was done without altering, in any way, the total number of pulses delivered, the interval between pulses, or any other parameter of stimulation. Increases in transmitter release, studied over a wide band of progressively lengthening pulse durations, permitted a precise and critical test of the presynaptic receptor hypothesis, particularly when coupled with a selective α_2-antagonist.

Yohimbine-induced increases in the transmitter efflux associated with field stimulation, should manifest a predictable pattern if due to the interruption of a negative-feedback system. The increases in efflux should be proportional to the level of ongoing feedback, in the absence of yohimbine, and explicable in terms of the amounts of noradrenaline in the neuroeffector cleft, to activate presynaptic receptors during stimulation. It is evident from the data presented here, obtained in a variety of tissues and from three species, that the enhancement of efflux by yohimbine is not positively correlated with the amounts of transmitter in the synapse. In fact, yohimbine had its most pronounced effects to magnify efflux, during stimulation with the shortest pulse durations when synaptic transmitter levels were relatively low. This fundamental contradiction to presynaptic theory points to a mechanism of action unrelated to extracellular transmitter levels and inhibitory presynaptic receptors.

The effect of yohimbine on stimulation-induced efflux appears to be, in one important respect, essentially 'all-or-none'. The absolute total efflux with 100 pulses, was elevated to roughly the same d.p.m. value by yohimbine at each of the pulse durations between 50 and 1,000 μs in any given tissue, except for the rabbit ear artery. In the latter case, a similar trend was evident, but incomplete. The declining percentage effect of yohimbine on tritium efflux, as the pulse duration is enlarged between 50 and 1,000 μs, is clearly the consequence of rising efflux values in the controls not matched by proportionally similar increases in the yohimbine-treated tissues.

Efflux values during stimulation in the presence of yohimbine, at pulse lengths between 50 and 1,000 μs, were all in the range of values achieved in the absence of yohimbine with long pulse lengths (1,000–2,000 μs), as was suggested previously (Kalsner, 1983a). Prolongation of the pulse duration and exposure to presynaptic antagonists clearly do not have additive effects. It seems instead, that prolongation of the pulse duration from 50 to a value of 1,000 μs and the exposure of tissues to presynaptic antagonists, such as yohimbine and phenoxybenzamine, involve a common mechanism to alter efflux. Thus, when pulse durations are inordinately prolonged (2,000–5,000 μs), the presynaptic antagonists are virtually ineffective.

The possibility that a mechanism, independent of feedback, limits stimulation-induced efflux in the presence of yohimbine (absence of feedback) at pulse durations of 50–1,000 μs, accounting for the present findings, can probably be dismissed. If such an efflux ceiling operated, it is already called into use with pulses of 50 μs and consequently the efflux at all durations between 50–1,000 μs, in the absence of yohimbine (presence of feedback), should be the same, reflecting a uniform degree of feedback inhibition. This clearly is not the case. Efflux per pulse increases with increasing pulse durations (Table 1). The data with phenoxybenzamine also argue against a ceiling on efflux in the absence of feedback regulation. The effects of the haloalkylamine antagonist were substantially greater than those of yohimbine, with stimulation-induced efflux values raised to almost double those seen in the presence of yohimbine (note required correction for strip length described in Methods). Tetraethylammonium had an even greater effect than phenoxybenzamine on efflux.

The process of transmitter release from the sympathetic nerve terminals (varicosities) is believed to be related to the duration of the action potential. Prolongation of the length of the stimulation pulse allows the calcium channels to stay open longer, permitting a greater entry of the ion and an increase in the amount of liberated transmitter (e.g. Baker, 1982). It was proposed previously (Kalsner, 1983a)

that the presynaptic action of yohimbine is linked to a prolongation of the duration of depolarization and consequently most manifest with short pulse durations.

Inactivation of the calcium gating mechanism is linked to the switching on of the outward potassium current (repolarization) and it appears that presynaptic antagonists interfere with the process. This possibility is substantiated by the finding, made earlier in guinea-pig atria, and extended now to rat spleen, that tetraethylammonium, a selective blocker of outward potassium channels (Hille, 1977; Szurszewski, 1978) known to prolong the duration of the action potential and the dependent neurosecretion, acts to increase ^{3}H-efflux similarly to presynaptic antagonists and to stimulator-induced prolongations of the pulse duration. It appears that the major component of the evidence in favour of functional presynaptic receptors mediating feedback, namely the enhancement of transmitter efflux by antagonists, should be re-interpreted.

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