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Yohimbine and prolongation of stimulation pulse duration alter similarly ^{3}H-transmitter efflux in heart: an alternative to the negative feedback hypothesis

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1 The hypothesis of negative feedback regulation of noradrenaline release was studied in guinea-pig left atrial halves mounted *in vitro*.

2 Tissues were transmurally stimulated with 30, 100 or 300 pulses at 2 Hz with pulse durations ranging from 50 μs to 2,000 μs, and the efflux of ^{3}H-transmitter determined.

3 The efflux of tritium increased with increasing pulse duration as was anticipated, but the effects of supposed presynaptic antagonism by yohimbine were opposite to expectations for a negative feedback system. The magnification of efflux by yohimbine, compared to untreated controls was less rather than more as stimulation-induced transmitter efflux climbed with increases in pulse duration, and with all other parameters of stimulation held constant.

4 It is concluded that the neuronal effect of yohimbine is not linked to negative feedback or to any other system sensing the perineuronal concentration of previously released transmitter.

5 Analysis of the effects on tritium efflux of yohimbine and of prolongation of the stimulation pulse duration, reveals a similarity in the way that they promote transmitter release. Yohimbine increased efflux to approximately the same value at all pulse durations between 50 and 1,000 μs and the value reached was equivalent to that obtained in untreated atria during stimulation with very long pulses (2,000 μs duration). It is suggested that yohimbine prolongs the outward current attributable to the efflux of potassium from axon terminals, and by this means prolongs depolarization and the period of transmitter release.

6 Tetraethylammonium (TEA), a quaternary ion known to plug potassium efflux channels, had an effect on transmitter efflux that was, in some ways, similar to that of yohimbine but of greater magnitude. The present findings provide, for the first time, an alternative to the hypothesis of negative feedback, that might explain the presynaptic effects of adrenoceptor antagonists and possibly other compounds.

Introduction

It has been proposed that presynaptic inhibitory receptors, located on nerve terminals, modulate neurotransmitter output. The main evidence in support of such a hypothesis is that noradrenaline and some other α-adrenoceptor agonists depress stimulation-induced efflux of ^{3}H-transmitter whereas α-adrenoceptor antagonists increase efflux. The enhancement of release is postulated to represent blockade of ongoing auto-inhibition, or negative feedback, mediated by neurally liberated noradrenaline. However, a number of studies now question seriously the credibility of feedback regulation (e.g. Kalsner, 1979a,b; 1982a,b; Angus & Korner, 1980; Drew, 1980; Holman & Surprenant, 1980; Robie, 1980; Blakeley, Cunnane & Peterson, 1982; Hamilton, Reid & Zamboulis, 1982).

In the present experiments, the question of negative feedback of neurotransmitter release was examined in guinea-pig cardiac tissue, *in vitro*, by assessing if substantial modifications to the amount of neurotransmitter released from the neuronal varicosities within the tissue with each impulse, with all other parameters of field stimulation, such as pulse number, frequency and voltage, fully controlled, modify the presynaptic effects of yohimbine. Clearly, they do not. Instead the junctional effects of yohim-

bine, a presumed potent and selective presynaptic antagonist, are obviously due to interactions with neuronal tissue which are independent of the ambient concentration of extracellular transmitter and appear to involve instead the gating mechanisms which control neuronal membrane depolarization and repolarization.

Tissue preparation

Albino guinea-pigs of either sex (300–600 g) were killed by cervical dislocation and their hearts were immediately removed and placed in cold (4°C) and oxygenated (95% O_2 plus 5% CO_2) Krebs-Henseleit (Krebs) solution (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 mM). Left atria were removed, bisected from base to apex as previously described (Furchgott, Garcia, Wakade & Cervoni, 1971; Kalsner, Suleiman & Dobson, 1980) and 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 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 (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M) to block neuronal and extraneuronal uptake.

Drugs and radiochemicals

The drugs used and their sources were: cocaine hydrochloride (BDH Ltd), (±)-normetanephrine hydrochloride (Calbiochem), tetraethylammonium bromide (Aldrich) or chloride (Eastmann Kodak) 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 min^{-1}) 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 the total d min^{-1} in the 3 min samples collected during and immediately after stimulation.

