Br. J. Pharmac. (1974), 52, 549-557

PROTECTION BY PHENTOLAMINE AGAINST THE EFFECTS OF PHENOXYBENZAMINE ON TRANSMITTER RELEASE ELICITED BY NERVE STIMULATION IN THE PERFUSED CAT HEART

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1 The effects of cocaine, phentolamine and phenoxybenzamine on neuronal uptake of [^{3}H]-noradrenaline and on ^{3}H-transmitter and noradrenaline overflow elicited by nerve stimulation were determined in the perfused heart of the cat.

2 During perfusion with cocaine 3.4×10^{-7}M, there was a 2-fold increase in transmitter overflow while neuronal uptake of [^{3}H]-noradrenaline was inhibited by 31.3 ± 2.1%.

3 After exposure to phenoxybenzamine 8.7×10^{-7} M for 20 min and washing with drug-free solution for 165 min there was an 8-fold increase in transmitter overflow during nerve stimulation. Under these conditions neuronal uptake of [^{3}H]-noradrenaline was inhibited by only 17.5 ± 5.4%.

4 There was no significant change in transmitter overflow or in neuronal uptake of [^{3}H]-noradrenaline, 155 min after a 30 min exposure to phentolamine (3.2×10^{-5}M).

5 Perfusion with phentolamine (3.2×10^{-5}M) before and during exposure to phenoxybenzamine (8.7×10^{-7}M), prevented the increase in transmitter overflow observed after perfusion with phenoxybenzamine alone.

6 Protection by phentolamine against the effects of phenoxybenzamine supports the view that the effects on transmitter release obtained after perfusion with phenoxybenzamine are due to the blockade of presynaptic α-adrenoceptors which regulate transmitter release through a negative feed-back mechanism.

Introduction

The release of noradrenaline by nerve stimulation appears to be regulated by a negative feed-back mechanism mediated by presynaptic α-adrenoceptors (Langer, Adler, Enero & Stefano, 1971; Farnebo & Hamberger, 1971; Starke, 1971; Kirpekar & Puig, 1971; Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972; Enero & Langer, 1973; Langer, 1974). In support of this hypothesis it has been demonstrated that the α-receptor blocking agents phentolamine and phenoxybenzamine increase transmitter release in concentrations at which neither neuronal nor extraneuronal uptake are inhibited (Starke, Montel & Schümann, 1971a; Starke, Montel & Wagner, 1971b; Farnebo & Hamberger, 1971; Enero *et al.*, 1972; Dubocovich & Langer, 1974).

However, the increase in transmitter overflow obtained with phenoxybenzamine is much more pronounced than that observed with phentolamine (Farnebo & Hamberger, 1971; Farah & Langer, 1972; Adler-Graschinsky & Langer, 1972). Consequently, it is possible that the increase in transmitter overflow obtained in the presence of phenoxybenzamine is not due entirely to the blockade of the α-adrenoceptors but may reflect other sites of action of this drug.

The aim of the present experiments was to test whether phentolamine and phenoxybenzamine increase transmitter overflow by acting on the same presynaptic α-adrenoceptors in the perfused heart of the cat. Consequently, studies on transmitter release were carried out in which the reversible competitive antagonist, phentolamine, was tested for protection against the irreversible block of these α-adrenoceptors by phenoxybenzamine.

Methods

Studies of [^{3}H]-noradrenaline overflow during nerve stimulation

Cats of either sex (2-4 kg body weight) were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.). In each the trachea was cannulated and 2500 i.u. heparin was injected intravenously. The heart with intact left and right sympathetic supply was dissected out and the coronary vessels were perfused with Krebs solution through an aortic cannula according to the Langendorff technique. 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; disodium edetate (EDTA) 0.004 and ascorbic acid 0.11. Atropine 1.4×10^{-6}M was added to the Krebs solution, which was bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C. Chronotropic responses were measured with the aid of a small latex balloon introduced through an incision in the left atrium up to the left ventricle cavity. The balloon was connected to a Statham Model P23AC Pressure Transducer and the frequency of the heart was recorded on a Grass Polygraph. Thirty min after perfusion of the heart was started, an infusion of (−)-[7-^{3}H]-noradrenaline (New England Nuclear, S.A., 6.4 Ci/mmol) was given through the aortic cannula during 20 min at a rate of 2.5 μCi/min (total infused: 50 μCi). The heart was perfused for a further 60 min before the periods of nerve stimulation began. The right postganglionic sympathetic nerves were prepared as described by Huković & Muscholl (1962) and a pair of platinum electrodes was hooked to the nerves. Stimulation was carried out with an S−44 Grass Stimulator; square pulses of 0.5 ms duration and supramaximal voltage were applied. The nerves were stimulated at 5 Hz for periods of 60 s (total of 300 shocks).

