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Release of [^{3}H]-amezinium from cortical noradrenergic axons: a model for the study of the α-autoreceptor hypothesis

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1 [^{3}H]-amezinium is taken up selectively into noradrenergic axons and their transmitter-storing vesicles and is released from these axons by action potentials. We used it as a non-α-adrenergic marker in order to study the α-adrenergic autoinhibition of noradrenaline release.

2 Rat occipitocortical slices were preincubated with [^{3}H]-amezinium 0.03 μM and then superfused and stimulated electrically (3 Hz for 3 min). The stimulation-evoked overflow of tritium was measured in six groups of slices: from saline-pretreated rats; from saline-pretreated rats, the slices being exposed to exogenous noradrenaline before preincubation with [^{3}H]-amezinium; from saline-treated rats, slices from which were exposed simultaneously to noradrenaline and cocaine before preincubation with [^{3}H]-amezinium; from rats in which noradrenaline stores had been depleted by pretreatment with α-methyltyrosine (α-MT); from α-MT-treated rats, the slices being exposed to noradrenaline before preincubation with [^{3}H]-amezinium; and from α-MT-treated rats, slices from which were exposed to noradrenaline plus cocaine before preincubation with [^{3}H]-amezinium.

3 The stimulation-evoked overflow of tritium, expressed as a percentage of the tritium content of the tissue, was 1.15% in slices from saline-pretreated rats, and was similar in slices from saline-pretreated rats after exposure to noradrenaline or noradrenaline plus cocaine. It was 2.56% in slices from α-MT-treated rats, 1.20% from α-MT-treated rats after exposure to noradrenaline, and 2.88% from α-MT-treated rats after exposure to noradrenaline plus cocaine.

4 Yohimbine 0.1 and 1 μM increased the stimulation-evoked overflow of tritium in slices from all groups of saline-pretreated rats and in those slices from α-MT rats that had been in contact with exogenous noradrenaline. Yohimbine did not change the evoked overflow in slices from α-MT rats that had not been exposed to noradrenaline, or had been exposed to noradrenaline plus cocaine.

5 Clonidine 0.01 – 1 μM decreased the stimulation-evoked overflow of tritium moderately in slices from saline-pretreated rats, markedly in slices from α-MT-treated rats, and moderately again when the latter slices had been exposed to noradrenaline.

6 It is concluded that the action potential-evoked release of [^{3}H]-amezinium as well as the modulation of this release by yohimbine and clonidine depend on the presence or absence of α-adrenergic autoinhibition caused by the co-secretion of noradrenaline. When there is co-secretion of noradrenaline, the evoked release of [^{3}H]-amezinium is relatively small, yohimbine increases the release, and clonidine can cause only moderate inhibition. When there is no or very little co-secretion of noradrenaline, the evoked release of [^{3}H]-amezinium is at least doubled, yohimbine causes no further increase and clonidine produces strong inhibition.

Introduction

Amezinium is a new indirectly acting sympathomimetic compound. Except perhaps at extremely high concentrations, it lacks direct effects on α- or β-adrenoceptors (Steppeler, Pfändler, Hedler & Starke, 1980; Lenke, Gries & Kretzschmar, 1981; Traut, Brode & Hoffmann, 1981). It is taken up into postganglionic sympathetic neurones via the noradrenaline carrier, and is subsequently incorporated into the transmitter-storing vesicles. Action potentials release it from the axons in a calcium-

dependent, tetrodotoxin-sensitive manner (Steppeler & Starke, 1982). Amezinium is similarly transported into the noradrenergic neurones of rat brain (Steppeler, Döring, Hedler & Starke, 1982). The experiments described below show that, as in the periphery, previously taken-up [^{3}H]-amezinium is released from occipitocortical preparations by electrical pulses, and that the stimulation-evoked overflow is blocked by tetrodotoxin as well as in calcium-free medium. [^{3}H]-amezinium is, hence, a marker for transmitter release from occipitocortical noradrenergic axons which, unlike noradrenaline itself, is devoid of affinity for adrenoceptors.

