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Experimental conditions required for the enhancement by α-adrenoceptor antagonists of noradrenaline release in the rabbit ear artery

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1 Segments of the rabbit ear artery were preincubated with (−)-[^{3}H]-noradrenaline and then perfused/superfused and stimulated by transmural electrical pulses. The outflow of [^{3}H]-noradrenaline, separated from its metabolites by column chromatography, was determined.

2 Tetrodotoxin abolished, cocaine increased, and clonidine reduced the overflow of [^{3}H]-noradrenaline elicited by 10 shocks at 0.2 Hz, 10 shocks at 2 Hz or 100 shocks at 2 Hz.

3 The effects of yohimbine (0.1 or 1 μM), phentolamine (1 μM) and piperoxan (1 or 10 μM) depended on the stimulation conditions. No antagonist increased the overflow of [^{3}H]-noradrenaline evoked by 10 pulses at 0.2 Hz, but all markedly increased the overflow evoked by 100 pulses at 2 Hz. Only piperoxan (10 μM) slightly enhanced the overflow at 10 pulses, 2 Hz. The effects of yohimbine and piperoxan were similar in arteries not exposed to cocaine and in those that were perfused/superfused with medium containing cocaine (10 μM) after preincubation.

4 It is concluded that yohimbine, phentolamine and piperoxan increase the release of noradrenaline only when the concentration of noradrenaline in the biophase of the ear artery is sufficiently high. The effect is, hence, an anti-noradrenaline effect and due to the blockade of presynaptic α-adrenoceptors. A second prerequisite for the release-enhancing effect appears to be a sufficient length of the pulse train, indicating that the α-adrenergic auto-inhibition develops relatively slowly.

Introduction

The presynaptic autoreceptor hypothesis claims that near or on the axon terminals of many neurones, receptors exist for the neurone's own transmitter, and that activation of these receptors by previously released transmitter leads to inhibition (rarely facilitation) of subsequent action potential-evoked transmitter release (rarely of transmitter synthesis). The hypothesis has been studied most extensively for noradrenergic neurones where the release-inhibiting sites are α-adrenoceptors (see reviews by Stjärne, 1975; Bacq, 1976; Starke, 1977; Westfall, 1977; Vizi, 1979; Gillespie, 1980; Rand, McCulloch & Story, 1980; Langer 1981).

Although widely accepted, the presynaptic α-autoreceptor concept has recently been questioned by Kalsner and his colleagues (Kalsner, 1979a,b; 1980; 1981; Chan & Kalsner, 1979; Kalsner, Suleiman & Dobson, 1980). The arguments of these authors are based mainly on observations with phenoxybenzamine. Phenoxybenzamine has often been shown to enhance the action potential-evoked release of noradrenaline, the interpretation being, in terms of the autoreceptor concept, that the antagonist nullifies the normal α-adrenergic restraint on release. Kalsner and coworkers studied the effects of phenoxybenzamine (33 μM) in guinea-pig vasa deferentia (Kalsner, 1979a,b; 1980) and cattle renal arteries (Chan & Kalsner, 1979), and of phenoxybenzamine (10 μM) in guinea-pig atria (Kalsner *et al.*, 1980; Kalsner, 1981). The tissues were preincubated with [^{3}H]-noradrenaline. In the vas deferens, phenoxybenzamine increased the release of noradrenaline (measured as overflow of total tritium) elicited by a single electrical pulse in spite of the fact that, under these conditions, there should be no auto-inhibition. In all tissues, when the neurones were stimulated with fixed numbers of pulses at various frequencies, phenoxybenzamine tended to increase the release of noradrenaline most at the low and least at the high frequencies, although the perineuronal concentration of noradrenaline increased with increasing frequency and, hence, from the standpoint of the autoreceptor hypothesis, a direct relationship between frequency and enhance-

ment by phenoxybenzamine would be expected. The authors concluded that phenoxybenzamine did not act by blocking presynaptic noradrenaline receptors but by a different mechanism; that there was no α-adrenergic auto-inhibition in sympathetically innervated tissues; and even, 'that the hypothesis of specific functional presynaptic adrenoceptors itself has been prematurely acknowledged' (Chan & Kalsner, 1979), i.e., that presynaptic α-receptors at noradrenergic neurones did not exist at all.

