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Presynaptic α-adrenoceptors: do exogenous and neuronally released noradrenaline act at different sites?

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1 The effects of dopamine receptor and α-adrenoceptor agonists and antagonists on the stimulation-evoked overflow of radioactivity from strips of dog saphenous vein previously loaded with [^{3}H]-noradrenaline have been examined alone and in combination.

2 In the presence of neuronal and extraneuronal catecholamine uptake inhibitors, noradrenaline ($0.1-1 \times 10^{-6}$M) and dopamine ($0.01-1 \times 10^{-6}$M) both inhibited the stimulation-evoked overflow of radioactivity. Sulpiride (1×10^{-6}M) was without effect and prazosin (1×10^{-7}M) had little effect on stimulation-evoked overflow but yohimbine enhanced it approximately 2 fold; the effect of yohimbine was similar at concentrations of 1×10^{-7} and 1×10^{-6}M.

3 Sulpiride abolished the inhibitory effect of dopamine on stimulation-evoked overflow, but was without effect against noradrenaline. When allowance was made for the effects of yohimbine, alone, on overflow, yohimbine (1×10^{-7}M) had no effect against dopamine and minimal effects against noradrenaline. A similar result was obtained when the concentration of yohimbine was increased to 1×10^{-6}M. Prazosin did not antagonize the effect of noradrenaline.

4 In the absence of the uptake inhibitors, clonidine ($0.01-1 \times 10^{-5}$M) inhibited stimulation-evoked overflow of radioactivity. Yohimbine (1×10^{-6}M) was without effect on its own and antagonized the effects of clonidine at a concentration of 0.1×10^{-5}M, but not at 0.01 or 1.0×10^{-5}M.

5 These findings suggest that dopamine inhibits overflow by stimulating presynaptic dopamine receptors on the terminals of the noradrenergic nerves supplying the dog saphenous vein. The interaction between yohimbine and noradrenaline is discussed in terms of the current concepts of control of transmitter release mediated via presynaptic α_2-adrenoceptors.

Introduction

The concept of presynaptic α-adrenoceptor mediated regulation of transmitter release from noradrenergic nerves has been developed from the observations that exogenous α-adrenoceptor agonists inhibit, and antagonists enhance, the overflow of noradrenaline during nerve stimulation (Langer, 1980). Recently, however, this concept has been challenged by Kalsner and his associates (see Kalsner, 1982a,b) who have shown that many of the predictions made from this concept do not seem to be substantiated by experimental investigation. Lately Kalsner (1982b) has shown that concentrations of yohimbine that seem to interrupt this inhibitory feedback process (because they enhance the overflow of [^{3}H]-noradrenaline from guinea-pig ureters during transmural stimulation) do not antagonize the ability of exogenously administered, non-radioactive noradrenaline to inhibit the stimulation-evoked overflow of [^{3}H]-noradrenaline. Kalsner concluded that the exogenous noradrenaline and yohimbine act at different sites to modulate the release of neuronal noradrenaline stores, and that these findings do not, therefore, support the unified hypothesis of feedback regulation of transmitter release.

This paper describes the results of experiments originally designed to determine whether noradrenaline and dopamine inhibit the release of [^{3}H]-noradrenaline from noradrenergic nerves supplying the dog saphenous vein, through the same or different types of presynaptic receptors. Although this question was answered, the results can also be viewed in a way that adds to the current controversy concerning the proposed presynaptic feedback hypothesis.

Methods

The details of the experimental procedure have been extensively described by Sullivan & Drew (1980). In brief, saphenous veins were removed from beagle dogs, cut spirally into strips and divided into 4 approximately equal segments. Each segment was then incubated in modified Krebs solution at 37°C containing (−)-[7-^{3}H]-noradrenaline (10 μCi ml^{-1}; specific activity 15 Ci mmol^{-1}; 6.7×10^{-7} M) for 2 h to label the nerve terminal noradrenaline stores. At the end of this period each strip was mounted separately under an initial tension of 0.6 g in a 1 ml jacketed organ bath. Krebs solution was then pumped continuously over each of the preparations at a rate of 2 ml min^{-1} for a period of 2 h to remove extraneuronally bound noradrenaline.