After a 90 min equilibration period, including one primer stimulation (10 pulses, 2 Hz) at 30 min following suspension, each pair of atria was stimulated

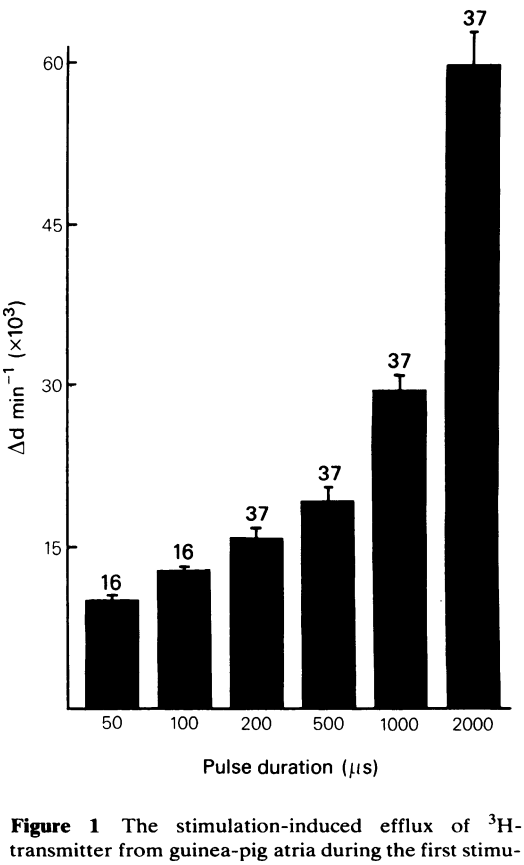

Figure 1 The stimulation-induced efflux of ^{3}H-transmitter from guinea-pig atria during the first stimulation period (S_1) with 100 pulses of varying pulse duration. Columns are means with s.e.mean bars and number of values in each group are above each column. All means significantly different from each other, $P < 0.05$, except 200 vs 100 μs, NS.

transmurally with a train of monophasic pulses of the desired number at 2 Hz and supramaximal voltage. In one major set of experiments, for example, the atria were stimulated in succession with 100 pulses at each of 4 different pulse durations, mainly 200, 500, 1,000 and 2,000 μs, with 9 min between each stimulation. The sequence of administration was reversed in alternate experiments (2,000 μs, 1,000 μs, 500 μs and 100 μs). In other experiments the pulse durations were 50 μs and 100 μs. After the initial stimulations (S_1), one of each pair of atria was exposed to yohimbine (3×10^{-6} M) or tetraethylammonium (3×10^{-3} M) for 30 min, followed without washout, by a repetition of the identical stimulation cycle (S_2) in both the control and treated preparations. Other specific protocols with 30 or 300 pulses are described below in the context of their use. Mean data are presented with their standard errors and Student's *t* test was used for all comparisons; a *P* value of less than 0.05 was considered significant. When intra-atrial comparisons were made a paired analysis was used, otherwise the unpaired *t* test was employed.

Results

Stimulation-induced efflux

Guinea-pig left atrial halves stimulated with 100 pulses at 2 Hz showed overflows of ^{3}H-transmitter which increased progressively with increasing pulse duration from 50 to 2,000 μs (Figure 1). The mean overflow with 100 pulses, when the pulse duration was 2,000 μs, was about 6.0 times that obtained when the duration was only 50 μs. In the absence of drug treatment, repetition of the stimulation cycle (S_2), after a 30 min interval, led to efflux values slightly reduced from those of the initial stimulations, yielding S_2/S_1 ratios between 0.74 and 1.0 (Table 1).

Yohimbine at 3×10^{-6} M, a concentration determined previously to produce a marked increase in ^{3}H-transmitter overflow (Kalsner, 1982c), increased the overflow of tritium at each of the stimulation pulse durations when present during the second run (S_2) in one of each set of atrial halves (Table 1). This is apparent if second run efflux values are com-

Table 1 The effect of yohimbine[a] on stimulation-induced efflux of [^{3}H]-noradrenaline in guinea-pig atria