If α-adrenoceptor antagonists increased the release of noradrenaline solely by mechanisms different from α-blockade, this would indeed be incompatible with the autoreceptor hypothesis. Since, however, Kalsner and his colleagues tested only a single antagonist in the experiments mentioned above, and only at a single high concentration per tissue, we decided to reinvestigate the issue using an approach similar to theirs. The sympathetic nerves of rabbit isolated ear arteries preincubated with [^{3}H]-noradrenaline were stimulated with 10 pulses at 0.2 Hz, with 10 pulses at 2 Hz or with 100 pulses at 2 Hz. The α-antagonists chosen were yohimbine, phentolamine and piperoxan. The basal outflow of tritium from noradrenergically innervated tissues pretreated with [^{3}H]-noradrenaline consists mostly of ^{3}H-metabolites, whereas the stimulation-evoked overflow consists largely of [^{3}H]-noradrenaline (see Langer, 1974). Therefore, in order to obtain a better signal (evoked overflow) to noise (basal outflow) ratio, we determined [^{3}H]-noradrenaline rather than total tritium.

Methods

Male or female rabbits were decapitated. The proximal 20–50 mm (7–20 mg) of the central ear artery were dissected free, the proximal end was cannulated, and a thread fixed to the distal end. The isolated segment was tested for leakage while being flushed with 15 ml medium. It was then preincubated for 60 min at 37°C in 1 ml medium containing 0.5 μM [^{3}H]-noradrenaline, sp. act. 41.2 or 44.7 Ci/mmol, under an atmosphere of 95% O_2 and 5% CO_2. After rinsing, the arteries were mounted vertically for perfusion/superfusion (henceforth called perfusion) with [^{3}H]-noradrenaline-free medium as in Figure 2 of Allen, Rand & Story (1973). In contrast to that figure, two platinum wire electrodes were placed parallel to and on opposite sides of the whole length of the tissue. The medium entered the arteries through the cannulae at the lower end, left them at the upper end and trickled down over their exterior surface and the electrodes. The arteries were stretched to a longitudinal tension of about 0.5 g. The perfusion rate was maintained at 6 ml/min with a roller pump. In most experiments, the perfusion pressure was monitored by means of a Statham P23Db transducer and a Rikadenki recorder.

Perfusion lasted for 231 min. Electrical pulses of 0.3 ms width and 200 mA current strength (supramaximal with respect to the tetrodotoxin-sensitive evoked overflow of [^{3}H]-noradrenaline) were delivered from a Stimulator I (Hugo Sachs Elektronik). The following periods of electrical stimulation were applied. An initial stimulation at 2 Hz for 3 min from 129 to 132 min of perfusion served to test the viability of the preparation. Then followed a cycle of three stimulation periods beginning after 153, 164 and 175 min of perfusion. The periods consisted of 10 shocks at 0.2 Hz (45 s), 10 shocks at 2 Hz (4.5 s) and 100 shocks at 2 Hz (49.5 s) and were applied either in this or in the reverse order. Finally, the three stimulation periods were repeated in a second cycle, beginning after 206, 217 and 228 min of perfusion and, in each experiment, in the order of the first cycle. For tritium determination, the perfusate was collected from 151 min onwards. At the end of the experiments, the arteries were solubilized in 1 ml Soluene 100 (Packard Instrument, Frankfurt am Main).

[^{3}H]-noradrenaline was determined in the five 1 min perfusate samples collected from 2 min before to 3 min after the onset of each of the six stimulation periods of the two cycles, yielding $6 \times 5 = 30$ samples per experiment. Total tritium was measured only in six pooled perfusates so as to allow the calculation of the tritium content of the arteries at the beginning of each stimulation period. The method of Graefe, Stefano & Langer (1973) was used to separate [^{3}H]-noradrenaline from its metabolites. Briefly, 5 ml of perfusate was chromatographed on an alumina column in order to separate catechols from *O*-methylated metabolites. [^{3}H]-noradrenaline and [^{3}H]-3,4-dihydroxyphenylglycol were eluted with 0.2 M acetic acid and separated on a Dowex 50 WX 4 column which adsorbed the amine only. [^{3}H]-noradrenaline was eluted with a mixture of equal volumes of ethanol and 12 M HCl. The recovery of authentic [^{3}H]-noradrenaline averaged 86%. Values are corrected for recovery. In the method of Graefe *et al.* (1973), the [^{3}H]-noradrenaline fraction is practically uncontaminated with other ^{3}H-compounds. Tritium was measured by liquid scintillation spectrometry.