It occurred to us that this marker might well be used to investigate the consequences of depletion and refilling of noradrenaline on the α-adrenergic autoinhibition of noradrenaline release (see reviews by Stjärne, 1975; Bacq, 1976; Starke, 1977; Westfall, 1977; Vizi, 1979; Gillespie, 1980; Rand, McCulloch & Story, 1980; Langer, 1981). Changes in the autoinhibition after noradrenaline depletion by reserpine or α-methyltyrosine (α-MT) have been analyzed previously. In those studies, depleted tissues were either treated with small doses of [^{3}H]-noradrenaline so that the stimulation-evoked overflow of the label could be measured (Enero & Langer, 1973; Cubeddu & Weiner, 1975), or the evoked overflow of dopamine-β-hydroxylase was used as an index of transmitter release (Cubeddu & Weiner, 1975; Dixon, Mosimann & Weiner, 1979). The advantage of [^{3}H]-amezinium is that, unlike [^{3}H]-noradrenaline, it is neutral with respect to the α-feedback-mechanism, and unlike dopamine-β-hydroxylase, its outflow is easily detected even with a few milligrams of brain slices.

We have examined the release of [^{3}H]-amezinium in rat occipitocortical slices, and effects on this release of the α_2-adrenoceptor antagonist yohimbine and the α_2-agonist clonidine, under various conditions of depletion of noradrenaline by *in vivo* pretreatment with α-MT and refilling by subsequent exposure of the slices to exogenous noradrenaline. The α-receptors in rat occipital cortex that modulate the release of noradrenaline have previously been subclassified as α_2 (Hedler, Stamm, Weitzell & Starke, 1981).

Methods

Male Wistar rats, weighing 200–300 g and in most experiments pretreated with α-MT or saline, were decapitated, the brain quickly removed and chilled. Round slices (0.4 mm thick, 5 mm diameter) were prepared from the occipital cortex after the superficial 0.3 mm layer had been removed (Reimann, Steinhauer, Hedler, Starke & Hertting, 1981).

Experiments with [^{3}H]-amezinium

Two to four slices were incubated in 10 ml medium containing [^{3}H]-amezinium 0.03 μM, sp. act. 7.8 Ci/mmol, for 60 min at 37°C under an atmosphere of 95% O_2 and 5% CO_2. The slices were then washed in 30 ml medium, transferred into superfusion chambers (one slice per chamber), and superfused with [^{3}H]-amezinium-free medium at 37°C for 110 min at 1 ml/min. Consecutive 5-min fractions of the superfusate were collected from 50 min of superfusion onwards. At the end of superfusion, each slice was solubilized in 0.5 ml Soluene 100 (Packard Instrument, Frankfurt am Main).

The slices were stimulated electrically three times for 3 min each (S_0, S_1, S_2). Stimulation periods began after 35, 60 and 95 min of superfusion. Rectangular pulses of 2 ms duration, a frequency of 3 Hz, and a current strength of 24 mA were delivered from a Stimulator T (Hugo Sachs Elektronik, Hugstetten). The superfusate was not collected during S_0.

The outflow of tritium was expressed as a fractional rate (min^{-1}), i.e., (nCi tritium outflow per 5 min)/5 · (nCi tritium content of the tissue at the beginning of this 5-min period). The stimulation-evoked overflow was calculated as the difference of the total tritium outflow in the 5 min after onset of stimulation, and the estimated basal outflow during this time; the basal outflow was assumed to be the average of the outflows in the 5-min sample collected before stimulation and in the sample collected 5–10 min after onset of stimulation; the difference total minus estimated basal outflow (nCi) was expressed as a percentage of the tritium (nCi) in the tissue at the onset of stimulation.

In some experiments, freshly prepared slices were first incubated for 15 min in 10 ml medium containing 0.1 μM noradrenaline. In other experiments, fresh slices were first incubated for 5 min in 10 ml of medium containing cocaine 10 μM, noradrenaline was added to give a final concentration of 0.1 μM and incubation was continued for a further 15 min. The slices were then washed four times for 2 min each in noradrenaline- and cocaine-free medium before being incubated with [^{3}H]-amezinium and treated further as described above.

Tritium was determined by liquid scintillation spectrometry. Counting efficiencies were measured with internal standards of tritiated toluene or water.

Experiments with [^{3}H]-noradrenaline

Six slices were incubated in 2 ml medium containing [^{3}H]-noradrenaline 0.2 μM, sp. act. 10.3 Ci/mmol, for 30 min. The further procedure was as for [^{3}H]-amezinium, but slices were superfused for 115 (not 110) min, and the length of the stimulation periods

was 2 (not 3) min. The stimulation-evoked overflow of tritium was calculated as the difference of the total tritium outflow in the 15 min following the onset of stimulation, and the estimated basal outflow; the basal outflow was assumed to be three times the average of the outflows in the 5-min sample collected before stimulation and in the sample collected 15–20 min after onset of stimulation; the difference total minus estimated basal outflow (nCi) was expressed as a percentage of the tritium (nCi) in the tissue at the onset of stimulation.