Experimental group	*Pulse duration* (μs)	*No. of values*[b]	*Transmitter efflux* 1st stim. period (S_1) (100 pulses) ($\times 10^3$ d min^{-1})	2nd stim. period (S_2) (100 pulses)[c] ($\times 10^3$ d min^{-1})	*Efflux ratio* (S_2/S_1)	*Treated group as % of control*[d,e]
Control	50	8	9.9±0.6	9.8± 0.6	1.00±0.04	
Yohimbine	50	8	10.3±0.7	39.7± 6.4	3.73±0.50*	374.2±50.5
Control	100	8	12.5±0.8	12.2± 0.7	0.98±0.03	
Yohimbine	100	8	12.7±0.5	47.1± 5.7	3.73±0.30*	383.3±37.6
Control	200	7	20.8±2.5	19.1± 2.4	0.92±0.04	
Yohimbine	200	7	17.7±3.6	53.7±10.6	3.19±0.27*	351.2±33.6
Control	500	7	23.2±2.4	19.2± 2.4	0.84±0.08	
Yohimbine	500	7	20.5±4.0	47.4± 8.7	2.43±0.21*	308.2±44.2
Control	1000	7	32.4±2.6	25.7± 2.5	0.80±0.05	
Yohimbine	1000	7	27.6±4.4	47.3± 7.7	1.76±0.14*	227.5±26.0
Control	2000	7	60.1±4.1	41.0± 4.7	0.74±0.03	
Yohimbine	2000	7	58.0±9.2	64.0± 8.7	1.21±0.14*	166.7±21.5

[a] Yohimbine hydrochloride (3×10^{-6} M) was administered 30 min prior to S_2 to one of each pair of half-atria and maintained throughout S_2.

[b] Values shown represent data from 4 sets of atria at 50–100 μs pulses and from 7 sets of atria at 200–2,000 μs duration.

[c] Yohimbine-treated S_2 values were not significantly different from each other between 1,000, 500, 200, 100 and 50 μs durations, except for 500 vs 200 μs, $P < 0.05$; 2,000 μs vs 1,000 & 500 μs, $P < 0.05$, but 2,000 μs vs 200, 100 & 50 μs not significantly different (NS). All control efflux values (S_2) significantly different from each other except for 500 vs 200 μs.

[d] Obtained by comparison of individually determined ratios for paired control and treated atrial halves taken from the same animals.

[e] Percentage value at 2,000 μs vs all others, $P < 0.01$; 1,000 μs vs others, $P < 0.05$.

* Indicates ratios of treated groups significantly different from those of corresponding control groups.

pared with those of the first run in the same tissues (S_2/S_1 ratios), or with S_2 values in matched untreated half-atria from the same hearts.

Pattern of efflux enhancement

Increases in ^{3}H-transmitter overflow induced by yohimbine, relative to matching controls, were more pronounced during stimulation with pulses of short duration than with those of long duration. This is easily seen if the S_2/S_1 ratios from treated tissues are expressed as percentages of the ratios obtained in matched control atrial halves at each of the test pulse durations (Table 1). Tritium efflux during field stimulation in the presence of yohimbine ranged from 383% to 351% that of control values at 50–200 μs pulse durations, but decreased to only 167% of matching control values when the pulse duration was 2,000 μs.

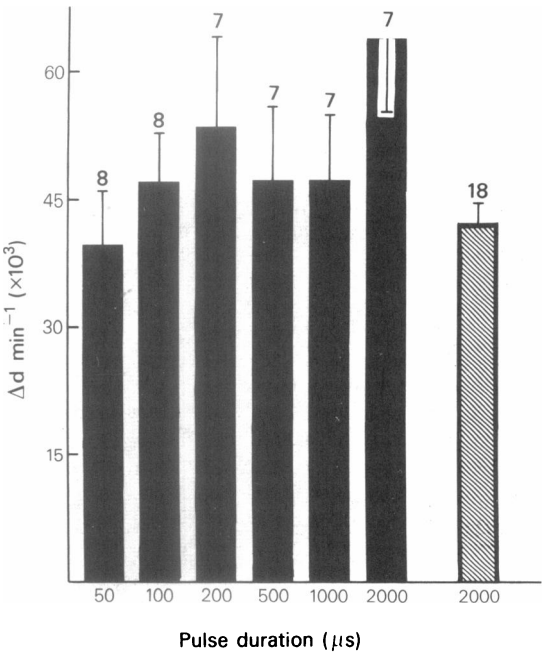

Figure 2 The stimulation-induced efflux of ^{3}H-transmitter from guinea-pig atria during the second stimulation period (S_2) with 100 pulses of varying pulse duration in the presence of yohimbine. The hatched column on extreme right shows mean efflux during S_2 with 100 pulses of 2,000 μs duration in the absence of yohimbine, for comparative purposes. Columns are means with s.e.mean bars and the number of values in each group are above each column. Yohimbine-treated values were not significantly different from each other between 1,000, 500, 200, 100 and 50 μs durations except for 500 vs 200 μs, $P<0.05$; 2,000 μs vs 1,000 and 500 μs, $P<0.05$; 2,000 μs vs 200, 100 and 50 μs, NS.

