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PRESYNAPTIC RECEPTORS AND THEIR ROLE IN THE REGULATION OF TRANSMITTER RELEASE

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Introduction

It is with deep gratitude that I receive the honour of having been selected by the Trustees of the Gaddum Memorial Fund to deliver the Sixth Gaddum Memorial Lecture. Among many other topics, Sir John Gaddum was interested in neurotransmission and he made important contributions in the field of cholinergic (Chang & Gaddum, 1933; Feldberg & Gaddum, 1934) and noradrenergic transmission (Gaddum & Kwiatowski, 1939; Gaddum, Peart & Vogt, 1949; Gaddum, 1950). The work of Sir John Gaddum is also linked to the discovery of other putative neurotransmitters like Substance P and 5-hydroxytryptamine (von Euler & Gaddum, 1931; Gaddum, 1953; Amin, Crawford & Gaddum, 1954). Although I never had a chance to meet Sir John Gaddum personally, as a pharmacologist I always admired his work and his important contributions. In addition, I had the privilege of working for two years with one of Gaddum's collaborators, Dr Marthe Vogt. The time spent with Dr Marthe Vogt in Babraham (1967 and 1968) was a stimulating and rewarding experience. It was during these two years that my interest in noradrenaline release during nerve stimulation started and this has developed further during the last eight years.

The topic of the lecture will be the presence of presynaptic receptors in noradrenergic nerve endings and their role in the regulation of the release of the neurotransmitter upon arrival of nerve impulses.

Presence of presynaptic α-adrenoceptors in noradrenergic nerve endings

Until a few years ago our knowledge of the role of noradrenergic nerve endings in neurotransmission was focused on the synthesis, storage, release and neuronal uptake of the neurotransmitter. During release elicited by the arrival of nerve impulses, the neurotransmitter interacts with specific receptors located in the membrane of the postsynaptic effector cell triggering the typical response of the effector organ: contraction or relaxation of a smooth muscle, positive chronotropic and intropic effects, or secretion of salivary glands. Until recently there were no indications that specific receptors might also be present in the outer surface of the membrane of nerve endings.

In 1957, Brown & Gillespie reported that phenoxybenzamine, an α-adrenoceptor blocking agent, increased the overflow of noradrenaline, elicited by nerve stimulation in the perfused cat spleen. These authors postulated that the α-adrenoceptor of the effector organ was an important site of loss for the released transmitter. In other words, when the α-adrenoceptors of the effector cell were occupied by the blocking agent, the transmitter released during nerve stimulation would not be able to combine with these receptors. Therefore, a larger fraction of the transmitter released by nerve stimulation would be collected in the venous effluent of the perfused spleen, resulting in an increase of the overflow of noradrenaline.

The main sites of loss for noradrenaline released by nerve stimulation are shown schematically in Figure 1: (a) recapture of the released transmitter through neuronal uptake; (b) extraneuronal uptake of noradrenaline; (c) the metabolizing enzymes: monoamine oxidase (coupled with either aldehyde reductase or aldehyde dehydrogenase) and catechol-*O*-methyltransferase, and (d) receptors and other binding sites.

It is clear from Figure 1 that an increase in transmitter overflow can result from the blockade of one or several sites of loss. Alternatively, an increase in transmitter overflow can be due to an actual increase in the release of noradrenaline, whether or not the sites of loss are affected.

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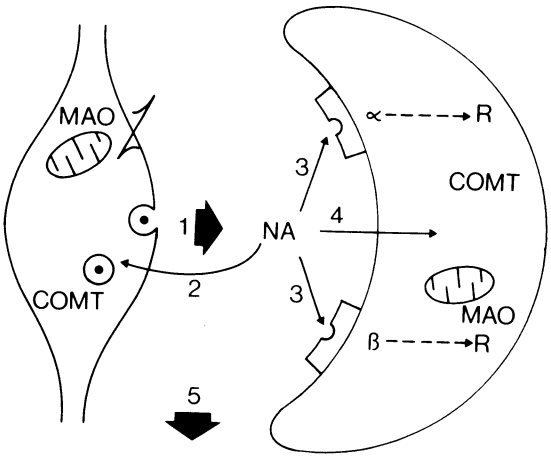

Figure 1 Main sites of loss for the transmitter released during sympathetic nerve stimulation. (1) Total amount of transmitter released by nerve stimulation. (2) Noradrenaline recaptured by neuronal uptake, subsequently deaminated or stored in vesicles. (3) Fraction of the transmitter released available for activation of the α- or β-postsynaptic receptors, leading to the response (R) of the effector organ. (4) Noradrenaline taken up at extraneuronal sites and subsequently metabolized, predominantly by catechol-*O*-methyltransferase. (5) Overflow: noradrenaline collected during and after the period of nerve stimulation. NA: noradrenaline; MAO: monoamine oxidase; COMT: catechol-*O*-methyltransferase.

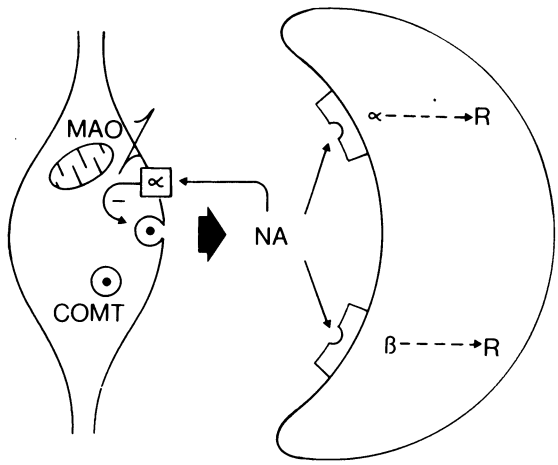

Figure 2 Schematic representation of the negative feed-back mechanism for noradrenaline released by nerve stimulation, mediated by presynaptic α-adrenoceptors. Noradrenaline (NA) released by nerve stimulation once it reaches a threshold concentration in the synaptic cleft activates presynaptic α-adrenoceptors leading to inhibition of transmitter release. The presynaptic negative feed-back mechanism is present both in tissues where the response (R) of the effector organ is mediated throuth α- or through β-adrenoceptors. MAO: monoamine oxidase; COMT: catechol-*O*-methyltransferase.

The causal relationship between the block of the α-adrenoceptors of the effector organ and the increase in transmitter overflow, proposed by Brown & Gillespie (1957) was challenged when it was discovered that phenoxybenzamine was also able to inhibit neuronal uptake (Hertting, 1965; Iversen, 1965) and extraneuronal uptake of noradrenaline (Iversen, 1967; Eisenfeld, Axelrod & Krakoff, 1967; Iversen & Langer, 1969).

However, when a similar degree or even maximal inhibition of neuronal uptake of noradrenaline was obtained with agents which do not block the α-adrenoceptors (like cocaine or desipramine) little or no increase in transmitter overflow was observed during nerve stimulation (Blakeley, Brown & Ferry, 1963; Geffen, 1965; Boullin, Costa & Brodie, 1967; Dubocovich & Langer, 1973; Langer & Enero, 1974).

