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α-Adrenoceptor antagonists and the release of noradrenaline in rabbit cerebral cortex slices: support for the α-autoreceptor hypothesis

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1 Slices of rabbit cerebral cortex were preincubated with [^{3}H]-noradrenaline and then superfused and stimulated electrically twice for 2 min each (S_1, S_2) at various frequencies (0.2–3 Hz). The stimulation-evoked overflow of tritium (S_1) increased with increasing frequency and was higher when cocaine (10 μM) was present.

2 In the absence of cocaine, tetraethylammonium (TEA; 100 and 300 μM), added before S_2, increased the stimulation-evoked overflow of tritium to about the same extent, irrespective of the frequency. In contrast, rauwolscine (0.1 and 1 μM) and idazoxan (0.1–10 μM) increased the evoked overflow much more, the higher the frequency of stimulation. Phentolamine (0.1 and 1 μM) reduced the overflow elicited at 0.3 and 1 Hz, and (1 μM) caused an increase only at 3 Hz.

3 In slices superfused throughout with cocaine 10 μM, rauwolscine (1 μM) and idazoxan (1 and 10 μM) again increased the evoked overflow of tritium more, the higher the frequency of stimulation. For a given frequency, rauwolscine and idazoxan enhanced the evoked overflow to a greater extent in the presence than in the absence of cocaine.

4 Idazoxan (1 and 10 μM) and rauwolscine (1 μM) counteracted the inhibition that phentolamine (0.1 μM) produced at low frequency. The increases caused by rauwolscine (1 μM) and TEA (300 μM) were approximately additive, but those caused by rauwolscine (1 μM) and idazoxan (10 μM) were not.

5 The effects of rauwolscine, idazoxan and phentolamine depend on the experimental conditions (frequency, cocaine) in a manner compatible with the operation of a presynaptic α_2-adrenoceptor-mediated autoinhibition of noradrenaline release. When given at sufficient concentrations, these antagonists enhance the release of noradrenaline more, the higher the biophase concentration of the transmitter and the stronger, hence, the autoinhibition. In the case of the partial α_2-adrenoceptor agonist phentolamine, a low perineuronal noradrenaline concentration even reverses facilitation to inhibition. This pattern differs markedly from the pattern of effects of TEA which increases the release of noradrenaline by a mechanism other than α-adrenoceptor blockade.

Introduction

A tenet in Kalsner's stimulating criticism of the α-autoreceptor hypothesis is that α-adrenoceptor antagonists increase the release of noradrenaline, not by blocking presynaptic α-adrenoceptors and preventing a release-inhibiting effect of noradrenaline, but by some other mechanism (Chan & Kalsner, 1979; Kalsner, 1982; 1983; for review of the hypothesis see Starke, 1977; 1981; Vizi, 1979; Gillespie, 1980; Langer, 1981). The most far-reaching inference has been, 'that the hypothesis of specific functional presynaptic adrenoceptors itself has been prematurely acknowledged' (Chan & Kalsner, 1979), i.e., that possibly there are no presynaptic α-adrenoceptors at all. More recently, Kalsner (1982) has explained the depression by exogenous noradrenaline of the release of intraneuronal [^{3}H]-noradrenaline as 'activation of inhibitory presynaptic α-sites by the exogenous amine', thus accepting the existence of the receptors. However, the release-enhancing effect of antagonists is still believed to be unrelated to the receptors. A recent suggestion has been that α-adrenoceptor antagonists directly or indirectly block the repolarizing potassium current and, hence, prolong axonal depolarization and the

period of transmitter release; they would then resemble the potassium channel inhibitor, tetraethylammonium (TEA; Kalsner, 1983).

In previous work in the rabbit isolated ear artery we have tried to distinguish between α-adrenoceptor blockade and an independent mechanism by studying the effect of α-antagonists on the release of [^{3}H]-noradrenaline under various conditions of electrical stimulation (Auch-Schwelk *et al.*, 1983; Limberger & Starke, 1983; 1984). Within the limits chosen, yohimbine, rauwolscine, phentolamine, idazoxan and piperoxan all increased the release more, the higher the frequency of stimulation and, hence, the biophase concentration of noradrenaline. At low frequency (0.2–0.25 Hz), the drugs did not cause any increase; phentolamine and idazoxan even caused a decrease. These findings support an α-adrenolytic mode of action: yohimbine, rauwolscine, phentolamine, idazoxan and piperoxan enhance the release of noradrenaline only when a sufficient biophase concentration of transmitter has led to significant α-adrenergic autoinhibition. A low biophase concentration of noradrenaline may even reverse the effect of the partial α_2-agonists phentolamine and idazoxan from disinhibition (antagonism) to inhibition (agonism).