An examination of the absolute amount of ^{3}H-transmitter leaving the tissues during S_2, in the presence of yohimbine, revealed that an approximately equivalent amount of tritium was released between 50 and 1,000 μs (correlation coefficient, $r=0.18$, $P>0.1$) (Figure 2) although a clearly higher mean efflux value was recorded with pulses of 2,000 μs duration, yielding an overall correlation coefficient of $r=0.79$ ($P<0.1>0.05$). This is in distinct contrast to the progressive increase in ^{3}H-transmitter efflux during S_2 with 100 pulses in the matching control tissues, as the pulse duration was lengthened from 50 to 1,000 μs ($r=0.91$, $P<0.02$) or 50 to 2,000 μs ($r=0.98$, $P<0.001$) (Table 1). Such an observation explains the declining percentage magnification of efflux by yohimbine with incremental enlargement of the pulse duration (Table 1). This lack of correlation between pulse durations of 50 to 1,000 μs and ^{3}H-efflux in the presence of yohimbine is apparent even if values are corrected for transmitter depletion, as reflected in the declining S_2/S_1 ratios ($r=0.53$, $P>0.1$).

Mechanism of enhancement

To examine further the observation that yohimbine, and extension of the pulse duration to 2,000 μs, alter tritium efflux quantitatively alike, and are less than additive in their effects, experiments were done using trains of 30 and 300 pulses instead of the customary 100 pulses. The stimulation pulse durations utilized were 100 and 1,000 μs. In control preparations, but not in those treated with yohimbine, the total tritium efflux during stimulation with either 30 or 300 pulses, each of 1,000 μs duration, was significantly greater than with pulses of 100 μs duration, in keeping with findings made with 100 pulses (Table 2).

It was noted that the ratio of the per pulse efflux of tritium with durations of 100 and 1,000 μs approximated 1.0 in the presence, but not the absence, of the antagonist, at all of the train lengths tested (30, 100 or 300) (Table 3). The per pulse efflux during S_2 approximated to the same absolute value in the presence of yohimbine, regardless of train length or duration (100 or 1,000 μs) but ranged 2.9 fold in value in controls (Table 3). Additionally, the effect of yohimbine expressed in percentage terms, relative to controls, was greatest with pulses of short duration, contrary to the quantitative differences in the amounts of noradrenaline released during such stimulations (Table 2).

Efflux in reduced calcium

The extracellular calcium was reduced to near zero by omission of $CaCl_2$ from the Krebs superfusion medium, commencing 30 min prior to the onset of S_2.

Table 2 The comparative effects of yohimbine[a] and pulse duration changes on the efflux of [^{3}H]-noradrenaline in guinea-pig atria

Experimental group	*Pulse duration* (μs)	*No. of values*	*Transmitter efflux* 1st stim. period (S_1) ($\times 10^3$ d min^{-1})	2nd stim. period (S_2)[b] ($\times 10^3$ d min^{-1})	*Efflux ratio* (S_2/S_1)	*Treated group as % of control*[c,d]
30 pulses						
Control	100	6	7.5 ± 0.9	6.2 ± 0.8	0.82 ± 0.03	
Yohimbine	100	6	6.2 ± 0.7	15.7 ± 2.5	2.34 ± 0.28	285 ± 33.0
Control	1000	6	14.2 ± 1.7	9.7 ± 1.2	0.68 ± 0.03	
Yohimbine	1000	6	14.3 ± 2.3	16.3 ± 2.5	1.15 ± 0.06	169 ± 8.6
300 pulses						
Control	100	6	82.3 ± 4.6	72.4 ± 5.2	0.88 ± 0.03	
Yohimbine	100	6	76.5 ± 6.0	128.5 ± 11.3	1.71 ± 0.15	198 ± 22.0
Control	1000	6	117.2 ± 6.3	93.5 ± 6.6	0.77 ± 0.03	
Yohimbine	1000	6	126.7 ± 8.0	132.1 ± 11.6	1.04 ± 0.04	134 ± 3.4