After the end of this washout period the superfusate was collected over successive 3 min periods into counting vials. Tissues were stimulated with rectangular pulses, 0.5 ms in duration at 2 Hz for 3 min on 7 successive occasions (S_1 to S_7) at intervals of 18 min. The stimulation evoked overflow of radioactivity was calculated as the overflow obtained during each period of stimulation plus that in the following three collection periods minus the estimated basal overflow, assuming a linear decline between the collection period immediately before stimulation and the fourth period after.

Experiments were carried out in which the effects of sulpiride, yohimbine or prazosin on stimulation-evoked overflow of radioactivity, and on the inhibitory effects of dopamine, noradrenaline or clonidine on this overflow, were examined. Four saphenous vein strips were used simultaneously and the experimental protocol was as follows:

Strip 1: received no drug treatment.
Strip 2: received sulpiride, yohimbine or prazosin, starting from 12 min before S_4 and throughout the rest of the experiment.
Strip 3: received dopamine, noradrenaline or clonidine, in three increasing concentrations, each concentration being introduced into the superfusion fluid 12 min before S_5, S_6 and S_7, respectively, and being kept in contact with the strip until the next increment in concentration.
Strip 4: received antagonist as in strip 2, plus three increasing concentrations of agonist as in strip 3.

The experimental protocol is shown diagrammatically in Figure 1. Thus the effects of the agonists and antagonists, alone or in combination, on the stimulation-evoked overflow of radioactivity were determined and compared with the effects of no treatment in a time-matched control preparation from the same animal.

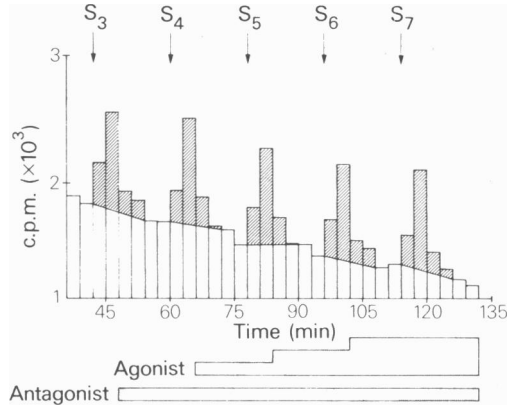

Figure 1 The experimental protocol. Each column represents the overflow of radioactivity collected in a 3 min period in one untreated preparation. Stimulation at 2 Hz was applied for 3 min at intervals of 18 min, as indicated by S_3, S_4, S_5, S_6 and S_7. The shaded portion of each column represents the stimulation-evoked overflow of radioactivity obtained from each period of stimulation and the open area reflects the basal overflow. (The overflows obtained from S_1 and S_2 are not shown because the data were not used in evaluating the effects of drugs.) The areas outlined below the figure illustrate the time periods during which agonists or antagonists were administered.

The radioactivity collected in each sample of superfusate was determined by liquid scintillation counting; counting efficiency was determined by an external standard channels ratio technique.

Comparisons between treated and untreated preparations at corresponding stimulation periods were made using Student's t test. Differences were considered to be statistically significant if $P < 0.05$.

Krebs solution

The composition of the Krebs solution used throughout these experiments was as follows (mM): Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.5, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0 and glucose 11.1. The solution was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Except during incubation with radioactive noradrenaline, cocaine (30 μM), corticosterone (40 μM), propranolol (1 μM) and indomethacin (3 μM) were normally incorporated in the Krebs solution to inhibit neuronal and extraneuronal uptake, to block β-adrenoceptors and to prevent the synthesis of endogenous prostaglandins. Ascorbic acid (1.1×10^{-4} M) was also incorporated to inhibit catecholamine oxidation. Cocaine and corticosterone were omitted from the Krebs

solution used in those experiments in which the interactions between yohimbine and clonidine on stimulation-evoked overflow of radioactivity were examined. During incubation of the saphenous vein strips with radioactive noradrenaline, ascorbic acid (1.1×10^{-4}M) and disodium edetate (EDTA) (4×10^{-6}M) were incorporated into the Krebs solution to prevent oxidation of the noradrenaline.