The possible significance of the inhibition by phenoxybenzamine of extraneuronal uptake became apparent after it was reported in studies on transmitter release carried out with [^{3}H]-noradrenaline, that a significant fraction of the ^{3}H-transmitter released by nerve stimulation was collected as [^{3}H]-noradrenaline metabolites (Langer, 1970; Langer, Stefano & Enero, 1972; Langer, 1974a; Cubeddu, Barnes, Langer & Weiner, 1974; Langer & Enero, 1974; Luchelli-Fortis & Langer, 1975). Although phenoxybenzamine prevents the metabolism of [^{3}H]-noradrenaline released by nerve stimulation, this effect does not fully account for the increase in transmitter overflow elicited by phenoxybenzamine (Langer, 1970; Langer & Vogt, 1971; Langer, 1974b).

In support of the view that phenoxybenzamine and other α-receptor blocking drugs like phentolamine increase the release of the neurotransmitter during nerve stimulation, it was reported that these agents enhance transmitter overflow in concentrations which do not inhibit either neuronal or extraneuronal uptake of noradrenaline (Starke, Montel & Schumann, 1971; Enero, Langer, Rothlin & Stefano, 1972). In addition, it was reported that the release of dopamine-β-hydroxylase was increased when neurotransmission in the perfused spleen was studied in the presence of phenoxybenzamine or phentolamine (de Potter, Chubb, Put & De Schaepdryver, 1971; Cubeddu *et al.*, 1974). Since dopamine-β-hydroxylase is a rather large molecule which is not taken up by noradrenergic nerve endings or inactivated by the tissue after its exocytotic release, an increase in overflow of the enzyme does indeed represent an actual increase in release.

Although the increase in transmitter release observed in the presence of α-receptor blocking agents was obtained within the range of drug concentrations eliciting α-receptor blockade (Enero *et al.*, 1972; Dubocovich & Langer, 1974) a causal relationship between the block of the responses of the effector organ and the increase in transmitter release was excluded because similar results were obtained in guinea-pig isolated atria (Langer, Adler, Enero & Stefano, 1971; McCulloch, Rand & Story, 1972), in the perfused rabbit heart (Starke *et al.*, 1971) and the perfused cat heart (Farah & Langer, 1974) where the adrenoceptors that mediate the response of the effector organ are of the β-type.

These results led to the hypothesis that α-adrenoceptors are present in the outer surface of noradrenergic nerve endings. According to this hypothesis presynaptic α-adrenoceptors are involved in the regulation of noradrenaline release through a negative feed-back mechanism mediated by the neurotransmitter itself. As shown schematically in Figure 2, noradrenaline released by nerve stimulation, once it reaches a threshold concentration in the synaptic gap, activates presynaptic α-adrenoceptors, triggering a negative feed-back mechanism that inhibits further release of the transmitter (Langer *et al.*, 1971; Farnebo & Hamberger, 1971a; Enero *et al.*, 1972; Starke, 1972a, b; Rand, Story, Allen, Glover & McCulloch, 1973; Langer, 1973; Langer, 1974b).

In support of this hypothesis it has been demonstrated that α-adrenoceptor agonists inhibit transmitter release during nerve stimulation (Langer, Enero, Adler-Graschinsky & Stefano, 1972b; Starke, 1972b; Starke, Montel, Gay & Merker, 1974; Starke, Endo & Taube, 1975a; Langer, Dubocovich & Celuch, 1975a). As shown in Table 1, the inhibition of [^{3}H]-noradrenaline release in the perfused cat spleen, obtained by exposure to exogenous noradrenaline in the presence of cocaine, was more pronounced at low frequencies of nerve stimulation. Similar results were obtained in other tissues: the magnitude of the inhibition of [^{3}H]-transmitter release obtained by exposure to α-receptor agonists was inversely related to the frequency of nerve stimulation (Starke *et al.*, 1975a).

The frequency-dependence of the inhibition of noradrenaline release obtained with clonidine was also demonstrated under *in vivo* conditions in the rat (Armstrong & Boura, 1973) and in the dog (Scriabine & Stavorski, 1973; Robson & Antonaccio, 1974; Yamaguchi, de Champlain & Nadeau, 1977).

The reduction in transmitter release obtained by exposure to α-receptor agonists was equally observed in tissues in which the response of the effector organ is mediated by α-receptors (Kirpekar, Furchgott, Wakade & Prat, 1973; Starke *et al.*, 1974; Starke *et al.*, 1975a; Langer *et al.*, 1975a) or by β-receptors (Starke, 1972b; Langer *et al.*, 1972b; Rand, McCulloch & Story, 1975).

Further support for the hypothesis that noradrenaline release is regulated through a presynaptic negative feed-back mechanism mediated by α-adrenoceptors was obtained in experiments in which an increase in noradrenaline release during nerve stimulation was obtained in the presence of α-receptor blocking agents. The enhancement in transmitter release obtained by α-receptor blocking agents was observed regardless of the α- or β-type of

Table 1 Influence of the frequency of stimulation on the inhibition by exogenous noradrenaline of ^{3}H-transmitter overflow elicited by nerve stimulation in the perfused cat spleen

	Experimental group	n	*Fractional release per shock* ($\times 10^{-5}$) S_1	S_2	*Ratio* S_2/S_1
1 Hz	Control	3	8.26 ± 3.78	8.34 ± 3.31	1.11 ± 0.13
	Noradrenaline 0.18 μM	5	6.64 ± 0.85	1.92 ± 0.26*	0.32 ± 0.07**
2 Hz	Control	3	10.22 ± 5.51	10.61 ± 6.21	1.01 ± 0.08
	Noradrenaline 0.18 μM	5	8.97 ± 0.94	5.63 ± 0.62*	0.64 ± 0.06**
5 Hz	Control	5	8.64 ± 2.53	7.07 ± 2.43	0.84 ± 0.12
	Noradrenaline 0.18 μM	4	14.14 ± 1.29	14.51 ± 1.37	1.03 ± 0.03

Fractional release per shock: total nCi released per shock divided by the total nCi remaining in the tissue at the onset of nerve stimulation (a total of 300 shocks was delivered at each frequency of stimulation). All experiments were carried out in the presence of cocaine (29 μM); (−)-noradrenaline was added 10 min before the second period of nerve stimulation (S_2). Mean values are shown ± s.e. mean. n = number of experiments. *$P < 0.025$ when compared against S_1 in the same group; **$P < 0.005$ when compared against the ratio of the control group.

the postsynaptic receptor that mediates the response of the effector organ (Langer, 1970; De Potter *et al.*, 1971; Langer *et al.*, 1971; Farnebo & Hamberger, 1971a; Starke *et al.*, 1971; Enero *et al.*, 1972; Dubocovich & Langer, 1974; Farah & Langer, 1974; Cubeddu *et al.*, 1974; Langer, 1974b; Langer *et al.*, 1975a; Langer, Enero, Adler-Graschinsky, Dubocovich & Celuch, 1975b; Langer, Adler-Graschinsky & Giorgi, 1977).

The negative feed-back mechanism for noradrenaline release during nerve stimulation would be expected to operate most effectively when the transmitter released by nerve impulses reaches a threshold concentration in the synaptic gap. In agreement with this view, it has been shown that when the endogenous noradrenaline stores are depleted either by pretreatment with reserpine or by inhibition of the synthesis of the transmitter by α-methyl-*p*-tyrosine, the effectiveness of phenoxybenzamine in increasing the release of [^{3}H]-noradrenaline and dopamine-β-hydroxylase during nerve stimulation is almost completely lost (Enero & Langer, 1973; Cubeddu & Weiner, 1975). Consequently it appears that when the concentration of released noradrenaline in the synaptic cleft falls below a certain threshold, it fails to trigger the presynaptic negative feed-back mechanism that regulates noradrenaline release.