In release experiments, the sympathetic nerves were stimulated for each of three periods (S_1, S_2, S_3); S_2 was applied 25 min after S_1 and S_3 185 min after S_2. Drugs were infused for 20 min (phenoxybenzamine) or 30 min (phentolamine) immediately following S_2. When the two α-receptor blocking agents were perfused, phentolamine was added to the Krebs solution 10 min before the addition of phenoxybenzamine and then both drugs were perfused simultaneously for 20 minutes. One min samples of the venous effluent were collected before, during and after each period of stimulation. An aliquot of 10 ml of each sample was adjusted to pH 2-3 with 1 N HCl; 0.2 ml of 10% EDTA and 0.4 ml of 12.5% Na_2SO_3 were added to each sample before storage at 0°C. At the end of each experiment the heart was removed, and portions of both atria and both ventricles were dissected, blotted on filter paper and weighed. These tissues were homogenized in 10 ml of cold 0.4N perchloric acid per g of tissue, containing 1 mg of EDTA and 1.25 mg of Na_2SO_3 per ml. The homogenates were kept at 4°C for 60 min and then centrifuged at 5000 rev/min for 15 minutes.

Separation of noradrenaline in tissue and in perfusate samples was performed according to the methods described by Graefe, Stefano & Langer (1973). The fluorimetric determination of noradrenaline was carried out according to Laverty & Taylor (1968) on 1 ml of the 0.2N acetic acid fraction from the alumina columns. Total radioactivity was measured in 1 ml aliquots of the venous effluent or of the tissue homogenate by liquid scintillation counting (Graefe *et al.*, 1973).

The means ($n = 4$) of the endogenous noradrenaline contents of the four chambers of the heart did not differ significantly for the four treatment groups: 2.01 ± 0.22 μg noradrenaline/g in the control, 2.04 ± 0.24 μg/g after phenoxybenzamine, 1.69 ± 0.21 μg/g after phentolamine and 2.33 ± 0.17 μg/g after phentolamine plus phenoxybenzamine. In each group more than 90% of the total radioactivity remaining in the heart at the end of the experiment was accounted for by [^{3}H]-noradrenaline.

Overflow induced by nerve stimulation was calculated by subtracting from the values of the samples obtained during and after the period of stimulation the spontaneous outflow assumed to have occurred in each sample. The value of the spontaneous outflow subtracted was the basal resting release obtained in the period immediately before stimulation. The 'overflow of the transmitter' was the sum of all increases above spontaneous levels induced by the period of nerve stimulation. There was considerable scatter in the absolute values for transmitter overflow in the different experiments. However, the variability was reduced when the overflow of radioactivity caused by S_3 was expressed as percent of the mean value of overflow obtained during the two control periods of stimulation in the same preparation (Table 1). The values for S_3 after treatment with drugs were adjusted in proportion to the 50% decay in the control group (Table 1, Figure 1).

Studies of [^{3}H]-noradrenaline uptake

In separate experiments (in which the accelerans nerve was not stimulated) the ability of the heart to take up exogenous noradrenaline was tested by infusion of (−)-[7-^{3}H]-noradrenaline for 3 min at a rate of 1.5 nCi/min (0.04 ng/min) with a Harvard

