Br. J. Pharmac. (1975), 53, 43-50

POSSIBLE ROLE OF A β-ADRENOCEPTOR IN THE REGULATION OF NORADRENALINE RELEASE BY NERVE STIMULATION THROUGH A POSITIVE FEED-BACK MECHANISM

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1 The effects of isoprenaline, propranolol and phentolamine, were studied on tritiated noradrenaline overflow elicited by postganglionic nerve stimulation in guinea-pig isolated atria.

2 Isoprenaline (1.2×10^{-8}M) increased while propranolol (1.0×10^{-7}M) reduced the overflow of tritiated noradrenaline evoked by nerve stimulation. These effects were less than those of phentolamine (3.1×10^{-6}M), which increased by approximately three-fold the overflow of [^{3}H]-noradrenaline elicited by nerve stimulation.

3 Neuronal accumulation of tritiated noradrenaline in guinea-pig atria was not affected by isoprenaline, propranolol or phentolamine at the concentrations employed in this study.

4 Isoprenaline (1.2×10^{-8}M) induced a positive chronotropic effect of about 80% of the maximum. On the other hand, propranolol produced a shift to the right in the frequency-response curve to nerve stimulation and in the concentration-response curve to exogenous noradrenaline in guinea-pig atria.

5 In the isolated nictitating membrane of the cat, the frequency-response curve to nerve stimulation was not modified by propranolol, while in the presence of 3.9×10^{-6}M of *N*,-2-(2,6-dimethylphenoxy)propyl-*N*,*N*,*N*-trimethylammonium (β-methyl-TM 10) there was a shift to the right and a depression of slope. Neither propranolol nor β-methyl-TM 10 affected responses to exogenous noradrenaline.

6 The effects of isoprenaline and of propranolol on transmitter release are compatible with the view that in addition to the presynaptic negative feed-back mechanism for noradrenaline release by nerve stimulation mediated via α-adrenoceptors a positive feed-back mechanism exists in adrenergic nerve endings which is triggered through the activation of presynaptic β-adrenoceptors.

Introduction

The release of noradrenaline by nerve stimulation appears to be regulated through a negative feed-back mechanism mediated by presynaptic α-adrenoceptors (Langer, Adler, Enero & Stefano, 1971; Farnebo & Hamberger, 1971; Kirpekar & Puig, 1971; Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972a). According to this hypothesis, once the transmitter released by stimulation reaches a threshold concentration in the synaptic gap, it activates presynaptic α-adrenoceptors, triggering a negative feed-back mechanism that inhibits further release of the transmitter. The experimental evidence that led to postulation of this mechanism is based on the inhibition of transmitter release in the presence of α-adrenoceptor agonists (Starke, 1972a; Starke, 1972b) and the increase in transmitter release obtained in the presence of α-adrenoceptor blocking agents (Langer, 1970; Farnebo & Hamberger, 1971; Starke, Montel & Schümann, 1971; Enero *et al.*, 1972).

On the other hand, there is little information about the effects of β-adrenoceptor agonists and antagonists on transmitter released by nerve stimulation, although propranolol and other β-adrenoceptor blocking agents are used in the treatment of hypertension (Dorph & Binder, 1969; Prichard & Gillam, 1969; Tibblin & Åblad, 1969; Zacharias & Cowan, 1970; Thorpe, 1972) and it appears that inhibition of release of adrenergic transmitter may contribute to their hypotensive effect (Mylecharane & Raper, 1970; Eliash & Weinstock, 1971).

Consequently, it was of interest to study the

effects of isoprenaline and propranolol on [^{3}H]-noradrenaline release evoked by accelerans nerve stimulation in guinea-pig isolated atria.

Methods

Guinea-pig atria

Guinea-pigs of 300 to 500 g of either sex were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.). The hearts were removed and both atria were dissected together with the accelerans nerve, in Locke solution of the following composition (mM): NaCl, 154.0; KCl, 5.6; $CaCl_2$, 2.2; $NaHCO_3$, 6.0; glucose, 11.1; ethylenediamine tetraacetic acid disodium salt (EDTA), 0.04 and ascorbic acid, 0.11. Atropine, 1.4×10^{-6}M was added to the Locke solution which was bubbled continuously with O_2. The atria, together with the accelerans nerve were set up in an isolated organ bath fitted with platinum electrodes for nerve stimulation. The tissue was attached to a force displacement transducer connected to a Grass polygraph to record spontaneous contractions of the preparation. All experiments were carried out in oxygenated Locke solution at 37°C.