In support of the view that a threshold concentration of released noradrenaline is required for the activation of the presynaptic negative feed-back mechanism, Rand *et al.* (1975) demonstrated that phenoxybenzamine did not enhance ^{3}H-transmitter release from guinea-pig atria elicited by a single pulse, but elicited a 4.5-fold increase in release when a train of 16 pulses was applied.

As already shown for the inhibition of release induced by α-receptor agonists, there was a relationship between the frequency of nerve stimulation and the magnitude of the increase in transmitter release obtained in the presence of α-receptor blocking agents. As the frequency of stimulation is increased, the effectiveness of the α-receptor blocking agents in enhancing noradrenaline release during nerve stimulation is progressively reduced (Brown & Gillespie, 1957; Kirpekar & Cervoni, 1963; Haefely, Hurlimann & Thoenen, 1965; Langer, 1970; Dubocovich & Langer, 1974; Langer *et al.*, 1975a; Dubocovich & Langer, 1976).

An example of the inverse relationship between the frequency of nerve stimulation and the increase in transmitter release induced by α-adrenoceptor blockade in the perfused cat spleen is shown in Table 2. While the increase in transmitter release obtained with phenoxybenzamine at 5 Hz was 6.5-fold, at 30 Hz the increase in release was only 2.4-fold. As shown recently by Dubocovich & Langer (1976) the decreased effectiveness of α-receptor blocking agents in enhancing release of noradrenaline at high frequencies of stimulation cannot be attributed to the attainment of maximal values of transmitter output per shock. It is likely that at high frequencies of nerve stimulation the negative feed-back regulatory mechanism which is mediated by presynaptic α-receptors, does not play an important role in the regulation of transmitter release (Dubocovich & Langer, 1974; Langer *et al.*, 1975a).

Tyramine elicits release of noradrenaline by displacing the transmitter from vesicular binding sites. In contrast to release elicited by potassium or by nerve stimulation, the noradrenaline release induced by tyramine is not calcium-dependent. The presynaptic negative feed-back mechanism that regulates noradrenaline release is operative for release elicited by nerve stimulation or by potassium while this mechanism is not involved in the regulation of transmitter release elicited by tyramine (Starke &

Table 2 Influence of the frequency of stimulation on the increase in ^{3}H-transmitter overflow elicited by phenoxybenzamine in the perfused cat spleen

	Experimental group	n	*Fractional release per shock* ($\times 10^{-5}$) S_1	S_2	*Ratio* S_2/S_1
5 Hz	Control	4	10.80 ± 2.86	8.68 ± 2.26	0.83 ± 0.05
	Phenoxybenzamine	4	8.80 ± 1.14	55.89 ± 6.75*	6.53 ± 0.96**
30 Hz	Control	4	18.00 ± 4.51	17.79 ± 4.39	1.01 ± 0.17
	Phenoxybenzamine	4	19.64 ± 3.39	45.50 ± 5.88*	2.42 ± 0.26**

Fractional release per shock: total nCi released per shock divided by the total nCi remaining in the tissue at the onset of stimulation (a total of 300 shocks was delivered at each frequency of stimulation). Phenoxybenzamine 29 μM was added 22 min before S_2. Mean values are shown ± s.e. mean. *n*: number of experiments. * $P < 0.01$ when compared against S_1 in the same group; ** $P < 0.005$ when compared against the ratio in the control group. (From Dubocovich & Langer, 1976).

Montel, 1973a). It is possible that the presynaptic feed-back mechanism modifies the availability of calcium ions for the release process and that this is the reason why release elicited by tyramine is not influenced by this regulatory mechanism.

It has recently been reported that the inhibition of transmitter release obtained by exposure to exogenous noradrenaline is more pronounced when the calcium concentration in the medium is reduced from 2.6 to 0.25 mM (Langer *et al.*, 1975a). The potentiation of the inhibitory effects of α-receptor agonists on neurotransmission by a reduction in the external calcium concentration indicates that activation of presynaptic α-receptors may reduce the availability of calcium for the excitation-secretion coupling.

Although both the pre- and postsynaptic α-adrenoceptors are stimulated by α-receptor agonists and blocked by α-receptor antagonists, it appears that the postsynaptic α-adrenoceptors that mediate the responses of the effector organ are not identical with the presynaptic α-adrenoceptors which regulate the release of noradrenaline during nerve stimulation. When the potency of phenoxybenzamine in blocking the pre- and postsynaptic α-receptors was tested in the perfused cat spleen it was found that a significant reduction in responses to nerve stimulation was obtained with low concentrations of the α-blocking agent, although transmitter release was not increased under these experimental conditions. As shown in Figure 3, phenoxybenzamine is about 30 times more potent in blocking the postsynaptic α-receptors that mediate the responses of the effector organ than it is in blocking the presynaptic α-receptors that regulate the release of noradrenaline during nerve stimulation. These results led to the postulate that the pre- and postsynaptic α-adrenoceptors are not identical (Dubocovich & Langer, 1974; Langer, 1974b).

These results led to the suggestion that the postsynaptic α-adrenoceptors should be referred to as α_1 while the presynaptic α-adrenoceptors should be referred to as α_2 (Langer, 1974b).

In support of this view it was found that in experiments in which the overflow of dopamine-β-hydroxylase was determined in the perfused cat spleen, phenoxybenzamine was 30 to 100 times more potent in blocking the postsynaptic α-receptors than the presynaptic receptors (Cubeddu *et al.*, 1974). On the other hand, these authors found only a very small difference between the potency of phentolamine in blocking the pre- and the postsynaptic α-adrenoceptors in the perfused cat spleen.

In further support of the view that the pre- and the postsynaptic α-adrenoceptors are not identical, it has recently been shown that the α-receptor blocking agent yohimbine was more potent in blocking the presynaptic α-receptor than the postsynaptic α-receptor in the rabbit main pulmonary artery (Starke, Borowski & Endo, 1975b).

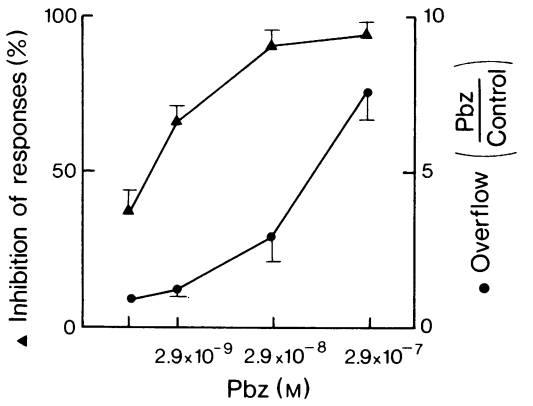

Figure 3 Differences in potency of phenoxybenzamine in blocking the pre- and postsynaptic adrenoceptors in the perfused cat spleen. Abscissa scale: molar concentration of phenoxybenzamine (Pbz). Left ordinate scale: (▲) percent inhibition of the postsynaptic responses (increase in perfusion pressure induced by nerve stimulation in the presence of different concentrations of phenoxybenzamine). Right ordinate scale: (●) increase in noradrenaline overflow induced by nerve stimulation (5 Hz, during 60 s, supramaximal voltage), expressed as the ratio phenoxybenzamine (Pbz) over control: 1 (no change in transmitter overflow), 5 (five-fold increase in transmitter overflow). Mean values of at least 4 experiments per group are shown. Vertical lines show s.e. means. Results from Dubocovich & Langer (1974).