[a] Yohimbine hydrochloride (3×10^{-6} M) was administered for 30 min prior to S_2 to one of each pair of half-atria and maintained throughout S_2.
[b] S_2 control efflux values at 30 pulses, 100 vs 1,000 μs $P < 0.01$; at 300 pulses 100 vs 1,000 μs $P < 0.001$. Yohimbine-treated values 100 vs 1,000 μs at either 30 or 300 pulses NS.
[c] Obtained by comparison of individually determined ratios for paired control and treated atrial halves taken from the same animals.
[d] Percentage increase due to yohimbine at 100 μs vs 1,000 μs with either 30 or 300 pulses, $P < 0.05$.

This was done to confirm that the efflux of tritium recorded in the present experiments was linked to neurosecretion. Half-atria were stimulated with 100 pulses at 2 Hz with pulse durations of 100, 500 and 2,000 μs, first in standard (S_1) and then in low calcium Krebs (S_2). The absence of calcium depressed severely tritium efflux under all test conditions. The S_2/S_1 ratios in the 2 tissues exposed to the low calcium medium during S_2 were 0.01, 0.03 and 0.02 at pulse durations of 100, 500 and 2,000 μs respectively, and the corresponding ratios ranged between 0.6–0.9 in the matching control tissues, maintained in standard Krebs during S_2.

Table 3 The effects of pulse duration changes and yohimbine on per pulse efflux of [^{3}H]-noradrenaline

Experimental group	*No. of pulses*	*Per pulse efflux* 100 μs *duration* ($\times 10^2$ d min^{-1})	1,000 μs *duration* ($\times 10^2$ d min^{-1})	*Efflux ratio* (1,000 μs/100 μs)
Control	30	2.19 ± 0.26(6)	3.50 ± 0.39(6)	1.59
Yohimbine	30	5.23 ± 0.82(6)	5.43 ± 0.84(6)	1.03
Control	100	1.21 ± 0.06(8)	2.19 ± 0.14(7)	1.80
Yohimbine	100	4.71 ± 0.56(8)	4.72 ± 0.76(7)	1.00
Control	300	2.41 ± 0.17(6)	3.12 ± 0.22(6)	1.29
Yohimbine	300	4.29 ± 0.38(6)	4.40 ± 0.39(6)	1.02

Number of values are shown in parentheses to the right of efflux values. Per pulse efflux values shown are for S_2 runs in untreated and antagonist-treated atria. Yohimbine hydrochloride (3×10^{-6} M), when administered, was given 30 min prior to S_2 to one of each pair of half atria and maintained throughout S_2. Per pulse efflux values in controls, 100 vs 1,000 μs: with 30 pulses, $P < 0.02$; with 100 pulses, $P < 0.01$; with 300 pulses, $P < 0.001$; values in yohimbine-treated atria, 100 vs 1000 μs, NS at 30, 100 or 300 pulses.

Table 4 The effect of tetraethylammonium[a] on stimulation-induced efflux of [^{3}H]-noradrenaline in guinea-pig atria

Experimental group	Pulse duration (μs)	No. of values[b]	Transmitter efflux: 1st stim. period (S_1) (100 pulses) ($\times 10^3$ d min^{-1})	Transmitter efflux: 2nd stim. period (S_2) (100 pulses)[c] ($\times 10^3$ d min^{-1})	Efflux ratio (S_2/S_1)	Treated group as % of control[d,e]
Control	50	6	15.0 ± 1.2	14.4 ± 0.9	0.97 ± .04	421 ± 24
TEA	50	10	16.5 ± 0.8	64.8 ± 4.3	3.94 ± .16	
Control	100	6	17.8 ± 1.4	16.0 ± 1.1	0.91 ± .03	532 ± 23
TEA	100	10	19.4 ± 1.0	84.7 ± 2.9	4.43 ± .17	
Control	200	5	26.9 ± 2.5	22.2 ± 2.4	0.83 ± .06	532 ± 59
TEA	200	6	28.8 ± 2.5	115.7 ± 9.6	4.18 ± .53	
Control	500	5	35.2 ± 5.0	25.5 ± 2.5	0.81 ± .10	480 ± 62
TEA	500	6	35.0 ± 3.2	114.6 ± 12.7	3.44 ± .51	
Control	1000	5	50.5 ± 6.1	34.9 ± 2.3	0.72 ± .07	396 ± 61
TEA	1000	6	50.0 ± 3.2	126.5 ± 15.5	2.58 ± .37	
Control	2000	5	76.8 ± 6.2	57.9 ± 3.0	0.77 ± 0.4	240 ± 23
TEA	2000	6	85.7 ± 4.6	148.8 ± 15.1	1.57 ± .21	