In experiments in which the noradrenaline stores were labelled, the atria were incubated for 30 min with 10 μCi/ml of (±)-[7-^{3}H]-noradrenaline (New England Nuclear Corporation, Boston, Mass) as described previously by Adler-Graschinsky, Langer & Rubio (1972).

The accelerans nerves were stimulated at 4 Hz for 60 s with square pulses of 0.5 ms duration and supramaximal voltage. In these experiments, two periods of nerve stimulation were applied. The first period of stimulation (S_1) was applied 85 min after the end of the incubation of the atria with tritiated noradrenaline. The second stimulation (S_2) was applied 40 min later.

The total increase in outflow of tritium elicited by nerve stimulation was expressed as the fractional release per shock (FR), i.e. the total nCi released per shock divided by the total nCi remaining in the tissue at the onset of stimulation (Enero & Langer, 1973). The radioactivity in the bathing solution and in the tissue was measured by scintillation counting as described by Adler-Graschinsky *et al.* (1972).

In separate experiments, the accelerans nerves were stimulated for 20 s at different frequencies at supramaximal voltage, and the maximal increase in atrial rate was determined. The interval between each period of nerve stimulation at different frequencies was 5 min, this being sufficient for the atrial rate to return to the resting values. The following frequencies of stimulation were applied: 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.5, 25 and 50 Hz. Two consecutive frequency-response curves were determined in each preparation, the second starting 30 min after the end of the first.

Cumulative concentration-response curves to noradrenaline were determined once only in each of several preparations by stepwise increase in the concentration by a factor of three as soon as the response to the previous administration had levelled off (i.e. at intervals of 2 to 4 minutes).

For studies of uptake and retention of [^{3}H]-noradrenaline, the atria were dissected as described above, placed in a separate beaker containing 5 ml of Locke solution, set in a Dubnoff metabolic incubator at 37°C and gassed with O_2. The drugs tested were added to the Locke solution which was then pre-incubated for 30 minutes. Then 20 ng/ml of [^{3}H]-noradrenaline was added and incubation was continued for a further 20 min after which the tissue was washed for 5 min in fresh Locke solution, blotted, weighed and homogenized with 5 ml of cold 0.4N perchloric acid containing EDTA 1.0 mg/ml and Na_2SO_3 1.25 mg/ml. Tritiated and cold noradrenaline were isolated by chromatography in alumina columns (Graefe, Stefano & Langer, 1973). Endogenous noradrenaline was determined according to Laverty & Taylor (1968) and [^{3}H]-noradrenaline was measured by scintillation counting.

Isolated cat nictitating membranes

Cats of 1.8 to 3.6 kg body weight and of either sex were used. The animals were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.) and the trachea was cannulated. The eyeball was excised and the nictitating membrane with all the adjoining tissue was removed from the orbit. The tissue was placed in a Petri dish with modified Krebs solution previously equilibrated with 95% O_2 and 5% CO_2. The composition of the Krebs solution was as follows (mM): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.6; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; glucose, 11.1; EDTA, 0.004 and ascorbic acid, 0.11. Under a binocular microscope, the medial muscle was dissected together with the postganglionic sympathetic fibres arising from the infratrochlear nerve which innervates this muscle of the nictitating membrane (Thompson, 1958). The cartilage on which the fibres of the medial muscle are inserted was fixed to the bottom of a 10 ml organ bath. The upper end of the muscle was connected to a force-displacement transducer and the tension developed by the muscle was recorded with a Grass polygraph. The temperature was maintained at 37°C and the organ bath was bubbled with 95% O_2 and 5% CO_2. The