Drugs

The following drugs were used: clonidine hydrochloride (Boehringer-Ingelheim), cocaine hydrochloride (Macfarlan Smith), corticosterone (Sigma), dopamine hydrochloride (Sigma), indomethacin (Merck, Sharpe & Dohme), (−)-noradrenaline bitartrate (Koch-Light), prazosin hydrochloride (Pfizer), (±)-propranolol hydrochloride (I.C.I.), (±)-sulpiride (Chemitechna) and yohimbine hydrochloride (Sigma). Indomethacin was dissolved (3×10^{-3}M) in 1 M sodium bicarbonate and corticosterone was dissolved (3×10^{-1}M) in a minimum quantity of absolute alcohol before adding to the Krebs solution. Other drugs were dissolved in saline or distilled water shortly before use to give stock solutions of 1×10^{-3}M. Appropriate dilutions were made in Krebs solutions. Clonidine, prazosin, propranolol and sulpiride were generously donated by Boehringer, Pfizer U.K., I.C.I. and Chemitechna, respectively, and (−)-[7-^{3}H]-noradrenaline was purchased from New England Nuclear.

Results

As previously demonstrated (Sullivan & Drew, 1980) field stimulation of dog saphenous vein strips, pre-incubated with [^{3}H]-noradrenaline, resulted in an increase in the overflow of radioactivity from the preparations. There was only a small decline in this stimulation-evoked overflow over the seven stimulation periods in untreated preparations.

Experiments carried out in the presence of catecholamine uptake inhibitors

The effects of agonists and antagonists on stimulation-evoked radioactivity overflow Sulpiride (1×10^{-6}M) and prazosin (1×10^{-7}M) had little effect on stimulation-evoked overflow, but yohimbine (1×10^{-7}M) considerably increased it and this effect of yohimbine was generally well sustained from S_4 to S_7. The magnitude of these changes is best expressed as the ratio of the stimulation-evoked increase in overflow obtained in S_4 to that obtained in S_3 (i.e. S_4/S_3) in the same strip. This ratio in yohimbine treated preparations was 2.36 ± 0.20 ($n = 16$); in prazosin and in sulpiride treated strips the ratios were 1.17 ± 0.05 ($n = 6$) and 1.01 ± 0.02 ($n = 14$), respectively. In the corresponding untreated strips the ratio was 1.04 ± 0.03 ($n = 36$).

In the absence of any antagonist, noradrenaline (0.1, 0.3 and 1×10^{-6}M) and dopamine (0.01, 0.1 and 1×10^{-6}M) caused a concentration-dependent inhibition of stimulation-evoked overflow of radioactivity. Dopamine and noradrenaline were approximately equipotent in this respect and, at the highest concentration used, reduced overflow by 70 – 80%

The interactions between agonists and antagonists on stimulation-evoked overflow of radioactivity Because sulpiride and prazosin had little or no effect on their own on stimulation-evoked overflow of radioactivity their interaction with the catecholamines was easy to