[a] Tetrathylammonium bromide or chloride (3 mM) was administered 30 min prior to S_2 to one of each pair of half-atria, and maintained throughout S_2.
[b] Values shown represent data from 3–5 atria at 50 to 100 μs and from 5–6 atria at 200–2,000 μs.
[c] TEA-treated S_2 values were significantly different from each other between 2,000, 1,000, 500, 200, 100 and 50 μs durations, but not 1,000 vs 200 μs and 500 vs 200 μs.
[d] Obtained by comparison of individually determined ratios for control and treated atria from the same experiment.
[e] Percentage value at 2,000 μs vs all others $P < 0.05$; 100 vs 50 μs $P < 0.05$; all other comparisons NS.

TEA and ^{3}H-transmitter efflux

In other experiments, tissues were stimulated with 100 pulses at 2 Hz with pulse durations between 50 and 2,000 μs, first in the absence and then in the presence of tetraethylammonium (TEA) (3×10^{-3} M). The quaternary ion, in the concentration used, increased the stimulation-induced efflux of ^{3}H-transmitter to a greater extent than did yohimbine at all test pulse durations, as is evident from the S_2 values in Table 4. In terms of percentage increase, TEA, like yohimbine, had its greatest effect at the short pulse durations (Table 4). The magnitude of enhancement declined from 532% to 240% of corresponding control values, for example, between pulse durations of 200 and 2,000 μs. The correlation coefficient (r) for pulse duration (50–1,000 μs) and ^{3}H-efflux, with TEA present in S_2, was 0.78 ($P < 0.1 > 0.05$) and when corrected for the decline in efflux indicated by the S_2/S_1 ratios of untreated preparations, r was 0.83 ($P < 0.05$). The tritium efflux with a pulse duration of 200 μs during S_2 differed from that at 1,000 μs duration in untreated but not in TEA-treated preparations. In untreated preparations during S_2 the efflux during stimulation at 2,000 μs was 160% more than at 200 μs but the increment was only 30% in TEA-treated tissues.

Basal efflux of tritium

The basal efflux of tritium from atria, in the absence of nerve stimulation, was very slightly but significantly increased by yohimbine. In 12 preparations exposed to the antagonist (3×10^{-6} M) the basal efflux during S_2 averaged $10.71 \pm 0.69 \times 10^3$ d min^{-1} and in 12 matching untreated half-atria the corresponding value was $8.91 \pm 0.63 \times 10^3$ d min^{-1} ($P < 0.05$). TEA also increased basal efflux slightly. The values during S_2 in the presence of the quaternary ion averaged $13.56 \pm 0.51 \times 10^3$ d min^{-1} in 8 tissues compared with $10.51 \pm 0.58 \times 10^3$ d min^{-1} in 8 matched untreated controls ($P < 0.02$).

Discussion

Transmitter release in noradrenergic systems does not appear to be regulated by α-receptor-mediated feedback, nor can such a process explain neuronal events involving compounds designated by some investigators as specific presynaptic α-receptor antagonists.

The experiments described here examined the presynaptic actions of a prototypal α-antagonist, yohimbine, in a previously untested way. The amount of transmitter released from each excited varicosity was

progressively increased by setting the duration of the individual impulse, to range between 50 μs and 2,000 μs. The total number of impulses, the length of the pulse train, the interval between impulses, were intentionally all held uniform and pulses of supramaximal voltage were used. Since blockade by adrenoceptor antagonists is hypothesized to do away with auto-inhibition of neurosecretion mediated by noradrenaline released during previous impulses, a predictable pattern to the antagonist-induced increases in transmitter efflux should be evident. That pattern should be decipherable in terms of the amounts of neurotransmitter released during stimulation and hence available in the neuroeffector cleft to excite presynaptic inhibitory α-receptors. The magnitude of the yohimbine effect, relative to matched controls similarly stimulated, but not exposed to yohimbine, should be in direct proportion to the extent of the interrupted auto-inhibition. With little active auto-inhibition, the effect of the antagonist should be meagre, and with auto-inhibition at a high level, its interruption should lead to a striking enhancement.