Pretreatment with α-methyltyrosine

Rats received nine i.p. injections at intervals of 3 h. Two injections of α-methyltyrosine methylester HCl 200 mg/kg in saline were followed by seven injections of 125 mg/kg. Control rats received corresponding saline injections. The animals were killed 1 h after the last injection.

Catecholamine determination

Portions of the occipital cortex that remained after slices had been punched out were extracted for 15 h with 1 ml of 0.1 M $HClO_4$ containing 1 mg Na_2EDTA and 1.2 mg Na_2SO_3. Noradrenaline and α-methylnoradrenaline were determined in the extracts, without further purification, by reverse-phase high pressure liquid chromatography with electrochemical detection. μ Bondapak C18 columns (Waters, Königstein) were used. The mobile phase was a mixture of 950 ml 0.05 M sodium acetate/0.03 M citric acid buffer, 50 ml methanol and 300 mg sodium octyl sulphate. Similar results were obtained when, in a few experiments, the catecholamines in the $HClO_4$ extracts were purified by adsorption to and elution from alumina prior to high pressure liquid chromatography.

Media, drugs, statistics

The incubation and superfusion medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, ascorbic acid 0.6, Na_2EDTA 0.03, glucose 11; it was saturated with 5% CO_2 in O_2 and warmed to 37°C. In all experiments on slices from α-MT-pretreated rats, the incubation and superfusion medium contained in addition α-MT 0.1 mM.

The drugs used were [methyl-^{3}H]-amezinium chloride, sp. act. 7.8 Ci/mmol, and unlabelled amezinium methylsulphate (Knoll, Ludwigshafen); (−)-[ring-2,5,6-^{3}H]-noradrenaline, sp. act. 41.2 Ci/mmol (NET-678, NEN, Dreieich) was diluted with unlabelled (−)-noradrenaline HCl (Hoechst, Frankfurt am Main) to a final sp. act. of 10.3 Ci/mmol; clonidine HCl (Boehringer, Ingelheim); (±)-α-methyl-*p*-tyrosine methylester HCl (α-MT; Labkemi, Göteborg); cocaine HCl (Merck, Darmstadt); yohimbine HCl (Roth, Karlsruhe); tetrodotoxin (Sigma, München).

Means ± s.e. are given throughout this paper. Differences between means were examined for significance with the two-tailed Student's *t* test.

Results

Experiments with [^{3}H]-amezinium

Occipitocortical slices from rats were preincubated with [^{3}H]-amezinium and then superfused. The outflow of tritium and the effect of yohimbine were studied in six groups of slices: (1) from saline-pretreated rats; (2) from saline-pretreated rats and exposed to noradrenaline 0.1 μM before preincubation with [^{3}H]-amezinium; (3) from saline-pretreated rats and exposed to noradrenaline 0.1 μM in the presence of cocaine 10 μM before preincubation with [^{3}H]-amezinium; (4) from rats pretreated with α-MT; (5) from α-MT-treated rats and exposed to noradrenaline 0.1 μM before preincubation with [^{3}H]-amezinium; and (6) from α-MT rats and exposed to noradrenaline 0.1 μM in the presence of cocaine 10 μM before preincubation with [^{3}H]-

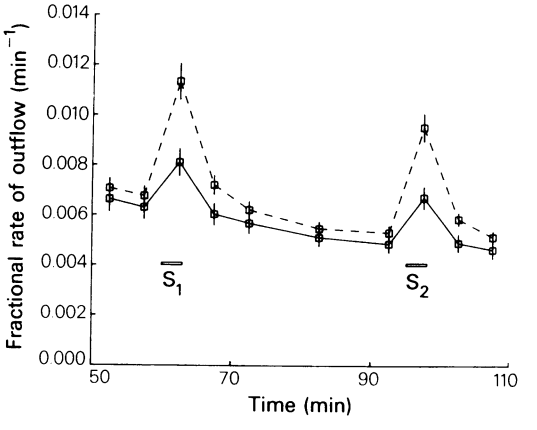

Figure 1 Outflow of tritium from rat occipitocortical slices preincubated with [^{3}H]-amezinium. After preincubation, the slices were superfused with [^{3}H]-amezinium-free medium. They were stimulated three times for 3 min each at 3 Hz (S_0, S_1, S_2). The superfusate during S_0 was discarded. Abscissa scale, min of superfusion. Ordinate scale, fractional rate of tritium outflow (min^{-1}). Continuous lines, slices from saline-pretreated rats ($n = 9$); dashed lines, slices from rats pretreated with α-MT as described in Methods ($n = 9$). Mean values are shown with s.e. indicated by vertical lines.