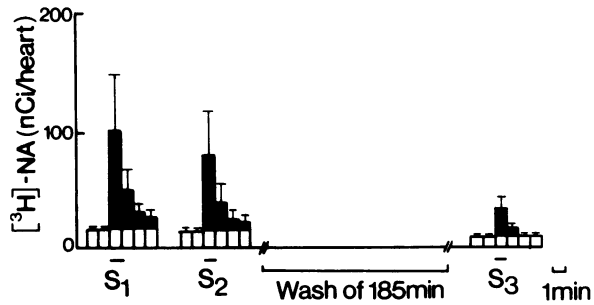

Fig. 1 Overflow of the tritiated transmitter ([^{3}H]-NA) elicited by nerve stimulation in the perfused cat heart. Ordinates: nCi per heart. The bars beneath the abscissa scale indicate the 1 min periods of nerve stimulation (S_1, S_2, S_3). The open columns represent the spontaneous outflow of radioactivity in consecutive 1 min samples. The black portion indicates the increase in outflow of radioactivity above the resting levels produced by nerve stimulation. S_1 and S_2 are two consecutive periods of nerve stimulation with an interval between of 25 minutes. After the second period of stimulation the heart was perfused for 185 min before the third period of stimulation (S_3). Nerve stimulation: 5 Hz, 0.5 ms duration and supramaximal voltage. The values shown are the means of six experiments. Vertical bars indicate s.e. mean.

pump. The amount of noradrenaline removed (R) from the perfusion was calculated as the percent of the arterio-venous difference:

$$R = \frac{A - V}{A} \times 100,$$

where A is the arterial and V the venous concentration of [^{3}H]-noradrenaline.

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

Drugs

The following drugs were used: (−)-noradrenaline bitartrate monohydrate; phenoxybenzamine hydrochloride; phentolamine hydrochloride; cocaine hydrochloride and atropine sulphate.

Results

Effects of phenoxybenzamine and phentolamine on transmitter overflow elicited by nerve stimulation in the perfused cat heart

Under control conditions there was no difference between the overflows with two consecutive periods of nerve stimulation separated by an interval of 25 min (Fig. 1 and Table 1). The results obtained with [^{3}H]-noradrenaline were similar to those observed for endogenous noradrenaline. After the second period of stimulation (S_2) the heart was perfused for 185 min before the third period of stimulation (S_3) was applied. Figure 1 and Table 1 show that the overflow of transmitter elicited by the third period of nerve stimulation was approximately one half of the corresponding

Table 1 Overflow of noradrenaline and [^{3}H]-noradrenaline elicited by nerve stimulation in the perfused cat heart

		Overflow elicited by nerve stimulation				
				S_2		S_3
	n	S_1	S_2	*% of* S_1	n	*% of controls (c)*
(a) nCi	20	278.5 ± 49.7	249.0 ± 44.1	90.3 ± 2.9	6	43.7 ± 10.9
(b) ng NA	15	84.8 ± 21.6	78.2 ± 17.8	98.9 ± 6.7	6	56.3 ± 19.5

S_1, S_2, S_3, : first, second and third periods of nerve stimulation (for details of time intervals, see methods section).

(a) Total radioactivity released by nerve stimulation.
(b) Total nanograms of noradrenaline (NA) released by nerve stimulation.
(c) Transmitter release during S_3 expressed as percent of the average of the two control periods of stimulation.

Values are mean with s.e. mean. *n* = number of experiments.

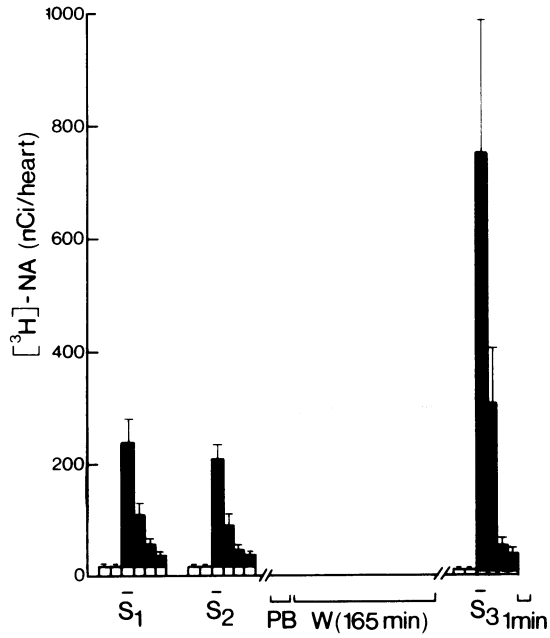

Fig. 2 Increase in the overflow of the tritiated transmitter ([^{3}H]-NA) elicited by nerve stimulation 165 min after exposure to phenoxybenzamine. For details see Figure 1. After the second period of stimulation (S_2) the heart was perfused for 20 min with phenoxybenzamine (PB) 8.7×10^{-7}M. Subsequently the perfusion continued without the drug (W) for 165 min before the third period of stimulation (S_3) was applied. Nerve stimulation: 5 Hz, 0.5 ms duration and supramaximal voltage. The values shown are the means of six experiments. Vertical bars indicate s.e. mean.

controls both for endogenous noradrenaline and for the tritiated transmitter.