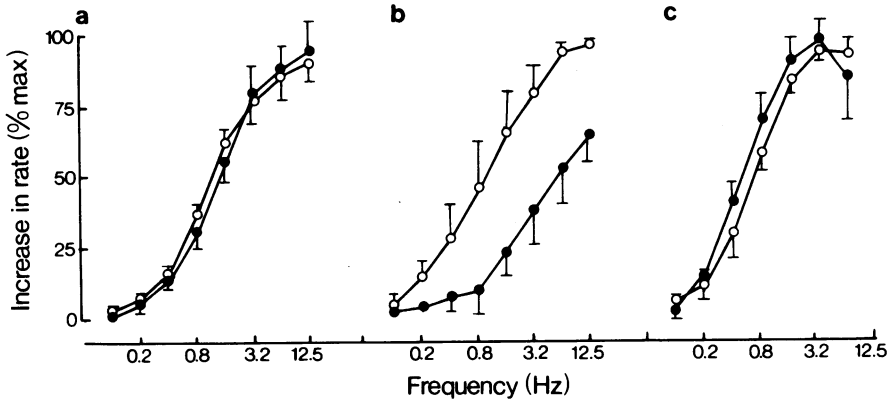

Figure 1 Effects of propranolol and of phentolamine on the positive chronotropic effects elicited by accelerans nerve stimulation in guinea-pig isolated atria. Ordinates: increase in atrial rate as % of the maximum of the first (before treatment) frequency-response curve. Abscissae: frequency of nerve stimulation (Hz). Frequency response curves obtained before (○) and 30 min after (●) treatment in (a), controls (n = 6); (b), atria exposed to propranolol (1.0×10^{-7}M) for 30 min before the second frequency-response curve (n = 3) and (c), atria exposed to phentolamine (3.1×10^{-6}M) for 30 min before the second frequency-response curve (n = 5). Mean values are shown. Vertical bars indicate s.e. mean. n = number of experiments.

infratrochlear nerve was pulled through shielded bipolar platinum electrodes for stimulation with monophasic square pulses of 0.5 ms duration and supramaximal voltage. Before starting experiments a period of 60 min was allowed to elapse during which the resting tension of the muscle was repeatedly adjusted to 2.5 g.

The postganglionic sympathetic fibres were stimulated at the progressively increasing frequencies of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.5, 25 and 50 Hz, the frequency being stepped up whenever the response of the nictitating membrane to the previous frequency of stimulation had levelled off. Two frequency-response curves were determined in each preparation, the interval between them being 40 minutes. Twenty min after the second determination, a concentration-response curve to (−)-noradrenaline was determined by adding the drug cumulatively in such a way that the final concentration in the bath was increased by a factor of about 3 whenever a steady response to the previous concentration was achieved.

Statistical calculations were performed according to conventional procedures (Snedecor & Cochran, 1967).

The following drugs were used: (−)-noradrenaline bitartrate monohydrate, (±)-propranolol hydrochloride, (±)-isoprenaline hydrochloride, [*N*-2-(2,6-dimethylphenoxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (β-methyl-TM 10) and phentolamine hydrochloride.

Results

Chronotropic responses to accelerans nerve stimulation and to exogenous noradrenaline

The frequency-response curve for the positive chronotropic effects of accelerans nerve stimulation is shown in Figure 1. The maximum increase in atrial rate (112 ± 21 beats/min) was usually obtained with a frequency of stimulation of 12.5 Hz and this did not differ significantly from that obtained with exogenous noradrenaline (130 ± 5 beats/minute).

There was no difference between two consecutive frequency-response curves under control conditions (Figure 1a). In the presence of propranolol (1.0×10^{-7}M) there was a marked shift to the right (Fig. 1b) and after exposure to phentolamine, (3.1×10^{-6}M) the curve tended to shift to the left but not to a significant degree (Figure 1c). In the presence of propranolol, there was a similar shift to the right in the concentration-response curve to exogenous noradrenaline (Figure 2).