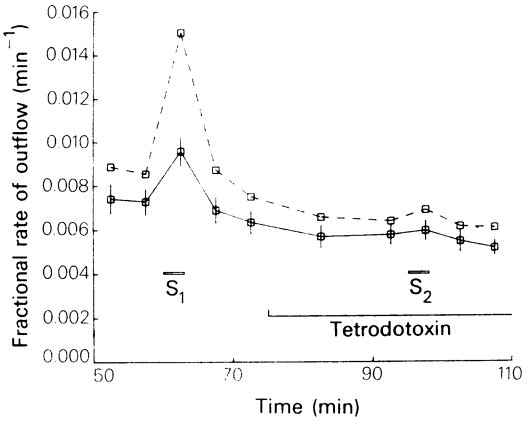

Figure 2 Effect of tetrodotoxin on the outflow of tritium from rat occipitocortical slices preincubated with [^{3}H]-amezinium. After preincubation, the slices were superfused with [^{3}H]-amezinium-free medium. They were stimulated three times for 3 min each at 3 Hz (S_0, S_1, S_2). The superfusate during S_0 was discarded. Tetrodotoxin 0.3 μM was added to the superfusion medium 20 min before S_2 as indicated. Abscissa scale, min of superfusion. Ordinate scale, fractional rate of tritium outflow (min^{-1}). Continuous line, slices from saline-pretreated rats ($n = 5$; mean values are shown with s.e.); dashed lines, slices from rats pretreated with α-MT as described in Methods ($n = 2$; mean values).

amezinium (see Methods for details). Clonidine was tested only in groups 1, 4 and 5. Figure 1 shows the outflow of tritium from slices from saline-pretreated rats (group 1) and α-MT-pretreated rats (group 4). The basal outflow of tritium declined over the 60 min period. Electrical stimulation increased the outflow. The response was reproducible when stimulation was repeated (S_1, S_2). It is evident that the stimulation-evoked overflow of tritium was higher in slices from α-MT rats than in slices from saline-pretreated rats. Figure 2 shows, in addition, that in both groups tetrodotoxin practically abolished the response to stimulation. The response to S_2 was also abolished when the slices were superfused with calcium-free medium from 20 min before S_2 onwards ($n = 3$ each for slices from saline- and α-MT-pretreated rats).

Table 1 summarizes the tritium content of the slices after 50 min of superfusion (at the beginning of sample collection, 10 min before S_1) as well as the magnitude of the basal and the stimulation-evoked overflow for all six groups. The tritium content (and, hence, presumably [^{3}H]-amezinium uptake and retention) and the basal outflow were similar in the six groups, although some of the small deviations were statistically significant. However, there were large differences in the stimulation-evoked overflow. Treatment with α-MT more than doubled the overflow evoked by S_1 (compare groups 4 and 1). Exposure of the slices from α-MT rats to noradrenaline before incubation with [^{3}H]-amezinium brought the evoked overflow back to normal (group 5). The presence of cocaine during contact with noradrenaline prevented the effect of noradrenaline, the evoked overflow being more than twice that in slices exposed to noradrenaline alone (compare groups 6

Table 1 Tritium content of, and outflow of tritium from, rat occipitocortical slices preincubated with [^{3}H]-amezinium

Treatment	*Tritium content after 50 min of superfusion* (nCi)	*Basal tritium outflow from 55–60 min of superfusion* (min^{-1})	*Tritium overflow evoked by S_1 (% of tissue tritium)*	n
(1) Saline	43.1 ± 2.1	0.0070 ± 0.0003	1.15 ± 0.05	47
(2) Saline, noradrenaline	44.5 ± 3.0	0.0075 ± 0.0003	1.02 ± 0.05	23
(3) Saline, noradrenaline + cocaine	40.8 ± 2.3	0.0072 ± 0.0004	1.04 ± 0.06	18
(4) α-MT	40.2 ± 1.9	0.0074 ± 0.0003	2.56 ± 0.11**	40
(5) α-MT, noradrenaline	40.7 ± 1.6	0.0082 ± 0.0003*	1.20 ± 0.08	27
(6) α-MT, noradrenaline + cocaine	36.7 ± 3.1	0.0086 ± 0.0005*	2.88 ± 0.03**	11