We now report a similar study on central noradrenergic axons, namely those innervating the rabbit brain cortex. These axons possess presynaptic α_2-adrenoceptors (Reichenbacher *et al.*, 1982). Rauwolscine, phentolamine and idazoxan were used as α-adrenoceptor antagonists, and the effect of TEA was examined for comparison. The biophase concentration of noradrenaline was modified by stimulation at different frequencies as well as by the absence or presence of cocaine. Some of these results have been reported to the German Pharmacological Society (Heepe & Starke, 1984).

Methods

The methods were those of Reichenbacher *et al.* (1982). Rabbits of either sex and weighing 1.8–2.7 kg were decapitated. The brain was quickly removed and chilled. Round slices (0.4 mm thick, 5 mm diameter) were prepared from the occipital and parietal cortices after the superficial layer (0.3 mm) had been removed. Seven to nine slices were preincubated with 0.1 μM (−)-[^{3}H]-noradrenaline (specific activity 10 Ci mmol^{-1}) in 2 ml medium at 37°C for 30 min. They were then washed three times with approx. 3 ml each of [^{3}H]-noradrenaline-free medium. One slice was transferred to each of six superfusion chambers equipped with platinum electrodes, and was superfused with medium for 125 min at 1 ml min^{-1}. Five min fractions of the superfusate were collected continuously from 50 min of superfusion onwards. Two 2 min periods of electrical stimulation were applied after 60 and 105 min of superfusion (S_1, S_2). The pulse width was 2 ms, current strength 24 mA, and frequency either 0.3, 1 or 3 (in a few experiments 0.2) Hz. After superfusion, each slice was solubilized in 0.5 ml Soluene-100 (Packard Instrument GmbH, Frankfurt, FRG). Tritium was determined by liquid scintillation spectrometry. Counting efficiencies were measured with internal standards of [^{3}H]-toluene or [^{3}H]-water.

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, glucose 11, ascorbic acid 0.57, disodium EDTA 0.03. It was saturated with 5% CO_2 in O_2.

Drugs were added either throughout superfusion (cocaine, idazoxan, rauwolscine) or 25 min before S_2 (TEA, rauwolscine, idazoxan, phentolamine). Controls without drug were always run in parallel to drug experiments so that each drug had its own control group.

The outflow of tritium was expressed as fractional rate (min^{-1}), i.e. (nCi tritium outflow per 5 min)/ 5 × (nCi tritium in the slice at the onset of the 5 min collection period). The stimulation-evoked overflow was calculated as the difference between the total overflow during and 13 min after stimulation and the estimated basal outflow; the basal outflow was assumed to decline linearly from the 5 min interval before to the interval 15–20 min after the onset of stimulation; the difference (nCi) was expressed as % of the tritium content (nCi) of the tissue at the onset of stimulation (cf. Taube *et al.*, 1977).

In order to quantify effects of drugs that were added 25 min before S_2 on the basal outflow of tritium, ratios were calculated between the fractional rate of outflow in the 5 min before S_2, and the fractional rate in the 5 min before S_1 (b_2/b_1). In order to quantify effects on the stimulation-evoked overflow, ratios were calculated between the overflow (as % tissue tritium) evoked by S_2 and the overflow evoked by S_1 (S_2/S_1).

(−)-[2, 5, 6,-^{3}H)-Noradrenaline was purchased from New England Nuclear Creieich, FRG (NET–678). Other drugs were phentolamine methanesulphonate (Ciba-Geigy, Basel, Switzerland), (−)-noradrenaline HCl (Hoechst, Frankfurt, FRG), cocaine HCl and tetraethylammonium chloride (Merck, Darmstadt, FRG), idazoxan HCl (RX 781094; Reckitt & Colman, Kingston-upon-Hull), and rauwolscine HCl (Roth, Karlsruhe, FRG). Stock solutions were made either in medium or (rauwolscine HCl) water or (noradrenaline HCl) 1 mM HCl.