Differences also exist for the α-adrenoceptor stimulating agents with regard to their relative potencies on the presynaptic or α_2 and the postsynaptic or α_1-receptors. Clonidine, α-methylnoradrenaline and oxymetazoline are more potent in reducing noradrenaline release during nerve stimulation than in stimulating the postsynaptic α-adrenoceptors (Starke *et al.*, 1974; Starke *et al.*, 1975a).

It is noteworthy that the sensitivity of the presynaptic α-adrenoceptors can be modified after exposure to a large concentration of an α-receptor agonist. In the cat spleen perfused with cocaine, there is a nearly 2-fold increase in transmitter release during nerve stimulation after 60 min of exposure to noradrenaline 0.59 μM (Langer & Dubocovich, 1977). This effect is probably due to a short lasting subsensitivity of the presynaptic α-adrenoceptors resulting from the exposure to noradrenaline. In support of this view, it has been found that this phenomenon is not observed in the presence of phentolamine (Langer & Dubocovich, 1977).These results are compatible with the view that chronic stimulation or blockade of presynaptic receptors may lead to changes in their

sensitivity to the neurotransmitter, as was already demonstrated for the postsynaptic receptor. Consequently, attention should be drawn to the fact that chronic stimulation or blockade of the presynaptic receptors may lead to changes in their sensitivity which may affect the regulation of neurotransmission.

In spite of the fact that the available evidence indicates that the negative feed-back mechanism for noradrenaline release is of a presynaptic nature, the possibility that a trans-synaptic regulatory mechanism is involved was suggested by several authors (Häggendal, 1970; Farnebo & Malmfors, 1971). Yet there are several pieces of evidence in favour of the presynaptic location of the α-adrenoceptor involved in the regulation of noradrenaline release during nerve stimulation.

In recently formed nerve endings from cultured rat superior cervical ganglia, phenoxybenzamine enhances [^{3}H]-noradrenaline release induced by potassium (Vogel, Silbertstein, Berv & Kopin, 1972). Under these experimental conditions, α-receptor block enhanced release from nerve endings in the absence of an effector postsynaptic cell.

After short term denervation of the cat nictitating membrane, and at a time when postsynaptic changes in sensitivity have not yet developed (Langer & Trendelenburg, 1966; Langer, Draskoczy & Trendelenburg, 1967; Langer, 1975) there are marked changes in the sensitivity of the presynaptic α-adrenoceptors that regulate noradrenaline release during nerve stimulation (Langer & Luchelli-Fortis, 1977). Eighteen hours after surgical denervation of the cat nictitating membrane phentolamine did not increase [^{3}H]-noradrenaline release by nerve stimulation and clonidine was significantly less effective in reducing ^{3}H-transmitter release. Yet, 18 h after surgical denervation the ability of phentolamine to block and of clonidine to stimulate the postsynaptic α-adrenoceptors did not differ from the controls (Langer & Luchelli-Fortis, 1977). In support of the view that the changes observed in neurotransmission are due to subsensitivity of the presynaptic α-adrenoceptors, it was found that 18 and 24 h after denervation the fractional release per stimulus was significantly higher than that obtained in the corresponding controls (Table 3).

Additional evidence in favour of the presynaptic location of the α-adrenoceptor involved in the regulation of noradrenaline release was recently obtained in the rat submaxillary gland. These experiments were carried out after atrophy of the secretory cells elicited by duct ligation 15 days before the experiment (Standish & Shafer, 1957). In these atrophied salivary glands, the secretory responses to adrenoceptor or cholinoceptor agonists are abolished at a time when there are no changes in either the cholinergic or the noradrenergic innervation (Filinger, Langer, Perec & Stefano, 1977). Exposure to phentolamine 3.1 μM, increased the release of [^{3}H]-noradrenaline from slices of the normal submaxillary gland elicited by 60 mM potassium 2.97 ± 0.59-fold; under these experimental conditions the increase in release obtained by phentolamine in atrophied salivary glands was 3.65 ± 0.78-fold. Consequently, the α-receptor blocking agent was equally effective in increasing transmitter release in normal as well as in atrophied glands. The effect of phentolamine was therefore mediated through a presynaptic mechanism which could be demonstrated whether or not the postsynaptic effector cell was physiologically or pharmacologically involved.

In connection with the physiological significance of the negative feed-back mechanism mediated by presynaptic α-adrenoceptors, it should be noted that an enhancement in transmitter release, as observed in the presence of α-receptor blocking agents, would be expected to result in the potentiation of the response of the effector organ to nerve stimulation. However, in tissues in which the response of the effector organ is mediated through postsynaptic α-adrenoceptors (spleen, nictitating membrane, blood vessels) the responses to nerve stimulation are reduced by the α-blocking agents. The only exception found so far concerns yohimbine, which is more potent in blocking

Table 3 Subsensitivity of the presynaptic α-adrenoceptors after short-term surgical denervation of the cat nictitating membrane

Experimental group	n	*Fractional release per shock ($\times 10^{-6}$)*	*Ratio Den/con*
Controls	9	1.01 ± 0.10	–
Denervated 18 h	16	1.83 ± 0.29*	1.57 ± 0.15**
Denervated 24 h	9	2.33 ± 0.39*	2.42 ± 0.44**

Fractional release per shock: total nCi released per shock divided by the total nCi remaining in the tissue at the onset of nerve stimulation (10 Hz, during 2 min, supramaximal voltage). Den: denervated. Mean values are shown $\pm$ s.e. mean. *n*: number of experiments.
* $P < 0.05$ when compared against the control group; ** $P < 0.025$ when compared against the ratio between right and left normal nictitating membranes. (From Langer & Luchelli-Fortis, 1977).

the presynaptic than the postsynaptic α-adrenoceptors in the rabbit main pulmonary artery (Starke *et al.*, 1975b). Low concentrations of yohimbine are thus able to enhance [^{3}H]-noradrenaline release and potentiate the contractile responses to nerve stimulation (Starke *et al.*, 1975b).

In tissues in which the response of the effector organ is mediated through β-receptors, exposure to α-adrenoceptor blocking agents would be expected to enhance the responses to nerve stimulation because of the increase in transmitter release observed under these experimental conditions. As expected, when the responses to accelerans nerve stimulation at *low* frequencies were determined in guinea-pig atria, phentolamine increased significantly the positive chronotropic responses to nerve stimulation (Langer *et al.*, 1977). Under these experimental conditions, the concentration of the α-receptor blocking agent produced a significant increase in ^{3}H-transmitter release during nerve stimulation (Langer *et al.*, 1977).

The significant increase in the positive chronotropic responses to nerve stimulation obtained under experimental conditions in which the release of noradrenaline was enhanced by exposure to phentolamine supports the view that the negative feed-back mechanism mediated by presynaptic α-adrenoceptors plays a major physiological role in noradrenergic neurotransmission. Similar results were obtained under *in vivo* conditions: Lokhandwala & Buckley (1976) demonstrated in the anaesthetized dog that phentolamine potentiated the positive chronotropic responses to cardioaccelerator nerve stimulation.