The outflow of [^{3}H]-noradrenaline is indicated in this paper as fmol/min. The stimulation-evoked overflow is any increase in [^{3}H]-noradrenaline outflow in the 3 min following the onset of stimulation above the average outflow in the 2 min before stimulation, and is expressed as % of the total tritium in the tissue at the onset of that stimulation period. (The results remain essentially the same when the stimulation-evoked overflow of [^{3}H]-noradrenaline

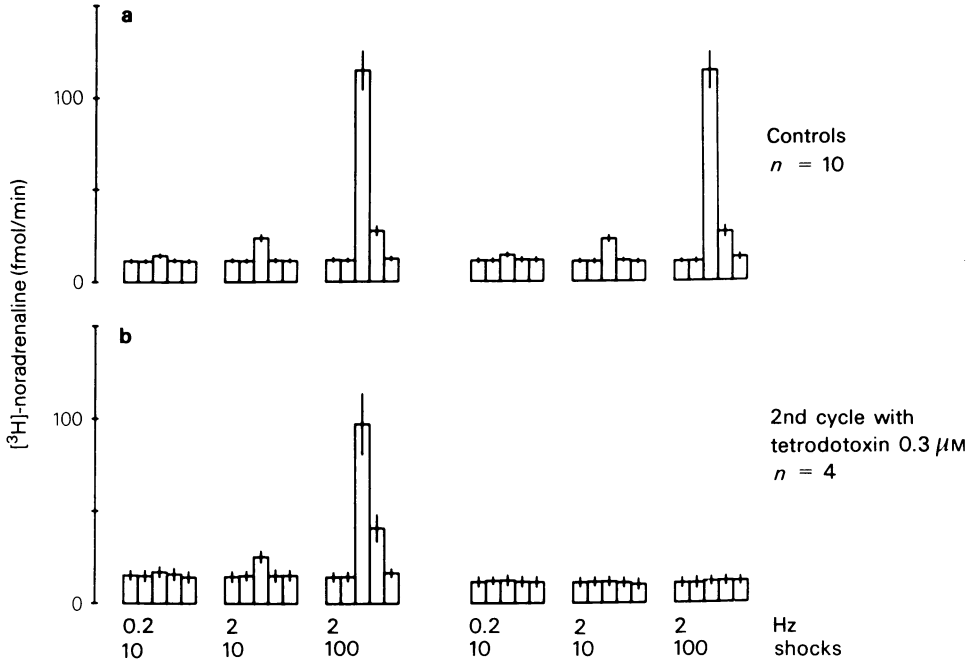

Figure 1 Outflow of [^{3}H]-noradrenaline from rabbit ear arteries preincubated with [^{3}H]-noradrenaline, and the effect of tetrodotoxin. The arteries were subjected to two cycles of three stimulation periods each, the first stimulation of the first cycle beginning after 153 min, the first stimulation of the second cycle beginning after 206 min of perfusion. Stimulation conditions are indicated below. Each group of five columns represents the outflow of [^{3}H]-noradrenaline in the 2 min before and in the 3 min after the onset of stimulation; stimulation was applied in the minute represented by the middle column. In the separate experiments in (b), tetrodotoxin was added after 181 min of perfusion, i.e., 25 min before the first stimulation of the second cycle. Means of n experiments with vertical lines indicating s.e.mean.

is expressed as fmol, i.e., not normalized with respect to tissue tritium.)

The medium used for preincubation and perfusion contained (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10.1, ascorbic acid 0.3, Na_2EDTA 0.03. It was saturated with 5% CO_2 in O_2 and warmed to 37°C.

The drugs used were (−)-[ring-2,5,6-^{3}H]-noradrenaline, sp. act. 41.2 or 44.7 Ci/mmol (NET-678, NEN, Dreieich); clonidine HCl and piperoxan HCl (Boehringer, Ingelheim); tetrodotoxin (Sigma, München); phentolamine methanesulphonate (Ciba-Geigy, Basel); cocaine HCl (Merck, Darmstadt); yohimbine HCl (Roth, Karlsruhe). Stock solutions of the drugs in water (tetrodotoxin: 0.1 M sodium acetate buffer pH 4.85) were diluted with medium and infused into the perfusion stream at 32 μl/min. In some experiments, cocaine was added directly to the perfusion fluid reservoir.

Means ± s.e. are given throughout. A one-way analysis of variance followed by the multiple range test of Duncan was used to examine differences between means for significance (Weber, 1967).