Transmitter overflow elicited by accelerans nerve stimulation

In a control group of atria there was no difference either between the chronotropic responses or between the fractional releases per shock observed with two consecutive periods of nerve stimulation

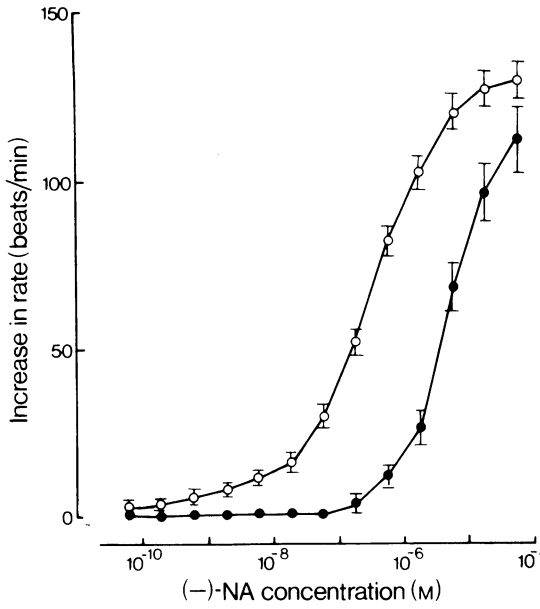

Figure 2 Effect of propranolol on the concentration-response curve to noradrenaline (NA) in spontaneously beating guinea-pig atria. (○) Controls (n = 16); (●) after exposure to propranolol (1.0×10^{-7}M) for 30 min (n = 7). Mean values are shown. Vertical bars indicate s.e. mean. n = number of experiments.

(Table 1). When isoprenaline (1.2×10^{-8}M) was added, a sustained positive chronotropic effect was obtained, the maximum being 98 ± 11 beats/min (mean ± s.e. mean of 6 observations). The increase in rate was still 84 ± 12 beats/min 20 min later when a second period of nerve stimulation was applied. The responses to nerve stimulation in the presence of isoprenaline were significantly reduced and this is attributable to the high atrial rate prior to nerve stimulation. Table 1 also shows that exposure to isoprenaline increased significantly the release of [^{3}H]-noradrenaline by nerve stimulation.

Propranolol, (1.0×10^{-7}M) did not affect the spontaneous rate but it decreased significantly the chronotropic responses to nerve stimulation. In the presence of the β-adrenoceptor blocking agent there was a significant reduction in the transmitter released by nerve stimulation as judged by the S_2/S_1 ratio (Table 1).

Exposure to phentolamine (3.1×10^{-6}M) reduced the spontaneous rate slightly but did not affect the positive chronotropic effect elicited by nerve stimulation. This concentration of the α-adrenoceptor blocking agent increased by more than three-fold the transmitter released by nerve stimulation (Table 1).

Effects of isoprenaline, propranolol and phentolamine on neuronal uptake of [^{3}H]-noradrenaline

The changes in transmitter overflow elicited by nerve stimulation in the presence of isoprenaline,

Table 1 Effects of isoprenaline, propranolol and phentolamine on atrial rate and on [^{3}H]-noradrenaline release elicited by nerve stimulation in guinea-pig isolated atria.

Experimental group	*No.*	*Resting rate (a) (beats/min)*	*Maximum increase in rate during stimulation (b) (beats/min)*	*Fraction released per shock ($\times 10^{-5}$) (c)*	*Release S_2/S_1*
Control	6	R_1 = 180 ± 9	S_1 = 82 ± 8	S_1 = 1.11 ± 0.18	
		R_2 = 187 ± 10	S_2 = 72 ± 8	S_2 = 1.12 ± 0.22	1.03 ± 0.11
Isoprenaline	6	R_1 = 193 ± 9	S_1 = 100 ± 9	S_1 = 1.07 ± 0.24	
1.2×10^{-8}M		R_2 = 276 ± 12***	S_2 = 25 ± 14***	S_2 = 1.66 ± 0.39	1.55 ± 0.19*
Propranolol	10	R_1 = 187 ± 7	S_1 = 92 ± 9	S_1 = 0.93 ± 0.13	
1.0×10^{-7}M		R_2 = 187 ± 7	S_2 = 66 ± 7*	S_2 = 0.55 ± 0.10	0.59 ± 0.08**
Phentolamine	4	R_1 = 202 ± 6	S_1 = 80 ± 4	S_1 = 0.99 ± 0.23	
3.1×10^{-6}M		R_2 = 184 ± 4*	S_2 = 86 ± 4	S_2 = 3.02 ± 0.60	3.68 ± 1.13**

Mean values ± s.e. mean for: (a) Resting rates (R_1 and R_2) prior to each of two 1 min periods of nerve stimulation at 4 Hz (S_1 and S_2), (b) Maximum increase in atrial rate elicited by the first (S_1) and the second (S_2) period of nerve stimulation, (c) Fraction released per shock: total nCi released per shock divided by the total nCi remaining in the tissue at the onset of nerve stimulation. Isoprenaline, propranolol and phentolamine were added at 20, 40 and 30 min respectively before S_2. Significance of difference from the corresponding control value – *$P < 0.05$; **$P < 0.025$; ***$P < 0.005$.