Neuronal uptake of noradrenaline, by effectively reducing the concentration of the transmitter in the vicinity of the outer surface of the nerve ending (Figure 1) appears to modulate this presynaptic feed-back mechanism, by regulating the fraction of the transmitter released by stimulation which is available to activate the presynaptic α-adrenoceptors (Langer, 1974b). Accordingly, when neuronal uptake is inhibited by cocaine, a higher fraction of the noradrenaline released by nerve stimulation would become available for activation of the presynaptic inhibitory α-adrenoceptors. In support of this view, it was reported that, in the perfused cat spleen, during exposure to concentrations of cocaine ranging from 0.3 to 30 μM, there was no significant increase in the overflow of total tritium elicited by nerve stimulation (Cubeddu *et al.*, 1974). In addition, in the presence of 3 and 30 μM cocaine, there was a concentration-dependent reduction in the overflow of dopamine-β-hydroxylase during nerve stimulation (Cubeddu *et al.*, 1974). These results are compatible with the view that inhibition of neuronal uptake by cocaine leads to a decrease in transmitter output because of enhanced feed-back inhibition by the higher concentration of the transmitter achieved in the vicinity of the nerve ending (Enero *et al.*, 1972; Langer, 1974b; Langer & Enero, 1974).

It is well known that the importance of neuronal uptake in regulating the concentration of noradrenaline in the biophase is inversely related to the neuromuscular distance; in other words, the narrower the neuromuscular gap, the more important is neuronal uptake in the regulation of the concentration of the neurotransmitter in the biophase. The width of the neuromuscular distance would also be expected to modify the presynaptic negative feed-back mechanism because it influences the concentration of the released transmitter achieved in the synaptic cleft. Accordingly, the analysis of results obtained in different tissues with known neuromuscular distances showed that the magnitude of the increase in transmitter release elicited by nerve stimulation in the presence of phentolamine was the more pronounced the smaller the neuromuscular interval of the tissue (Langer *et al.*, 1975b). Consequently, it appears that in organs with narrow neuromuscular gaps the presynaptic feed-back inhibition for noradrenaline release during nerve stimulation plays a more important role as a regulatory mechanism than in tissues with wide synaptic gaps.

It is of interest to note that there is evidence in favour of the presence of neural α-adrenoceptors mediating the negative feed-back control of noradrenaline release in human vasoconstrictor nerves. These experiments were carried out in isolated superfused field stimulated biopsy specimens of human peripheral arteries and veins. An enhancement in ^{3}H-transmitter release was demonstrated in the presence of α-receptor blocking agents, as well as inhibition of release induced by exposure to exogenous noradrenaline (Stjärne & Gripe, 1973; Stjärne & Brundin, 1975).

Evidence for the presence of presynaptic β-adrenoceptors in noradrenergic nerve endings

It has recently been postulated that in addition to the presynaptic negative feed-back mechanism for noradrenaline released by nerve stimulation which is mediated via α-adrenoceptors, a positive feed-back mechanism exists in noradrenergic nerve endings that is triggered through the activation of presynaptic β-adrenoceptors (Langer, Adler-Graschinsky & Enero, 1974; Adler-Graschinsky & Langer, 1975; Langer *et al.*, 1975a; Stjärne & Brundin, 1975; Dahlöf, Ablad, Borg, Ek & Waldeck, 1975; Langer, 1976; Stjärne & Brundin, 1976a; Yamaguchi *et al.*, 1977).

This hypothesis is based on the fact that exposure to low concentrations of isoprenaline enhances the release of noradrenaline during nerve stimulation at low frequencies in several noradrenergically innervated organs: guinea-pig atria, perfused cat

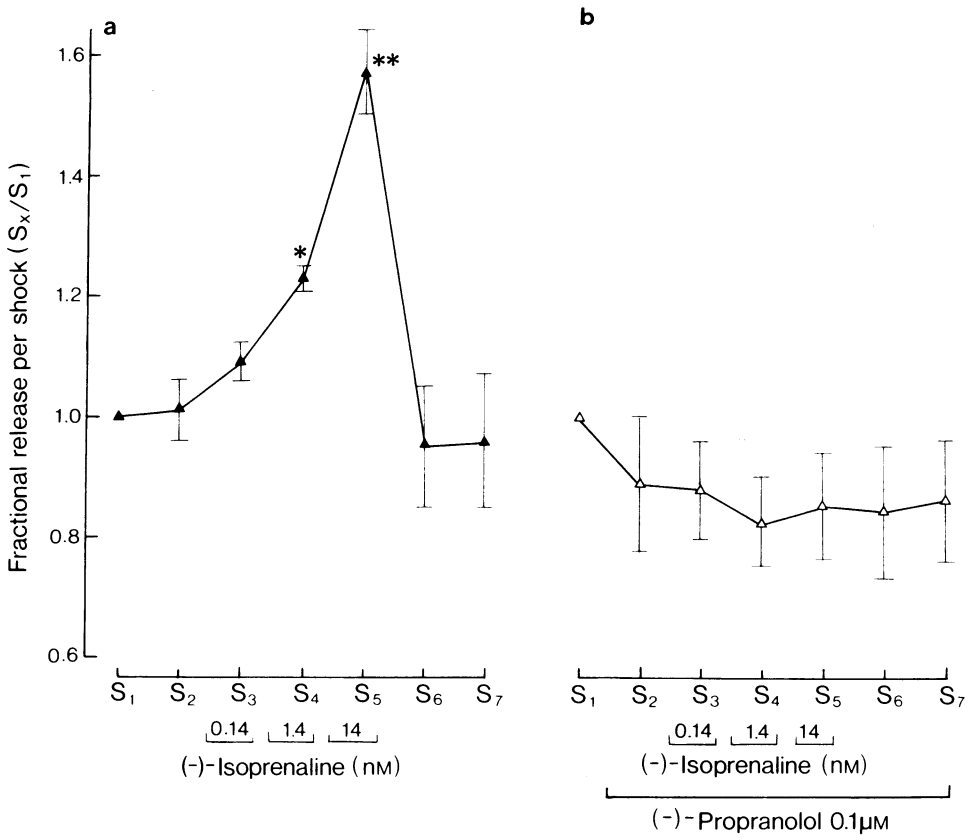

Figure 4 Increase by isoprenaline of ^{3}H-transmitter release elicited by nerve stimulation in the perfused cat spleen. Abscissa scale: S_1 to S_7 indicate the periods of nerve stimulation (1 Hz, during 120 s, with supramaximal voltage). The interval between each period of nerve stimulation was 20 minutes. Ordinate scale: ratio between the fractional release of ^{3}H-transmitter per shock obtained during a given period of nerve stimulation (S_x) and the first period (S_1). (a) (▲) Isoprenaline in the concentration which is indicated was added to the perfusion medium 10 min before S_3, S_4 and S_5; (b) (△) same as in (a) but propranolol 0.1 μM was added 10 min before S_2 and was present throughout. Note that isoprenaline induced a concentration-dependent increase in ^{3}H-transmitter release, which was reversible by washing. The effect of isoprenaline was prevented by exposure to propranolol. Mean values of at least 4 experiments in each group are shown. Vertical lines show s.e. means. (Celuch, Dubocovich & Langer, unpublished).
* $P<0.05$; ** $P<0.005$.

spleen, cat thoracic aorta, cat nictitating membrane, human oviduct and human vasoconstrictor nerves (Langer *et al.*, 1974; Adler-Graschinsky & Langer, 1975; Langer *et al.*, 1975b; Stjärne & Brundin, 1975; Hedqvist & Moawad, 1975; Stjärne & Brundin, 1976a). The increase in transmitter release obtained in the presence of isoprenaline is independent of the α or β nature of the response of the effector organ to the transmitter (Langer *et al.*, 1975b).