After preincubation, the slices were superfused with [^{3}H]-amezinium-free medium. The tissue tritium content after 50 min of superfusion was calculated from the measured content of the end of the experiment (110 min) and the cumulative outflow from 50–110 min. The basal outflow is expressed as a fractional rate (min^{-1}). The evoked overflow refers to the stimulation period S_1 after 60 min of superfusion. Treatments: (1) and (4) with saline and α-MT, respectively (Methods); (2) and (5) with saline and α-MT, respectively, and exposure to noradrenaline 0.1 μM for 15 min before preincubation with [^{3}H]-amezinium (Methods); (3) and (6) with saline and α-MT, respectively, and simultaneous exposure to noradrenaline 0.1 μM and cocaine 10 μM prior to preincubation with [^{3}H]-amezinium (Methods). Means ± s.e. of n experiments. Significant differences from group 1: *$P < 0.02$; **$P < 0.001$

Table 2 Effects of yohimbine on the stimulation-evoked overflow of tritium from rat occipitocortical slices preincubated with [^{3}H]-amezinium

Treatment	*Stimulation-evoked overflow (S_2/S_1) with yohimbine*		
	0 μM	0.1 μM	1 μM
(1) Saline	0.93 ± 0.03	1.38 ± 0.07**	1.55 ± 0.03**
	(9)	(6)	(8)
(2) Saline, noradrenaline	0.97 ± 0.06	1.39 ± 0.05**	1.57 ± 0.06**
	(7)	(8)	(8)
(3) Saline, noradrenaline + cocaine	0.93 ± 0.06	1.33 ± 0.07*	1.71 ± 0.13**
	(6)	(6)	(6)
(4) α-MT	0.94 ± 0.04	0.93 ± 0.03	1.01 ± 0.06
	(9)	(5)	(6)
(5) α-MT, noradrenaline	0.98 ± 0.03	1.48 ± 0.06**	1.52 ± 0.03**
	(6)	(6)	(5)
(6) α-MT, noradrenaline + cocaine	0.95 ± 0.02		0.92 ± 0.06
	(6)		(5)

After preincubation, the slices were superfused with [^{3}H]-amezinium-free medium. They were stimulated three times for 3 min each at 3 Hz (S_0, S_1, S_2). Yohimbine 0.1 or 1 μM was added to the superfusion medium 20 min before S_2. Indicated are the ratios of the evoked overflows (S_2/S_1). Treatments: (1) and (4) with saline and α-MT, respectively; (2) and (5) with saline and α-MT, respectively, and exposure to noradrenaline 0.1 μM for 15 min before preincubation with [^{3}H]-amezinium; (3) and (6) with saline and α-MT, respectively, and simultaneous exposure to noradrenaline 0.1 μM and cocaine 10 μM prior to preincubation with [^{3}H]-amezinium. Means ± s.e. of (n) experiments. Significant differences from corresponding experiments with yohimbine 0 μM: *$P < 0.005$; **$P < 0.001$

and 5). Exposure to noradrenaline or noradrenaline plus cocaine caused no change in slices from saline-pretreated rats (groups 2 and 3).

Yohimbine 0.1 or 1 μM was added 20 min before S_2 (Table 2). Its effect was quantitated by calculating the ratio of the evoked overflows S_2/S_1. Control ratios (yohimbine concentration = 0) were close to 1, in agreement with the reproducibility already shown in Figure 1. The effect of yohimbine depended crucially on the pretreatment of the rats. In slices from saline-pretreated animals, both concentrations increased the evoked overflow of tritium (group 1). After pretreatment with α-MT, the increase was abolished (group 4). It reappeared, however, and was

Table 3 Effects of clonidine on the stimulation-evoked overflow of tritium from rat occipitocortical slices preincubated with [^{3}H]-amezinium