In a second experimental group phenoxybenzamine (8.7×10^{-7}M) was perfused for 20 min after the second period of nerve stimulation. Subsequently the perfusion continued without the drug for 165 min before the third period of stimulation (S_3) was applied. Under these experimental conditions the overflow of the labelled transmitter during S_3 was increased nearly 8-fold (Fig. 2, Table 2) in proportion to the decline observed in the controls. The corresponding increase in overflow for endogenous noradrenaline in this group, exposed to phenoxybenzamine, was 5.69 ± 0.96 fold (mean with s.e. mean of 6 experiments).

Similar experiments were carried out in which the competitive α-receptor blocking agent, phentolamine (3.2×10^{-5}M) was perfused for 30 min after S_2, after which the preparation was washed for 155 min before the third period of nerve stimulation was applied. Under these experimental conditions the overflow of the tritiated transmitter during S_3 did not differ significantly from that in the S_1 and S_2 periods (Fig. 3) but a decline like that in the control group (Fig. 1) did not occur. The overflow of endogenous noradrenaline during S_3 tended to exceed that in S_1 and S_2 but not significantly: 1.72 ± 0.86-fold (mean with s.e. mean of 4 experiments).

It was thought that the increase in transmitter overflow obtained after the short exposure to phenoxybenzamine might be due to the persistent blockade of the α-adrenoceptors involved in the regulation of the release of noradrenaline. If this

Table 2 Comparison of the effects of cocaine and of phenoxybenzamine on neuronal uptake and on transmitter overflow in the perfused cat heart

Exposure	n	*% inhibition of uptake of [^{3}H]–NA*	n	*Transmitter overflow by stimulation (% of controls)*
Cocaine (3.4×10^{-7}M) for 15 min	4	31.3 ± 2.1	7	196.6 ± 19.8
PB (8.7×10^{-7}M) for 20 min then washed for 165 min	4	17.5 ± 5.4	6	788.6 ± 159.6*

Inhibition of neuronal uptake of [^{3}H]-noradrenaline (NA) and the overflow of total ^{3}H elicited by nerve stimulation are expressed as percentages of the control values obtained in the same preparation. Mean values with s.e. mean.

PB = phenoxybenzamine
n = number of experiments
* indicates significant difference from control value ($P < 0.005$)

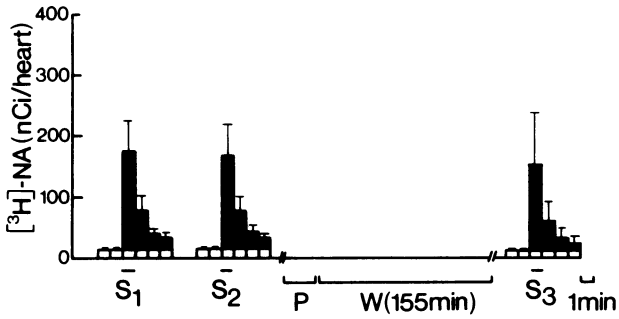

Fig. 3 Overflow of the tritiated transmitter ([^{3}H]-NA) elicited by nerve stimulation 155 min after exposure to phentolamine. For details see Figure 1. After the second period of stimulation (S_2) the heart was perfused during 30 min with phentolamine (P) 3.2 x 10^{-5} M. Subsequently the perfusion continued without the drug (W) for 155 min before the third period of stimulation (S_3) was applied. Nerve stimulation: 5 Hz, 0.5 ms duration and supramaximal voltage. The values shown are the means of four experiments. Vertical bars indicate s.e. mean.