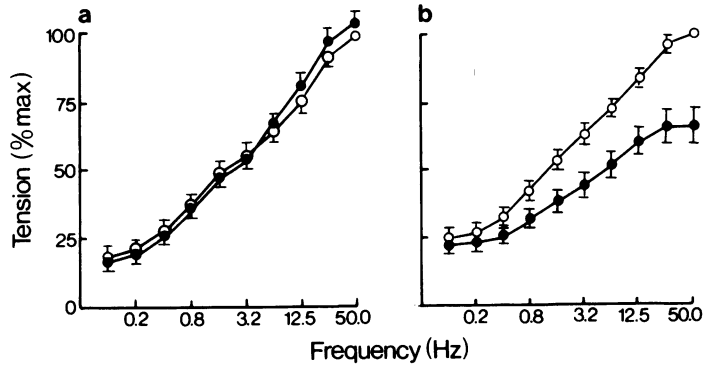

Figure 3 Effects of propranolol and of β-methyl-TM 10 on responses of the isolated medial muscle of the cat nictitating membrane to nerve stimulation. The relationship between the frequency of nerve stimulation and the percent of maximum response was determined before (○) and 30 min after (●) exposure to propranolol (1.0×10^{-7}M) in 6 preparations (a) and to β-methyl-TM 10 (3.9×10^{-6}M) in 7 preparations (b). Mean values are shown. Vertical bars indicate s.e. mean.

propranolol or phentolamine could be due to an effect of these drugs on neuronal uptake of noradrenaline. This possibility was explored in experiments in which the uptake and retention of [^{3}H]-noradrenaline was determined after exposure to these drugs.

Table 2 shows that there was no difference between the endogenous noradrenaline contents of the groups studied. Exposure to isoprenaline, propranolol or phentolamine in the concentrations at which these drugs modified transmitter overflow during nerve stimulation, did not affect the accumulation of [^{3}H]-noradrenaline in the atria. Consequently, the effects of these drugs on transmitter release were not related to changes in neuronal uptake of noradrenaline.

Effects of propranolol or β-methyl-TM 10 on responses of the isolated nictitating membrane of the cat to nerve stimulation and to exogenous noradrenaline

It has been reported that propranolol reduces the responses to nerve stimulation by causing adrenergic neurone blockade (Mylecharane & Raper, 1970; Eliash & Weinstock, 1971). Consequently, it was of interest to determine whether the concentration of propranolol employed in our experiments affected responses to sympathetic nerve stimulation in the isolated nerve muscle preparation of the cat nictitating membrane.

There was no difference between the two consecutive frequency-response curves either in controls ($n = 9$) or in preparations treated with propranolol (1.0×10^{-7}M) (Figure 3a). In contrast, exposure to the adrenergic neurone blocking agent β-methyl-TM 10 (3.9×10^{-6}M) decreased the slope of the frequency-response curve to nerve stimulation producing a shift to the right and a depression of the maximum (Figure 3b). At these concentrations, neither β-methyl-TM 10 (7 preparations), nor propranolol (6 preparations) modified the pD_2 values or the maximum development of tension in response to exogenous noradrenaline by comparison with controls (35 preparations).

Table 2 Effects of isoprenaline, propranolol and phentolamine on neuronal uptake of [^{3}H]-noradrenaline in guinea-pig isolated atria.