Results

Stimulation-evoked overflow of [^{3}H]-noradrenaline

As shown in Figure 1, electrical stimulation increased the outflow of [^{3}H]-noradrenaline from rabbit ear arteries preincubated with [^{3}H]-noradrenaline. The increase was small when 10 shocks were delivered at 0.2 Hz, larger when 10 shocks were delivered at 2 Hz, and largest for 100 shocks at 2 Hz. When, in control experiments, the three stimulation periods of the first cycle were repeated in a second cycle, the overflows obtained were similar (Figure 1a). Tetrodotoxin abolished the response to electrical stimulation (Figure 1b).

In order to exclude an influence of the sequence of the stimulation conditions, half of the arteries were stimulated in the order 10 shocks, 0.2 Hz – 10 shocks, 2 Hz – 100 shocks, 2 Hz, and the other half in the reverse order, in each experimental group. When the results of all experiments in which arteries were perfused with drug-free medium during the first cycle were averaged, the overflow of [^{3}H]-noradrenaline

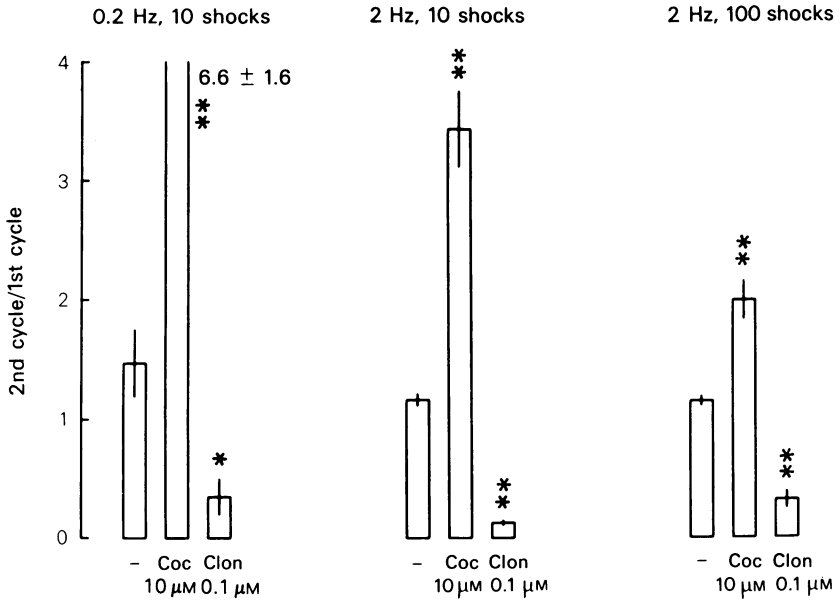

Figure 2 Effects of cocaine (Coc) and clonidine (Clon) on the stimulation-evoked overflow of [^{3}H]-noradrenaline. For each of the three stimulation conditions specified at the top, the ratio of the overflow evoked during the second stimulation cycle and the overflow during the first cycle was calculated (ordinate scale). Cocaine 10 μM ($n = 4$) or clonidine 0.1 μM ($n = 6$) was added after 181 min of perfusion, i.e., 25 min before the first stimulation of the second cycle. Means of n experiments with vertical lines indicating s.e.mean. Significant differences from controls (−; $n = 10$): $^*P < 0.05$; $^{**}P < 0.01$.