Results are expressed as arithmetic means ± s.e.mean. Differences between means of

b_2/b_1 and S_2/S_1 ratios were tested for significance by an analysis of variance and the Bonferroni method (Wallenstein *et al.*, 1980). For the analysis of variance, ratios were transformed logarithmically because this yielded standard deviations that did not differ between groups. *n* is the number of observations (one observation = one brain slice).

Results

Time course of tritium outflow

By the time the collection of superfusate began, the efflux of tritium had reached a low and (when expressed as fractional rate) quite constant level (Figure 1). Electrical pulses accelerated the outflow of tritium. The higher the frequency of stimulation, the greater was the acceleration. In control experiments, the two stimulation periods S_1 and S_2 elicited similar responses. Rauwolscine 1 μM, added 25 min before S_2, enhanced the stimulation-evoked overflow at all frequencies, but to a much greater extent at 3 than at 1 Hz, and even less at 0.3 Hz (Figure 1).

Initial tritium outflow (b_1, S_1)

Values for the basal outflow of tritium at b_1 (immediately before S_1), and for the overflow elicited by S_1, are summarized in Table 1. All appropriate experiments were pooled. The stimulation-evoked overflow of tritium increased with increasing frequency, but less than would be expected from simple proportionality. In fact, division of the S_1 values of Table 1 by the number of pulses shows that the overflow per pulse decreased with increasing frequency. Cocaine 10 μM, when present throughout superfusion, enhanced the stimulation-evoked overflow at all frequencies (by 45–67%). The inverse relationship between frequency and per pulse over-

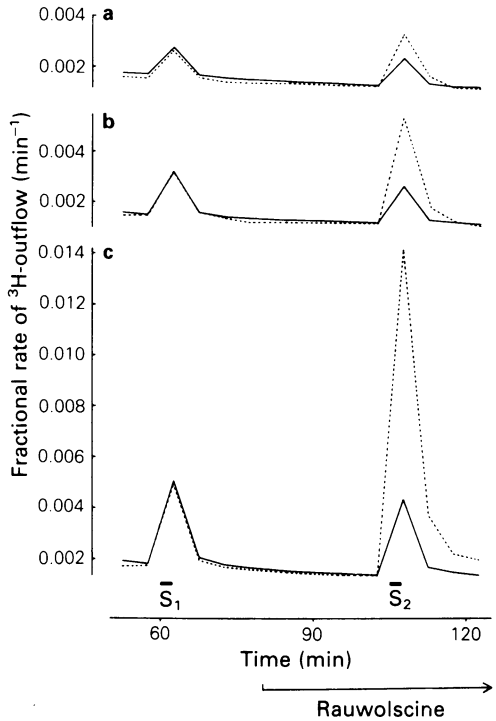

Figure 1 Time course of the outflow of tritium from slices of rabbit cerebral cortex preincubated with [^{3}H]-noradrenaline. After preincubation, the slices were superfused with [^{3}H]-noradrenaline-free medium. They were stimulated electrically twice (S_1, S_2) for 2 min each at either 0.3 (a), 1 (b) or 3 Hz (c). Abscissa scale: time elapsed since the beginning of superfusion (min). Ordinate scale: fractional rate of tritium outflow (min^{-1}). Solid lines represent controls (no drug added), dashed lines experiments in which rauwolscine 1 μM was added at 80 min (25 min before S_2). Means of 6–16 experiments. Standard errors have been omitted for clarity; they were 4–18% of corresponding means.

Table 1 Basal and evoked tritium outflow from slices of rabbit cerebral cortex preincubated with [^{3}H]-noradrenaline

Drug present throughout superfusion	*Stimulation frequency*	*Basal tritium outflow at* b_1 (min^{-1})	*Overflow evoked by* S_1 (% of tissue tritium)	n
—	0.3 Hz	0.0017 ± 0.0001	0.51 ± 0.02	126
	1 Hz	0.0017 ± 0.0001	0.95 ± 0.03	117
	3 Hz	0.0018 ± 0.0001	1.98 ± 0.06	105
Cocaine 10 μM	0.3 Hz	0.0016 ± 0.0001	0.81 ± 0.04	67
	1 Hz	0.0017 ± 0.0001	1.38 ± 0.06	66
	3 Hz	0.0020 ± 0.0001	3.31 ± 0.10	64

After preincubation, the slices were superfused with [^{3}H]-noradrenaline-free medium. In some experiments, the medium contained cocaine 10 μM throughout superfusion. Basal tritium outflow at b_1 is the outflow in the 5 min immediately before S_1 (i.e., from 55–60 min of superfusion). Means ± s.e.mean of *n* experiments.

flow persisted in the presence of cocaine. A similar inverse relationship has been found for the release of noradrenaline in rat cerebro-cortical slices (Montel *et al.*, 1974).