As shown in Figure 4, the effects of (−)-isoprenaline on transmitter release obtained in the perfused cat spleen were concentration-dependent, and readily reversed by washing. The enhancement in transmitter release obtained with (−)-isoprenaline was stereospecific, since it was not obtained with (+)-isoprenaline (Langer, 1976).

The effects of isoprenaline on transmitter release can be prevented by preincubation with 0.1 μM propranolol (Figure 4). However, acute exposure to propranolol 0.1 μM did not always reduce significantly noradrenaline release during nerve stimulation. Propranolol reduced transmitter release elicited by nerve stimulation in guinea-pig isolated atria (Adler-Graschinsky & Langer, 1975) and in the calf muscle of the cat pretreated with phenoxybenzamine (Dahlöf *et al.*, 1975). Yet, exposure to propranolol 0.1 μM did not reduce ^{3}H-transmitter release from human omental arteries and veins (Stjärne & Brundin, 1975).

Considerably higher concentrations of propranolol reduce noradrenaline release during nerve stimulation in several tissues but this effect is related to the local anaesthetic properties of propranolol (Barret & Nunn, 1970; Hughes & Kneen, 1976).

Recently Yamaguchi *et al.* (1977) demonstrated that in the anaesthetized dog an infusion of isoprenaline enhanced the release of noradrenaline elicited by right cardioaccelerator nerve stimulation at low frequencies. These authors also reported that the administration of the β-receptor blocking agent, sotalol reduced significantly the release of noradrenaline at stimulation frequencies between 1 and 5 Hz. Consequently, the physiological role of the presynaptic β-adrenoceptors in noradrenergic neurotransmission has also been demonstrated under *in vivo* conditions.

It is possible that the long-term β-receptor blockade may be more effective in reducing the amount of transmitter released per impulse from noradrenergic nerves. Ljung, Äblad, Dählof, Henning & Hultberg (1975) reported that prolonged administration of propranolol or metoprolol to spontaneously hypertensive rats resulted in a reduction of the responses to postganglionic nerve stimulation in the portal vein preparation without concomitant changes in sensitivity to exogenous noradrenaline. In addition, Lewis (1974) reported that in the pithed rat preparation, chronic, but not acute administration of practolol reduced the pressor responses to preganglionic sympathetic stimulation.

It is noteworthy that Stjärne & Brundin (1975) have shown that isoprenaline increases [^{3}H]-noradrenaline release elicited by field stimulation from strips of human omental arteries and veins. A similar effect was obtained with low concentrations of adrenaline (Stjärne & Brundin, 1975) although a further increase in the concentration of adrenaline led to inhibition of transmitter release due to stimulation of α-presynaptic receptors. Stjärne & Brundin (1975) suggested that the presynaptic β-adrenoceptors can be activated by the levels of circulating catecholamines and may subserve the function of enhancing the secretion of sympathetic transmitter during conditions of increased secretion of adreno-medullary hormone. Isoprenaline also increases the release of [^{3}H]-noradrenaline during nerve stimulation at low frequency in the human oviduct (Hedqvist & Moawad, 1975).

These results are compatible with the existence of β-adrenoceptors in noradrenergic nerve endings. Activation of this mechanism by β-adrenoceptor agonists leads to an increase in transmitter release during nerve stimulation (Figure 5). The increase in transmitter release elicited by β-adrenoceptor activation is more pronounced at low frequencies of nerve stimulation (Langer *et al.*, 1975b).

The presynaptic β-adrenoceptors appear to mediate a positive feed-back mechanism for noradrenaline released at low frequencies of nerve stimulation

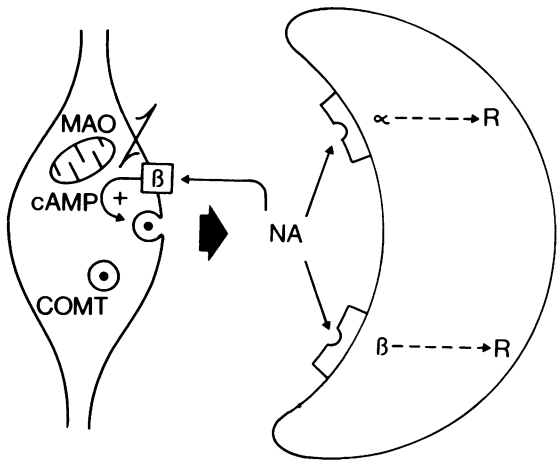

Figure 5 Schematic representation of the positive feed-back mechanism for noradrenaline released by nerve stimulation, mediated by presynaptic β-adrenoceptors. Noradrenaline (NA) released by low frequencies of nerve stimulation activates presynaptic β-adrenoceptors, leading to an increase in transmitter release. This effect appears to be mediated through an increase in the levels of cyclic AMP (cAMP) in noradrenergic nerve endings. The presynaptic positive feed-back mechanism is present both in tissues where the response (R) of the effector organ is mediated through α- or through β-adrenoceptors. MAO: monoamine oxidase; COMT: catechol-*O*-methyltransferase.

(Figure 5). The transmitter released at low frequencies would then facilitate its own release through the activation of presynaptic β-adrenoceptors.

The β_1 or β_2 nature of the presynaptic β-adrenoceptors is still somewhat controversial. According to Dahlöf *et al.* (1975) the presynaptic receptors are of the β_1-type because they are blocked by metoprolol, a selective β_1-receptor blocking agent. Yet, a recent publication by Stjärne & Brundin (1976a) suggests that the presynaptic β-adrenoceptors involved in the facilitation of noradrenaline release are of the β_2-type because terbutaline and salbutamol enhanced transmitter release, while a β_1-agonist, H 110/38, was without effect.

Since the results of Dahlöf *et al.* (1975) were obtained in the rat portal vein while those of Stjärne & Brundin (1976a) were obtained with human omental arteries and veins, it is possible that the different results reflect species and tissue differences.

The facilitation of transmitter release triggered by the activation of presynaptic β-receptors may be mediated through an increase in the levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in noradrenergic nerve endings (Figure 5). Wooten, Thoa, Kopin & Axelrod (1973) reported that dibutyryl cyclic AMP and theophylline increase the

release of noradrenaline and of dopamine-β-hydroxylase elicited by nerve stimulation in the guinea-pig vas deferens. Papaverine and other phosphodiesterase inhibitors enhance noradrenaline release during nerve stimulation (Langer *et al.*, 1975b; Cubeddu, Barnes & Weiner, 1975). In the perfused cat spleen several cyclic nucleotide analogues enhance both noradrenaline and dopamine-β-hydroxylase release during nerve stimulation (Cubeddu *et al.*, 1975).

Papaverine produces a shift to the left in the concentration-effect curve for (−)-isoprenaline on transmitter release in the perfused cat spleen (Langer, 1976). In addition, the effect of papaverine on transmitter release is significantly reduced by exposure to 0.1 μM propranolol.

It is of interest to note that in the adrenal medulla, Serck-Hanssen (1974) postulated a β-adrenergic system confined to the adrenaline storing cell, which enhances release of the catecholamine and which appears to be mediated by an increase in the cellular concentration of cyclic AMP.