However, it is clear from the data provided here that the antagonist effect is not associated with the density of liberated transmitter in the synapse. Instead, the enhancement was least at the long pulse durations and most at the short pulse durations, as determined by intra-atrial comparisons between untreated and yohimbine-treated left atrial halves. This observation, made under precise experimental conditions in which all other stimulation parameters were held constant, is entirely contradictory to presynaptic theory.

The present experiments not only illuminate the inadequacies of current formulations about presynaptic receptors and their presumed function of negative feedback but, for the first time, provide sufficient experimental insight to allow a tentative alternative proposal to be put forward to account for the presynaptic action of antagonists.

The diminishing potentiation of tritium efflux by yohimbine, with increases in pulse duration, is primarily due to rising efflux values in the controls unmatched by proportionally similar increases in the yohimbine-treated tissues. The absolute total efflux of tritium with 100 pulses was raised to approximately the same value by yohimbine, to about $40-50 \times 10^3$ d min^{-1}, at all of the test durations from 50 μs to 1,000 μs. The increased efflux produced by yohimbine with pulse durations between 50 and 1,000 μs, is similar to that achieved in matched untreated atrial halves by increasing the duration of the stimulation pulse to 2,000 μs. Therefore, as the pulse duration lengthens, the gap between untreated and yohimbine-treated efflux values narrows. This interpretation was supported by experiments using 30 and 300 pulses in which efflux in the presence of yohimbine, but not in its absence, was identical at pulse durations of 100 and 1,000 μs, just as it was with 100 pulses. The combination of a long pulse duration (2,000 μs) and yohimbine increased efflux more than did either one alone, however, indicating that the overlap is less than complete.

The release of transmitter from sympathetic nerves is directly related to the duration of the action potential; prolongation of duration permits the calcium channels to stay open longer, leading to greater entry of calcium and to an increase in the amount of transmitter released (Rahamimoff, 1970; Baker, 1972). It is proposed here that the presynaptic action of yohimbine, most clearly manifest with short pulse durations, is linked to a prolongation of the duration of depolarization, by indirect modification of the calcium gating mechanism, delaying its inactivation.

Repolarization begins with the turning on of the potassium current and the whole of the maintained outward current appears due to an efflux of potassium ions (Katz, 1966; Hille, 1977). It is possible that interruption of this process, perhaps indirectly, is the primary means by which presynaptic antagonists such as yohimbine enhance ^{3}H-transmitter efflux. Such a suggestion is supported by experiments with tetraethylammonium (TEA), one of the few substances known to selectively block outward potassium permeability directly, by blocking potassium channels (Hille, 1977; Szurszewski, 1978). TEA prolongs the duration of the action potential and hence neurosecretion. The quaternary ion increased transmitter efflux in the present experiments in a way not unlike yohimbine, although the efflux values reached were higher and the congruence was not complete. Higher concentrations of TEA than used here can prolong inordinately or disrupt entirely neuronal function, leading to repetitive action potentials and discharge of transmitter, sufficient to exhaust neuronal stores (Wakade, 1980). It is clear that much additional work needs to be done to establish unequivocally the precise mechanism and locus of yohimbine action.

The results described here validate recent concerns, from several laboratories (e.g. Kalsner, 1979a,b, 1982a,b; Holman & Surprenant, 1980; Blakeley *et al.*, 1982) that the concept of feedback regulation of neurotransmitter release has been prematurely integrated into the corpus of accepted working hypotheses. Several studies in which frequency, pulse number, train length and effector response were systematically altered to determine the experimental predictability of presynaptic theory have brought to light its severe limitations (Chan & Kalsner, 1979; Kalsner & Chan, 1979; Kalsner 1979a,b; 1980; 1982c; 1983a; Kalsner *et al.*, 1980). Conceptual modifications to the hypothesis, in terms

of intermittent patterns of nodal release (Bevan, Tayo, Rowan & Bevan, 1983), do not resolve its basic shortcomings. Additionally, the hypothesis provides little clear advantage in the cycle of neurotransmission, since it does not seem to be at all linked to the contours of the effector response, as shown elsewhere (Kalsner, 1983a,b).

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