Effects on evoked tritium overflow in the absence of cocaine

TEA, rauwolscine, idazoxan and phentolamine were first studied in the absence of cocaine (Figure 2, 3). The drugs were added before S_2 and their effects quantified as the ratio S_2/S_1. In control experiments, these ratios were close to unity. TEA 100 and 300 μM caused a concentration-dependent increase. Each concentration augmented the stimulation-evoked overflow to a comparable extent at all frequencies, the greatest difference between frequencies being the 60% increase caused by TEA 300 μM at 0.3 Hz and the 114% increase at 3 Hz (Figure 2). In contrast, the effect of rauwolscine 0.1 and 1 μM rose markedly with rising frequency. For instance, rauwolscine 0.1 μM caused a 53% increase at 0.3 Hz but a 216% increase at 3 Hz, and rauwolscine 1 μM produced a 95% increase at 0.3 Hz and a 410% increase at 3 Hz. The effect of rauwolscine 0.01 μM did not change so clearly with the stimulation frequency (Figure 2).

A pronounced frequency-dependence was also found for idazoxan and phentolamine. None of three concentrations of idazoxan (0.1–10 μM) enhanced the response to 0.3 Hz, but all enhanced the response to 3 Hz; the response to 1 Hz was affected to an intermediate degree (Figure 3). In the case of phentolamine, the frequency change actually reversed the direction of the effect: phentolamine 0.1 and 1 μM reduced the overflow of tritium elicited by pulses at 0.3 Hz, reduced less the overflow elicited by pulses at 1 Hz, and (1 μM) augmented the overflow at 3 Hz (Figure 3).

Effects on evoked tritium overflow in the presence of cocaine

Rauwolscine and idazoxan were also examined in the presence of cocaine 10 μM throughout superfusion (Figure 4). As mentioned above, cocaine enhanced the overflow of tritium evoked by S_1. Without further drug addition, S_2/S_1 ratios again were close to unity. The marked frequency-dependence of the effects of rauwolscine 1 μM and idazoxan 1 and 10 μM also held true in the presence of cocaine (Figure 4). However, all increases in overflow produced by the α-adrenoceptor antagonists were larger. For instance, rauwolscine 1 μM produced a 233% increase at 0.3 Hz (instead of 95% without cocaine) and a 477% increase at 3 Hz (instead of 410% without cocaine); idazoxan, which had been ineffective at 0.3 Hz in the absence of cocaine, became significantly effective in its presence. As in the absence of cocaine, low concentrations of rauwolscine (0.003 and 0.01 μM) did not exhibit this typical frequency-dependence. In fact, rauwolscine 0.01 μM produced the greatest increase (by 100%) at the lowest frequency.

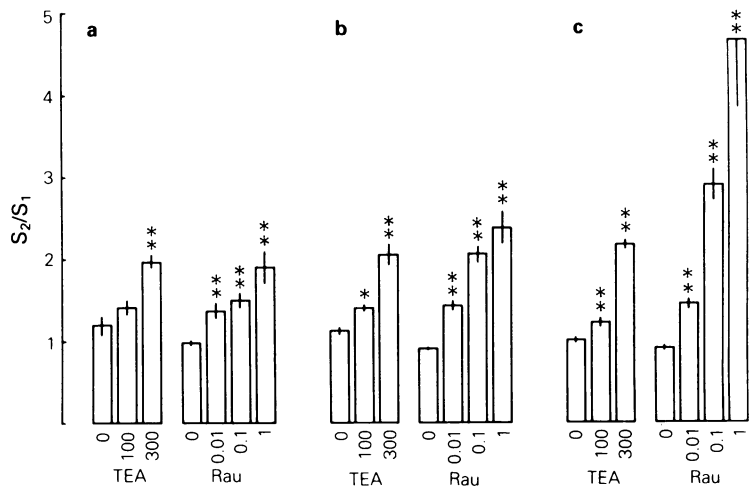

Figure 2 Effects of tetraethylammonium (TEA) and rauwolscine (Rau) on the overflow of tritium evoked by stimulation at different frequencies. Frequencies were 0.3 (a), 1 (b) and 3 Hz (c). Rauwolscine or TEA was added 25 min before S_2 at concentrations (μM) indicated. Ordinate scale: ratio between the overflow of tritium evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Means of 4–16 experiments; vertical lines show s.e.means. Significant differences from corresponding controls: * $P < 0.05$; ** $P < 0.01$ (analysis of variance; see Methods for details).