Treatment	*Stimulation-evoked overflow (S_2/S_1 and $S_2 - S_1$) with clonidine*			
	0 μM	0.01 μM	0.1 μM	1 μM
(1) Saline	0.93 ± 0.03	0.66 ± 0.03**	0.33 ± 0.02**	0.24 ± 0.06**
	− 0.05 ± 0.03	*− 0.40 ± 0.03***	*− 0.95 ± 0.30**	*− 0.90 ± 0.12***
	(9)	(5)	(5)	(6)
(4) α-MT	0.94 ± 0.04	0.45 ± 0.02**	0.19 ± 0.02**	0.17 ± 0.02**
	− 0.19 ± 0.09	*− 1.53 ± 0.16***	*− 2.37 ± 0.26***	*− 2.01 ± 0.11***
	(9)	(5)	(5)	(5)
(5) α-MT, noradrenaline	0.98 ± 0.03	0.65 ± 0.03**	0.29 ± 0.02**	
	− 0.01 ± 0.03	*− 0.40 ± 0.01***	*− 0.89 ± 0.11***	
	(6)	(5)	(5)	

After preincubation, the slices were superfused with [^{3}H]-amezinium-free medium. They were stimulated three times for 3 min each at 3 Hz (S_0, S_1, S_2). Clonidine 0.01, 0.1 or 1 μM was added to the superfusion medium 20 min before S_2. Indicated are the ratios S_2/S_1 (first lines) and the differences $S_2 - S_1$ (second lines, italicized) of the evoked overflows. Treatments: (1) and (4) with saline and α-MT, respectively; (5) with α-MT, and exposure to noradrenaline 0.1 μM for 15 min prior to preincubation with [^{3}H]-amezinium. Means ± s.e. of (n) experiments. Significant differences from corresponding experiments with clonidine 0 μM: *$P < 0.005$; **$P <$ P.001

as large as in the saline group, when slices from α-MT rats were initially incubated with noradrenaline (group 5). Cocaine prevented the effect of noradrenaline (group 6). Exposure to noradrenaline or noradrenaline plus cocaine did not change the effect of yohimbine in slices from saline-pretreated rats (groups 2 and 3).

In order to quantify the effect of clonidine, the difference between the evoked overflows S_2-S_1, each first expressed as a percentage of tissue tritium, was calculated in addition to the S_2/S_1 ratio, and is shown in italics in Table 3. Clonidine concentration-dependently decreased S_2/S_1 and $S_2 - S_1$ in all three groups, but with considerable quantitative differences. The clonidine-induced inhibition was enhanced by pretreatment with α-MT; after additional exposure to noradrenaline, it was similar to that in slices from saline-pretreated rats. Due to the higher evoked overflow (S_1) in the α-MT group (4) as compared to the saline group (1), this effect of pretreatment with α-MT was particularly obvious from the $S_2 - S_1$ differences. For instance, clonidine 0.01 μM decreased the evoked overflow of tritium by 0.40% of the tritium content of the tissue in slices from saline-pretreated rats, but by 1.53% of tissue tritium in slices from α-MT-pretreated rats (taking the control $S_2 - S_1$ values to be zero); clonidine 1 μM caused an overflow deficit of 0.90% of tissue tritium in group 1, but of 2.01% in group 4.

Neither yohimbine nor clonidine caused any consistent change in the basal outflow of tritium.

Experiments with [^{3}H]-noradrenaline

To study the effect of amezinium on the release of noradrenaline, occipitocortical slices from rats that had received no pretreatment were preincubated with [^{3}H]-noradrenaline and then superfused. Cocaine 10 μM was present throughout superfusion to block the neuronal uptake of amezinium (Steppeler *et al.*, 1982). The outflow of tritium in this type of experiment and the response to electrical stimulation have been described repeatedly (e.g. Hedler *et al.*, 1981). In the present series, the overflow of tritium evoked by S_1 amounted to 4.4 ± 0.2% of the tritium content of the tissue ($n = 18$). In control experiments without amezinium, the ratio S_2/S_1 was 1.00 ± 0.03 ($n = 5$). Amezinium was added 15 min before S_2. At concentrations of 0.01, 0.1 and 1 μM, it neither changed the ratio S_2/S_1 (0.99 ± 0.03, $n = 4$; 1.00 ± 0.03, $n = 5$; and 1.01 ± 0.01, $n = 4$, respectively), nor the basal outflow of tritium.