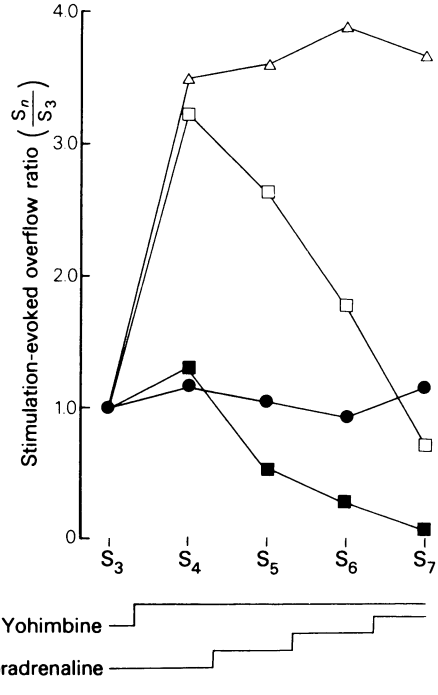

Figure 2 The effects of yohimbine and noradrenaline, alone and in combination, on the stimulation-evoked overflow of radioactivity from dog saphenous veins. Yohimbine (1×10^{-7}M) was infused continuously from 12 min before S_4. Noradrenaline, 1×10^{-7}, 3×10^{-7} and 1×10^{-6}M, was infused continuously from 12 min before S_5, S_6 and S_7, respectively. The same protocols were adopted when yohimbine and noradrenaline were combined. (●) Untreated preparation, (△) yohimbine treated, (■) noradrenaline treated and (□) yohimbine and noradrenaline treated preparations. All results are expressed as the ratios of the stimulation-evoked overflow obtained from S_n to that obtained from S_3.

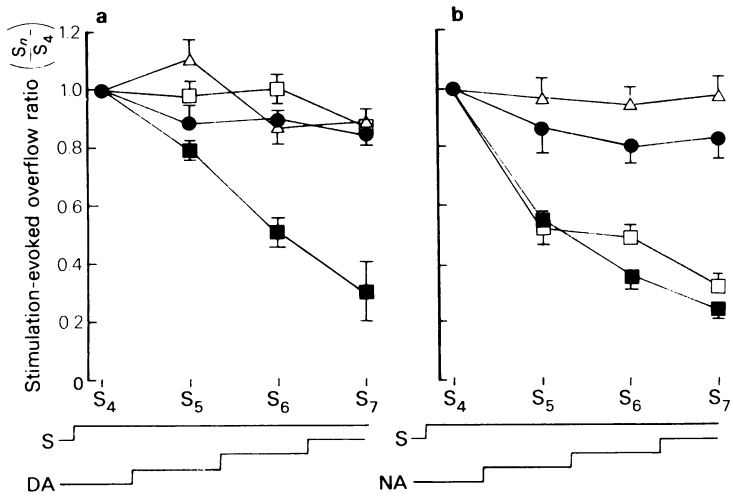

Figure 3 The effects of (a) dopamine (DA; 1×10^{-8}, 1×10^{-7} and 1×10^{-6} M; $n = 3$) and (b) noradrenaline (NA; 1×10^{-7}, 3×10^{-7} and 1×10^{-6} M; $n = 4$), alone (■) and in combination (□) with sulpiride (S; 1×10^{-6} M), on stimulation-evoked overflow of radioactivity from dog saphenous veins. Results are expressed as the ratio of the stimulation-evoked overflow obtained from S_n to that obtained from S_4. The corresponding overflow ratios obtained from untreated (●) and sulpiride, alone (△), treated preparations are also shown.

assess. However, this was not the case with yohimbine; both catecholamines clearly reduced stimulation-evoked overflow in the absence and in the presence of yohimbine but because yohimbine, itself, had increased stimulation-evoked overflow it was difficult to determine whether yohimbine displaced the dopamine and noradrenaline concentration-effect curves. Results, taken from a single experiment to illustrate this point, are shown in Figure 2. In order to overcome this problem it was decided that the effects of dopamine or noradrenaline, alone and in combination with yohimbine, would best be expressed as the ratios of the stimulation-evoked overflows obtained in S_5, S_6 or S_7 (i.e. S_n) to that obtained in S_4. In this way the effect of yohimbine, alone, on stimulation-evoked overflow