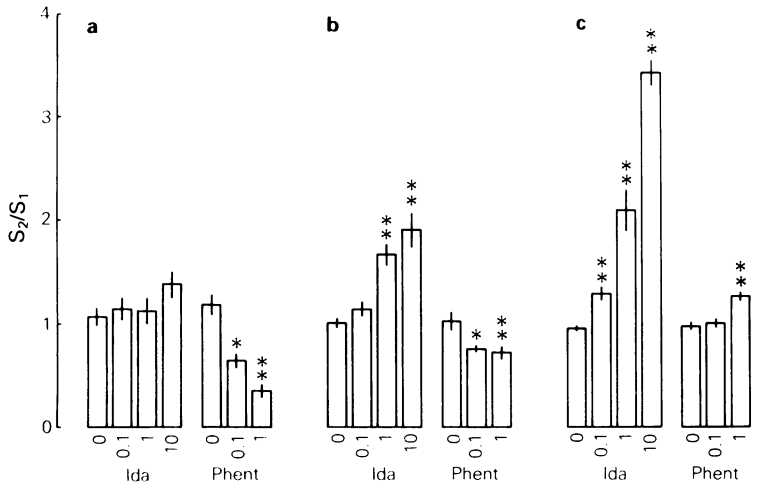

Figure 3 Effects of idazoxan (Ida) and phentolamine (Phent) on the overflow of tritium evoked by stimulation at different frequencies. Frequencies were 0.3 (a), 1 (b) or 3 Hz (c). Idazoxan or phentolamine was added 25 min before S_2 at concentrations (μM) indicated. Ordinate scale: ratio between the overflow of tritium evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Means of 5–15 experiments; vertical lines show s.e.means. Significant differences from corresponding controls: * $P < 0.05$; ** $P < 0.01$ (analysis of variance; see Methods for details).

Interactions on evoked tritium overflow

In order to characterize further the inhibitory effect of phentolamine, we administered the drug to slices that were superfused throughout with either idazoxan 1 or 10 μM or rauwolscine 1 μM; the stimulation frequency was 0.2 or 0.3 Hz (Table 2). Under these conditions, the overflow of tritium elicited by S_1 was close to that obtained at 0.3 Hz in drug-free medium. Phentolamine 0.1 μM which, given alone, reduced the

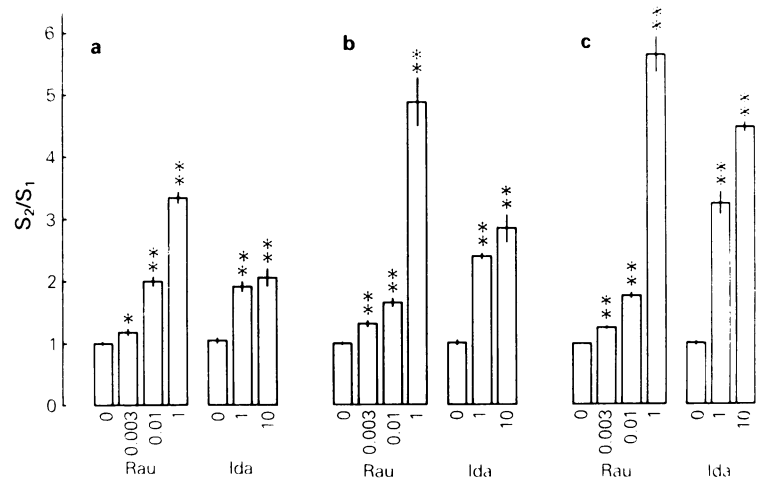

Figure 4 Effects of rauwolscine (Rau) and idazoxan (Ida) on the overflow of tritium evoked by stimulation at different frequencies; experiments in which cocaine 10 μM was present throughout superfusion. Frequencies were 0.3 (a), 1 (b) or 3 Hz (c). Rauwolscine or idazoxan was added 25 min before S_2 at concentrations (μM) indicated. Ordinate scale: ratio between the overflow of tritium evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Means of 5–23 experiments; vertical lines show s.e.means. Significant differences from corresponding controls: * $P < 0.05$; ** $P < 0.01$ (analysis of variance; see Methods for details).

overflow at 0.3 Hz by 46%, reduced the overflow less (by 35%) in the presence of idazoxan 1 μM and not at all in the presence of idazoxan 10 μM or rauwolscine 1 μM (Table 2).