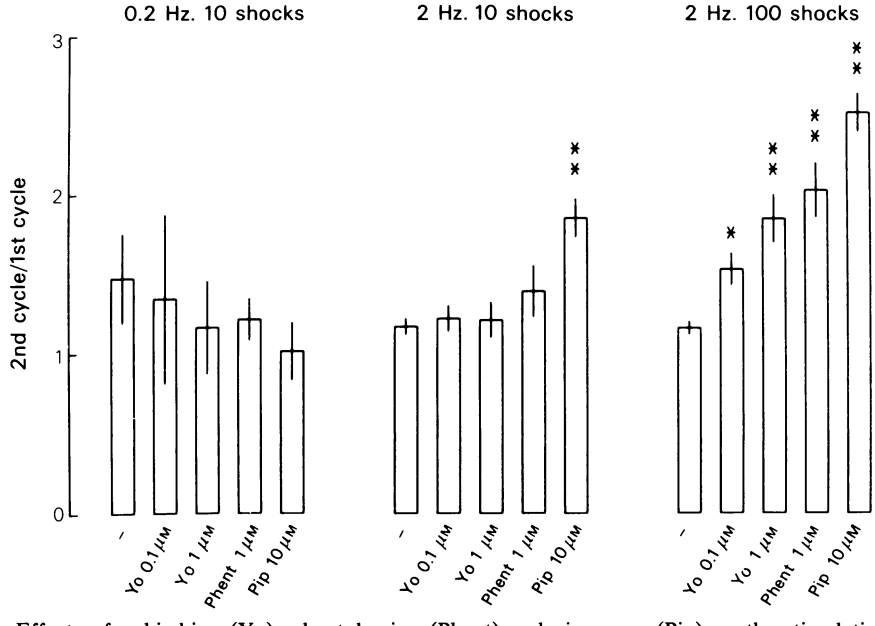

Figure 3 Effects of yohimbine (Yo), phentolamine (Phent) and piperoxan (Pip) on the stimulation-evoked overflow of [^{3}H]-noradrenaline. For each of the three stimulation conditions specified at the top, the ratio of the overflow evoked during the second stimulation cycle and the overflow during the first cycle was calculated (ordinate scale). Yohimbine 0.1 ($n = 6$) or 1 μM ($n = 6$), phentolamine 1 μM ($n = 6$) or piperoxan 10 μM ($n = 4$) was added after 181 min of perfusion, i.e., 25 min before the first stimulation of the second cycle. Means of n experiments, with vertical lines indicating s.e. mean. Significant differences from controls (−; $n = 10$): $^*P < 0.05$; $^{**}P < 0.01$.

was 0.0058 ± 0.0006% (10 shocks, 0.2 Hz), 0.021 ± 0.002% (10 shocks, 2 Hz) and 0.21 ± 0.02% (100 shocks, 2 Hz) of the tritium content of the tissue at the onset of stimulation, respectively ($n = 46$).

The effects of cocaine and clonidine, added before and during the second cycle of stimulation periods, are shown in Figure 2. Cocaine 10 μM increased the evoked overflow of [^{3}H]-noradrenaline at all stimulation conditions; its effect was greatest for 10 shocks, 0.2 Hz and smallest for 100 shocks, 2 Hz. Clonidine 0.1 μM reduced the evoked overflow under all conditions.

In contrast to the effects of these drugs, those of yohimbine, phentolamine and piperoxan depended markedly on the stimulation parameters (Figure 3). None of the antagonists caused any increase at 10 shocks, 0.2 Hz. Only piperoxan increased the overflow evoked by 10 shocks at 2 Hz. All, however, increased the overflow evoked by 100 shocks at 2 Hz; the effect of yohimbine was concentration-dependent, and the effect of piperoxan was much more marked than at 10 shocks, 2 Hz (Figure 3).

In the experiments described so far, the arteries were perfused with drug-free medium for 181 or even (controls) 231 min after preincubation with [^{3}H]-noradrenaline. In another series, cocaine 10 μM was present throughout the entire perfusion, i.e., for 231 min. When the results of all experiments of this series were averaged, the overflow of [^{3}H]-noradrenaline during the first cycle was 0.019 ± 0.002% (10 shocks, 0.2 Hz), 0.072 ± 0.007% (10 shocks, 2 Hz) and 0.47 ± 0.06% (100 shocks, 2 Hz) of the tritium in the tissue, respectively ($n = 24$).

The effects of yohimbine and piperoxan are shown in Figure 4. As in experiments without cocaine, neither yohimbine nor piperoxan increased the overflow at 10 shocks, 0.2 Hz. Only piperoxan 10 μM slightly enhanced the overflow evoked by 10 shocks at 2 Hz. Both drugs, however, caused a pronounced and concentration-dependent increase of the overflow of [^{3}H]-noradrenaline elicited by 100 shocks, 2 Hz.

Basal outflow of [^{3}H]-noradrenaline

As can be seen from Figure 1, the basal outflow of [^{3}H]-noradrenaline was fairly constant throughout control experiments. Effects of drugs were evaluated by comparing the outflow in the 2 min before the first stimulation period of the second cycle (204–206 min) with the outflow in the 2 min before the last stimulation period of the first cycle (173–175 min). In arteries perfused with drug-free