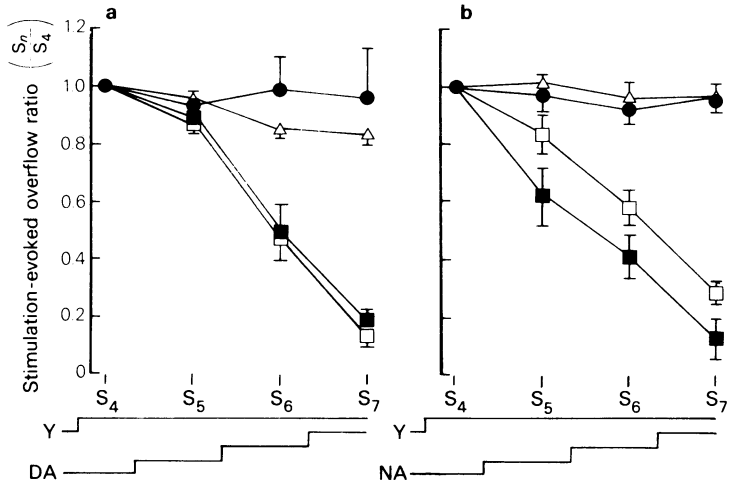

Figure 4 The effects of (a) dopamine (DA; 1×10^{-8}, 1×10^{-7} and 1×10^{-6} M; $n = 4$) and (b) noradrenaline (NA; 1×10^{-7}, 3×10^{-7} and 1×10^{-6} M; $n = 4$), alone (■) and in combination (□) with yohimbine (Y; 1×10^{-7} M), on stimulation-evoked overflow of radioactivity from dog saphenous veins. Results are expressed as the ratio of the stimulation-evoked overflow obtained from S_n to that obtained from S_4. The corresponding overflow ratios obtained from untreated (●) and yohimbine, alone (△), treated preparations are also shown.

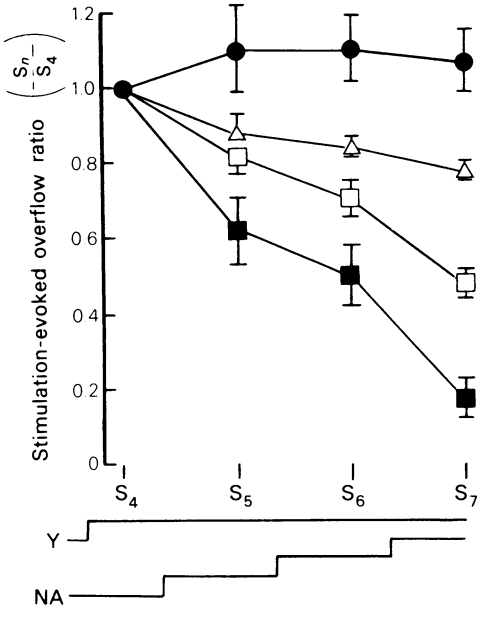

Figure 5 The effects of noradrenaline (NA; 1×10^{-7}, 3×10^{-7} and 1×10^{-6} M; $n = 5$), alone (■) and in combination (□) with yohimbine (Y; 1×10^{-6} M) on stimulation-evoked overflow of radioactivity from dog saphenous veins. Results are expressed as the ratio of stimulation-evoked overflow obtained from S_n to that obtained from S_4. The corresponding overflow ratios obtained from untreated (●) and yohimbine, alone (△), treated preparations are also shown. Yohimbine significantly reduced responses to noradrenaline in S_7 only.

would be nullified. Despite the minimal effects of sulpiride or prazosin, alone, on stimulation-evoked overflow the interactions between these antagonists and the catecholamines were evaluated in the same way for consistency. When expressed in this manner it is clear that sulpiride (1×10^{-6} M) abolished the effect of dopamine on stimulation-evoked overflow of radioactivity but had no effect on that produced by noradrenaline (Figure 3). In contrast, yohimbine (1×10^{-7} M) did not antagonize dopamine; it also had little effect on responses to noradrenaline (Figure 4).

In five further experiments, the effect of increasing the concentration of yohimbine to 1×10^{-6} M was examined. In these experiments, the S_4/S_3 ratio was 2.19 ± 0.20 in the presence of yohimbine and 1.03 ± 0.06 in the corresponding untreated strips ($n = 10$ for each). At this concentration, yohimbine displaced the noradrenaline concentration-effect curve about 3-fold to the right. However, yohimbine significantly reduced the response to the highest concentration of noradrenaline only. Results are shown in Figure 5.

Prazosin (1×10^{-7} M) did not antagonize the effect of noradrenaline on stimulation-evoked overflow of radioactivity; its effect against dopamine was not examined.