In order to differentiate further between TEA and the α-adrenoceptor antagonists, we added idazoxan and TEA to slices that were superfused throughout with rauwolscine 1 μM; the stimulation frequency was 3 Hz (Table 3). Rauwolscine greatly augmented the overflow of tritium elicited by S_1 (cf. Tables 3 and 1). Idazoxan 10 μM which, given alone, increased the overflow at 3 Hz by 257% (Figure 3), caused only a 16% further increase in the presence of rauwolscine. On the other hand, TEA 300 μM which given alone, increased the overflow at 3 Hz by 114%, caused a 95% further increase in the presence of rauwolscine (Table 3).

Effects on basal tritium outflow

TEA, rauwolscine, idazoxan and phentolamine, when added before S_2, did not change the basal efflux of tritium as quantified by the b_2/b_1 ratio. The one exception was idazoxan 10 μM which in most groups significantly ($P < 0.05$ or 0.01) decelerated the basal efflux. The reduction was observed when idazoxan was given alone as well as in the presence of cocaine 10 μM or rauwolscine 1 μM, and ranged between 5 and 24%.

Discussion

The discussion will be based on two premises; first, that the electrically-evoked overflow of tritium represented action potential-evoked release of [^{3}H]-noradrenaline from cortical noradrenergic axons; second, that TEA, rauwolscine, idazoxan and phentolamine modified the stimulation-evoked overflow by changing this release and not by changing the inactivation of released [^{3}H]-noradrenaline within the slices. In support of the first assumption it has been shown that, under the present conditions, the evoked overflow is Ca^{2+}-dependent and tetrodotoxin-sensitive and consists mainly of [^{3}H]-noradrenaline (Reichenbacher *et al.*, 1982; cf. for rat cortical slices Taube *et al.*, 1977). The second assumption is supported by the lack of effect of TEA (Thoenen *et al.*, 1967) and most α-adrenoceptor antagonists (see Starke, 1977) on inactivation mechanisms for catecholamines; moreover, in the present experiments rauwolscine and idazoxan enhanced the evoked overflow of tritium more rather than less in the presence of cocaine, indicating that their effect was not due to inhibition of [^{3}H]-noradrenaline re-uptake, the major pathway of inactivation.

When interpreted on this basis, the results indicate that the effect of rauwolscine, idazoxan and phentolamine on action potential-evoked release of [^{3}H]-noradrenaline depends on the experimental conditions (frequency, cocaine) in a characteristic manner that does not hold good for TEA. TEA increased the release of [^{3}H]-noradrenaline to about the same degree, irrespective of whether the neurones were stimulated at 0.3, 1 or 3 Hz (tested without cocaine only). In contrast, rauwolscine, idazoxan and phentolamine increased the release much less at 0.3 than at 1 Hz, and again much less at 1 than at 3 Hz; the effect of phentolamine was actually reversed from inhibition at 0.3 and 1 Hz to facilitation at 3 Hz. Moreover, for a given frequency the facilitation

Table 2 Interaction of phentolamine with idazoxan and rauwolscine on the stimulation-evoked overflow of tritium

Drug present throughout superfusion	*Stimulation frequency*	*Overflow evoked by S_1* (% of tissue tritium)	*Drug added before S_2*	S_2/S_1
—	0.3 Hz	0.43 ± 0.02 (15)	—	1.19 ± 0.11 (7)
			Phentolamine 0.1 μM	0.64 ± 0.08 (8)*
Idazoxan 1 μM	0.3 Hz	0.36 ± 0.03 (15)	—	1.13 ± 0.09 (7)
			Phentolamine 0.1 μM	0.73 ± 0.08 (8)*
Idazoxan 10 μM	0.3 Hz	0.61 ± 0.05 (22)	—	1.14 ± 0.06 (11)
			Phentolamine 0.1 μM	1.09 ± 0.05 (11)
Rauwolscine 1 μM	0.2 Hz	0.56 ± 0.07 (16)	—	1.17 ± 0.12 (7)
			Phentolamine 0.1 μM	1.28 ± 0.09 (9)