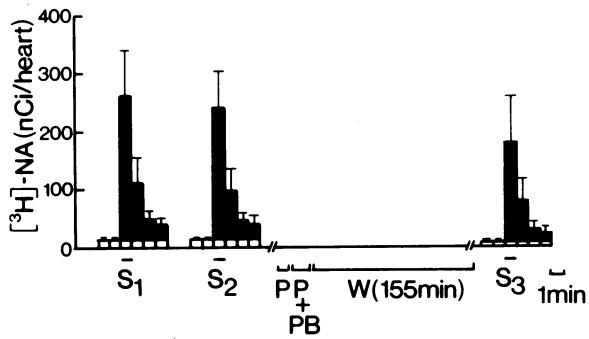

Fig. 4 Protection by phentolamine of the effects of phenoxybenzamine on transmitter release ([^{3}H]-NA) elicited by nerve stimulation. For details see Figure 1. After the second period of stimulation (S_2) the heart was perfused with 3.2 x 10^{-5} M phentolamine (P) 10 min before and then during a 20 min exposure to 8.7 x 10^{-7} M phenoxybenzamine (PB). Subsequently the perfusion continued without drugs for 155 min (W) until the third period of stimulation was applied. Nerve stimulation: 5 Hz, 0.5 ms duration and supramaximal voltage. The values shown are the means of four experiments. Vertical bars indicate s.e. mean.

were the case, phentolamine, in a higher concentration than phenoxybenzamine might be expected to protect the blockade of these receptor sites by the unsurmountable blocking agent. To test this hypothesis, experiments were carried out in which phentolamine, 3.2 x 10^{-5}M, was perfused 10 min before and then during the 20 min of exposure to phenoxybenzamine (8.7 x 10^{-7}M). Subsequently, the hearts were perfused for 155 min without drugs and then a third period of stimulation (S_3), was carried out. Figure 4 shows that perfusion with phentolamine before and during exposure to phenoxybenzamine prevented the increase in overflow of the labelled transmitter obtained when only phenoxybenzamine was added to the perfusion medium. Similar results were obtained for endogenous noradrenaline in this group, the overflow during S_3 did not differ significantly from the means for S_1 and S_2, the ratio being 1.08 ± 0.60 (mean with s.e. mean of 4 experiments).

Positive chronotropic responses to nerve stimulation.

In the controls there was practically no decrease in the heart rate throughout the experiments (Table 3). Twenty-five min after the addition of phentolamine (3.2 x 10^{-5}M) the heart rate decreased from 96 ± 8 to 74 ± 5 beats/min (mean with s.e. mean of 4 observations) but after removal of phentolamine from the perfusion

medium the heart rate returned to the previous values. While exposure to phenoxybenzamine did not affect basal rates prior to S_3, after both α-receptor blocking agents had been perfused simultaneously there was a decrease in the basal rate prior to the third period of nerve stimulation (Table 3).

The positive chronotropic responses elicited by nerve stimulation during three periods of stimulation in the control group did not differ significantly (Table 3) nor was a significant change in their intensity seen in the groups given the α-blocking agents. The increase in transmitter overflow obtained in the group exposed to phenoxybenzamine (Table 2) was reflected only by prolongation of the chronotropic responses to nerve stimulation. This apparent dissociation could be due to the experiments being carried out at a frequency of nerve stimulation (5 Hz) to which the response is nearly maximum. (In 5 separate experiments in which full frequency-response curves were determined the mean maximum increase in rate produced by nerve stimulation was 79 ± 10 beats/minute).

Effects of phenoxybenzamine and phentolamine on neuronal uptake of [^{3}H]-noradrenaline in the perfused cat heart

The two α-receptor blocking agents studied are known to inhibit neuronal uptake of noradrenaline (Iversen, 1967: Starke *et al.*, 1971a; Starke *et al.*, 1971b; Cubeddu, Langer & Weiner, 1974a). Consequently, it was of interest to determine the arterio-venous difference for infused [^{3}H]-noradrenaline under the experimental conditions in which the release experiments were carried out.

In 3 control experiments in which no drugs were added to the perfusion medium the arterio-venous difference for infused [^{3}H]-noradrenaline did not change significantly throughout the experiment but Fig. 5a shows that during exposure to phenoxybenzamine there was a marked inhibition of uptake of [^{3}H]-noradrenaline. Fourteen min after the perfusion with phenoxybenzamine (8.7×10^{-7}M) had begun, the uptake was reduced by 44.20 ± 8.48% (mean with s.e. mean of 4 experiments). During the washout period there was a progressive recovery in the uptake of the labelled transmitter. After 165 min (i.e. at the time corresponding to the third period of nerve stimulation in other experiments) a small inhibition in neuronal uptake was still observed: 17.52 ± 5.36% (mean of 4 experiments).