Experiments carried out in the absence of catecholamine uptake inhibitors

The interactions between yohimbine and clonidine on stimulation-evoked overflow of radioactivity In the presence of catecholamine uptake inhibitors, clonidine does not inhibit stimulation-evoked overflow of radioactivity from dog saphenous veins (Sullivan & Drew, 1980). However, as previously demonstrated (Sullivan & Drew, 1980) clonidine (0.01, 0.1 and 1×10^{-5} M) did inhibit stimulation-evoked overflow of radioactivity when cocaine and corticosterone were omitted from the Krebs solution, but the concentration-effect curve was shallow and the maximum inhibition obtained was only 50%.

Under these experimental conditions, yohimbine (1×10^{-6} M) had no effect on stimulation-evoked overflow of radioactivity; the S_4/S_3 ratios in yohimbine treated and untreated preparations were

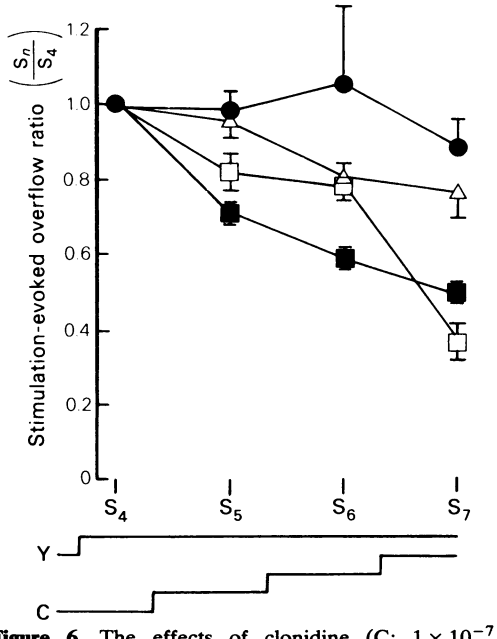

Figure 6 The effects of clonidine (C; 1×10^{-7}, 1×10^{-6} and 1×10^{-5} M; $n = 4$), alone (■) and in combination (□), with yohimbine (Y; 1×10^{-6} M) on stimulation-evoked overflow of radioactivity from dog saphenous veins. Results are expressed as the ratio of the stimulation-evoked overflow obtained from S_n to that obtained from S_4. The corresponding overflow ratios obtained from untreated (●) and yohimbine, alone (△), treated preparations are also shown. Yohimbine significantly reduced the response to clonidine in S_6 only

1.03 ± 0.06 and 0.99 ± 0.05 respectively ($n = 8$ for each). Yohimbine (1×10^{-6}M) reduced the inhibitory effect of clonidine, 0.01 and 0.1×10^{-5}M, but the effect was significant only at the concentration of 0.1×10^{-5}M. Yohimbine did not reduce the inhibitory effect of the highest concentration of clonidine (1×10^{-5}M). Results are shown in Figure 6.

At the concentrations used in these experiments none of the drugs altered the basal overflow of radioactivity.

Discussion

In the presence of neuronal and extraneuronal catecholamine uptake inhibitors, noradrenaline and dopamine inhibited the stimulation-evoked overflow of radioactivity from dog saphenous veins preincubated with [^{3}H]-noradrenaline. Previous work from these laboratories has shown that, under similar experimental conditions, approximately 80% of this radioactivity is in the form of noradrenaline, and that it is released from nerve terminals (Sullivan & Drew, 1980; Watts *et al.*, 1981).

Sulpiride (1×10^{-6}M) prevented the effects of dopamine but not of noradrenaline; since this concentration of sulpiride is too low to block presynaptic α_2-adrenoceptors but does block presynaptic dopamine receptors (Brown & O'Connor, 1981), these results indicate that the effects of dopamine are mediated via presynaptic dopamine receptors, whereas those of noradrenaline are mediated via other receptors, presumably presynaptic α_2-adrenoceptors. The failure of sulpiride, alone, to alter the overflow of radioactivity shows that the presynaptic dopamine receptors are not involved in regulating transmitter release under the present experimental conditions.