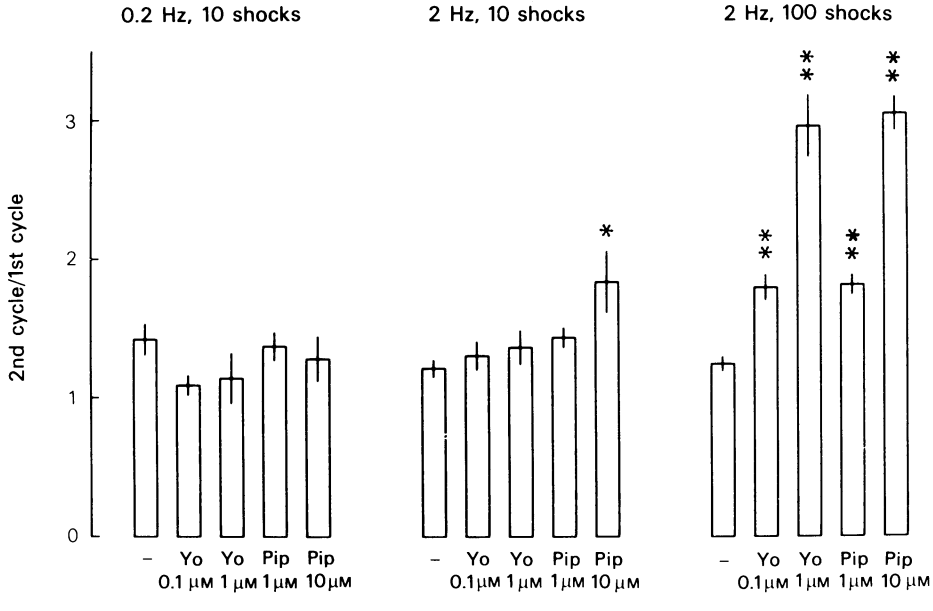

Figure 4 Effects of yohimbine (Yo) and piperoxan (Pip) on the stimulation-evoked overflow of [^{3}H]-noradrenaline; experiments in which cocaine 10 μM was present throughout perfusion. For each of the three stimulation conditions specified at the top, the ratio of the overflow evoked during the second stimulation cycle and the overflow during the first cycle was calculated (ordinate scale). Yohimbine 0.1 ($n = 6$) or 1 μM ($n = 4$) or piperoxan 1 ($n = 4$) or 10 μM ($n = 4$) was added after 181 min of perfusion, i.e., 25 min before the first stimulation of the second cycle. Means of n experiments, with vertical lines indicating s.e.mean. Significant differences from controls (−; $n = 6$): $^*P < 0.05$; $^{**}P < 0.01$.

medium during the first cycle (experiments of Figures 1–3), tetrodotoxin 0.3 μM and clonidine 0.1 μM decreased the basal outflow by 14% and 16%, respectively, whereas cocaine 10 μM and piperoxan 10 μM increased it by 40% and 13%, respectively (values corrected for any change in the corresponding controls). Piperoxan 10 μM also increased the basal outflow of [^{3}H]-noradrenaline by 15% in arteries perfused with cocaine 10 μM throughout (experiments of Figure 4). No changes except these were observed.

The vasoconstrictor response

The perfusion pressure was not recorded in all experiments. The basal perfusion pressure averaged 22 mmHg in the absence of cocaine (experiments of Figures 1–3) and 21 mmHg in its presence (experiments of Figure 4). Ten shocks given at 0.2 Hz did not elicit detectable vasoconstriction. In the absence of cocaine (experiments of Figures 1–3), stimulation with 10 shocks at 2 Hz increased the perfusion pressure by 26 ± 3 mmHg, and stimulation with 100 shocks at 2 Hz increased it by 50 ± 6 mmHg (responses during first cycle; $n = 28$). In the presence of cocaine (experiments of Figure 4), 10 shocks at 2 Hz and 100 shocks at 2 Hz increased the perfusion pressure by 53 ± 6 and 106 ± 13 mmHg, respectively (responses during first cycle; $n = 19$). In control experiments, the responses obtained during the second cycle were similar. As expected, tetrodotoxin abolished, cocaine, added during the second cycle, increased, and clonidine as well as phentolamine and piperoxan reduced the stimulation-evoked vasoconstriction. Yohimbine 0.1 μM approximately doubled the vasoconstrictor responses both to 10 shocks and to 100 shocks at 2 Hz. Yohimbine 1 μM marginally reduced the response to 10 pulses but increased the response to 100 pulses at 2 Hz. These effects of yohimbine were similar in the absence and in the presence of cocaine.