Experimental group	n	*Endogenous content (μg NA/g tissue)*	*Neuronal uptake (ng [^{3}H]-NA/μg NA) (a)*
Controls	21	3.91 ± 0.20	31.3 ± 1.48
Isoprenaline 1.2×10^{-8}M	12	3.72 ± 0.18	27.3 ± 1.52
Propranolol 1.0×10^{-7}M	6	3.80 ± 0.20	33.0 ± 2.18
Phentolamine 3.1×10^{-6}M	6	3.16 ± 0.25	35.8 ± 2.45

(a) Neuronal uptake is expressed as ng [^{3}H]-noradrenaline (NA) taken up per μg of endogenous noradrenaline. The atria were exposed to the drug during a 30 min preincubation period and during a further 20 min incubation with 20 ng of [^{3}H]-noradrenaline. Values are mean ± s.e. mean. n = number of experiments.

Discussion

The frequency of stimulation at which the release experiments (Table 1) were carried out (4 Hz, 60 s) elicits a chronotropic response of approximately 80% of the maximum (Figure 1). Under these experimental conditions, exposure to phentolamine, in a concentration which does not inhibit neuronal uptake of [^{3}H]-noradrenaline (Table 2) increased more than three-fold the transmitter released by nerve stimulation. This effect is attributed to the block of the presynaptic α-adrenoceptors which mediate the negative feed-back mechanism that regulates noradrenaline release by nerve stimulation (Langer, 1974).

In spite of the increase in transmitter overflow observed in the presence of phentolamine the positive chronotropic response obtained under these conditions was not appreciably enhanced. It is likely that the negative chronotropic effect of phentolamine offsets the effect of increase in transmitter release. A negative chronotropic effect was also observed with phentolamine in the perfused cat heart (Farah & Langer, 1974).

An increase in transmitter release was observed in the presence of isoprenaline but this was much less pronounced than that obtained with phentolamine. As was shown for phentolamine, the effect of isoprenaline could also be dissociated from inhibition of neuronal uptake of noradrenaline (Table 2). In addition, the effect of isoprenaline on transmitter release appears to be unrelated to the activation of β-adrenoceptors of the effector organ because this agent also increases the release of [^{3}H]-noradrenaline during nerve stimulation in the cat nictitating membrane (Enero & Langer, unpublished observations) where the responses of the organ are mediated through α-receptors.

The decrease in transmitter release observed in the presence of the concentration of propranolol used (1.0×10^{-7}M) appears to be unrelated to either an increase in neuronal uptake of noradrenaline (Table 2) or to an adrenergic neurone blocking effect of the drug (Figure 3) because this concentration did not affect uptake of [^{3}H]-noradrenaline by guinea-pig atria nor reduce responses to nerve stimulation of the cat nictitating membrane. In addition, the concentration of propranolol employed in our experiments produced a shift to the right of the frequency-response curve to nerve stimulation in isolated atria (Figure 1) which should be attributed predominantly to the blockade of β-adrenoceptors because a similar shift to the right was obtained in the concentration-response curve to exogenous noradrenaline (Figure 2).

A decrease in responses to adrenergic nerve stimulation has been described after the administration of propranolol in the guinea-pig vas deferens (Mylecharane & Raper, 1970) and in the cat nictitating membrane *in vivo* (Eliash & Weinstock, 1971). These effects were attributed to an adrenergic neurone blocking effect of propranolol. In addition, it has been reported that high concentrations of propranolol potentiate responses to adrenergic nerve stimulation (Eliash & Weinstock, 1971) or increase the output of noradrenaline by nerve stimulation (Werner, Wagner & Schümann, 1971). The latter is related to the ability of high concentrations of propranolol to inhibit reuptake of noradrenaline (Werner *et al.*, 1971).

The concentration of propranolol employed in our experiments did not reduce responses to nerve stimulation in the nictitating membrane and it reduced to nearly the same extent the responses to exogenous noradrenaline and to nerve stimulation in guinea-pig atria. Consequently, it is suggested that under our experimental conditions the effects of propranolol were not due to adrenergic neurone blockade.

The increase in transmitter release observed in the presence of isoprenaline and the decrease in transmitter release obtained during exposure to the β-receptor blocking agent, propranolol, indicate that these agents may influence transmitter release by acting on β-adrenoceptors. The results obtained are compatible with the existance in adrenergic nerve endings of a regulatory mechanism for noradrenaline release by nerve stimulation mediated through β-adrenoceptors. It is supposed that activation of this mechanism by β-adrenoceptor agonists leads to an increase in transmitter release during nerve stimulation.