As previously shown (Sullivan & Drew, 1980), yohimbine (1×10^{-7}M) substantially enhanced the stimulation-evoked overflow of radioactivity; this effect was attributed to interruption of the feedback control of transmitter release mediated via presynaptic α_2-adrenoceptors. The increase in stimulation-evoked overflow makes it difficult to assess the influence of yohimbine on the inhibitory effects of noradrenaline and dopamine but we have tried to compensate for the effect of yohimbine, alone, by expressing the effects of the agonists on radioactivity overflow from S_5, S_6 and S_7 as a percentage change in the overflow obtained from S_4. Using this means of assessment, yohimbine did not antagonize the inhibitory effect of dopamine on radioactivity overflow. This seems to confirm the impression, obtained with sulpiride, that the effect of dopamine is not mediated via presynaptic α_2-adrenoceptors. However, yohimbine did not antagonize the effects of noradrenaline either. It might be argued that the concentration of yohimbine (1×10^{-7}M) was too low to exert any appreciable antagonism of noradrenaline, yet it is generally found that the threshold blocking concentration of yohimbine at presynaptic α_2-adrenoceptors is $1 \times 10^{-8} - 3 \times 10^{-8}$M (Ruffolo *et al.*, 1981) and, anyway, the concentration of yohimbine used in the present experiments (1×10^{-7}M) was enough to increase markedly the stimulation-evoked overflow of radioactivity. Perhaps the yohimbine-induced increase in stimulation-evoked overflow was responsible for the apparent failure of yohimbine to antagonize the effects of exogenous noradrenaline. Thus, in the presence of yohimbine, the local concentration of endogenous noradrenaline in the vicinity of the presynaptic α-adrenoceptors would be higher than in untreated preparations because of the extra quantity released during stimulation, and would thus be able to overcome the yohimbine-induced blockade, leading to a smaller than expected shift to the right in the concentration-response curve to exogenous noradrenaline. This does not seem to be the whole explanation, however, for the following reason. A ten fold increase in the yohimbine concentration to 1×10^{-6}M caused no greater increase in the S_4/S_3 ratio of stimulation-evoked overflow of radioactivity than was seen with the lower concentration. This need not mean that the lower concentration of yohimbine was fully effective in preventing the feedback control of transmitter release because phentolamine can cause greater increases in overflow from this tissue (Sullivan & Drew, 1980). Instead, it may reflect the onset of some other effect of this high concentration of yohimbine opposing the consequence of more effective blockade of the feedback inhibition of transmitter release. Indeed, Kalsner & Chan (1979) have already demonstrated that high concentrations of yohimbine ($3 \times 10^{-6} - 3 \times 10^{-5}$M) considerably depressed stimulation-evoked overflow of radioactivity from bovine radial arteries, and that this effect was unaltered after blockade of the presynaptic α-adrenoceptors with phenoxybenzamine, implying some other site of action. Even so, the higher concentration of yohimbine used in the present experiments would still be expected to antagonize the effect of exogenous noradrenaline on stimulation-evoked overflow of radioactivity much more effectively than the lower concentration; in practice, the difference was minimal.

In an attempt to shed more light on this problem the interaction between yohimbine and clonidine on stimulation-evoked overflow of radioactivity was examined. In the dog saphenous vein, clonidine does not inhibit stimulation-evoked overflow in the presence of catecholamine uptake inhibitors. This observation has been tentatively explained by suggesting that clonidine has a lower efficacy at the presynaptic α_2-adrenoceptors than the endogenous noradrenaline with which it competes. Thus the net effect

of clonidine on overflow will depend upon the relative degrees to which it, and endogenous noradrenaline, activate the presynaptic α_2-adrenoceptors (Medgett *et al.*, 1978; Sullivan & Drew, 1980). In contrast, clonidine does inhibit stimulation-evoked overflow in the absence of the uptake inhibitors presumably because the concentration of endogenous noradrenaline at the presynaptic α-adrenoceptors is inadequate to operate the feedback process under these conditions (Sullivan & Drew, 1980). Even so, the interactions between clonidine and yohimbine in the present experiments were difficult to interpret. At the concentration used in these experiments, yohimbine (1×10^{-6}M) has commonly been shown to produce a 30 – 100 fold shift to the right of the clonidine concentration-effect curve at presynaptic α_2-adrenoceptors in many other isolated tissues, yet, in the saphenous vein, it only significantly antagonized the effect of clonidine at a concentration of 0.1×10^{-6}M. The failure of yohimbine to antagonize the response to 1×10^{-5}M clonidine may be attributable to a local anaesthetic action contributing to the effect of this high concentration of clonidine (Starke *et al.*, 1972) but this is unlikely to explain why yohimbine did not significantly reduce the response to the lowest concentration of clonidine. In short, these findings do not help to explain the interactions between yohimbine and exogenous noradrenaline but it is interesting to note that previous experiments have shown that clonidine can antagonize the presynaptic effects of exogenous noradrenaline in the dog saphenous vein indicating their affinity for a common group of receptors (Sullivan & Drew, 1980).