Recently, Roth, Morgenroth & Salzman (1975) have postulated that adenylate cyclase present in noradrenergic nerve endings is involved through increased cyclic AMP formation in the activation of tyrosine hydroxylase as a result of nerve stimulation. At present it is not yet clear as to whether a common presynaptic site of action is involved in the facilitation of noradrenaline release mediated by presynaptic β-adrenoceptors and the increase in tyrosine hydroxylase activity that occurs during and after sympathetic nerve stimulation. Both mechanisms appear to be mediated through an increase in cyclic AMP levels in nerve endings.

In rat striatal slices labelled with [^{3}H]-dopamine, dibutyryl cyclic AMP produces a concentration-dependent increase in [^{3}H]-dopamine release elicited by electrical stimulation (Westfall, Kitay & Wahl, 1976). These results add further support to the view that cyclic AMP may play a role in monoaminergic neurotransmission, acting presynaptically to increase transmitter release.

Recently, Stjärne & Brundin (1976b) demonstrated that isoprenaline enhanced [^{3}H]-noradrenaline release elicited by nerve stimulation of human omental blood vessels even after the local production of prostaglandins was blocked by the addition of 5,8,11,14-eicosatetranoic acid (ETA).

Working hypothesis for the participation of the presynaptic α- and β-adrenoceptors in the regulation of transmitter release during nerve stimulation

Two presynaptic mechanisms appear to be involved in the autoregulation of noradrenaline release during nerve stimulation. The first one, mediated by β-adrenoceptors, would be activated by low concentrations of noradrenaline (i.e. in the range of low frequencies of nerve stimulation) leading to an increase in transmitter release (Figure 6). The second one, mediated through α-adrenoceptors, is triggered when higher concentrations of the transmitter are reached in the synaptic cleft, leading to inhibition of transmitter release.

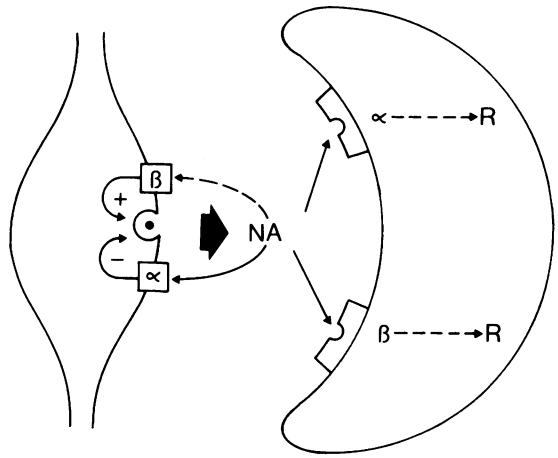

Figure 6 Role of the presynaptic α- and β-adrenoceptors in the regulation of noradrenaline release during nerve stimulation. During noradrenaline (NA) release at low frequencies of nerve stimulation (when the concentration of the released transmitter in the synaptic cleft is rather low) the positive feed-back mechanism mediated by presynaptic β-adrenoceptors is activated leading to an increase in transmitter release. As the concentration of released noradrenaline increases, a threshold is reached at which the negative feed-back mechanism mediated by presynaptic α-adrenoceptors is triggered, leading to inhibition of transmitter release. Both presynaptic feed-back mechanisms are present in nerves, irrespective of the α or β nature of the receptors that mediate the response (R) of the effector organ.

The positive feed-back mechanism which facilitates transmitter release appears to be mediated through an increase in the cyclic AMP levels in noradrenergic nerve endings. On the other hand, the negative feed-back mechanism which leads to inhibition of noradrenaline release operates by restricting the calcium available for the excitation-secretion coupling.

In support of this working hypothesis, it is of interest to note that, in most tissues, the concentrations of noradrenaline required for stimulation of postsynaptic α-adrenoceptors are about 100 times higher than those necessary to stimulate the postsynaptic β-receptors (Adler-Graschinsky & Langer, 1975; Langer *et al.*, 1975b). If the relative affinities of noradrenaline for the postsynaptic α- and β-adrenoceptors can be extrapolated to the presynaptic receptors, this difference in potency would be

compatible with the present hypothesis: low concentrations of released noradrenaline activating presynaptic β-receptors while the presynaptic α-adrenoceptors are activated when a higher, threshold concentration of the transmitter is achieved in the synaptic cleft (Figure 6).

In view of the fact that the most pronounced increases in transmitter release are obtained when the presynaptic α-adrenoceptors are blocked by drugs, it follows that the major regulatory mechanism for noradrenaline release by nerve stimulation under physiological conditions is mediated by presynaptic α-adrenoceptors.

Other types of presynaptic receptors in noradrenergic nerve endings

In addition to the presynaptic α- and β-adrenoceptors, a variety of presynaptic receptor sites have been described in noradrenergic nerve endings. As shown schematically in Figure 7, the following presynaptic receptors have been postulated: (a) muscarinic inhibitory cholinoceptors (Löffelholz and Muscholl, 1969; Steinsland, Furchgott & Kirpekar, 1973; Langer, Enero, Adler-Graschinsky, Dubocovich & Giorgi, 1976); (b) dopamine inhibitory receptors (Langer, 1973; McCulloch, Rand & Story, 1973; Enero & Langer, 1975; Long, Heintz, Cannon & Kim, 1975); (c) opiate inhibitory receptors, which are activated by morphine and also by the naturally occurring pentapeptides met and leu-enkephalin (Hughes, Kosterlitz & Leslie, 1975; Dubocovich & Langer, unpublished observations); (d) prostaglandin inhibitory receptors, which are activated by prostaglandin E_1 and E_2 (Hedqvist, 1970; Stjärne, 1973; Dubocovich & Langer, 1975; Hedqvist, 1976); (e) adenosine inhibitory receptors (Hedqvist & Fredholm, 1976); (f) angiotensin II facilitatory receptors (Starke, 1970, 1971; Hughes & Roth, 1971) and (g) nicotinic facilitatory receptors (Lindmar, Löffelholz & Muscholl, 1968; Löffelholz, 1970).

In connection with the multiplicity of presynaptic receptor sites (Figure 7) it should be noted that some of these receptors are not present in all noradrenergic nerve endings of the peripheral nervous system. The presynaptic opiate receptors are not present in the rabbit heart (Montel & Starke, 1973), or in the guinea-pig heart (Dubocovich & Langer, unpublished observations). Presynaptic angiotensin receptors are not present in the perfused cat spleen (Hertting & Suko, 1966; Langer & Enero, unpublished observations) and prostaglandin receptors of the E series are not present in the cat nictitating membrane (Enero & Langer, unpublished observations).

Except for the α- and β-presynaptic adrenoceptors, it appears that the other presynaptic receptors do not play a physiological role in noradrenergic neurotransmission and consequently are of pharmacological rather than physiological importance. However, as discussed below, these receptors can be acted upon by agonists or their analogues to modify sympathetic neurotransmission in the peripheral nervous system.