Discussion

It has been amply documented that low concentrations of α-adrenoceptor agonists and antagonists do not modify noradrenaline uptake or degradation mechanisms (see Starke, 1977; Westfall, 1977; Gillespie, 1980). Hence, the changes in stimulation-evoked overflow of [^{3}H]-noradrenaline that clonidine, yohimbine, phentolamine and piperoxan caused in our experiments will be taken to reflect changes in neuronal release. Blockade by tetrodotoxin shows that this release, like physiological release, was triggered by action potentials.

Our experiments confirm that α-adrenoceptor agonists reduce, whereas α-antagonists increase the action potential-evoked release of noradrenaline in the rabbit ear artery (Rand, Story, Allen, Glover & McCulloch, 1973; Hope, Law, McCulloch, Rand & Story, 1976; Hope, McCulloch, Rand & Story, 1978; Hieble & Pendleton, 1979). They also confirm that yohimbine augments vasoconstrictor responses to neuronal stimulation under certain conditions (Borowski, Starke, Ehrl & Endo, 1977). Part of this enhancement is probably due to the facilitation of noradrenaline release. Yet, yohimbine 0.1 μM increased the pressor response even to 10 pulses at 2 Hz when noradrenaline release was not increased. The reason may be a potentiation at the level of the smooth muscle, since yohimbine 0.1 μM also increased the response to small doses (3–10 ng) of exogenous noradrenaline (authors' unpublished observation; cf. Jang, 1941).

Our main finding is that the release-enhancing effect of α-adrenoceptor antagonists depends markedly on the conditions of electrical stimulation. No antagonist increased the release of [^{3}H]-noradrenaline evoked by 10 pulses at 0.2 Hz. Under these conditions, the evoked overflow was small and variable, and it might be thought that an existing increase was concealed by the large variability. However, enhancement did not occur even in arteries perfused with cocaine throughout, when the evoked overflow was larger and less variable; moreover, enhancement by cocaine and inhibition by clonidine were readily demonstrable; and finally, even the slightest indication of an enhancement was lacking, all mean second cycle/first cycle ratios with α-antagonists being in fact lower than the corresponding control ratios. On the other hand, yohimbine, phentolamine and piperoxan all caused pronounced increases of the release of [^{3}H]-noradrenaline evoked by 100 pulses at 2 Hz. Only piperoxan 10 μM slightly increased the release elicited by 10 pulses at 2 Hz. The increases were obtained both in the absence and in the presence of cocaine, showing again that the α-antagonists did not act by inhibiting neuronal re-uptake.

We would like to interpret this marked dependence on the stimulation conditions in terms of the α-autoreceptor concept. When 10 shocks were distributed over 45 s (0.2 Hz), the ensuing biophase concentration of noradrenaline was small. The evoked overflow of [^{3}H]-noradrenaline was only 0.0058% of the tissue tritium in the absence and 0.019% in the presence of cocaine. Under these circumstances, the α-autoreceptors presumably were not activated sufficiently for a measurable disinhibition by antagonists. When, on the other hand, 100 shocks were applied over approximately the same time (49.5 s; 2 Hz), the biophase concentration of noradrenaline was much higher. The evoked overflow of [^{3}H]-noradrenaline

averaged 0.21% in the absence and 0.47% in the presence of cocaine. The auto-inhibition operated and manifested itself in the release-enhancing effect of α-receptor antagonists.

However, a sufficient concentration of noradrenaline in the vicinity of the presynaptic receptors does not seem to be the only prerequisite. When we delivered 10 shocks at 2 Hz, the evoked overflow of [^{3}H]-noradrenaline was 0.021% in the absence of cocaine and 0.072% in its presence (the higher overflow as compared with 10 shocks, 0.2 Hz probably reflects the frequency-dependent facilitation that occurs in the rabbit ear artery: Rand *et al.*, 1973; see Starke, 1977). Since stimulation lasted for 4.5 s, i.e., 1/11 of the 100 shocks, 2 Hz period, the biophase concentration of noradrenaline was at least as high as under the latter conditions. Yet, only piperoxan 10 μM slightly enhanced the release elicited by 10 pulses at 2 Hz. Story, McCulloch, Rand & Standford-Starr (1981) have presented evidence to show that, in guinea-pig atria, a certain minimal time (> 1.5 s in their model at 2 Hz) elapses between the first pulse in a train and the beginning of auto-inhibition, perhaps because of a relatively slow receptor-coupled translation mechanism. Our experiments suggest that there is an analogous delay in the rabbit ear artery and that inhibition may just start 4.5 s after the first pulse.