Catecholamine content

The noradrenaline content of the occipital cortex from 10 saline-pretreated rats was 182 ± 12 ng/g. α-Methylnoradrenaline was not detected. Pretreatment with α-MT reduced noradrenaline to 45 ± 6 ng/g ($n = 20$), i.e., to about 25%. A small peak with the retention time of α-methylnoradrenaline was detected in 9 of these 20 experiments, corresponding to a tissue concentration of 17 ± 3 ng/g ($n = 9$). No α-methylnoradrenaline was detected in the remaining 11 experiments, indicating tissue levels below 7 ng/g (the detection limit).

Discussion

The electrically evoked overflow of tritium from rat occipitocortical slices preincubated with [^{3}H]-amezinium resembled the previously described evoked overflow from the isolated pulmonary artery of the rabbit (Steppeler & Starke, 1982): both were blocked by tetrodotoxin or omission of calcium, increased by yohimbine (as long as there was co-release of noradrenaline; see below) and diminished by clonidine. There is little doubt that the overflow originated from noradrenergic axons. In the periphery, amezinium, at least at low concentrations, accumulates exclusively in noradrenergic neurones (Steppeler & Starke, 1980; 1982); centrally, it has at least ten times higher affinity for uptake into noradrenergic fibres (K_i of amezinium for inhibition of synaptosomal noradrenaline uptake, 0.02 μM) than for uptake into dopaminergic or 5-hydroxytryptaminergic fibres (Traut *et al.*, 1981; Steppeler *et al.*, 1982). Moreover, dopaminergic axons are sparse in the rat occipital cortex, and transmitter release from 5-hydroxytryptaminergic axons is reduced rather than increased by yohimbine (Göthert, Huth & Schlicker, 1981). Again as in the periphery (Steppeler & Starke, 1982), thin layer chromatography indicated that the tritiated material retained in the brain slices and appearing in the superfusate was intact [^{3}H]-amezinium. Unlabelled amezinium, in the presence of cocaine, did not affect the release of [^{3}H]-noradrenaline; this confirms the absence of agonist or antagonist activity at (presynaptic) α-adrenoceptors (Steppeler *et al.*, 1980; Lenke *et al.*, 1981) and shows that [^{3}H]-amezinium is in fact a marker of transmitter release that, unlike noradrenaline itself, is neutral with respect to α-autoinhibition. Finally, since yohimbine and clonidine were used at relatively low concentrations which do not interfere with the neuronal uptake mechanism for noradrenaline and amezinium (Starke, Wagner & Schümann, 1972; Borowski, Starke, Ehrl & Endo, 1977), their effects on the evoked overflow were due to changes in the release of [^{3}H]-amezinium from the interior of the neurones.

Our main finding is that the release of this marker as well as the modulation by yohimbine and clonidine

depended crucially on the noradrenaline content of, and presumably noradrenaline release from, the occipital cortex. Admittedly, pretreatment with α-MT decreased the tissue noradrenaline by only 75%. However, α-MT was present throughout the entire α-MT experiments including preincubation and superfusion; hence, the synthesis of noradrenaline was blocked throughout, and since the main source of release is newly synthesized (or newly taken-up) noradrenaline (see Glowinski, 1975), it seems very likely that the release was depressed much more than the tissue level. In addition, little, if any, α-methylnoradrenaline was formed from α-MT. All facets of the interaction that we observed strongly suggest an explanation in terms of the α-autoreceptor hypothesis, as will now be discussed.

When the tissue noradrenaline stores were reduced by α-MT, and the release of noradrenaline presumably markedly depressed, the evoked release of [^{3}H]-amezinium, expressed as a percentage of the [^{3}H]-amezinium content of the tissue, was approximately doubled (from 1.15 to 2.56%). A similar increase was calculated when the evoked overflow was expressed as nCi per stimulation period (not shown). The interpretation from the standpoint of the autoreceptor hypothesis is that, once the noradrenaline input to the presynaptic receptors vanishes, the release of the (non-α-adrenergic) marker is disinhibited. The experiments with yohimbine support this view. The antagonist increased the release of [^{3}H]-amezinium when the natural agonist was present in the biophase, but failed to do so when noradrenaline had been depleted and, hence, release was *a priori* unrestrained.

So far, our experiments confirm, by another method, previous work in which the release of [^{3}H]-noradrenaline or dopamine-β-hydroxylase from noradrenaline-depleted tissues was studied (Farnebo, Hamberger & Jonsson, 1971; Enero & Langer, 1973; Cubeddu & Weiner, 1975; Dixon *et al.*, 1979). One minor difference is that we succeeded in abolishing the release-facilitating effect of the α-antagonist completely, perhaps because we used the non-α-adrenergic [^{3}H]-amezinium rather than small amounts of [^{3}H]-noradrenaline to measure transmitter release.