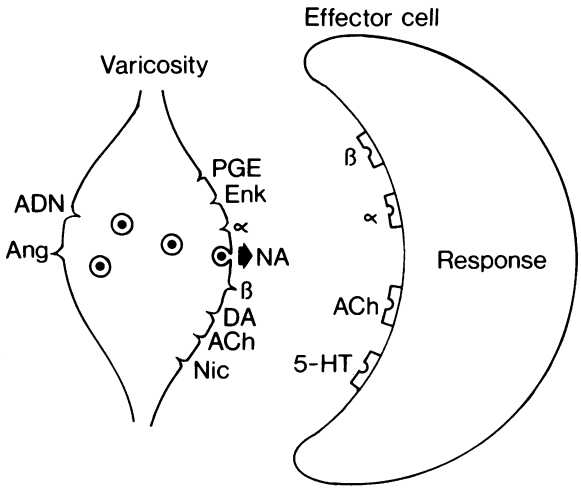

Figure 7 Schematic representation of the pre- and postsynaptic receptors in a noradrenergic neuroeffector junction in the peripheral nervous system. Noradrenergic varicosity. (1) PGE: prostaglandins of the E series, inhibition of noradrenaline (NA) release; (2) Enk: enkephalin opiate receptors: inhibition of NA release; (3) α-adrenoceptors: inhibition of noradrenaline release; (4) β-adrenoceptors, enhancement of noradrenaline release; (5) DA: dopamine receptors, inhibition of noradrenaline release; (6) ACh: muscarinic cholinoceptors, inhibition of noradrenaline release; (7) Nic: nicotinic receptors, enhancement of noradrenaline release; (8) ADN: adenosine receptors, inhibition of noradrenaline release; (9) Ang: angiotensin II receptors, enhancement of noradrenaline release.
Effector cell. Smooth muscle postsynaptic receptors. (1) β-adrenoceptors: relaxation; (2) α-adrenoceptors: contraction; (3) ACh: muscarinic cholinoceptors, contraction; (4) 5-HT: 5-hydroxytryptamine receptors, contraction. The smooth muscle of the cat nictitating membrane was used as an example.

The experimental evidence accumulated during recent years indicates that in addition to the multiple receptor sites in the postsynaptic effector cell there are also multiple receptor sites in the presynaptic membrane of nerve endings (Figure 7). Further research in the field of presynaptic receptors may clarify their physiological role and eventually open up new possibilities in therapeutics.

It has been suggested that activation of presynaptic α-adrenoceptors in the peripheral sympathetic system contributes to the antihypertensive effects of clonidine (Armstrong & Boura, 1973; Starke *et al.*, 1974; Langer *et al.*, 1975b; Langer, 1976).

More recently, it has been postulated that block of the presynaptic β-adrenoceptors in the periphery contributes significantly to the antihypertensive effects of β-receptor blocking agents (Langer *et al.*, 1975b; Adler-Graschinsky & Langer, 1975; Ljung *et al.*, 1975; Langer, 1976; Yamaguchi *et al.*, 1977).

Evidence for the presence of presynaptic receptors in the central nervous system

Results obtained on [^{3}H]-noradrenaline release in several areas of the central nervous system indicate that a similar negative feed-back mechanism mediated by presynaptic α-adrenoceptors is operative in noradrenergic nerve endings in the brain (Farnebo & Hamberger, 1971b; Starke & Montel, 1973b; Dismukes & Mulder, 1976). These conclusions are based on the observations that α-adrenoceptor agonists decrease while α-adrenoceptor blocking agents increase the release of [^{3}H]-noradrenaline induced by electrical field stimulation or by potassium in brain slices.

It has been suggested that activation by clonidine of *presynaptic* α-adrenoceptors in the central nervous system is causally related to its antihypertensive effects (Starke & Altman, 1973). Yet, Haeusler (1974) and Kobinger & Pichler (1976) demonstrated that clonidine and other imidazolines exert their sympathoinhibitory effects and thus hypotension and bradycardia, by stimulation of *postsynaptic* rather than *presynaptic* α-adrenoceptors in the central nervous system.

The α-adrenoceptors which mediate the decrease in central turnover of noradrenaline have been shown to differ from the central postsynaptic α-receptors and are probably presynaptic (Anden, Grabowska & Strömbom, 1976). In addition, it appears that presynaptic α-adrenoceptors in the central nervous system are involved in the analgesia and locomotor depression caused by clonidine (Paalzow & Paalzow, 1976; Strömbom, 1976).

The presence of presynaptic opiate receptors has been demonstrated in noradrenergic nerve endings of the rat cerebral cortex (Montel, Starke & Weber, 1974), although the physiological relevance of these presynaptic receptors and the role of the endogenous enkephalins remains to be clarified.

Presynaptic mechanisms in central dopaminergic neurones have also been described. These presynaptic dopaminergic receptors appear to be involved in the regulation of dopamine synthesis and release (Farnebo & Hamberger, 1971b; Carlsson, 1975; Roth, Walters, Murrin & Morgenroth, 1975; Iversen, Rogawski & Miller, 1976). Yet, the role of the presynaptic dopamine receptors in the regulation of transmitter release is still controversial. While Seeman & Lee (1975) reported that neuroleptic dopamine-blocking agents inhibited the electrically stimulated release of [^{3}H]-dopamine from rat striatal slices, Farnebo & Hamberger (1971b) found that the neuroleptics pimozide and chlorpromazine enhanced the release of [^{3}H]-dopamine under similar experimental conditions.

At present there is no evidence for the presence of presynaptic β-adrenoceptors in nerve endings of the central nervous system. Yet, the concentration-dependent increase in [^{3}H]-dopamine release during electrical stimulation of rat striatal slices obtained in the presence of dibutyryl cyclic AMP is compatible with the possibility that presynaptic β-adrenoceptors are involved in this phenomenon.

Szerb & Somogyi (1973) reported a negative feed-back mechanism for acetylcholine released by electrical stimulation from cerebral cortical slices which appears to be mediated by muscarinic presynaptic receptors.

It is possible that presynaptic regulatory mechanisms for transmitter release may be present for other neurotransmitters in the central nervous system, in addition to those described for noradrenaline, dopamine and acetylcholine. Further knowledge in this area may prove to be of great interest. Characterization of the types of presynaptic receptors involved in these regulatory mechanisms for transmitter release may prove to be very useful in designing new drugs with selective affinities for either the pre- or the postsynaptic receptors.

Conclusions

In addition to the classical receptors that mediate the responses of the effector organ, specific receptors are present in the outer surface of noradrenergic nerve endings. These presynaptic receptors are involved in the regulation of the release of the transmitter during nerve stimulation.

The presynaptic α-adrenoceptors mediate a negative feed-back mechanism which leads to inhibition of transmitter release probably by restricting the calcium available for the excitation-secretion coupling.

The presynaptic β-adrenoceptors mediate a positive feed-back mechanism which is activated at low frequencies of nerve stimulation leading to an increase in transmitter release. This mechanism appears to be mediated through an increase in the levels of cyclic AMP in noradrenergic nerve endings.

Comparison of the pre- and postsynaptic potencies of α-receptor agonists and antagonists indicates that the pre- and the postsynaptic α-adrenoceptors are not identical.

In addition to the presynaptic α- and β-adrenoceptors, other presynaptic receptors have been described: inhibitory dopamine, opiate and muscarinic receptors. Also the inhibition of release induced by adenosine and by prostaglandins of the E series may be linked to specific presynaptic receptors.

Some of these presynaptic receptors are also present in nerve endings in the central nervous system, like the α-adrenoceptor and opiate inhibitory receptors.

Agonists and antagonists with high affinity for presynaptic receptors may elicit pharmacological effects which are due to changes in transmitter release. Such drugs may open new possibilities in therapeutics.

I would like to thank all my colleagues who made the work described here possible. I would also like to express my gratitude to the National Research Council of Argentina (CONICET) for the support given to this research (1969–1976) and to the Wellcome Foundation for the stimulating scientific atmosphere in which the manuscript was prepared.

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