Idazoxan or rauwolscine, when given, was present throughout superfusion. Phentolamine, when given, was added 25 min before S_2. Indicated are the overflow of tritium evoked by S_1, expressed as % of tissue tritium, and ratios between the overflow evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Means ± s.e.mean of (n) experiments. Significant differences from corresponding experiments without phentolamine: * $P < 0.05$ (analysis of variance; see Methods for details). Experiments without idazoxan or rauwolscine are identical with those shown in Figure 3.

Table 3 Interaction of rauwolscine with idazoxan and TEA on the stimulation-evoked overflow of tritium

Drug added before S_2	S_2/S_1	n
—	1.01 ± 0.02	12
Idazoxan 10 μM	1.17 ± 0.06*	6
TEA 300 μM	1.97 ± 0.06*	6

Rauwolscine 1 μM was present throughout superfusion. The stimulation frequency was 3 Hz. The overflow of tritium evoked by S_1 was 8.77 ± 0.56% of the tritium content of the tissue ($n = 24$). Idazoxan and TEA, when given, were added 25 min before S_2. Values are the ratios of the overflow of tritium evoked by S_2 and the overflow evoked by S_1 (S_2/S_1). Means ± s.e.mean of n experiments. Significant differences from experiments without idazoxan or TEA: $^*P < 0.01$ (analysis of variance; see Methods for details).

caused by rauwolscine and idazoxan was enhanced by cocaine. The one exception to this typical frequency-dependence was the effect of low concentrations of rauwolscine (0.003 and 0.01 μM). In our opinion, the effects of rauwolscine, idazoxan and phentolamine are as one would expect if the drugs competed with released noradrenaline for presynaptic α-adrenoceptors and are, hence, in favour of the α-autoreceptor hypothesis, as will now be detailed.

First, the frequency-dependence at high antagonist concentrations: an increase in frequency increased the release of [^{3}H]-noradrenaline per unit time and, hence, the biophase concentration of the transmitter. The hypothesis predicts that this should intensify the autoinhibition and promote the release-enhancing effect of α-adrenoceptor antagonists (if administered at sufficiently high concentration). This was in fact found. Idazoxan and phentolamine act as partial agonists at some α_2 (or 'α_2-like') adrenoceptors (idazoxan: Goldstein *et al.*, 1983; Limberger & Starke, 1983; Angus & Lew, 1984; phentolamine: Broadhurst *et al.*, 1983; Angus & Lew, 1984; Limberger & Starke, 1984). Such compounds should enhance the release of noradrenaline only when the biophase concentration of transmitter is high, whereas at low perineuronal noradrenaline levels they should reveal their agonist character and depress release (Starke *et al.*, 1974). In the present experiments, phentolamine exhibited precisely this pattern of effect, and blockade by idazoxan and rauwolscine of the release-inhibiting effect of phentolamine at low frequency bears out the involvement of α_2-adrenoceptors. Idazoxan did not reduce the release of noradrenaline, in contrast to findings in the rabbit ear artery (Limberger & Starke, 1983). Perhaps the failure of idazoxan to cause an increase at 0.3 Hz in the absence of cocaine, which contrasts with the facilitatory effect of rauwolscine under the same conditions, reflects some intrinsic activity of idazoxan also in rabbit cerebral cortex.

Second, the effect of cocaine: for any given frequency, cocaine augmented the stimulation-evoked overflow of tritium; the increase in the biophase concentration of noradrenaline was probably greater than the 45–67% increase in total tritium overflow, because cocaine additionally reduces the fraction of total tritium that consists of [^{3}H]-metabolites (Farah *et al.*, 1977; Taube *et al.*, 1977). The autoreceptor hypothesis again predicts intensified autoinhibition and, hence, an increase in the release-enhancing effect of α-adrenoceptor antagonists (if given at sufficient concentration), and again this is what was found.