According to our working hypothesis, the positive feed-back mechanism mediated via presynaptic β-adrenoceptors is activated during noradrenaline release until the transmitter in the synaptic gap reaches the threshold concentration that triggers the negative feed-back mechanism mediated by presynaptic α-receptors, resulting in inhibition of release. Thus, noradrenaline release by nerve stimulation may be modulated by two different presynaptic mechanisms. The first one, mediated by β-adrenoceptors, would be activated by low concentrations of the transmitter, leading to an increase in the release of noradrenaline per stimulus. The second one, mediated via α-receptors, would be triggered when higher concentrations of noradrenaline are reached in the synaptic gap, leading to the inhibition of transmitter release.

Compatible with this working hypothesis is the finding that the concentrations of noradrenaline

Table 3 Potencies of (−)-noradrenaline on the α- and β-adrenoceptors in several tissues

Tissue	*Species*	*Adrenoceptor*	*pD_2*	*Reference*
Splenic strip	cat	α	5.02*	Granata & Langer (1973)
Nictitating membrane	cat	α	5.04 ± 0.04	Langer & Rubio (1973)
Vas deferens	rat	α	5.23	Patil, LaPidus & Tye (1967)
Vas deferens	guinea-pig	α	4.48 ± 0.15	Nedergaard & Westermann (1968)
Tracheal strips	guinea-pig	β	6.52*	Furchgott (1967)
Atria	guinea-pig	β	6.52 ± 0.07	Langer & Rubio (1973)
Left atria	cat	β	7.64*	Osorio, Stefano & Langer (1974)
Papillary muscle	cat	β	7.66*	Kaumann (1970)
Fat cells (lipolysis)	rat	β	7.30*	Fain (1967)

pD_2: mean negative log of the molar concentration producing 50% of the maximal response.
* Calculated values.

required to activate postsynaptic α-adrenoceptors in general are at least 30 to 100 times higher than those necessary for the activation of the β-adrenoceptors (Table 3).

The results obtained in the present experiments do not exclude the possibility that propranolol and other β-adrenoceptor antagonists have an adrenergic neurone blocking effect under certain experimental conditions but in the experiments by Mylecharane & Raper (1970) and by Eliash & Weinstock (1971) blockade by propranolol of presynaptic β-adrenoceptors concerned with a positive feed-back mechanism may have contributed to the decrease in noradrenaline release by nerve stimulation.

The mechanism of action of β-adrenoceptor antagonists in the treatment of essential hypertension is not clear (Dorph & Binder, 1969; Prichard & Gillam, 1969; Tibblin & Åblad, 1969; Zacharias & Cowan, 1970; Thorpe, 1972). It is possible that a decrease in transmitter release obtained with propranolol contributes significantly to the hypotensive effects of this agent and other β-receptor blocking agents in hypertensive patients.

Several effects elicited by the stimulation of β-receptors have been attributed to the activation of adenyl-cyclase and the consequent increase in the tissue levels of cyclic adenosine-3′,5′-monophosphate (AMP). It is of interest that Wooten, Thoa, Kopin & Axelrod (1973) reported that dibutyryl cyclic AMP and theophylline increase the release of noradrenaline elicited by nerve stimulation in the guinea-pig vas deferens. In addition, monobutyryl cyclic AMP and several phosphodiesterase inhibitors increase transmitter release in the perfused cat spleen (Cubeddu, Weiner & Langer, unpublished observations). Consequently, it is possible that the proposed positive feed-back mechanism which is triggered by the activation of presynaptic β-adrenoceptors may be mediated through an increase in the levels of cyclic AMP in adrenergic nerve endings.

The authors are indebted to Dr S. Archer of Sterling-Winthrop Research Institute, U.S.A., for the supply of (−)-noradrenaline and isoprenaline, to Dr R.A. McLean of Smith, Kline and French Laboratories, Philadelphia, U.S.A. for β-TM 10 and to Dr M. Brunner of Ciba-Geigy Laboratories, Basel, Switzerland for phentolamine.

The technical assistance of Mrs Elina de Di Nasso and Miss María J. Rodriguez is gratefully acknowledged.

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(Revised June 14, 1974)