Our other findings, however, go beyond previous investigations and further support our interpretation. It might still be thought that α-MT increased the release of [^{3}H]-amezinium and blunted the effect of yohimbine by some non-specific mechanism. This view can be refuted because refilling of the readily releasable noradrenaline pools with exogenous noradrenaline was followed by re-depression of the release of [^{3}H]-amezinium (1.20%) as well as restoration of the release-enhancing effect of yohimbine. Furthermore, to bring about this normalization, the exogenous noradrenaline had to enter (presumably noradrenergic) neurones, since the presence of cocaine nullified its effect (release at S_1 2.88%). Exposure to exogenous noradrenaline or noradrenaline plus cocaine did not change the release of [^{3}H]-amezinium or the effect of yohimbine when the slices contained normal stores of endogenous noradrenaline.

Finally, the presence or absence of releasable noradrenaline determined not only the facilitatory effect of yohimbine but the inhibitory effect of clonidine as well. In slices from saline-pretreated rats, when there was co-release of [^{3}H]-amezinium and noradrenaline and, hence, α-adrenergic autohibition and a small overflow at S_1, clonidine caused only a moderate depression of the S_2-S_1 difference (by 0.40, 0.95 and 0.90% of tissue tritium at 0.01, 0.1 and 1 μM, respectively; Table 3). When, however, noradrenaline was depleted and release disinhibited, the exogenous α-agonist acted over a much wider range (decrease of S_2-S_1 by 1.53, 2.37 and 2.01% of tissue tritium, respectively, Table 3). The refilling experiment indicates that this difference was indeed due to background autoinhibition: when exposure to exogenous noradrenaline had re-established the autoinhibition, the effect of clonidine was once more curtailed (decrease of S_2-S_1 by 0.40 and 0.89% of tissue tritium at 0.01 and 0.1 μM, respectively, Table 3).

These results combine to yield a consistent picture. The release of the non-α-adrenergic compound [^{3}H]-amezinium is subject to limitations imposed by the α-adrenergic autoinhibition of the co-secretion of noradrenaline, and the effects of exogenous antagonists and agonists are determined by the degree of autoinhibition with which they meet. As to yohimbine, the present results supplement those obtained in a parallel study in the rabbit ear artery in which we used an entirely different approach (Auch-Schwelk, Starke & Steppeler, 1983). The conclusion from either study is that the release-enhancing effect of yohimbine is indeed a specific anti-noradrenaline effect, requiring a sufficient biophase concentration of noradrenaline and revealing an α-adrenergic autoinhibition.

The present results confirm that α-adrenergic agonists inhibit the release of noradrenaline less, the higher the biophase concentration of noradrenaline, and *vice versa*. This observation provided one of the earliest arguments for the α-feedback concept that went beyond the simple qualitative effects of agonists and antagonists (Starke, 1972; Starke & Altmann, 1973; Reichenbacher, Reimann & Starke, 1982). Competition between exogenous agonist and liberated noradrenaline for one set of autoreceptors may also explain in part the inverse relationship that exists between the inhibition caused by the exogenous

agonist and the frequency of stimulation (Vizi, Somogyi, Hadházy & Knoll, 1973; Starke, Montel, Gayk & Merker, 1974; Langer, Dubocovich & Celuch, 1975). However, the influence of the stimulation frequency on the effectiveness of α-agonists is complex, and varying degrees of background inhibition by liberated noradrenaline are only one factor (see Starke, 1977, and the critical discussion by Kalsner, 1981). The present experiments in which the effect of clonidine could be increased or diminished by emptying or replenishment of noradrenaline stores, all at a constant frequency, demonstrate perhaps less ambiguously the interference of the exogenous agonist with an endogenous autoinhibitory system.

The [^{3}H]-amezinium model, taken together with previous observations such as the reliability and predictability with which all α_2-agonists and -antagonists tested modified the release of noradrenaline (Hedler *et al.*, 1981; Wemer, Frankhuyzen & Mulder, 1982), strongly supports the existence and *in vitro* operation of presynaptic α-autoreceptors at the noradrenergic axons of the occipital cortex of the rat.

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