Third, the effect of low concentrations of rauwolscine: as has been emphasized, an increase in α-adrenolytic facilitation with increasing perineuronal concentration of noradrenaline can be expected only when the antagonists are given at sufficient concentrations. Receptor blockade by low antagonist concentrations may be surmounted by a rising concentration of noradrenaline, and then the release-enhancing effect of the antagonists will decline rather than increase. Using rauwolscine 0.003 and 0.01 μM, we did not obtain a clear pattern of that kind. For instance, although rauwolscine 0.01 μM in the presence of cocaine increased [^{3}H]-noradrenaline release more at 0.3 than at 1 Hz, its effect did not decline further from 1 to 3 Hz (Figure 4). However, it was found consistently that the typical direct relationship between frequency and degree of α-adrenolytic facilitation disappeared at the low concentrations of rauwolscine, and this may be an indication of displacement of the antagonist from the receptor.

The present findings are in agreement with our previous experiments in the rabbit perfused ear artery in which yohimbine, rauwolscine, phentolamine, idazoxan and piperoxan were used as α-adrenoceptor antagonists (Auch-Schwelk *et al.*, 1983; Limberger & Starke, 1983; 1984). In the first of those studies, cocaine slightly augmented the release-enhancing effect of the α-antagonists only under one of three stimulation conditions (100 shocks at 2 Hz), presumably because under the two other conditions either the noradrenaline level remained subthreshold for autoinhibition (10 shocks at 0.2 Hz) or the pulse train was too short for autoinhibition to develop (10 shocks at 2 Hz; Auch-Schwelk *et al.*, 1983). Moreover, the present findings agree with several other recent neurochemical observations on α-adrenoceptor antagonists and the release of noradrenaline; in all studies, the biophase concentration of noradrenaline was found to determine crucially the

occurrence or magnitude of the release-enhancing effect of the antagonists (Baumann & Koella, 1980; Hedler *et al.*, 1983; Marshall, 1983; Angus *et al.*, 1984; Baker *et al.*, 1984; Fuder *et al.*, 1984). It seems relevant to point out that one group of authors who regard with scepticism the general physiological significance of α-autoreceptors, nevertheless explain the enhancement of release by α-antagonists as an anti-noradrenaline action at these receptors (Angus *et al.*, 1984).

When Kalsner examined the effect of α-adrenoceptor antagonists on the release of noradrenaline, his results seemed to contradict the autoreceptor hypothesis (e.g. Chan & Kalsner, 1979; Kalsner, 1982; 1983). Possible reasons have been discussed elsewhere (Starke, 1981; Auch-Schwelk *et al.*, 1983). A recent suggestion has been that α-adrenoceptor antagonists might increase noradrenaline release by blocking the repolarizing potassium outward current from terminal axons and, hence, prolonging the nerve action potential (Kalsner, 1983). We do not exclude this possibility. Yet, if α-antagonists block potassium efflux, we would maintain that they do so by competing with released noradrenaline for presynaptic α_2-adrenoceptors through which the transmitter would then normally promote potassium conductance, and not by a mechanism unrelated to α-adrenoceptors; promotion of potassium conductance is in fact one presently discussed ionic basis of α_2-adrenergic inhibition of noradrenaline release (Stjärne, 1978; Egan *et al.*, 1983). TEA enhances the release of noradrenaline by a decrease in potassium efflux, unrelated to α-adrenoceptors. Its pattern of effect differed markedly from that of the α-adrenoceptor antagonists. Moreover, the effects of TEA and rauwolscine were additive, but those of idazoxan and rauwolscine were not. These findings clearly dissociate the mechanisms of action, notwithstanding the possibility that ultimately (but via α-adrenoceptor blockade) the α-antagonists also impede a potassium outward flux.

In rabbit cortical slices, the inhibition of noradrenaline release by α_2-adrenoceptor agonists also depends on the stimulation conditions in a manner compatible with an endogenous autoinhibiton (Reichenbacher *et al.*, 1982). Moreover, dopaminergic and cholinergic neurones appear to possess presynaptic autoreceptors, and effects of agonists and antagonists are determined by the experimental conditions as one would expect from interference with an ongoing autoinhibition (Cubeddu & Hofmann, 1982; Kilbinger & Wessler, 1983). These findings might suggest a widespread occurrence of presynaptic autoinhibition but it must be admitted that, as emphasized 7 years ago (Starke, 1977), the basic physiological significance of the autoinhibition is still far from clear.

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