How can we explain the surprising finding that concentrations of yohimbine that enhance stimulation-evoked overflow of neuronally stored noradrenaline exert little or no antagonism of the presynaptic effects of exogenous noradrenaline? Three possibilities arise. Firstly, as suggested by Kalsner (1982b), yohimbine and exogenous noradrenaline may act at different sites to modulate stimulation-evoked overflow of radioactivity, but yohimbine acts at the same sites as the noradrenaline released from the nerve terminals. If this is so, why does yohimbine not block the receptors at which exogenous noradrenaline acts, and what sort of receptors are they? It should be noted that the apparent dissociation of the effects of yohimbine against exogenous and neuronally released noradrenaline is not peculiar to yohimbine; preliminary experiments with tolazoline (1×10^{-6} and 1×10^{-5}M) reveal a similar picture (unpublished observations). Furthermore, the idea that exogenous noradrenaline may inhibit transmitter release by stimulating a population of presynaptic α_1-adrenoceptors is improbable in view of the fact that prazosin did not prevent this effect. Secondly, the effect of yohimbine on stimulation-evoked overflow of radioactivity may be nothing to do with blockade of feedback control of transmitter release mediated via presynaptic α-adrenoceptors. Some support for this view may be provided by the finding that yohimbine (1×10^{-8} and 1×10^{-7}M) increased the overflow of radioactivity from the dog saphenous vein more effectively than the same concentrations of phentolamine (Sullivan & Drew, 1980), whereas it is almost invariably found that phentolamine is as potent, or more potent, than yohimbine at antagonizing the presynaptic effects of exogenous agonists, such as clonidine, in other isolated tissues. Against this view, however, is the finding that yohimbine did not enhance stimulation-evoked overflow obtained when the catecholamine uptake inhibitors were omitted from the superfusate. It seems, therefore, that yohimbine can only enhance stimulation-evoked overflow if enough noradrenaline accumulates in the neuroeffector junction to operate the feedback process, thus ruling out a non-specific action of yohimbine. Whatever the mechanism responsible for the effect of yohimbine on stimulation-evoked overflow the concentrations of yohimbine used in the present experiments were still high enough to exert substantial blockade at presynaptic α_2-adrenoceptors and ought, therefore, to have antagonized the inhibitory effects of the exogenous noradrenaline. Thirdly, the way of expressing the data may introduce an artefact that obscures an interaction between yohimbine and exogenous noradrenaline and wrongly suggests that there is no antagonism. If the latter explanation is correct, why does the same artefact not influence the interaction between yohimbine and dopamine, in such a way that it would appear that yohimbine enhances the inhibitory effect of dopamine on stimulation-evoked overflow?If this method of expressing the results does introduce such an artefact, how can they be better expressed in order to overcome this problem? Taken at face value, the present results add weight to the view of Kalsner (1982a) that the experimental results cannot be explained in terms of the prevailing assumption that yohimbine and exogenous noradrenaline modulate transmitter release by acting at the same presynaptic receptors. If this is indeed the case, it is pertinent to ask why endogenous noradrenaline does not also stimulate the yohimbine-insensitive receptors through which exogenous noradrenaline seems to exert its effect. It may to some extent: perhaps the finding that yohimbine increases stimulation-evoked overflow by only 2–3 fold can be explained if only part of the action of neuronally released noradrenaline is mediated via the receptors that yohimbine blocks, and the rest through those at which exogenous noradrenaline acts. It is important to resolve these questions to establish whether the current concept of feedback control of transmitter release is in need of modification.

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