We have not been able to measure the release of [^{3}H]-noradrenaline by a single pulse. However, from the finding that yohimbine, phentolamine and piperoxan failed to enhance the release evoked by trains of 10 widely spaced pulses (0.2 Hz) it is clear that α-adrenoceptor antagonists do not enhance the release caused by a single pulse in the rabbit ear artery. α-Antagonists also do not increase the release of noradrenaline evoked by single pulses in guinea-pig atria (Rand *et al.*, 1973), the mouse vas deferens (Markiewicz, Marshall & Nasmyth, 1980; Baker & Marshall, 1982) and the rabbit pulmonary artery (J. W. Constantine, personal communication).

There was an interesting parallel between the stimulation conditions required for detectable presynaptic α-adrenergic auto-inhibition and detectable postsynaptic α-adrenergic vasoconstriction. When 10 pulses were delivered at 0.2 Hz and presynaptic auto-inhibition was absent, the perfusion pressure did not change either. With 10 pulses at 2 Hz, auto-inhibition was perhaps just beginning, and there was moderate vasoconstriction. One hundred pulses at 2 Hz elicited both marked auto-inhibition and pronounced vasoconstriction.

To conclude the discussion thus far, the uniform release-enhancing effect of α-adrenoceptor antagonists, taken together with the uniform inhibitory effect of agonists (cf. Rand *et al.*, 1973; Hope *et al.*, 1976; 1978; Hieble & Pendleton, 1979), strongly supports the existence of presynaptic α-adrenoceptors in the rabbit ear artery as well as their activation by released noradrenaline. The effect of yohimbine, phentolamine and piperoxan does depend on the conditions of stimulation, and it does so in a manner consonant with the α-autoreceptor hypothesis. The antagonists enhance the release only when the biophase concentration of noradrenaline is sufficiently high. The effect is, hence, an anti-noradrenaline effect, due to the blockade of α-adrenoceptors. The parallelism between the conditions required for presynaptic inhibition and postsynaptic vasoconstriction is in accord with the view that the former, like the latter, is a physiological event in neurotransmission.

There may be several reasons for the discrepant results obtained by Kalsner and his colleagues who used phenoxybenzamine 10 or 33 μM as an α-antagonist (Kalsner, 1979a,b; 1980; 1981; Chan & Kalsner, 1979; Kalsner *et al.*, 1980). Phenoxybenzamine 10 or 33 μM accelerates the basal outflow of noradrenaline and its metabolites, presumably by a reserpine-like mechanism (Adler-Graschinsky, Langer & Rubio, 1972). In the rabbit ear artery, phenoxybenzamine 33 μM increased the basal outflow of [^{3}H]-noradrenaline by 427% (authors' unpublished observations), which contrasts with the maximal 15% increase caused by one α-adrenoceptor antagonist (piperoxan 10 μM) in the present study. The ability to augment the release of noradrenaline by action potentials is an inherent property of reserpine-like drugs (Cubeddu & Weiner, 1975). Hence, a reserpine-like action may be one mechanism by which phenoxybenzamine 10 or 33 μM increases action potential-evoked release of noradrenaline. This component will not obey predictions made for α-adrenoceptor blockade and makes such high concentrations of phenoxybenzamine doubtful tools for testing the α-autoreceptor hypothesis. There are further mechanisms unrelated to α-adrenoceptors by which high concentrations of phenoxybenzamine may act. Phenoxybenzamine potently and, after prolonged administration *in vitro*, non-competitively blocks the neuronal uptake of noradrenaline (Iversen & Langer, 1969). Although Kalsner and his colleagues added cocaine 8.8 μM throughout most of their experiments, phenoxybenzamine may have caused an additional inhibition of noradrenaline re-uptake and thereby have enhanced the evoked overflow of tritium. Depending on tissue and experimental details, these and other mechanisms (Starke, 1981) may mask any increase in noradrenaline release due to α-blockade.

Our experiments themselves caution against side-effects of α-adrenoceptor antagonists. Piperoxan 10 μM was the only antagonist that accelerated the basal outflow of tritium, presumably by a mechanism related to that of reserpine (Borowski *et al.*, 1977). It was also the only antagonist that increased the re-

lease of [^{3}H]-noradrenaline evoked by 10 pulses at 2 Hz, possibly also at least in part by the reserpine-like component. This side-effect contrasts to those effects that all three antagonists had in common, in particular the uniform enhancement of noradrenaline release which required a sufficiently high biophase concentration of noradrenaline and a sufficient time for that concentration to act.

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