During exposure to phentolamine (Fig. 5b) neuronal uptake of [^{3}H]-noradrenaline was inhibited by 58.25 ± 4.05% (mean of 4 experiments). However, after the drug was removed from the perfusion medium neuronal uptake recovered rapidly, reaching control values at the time of the third period of nerve stimulation (Figure 5b).

When phentolamine (3.2×10^{-5}M) was perfused before and during exposure to phenoxybenzamine (8.7×10^{-7}M) the degree of inhibition of neuronal uptake was similar to that obtained with phentolamine alone (Figure 5c). Fourteen min after the perfusion with phenoxybenzamine plus phentolamine began the inhibition of neuronal uptake was 63.53 ± 7.33% (mean of 4 experiments) and during the washout period there was a rapid and complete recovery such as was observed with phentolamine but not with phenoxybenzamine alone.

Table 3 Basal rates and chronotropic responses to nerve stimulation in perfused heart of cat

Treatment	n	Mean basal rates with s.e. mean (beats/min) (a)			Mean increase in rate during nerve stimulation with s.e. mean (b)		
		S_1	S_2	S_3	S_1	S_2	S_3
Controls	6	100 ± 8	97 ± 8	94 ± 5	79 ± 5	82 ± 8	80 ± 14
PB 8.7×10^{-7}M	6	103 ± 8	102 ± 8	91 ± 14	79 ± 9	78 ± 8	86 ± 12
Phentolamine 3.2×10^{-5} M	6	91 ± 6	92 ± 6	83 ± 5	80 ± 5	81 ± 5	92 ± 7
Phentolamine 3.2×10^{-5}M plus PB 8.7×10^{-7}M	4	88 ± 7	88 ± 4	65 ± 6	110 ± 6	105 ± 6	102 ± 15

(a) Heart rates prior to the first (S_1), second (S_2) and third (S_3) periods of nerve stimulation.
(b) Maximum increase in rate elicited by nerve stimulation (5 Hz, 60s, 0.5 ms and supramaximal voltage).

S_1 and S_2 represent control periods of nerve stimulation. S_3 was applied 165 min after perfusion with phenoxybenzamine (PB), 155 min after phentolamine or 155 min after both drugs.
n = number of experiments.

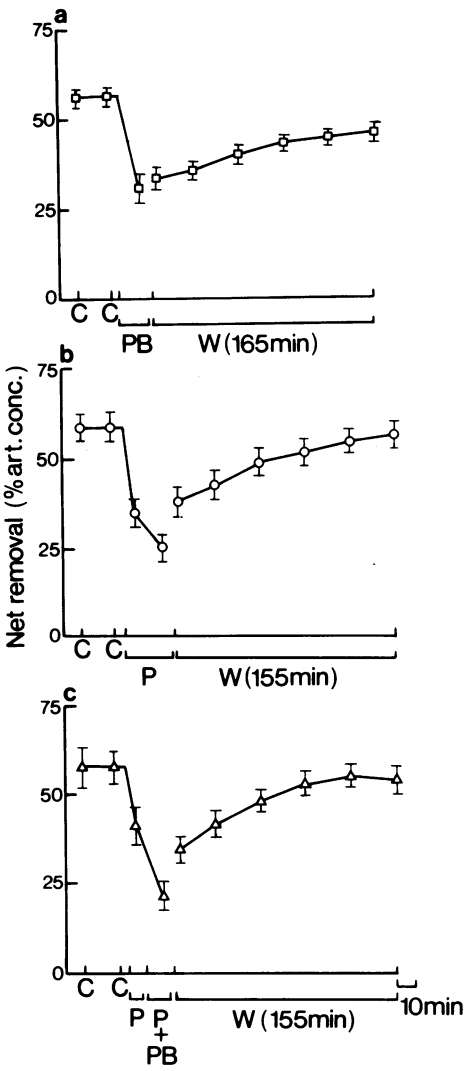

Fig. 5 Effects of phenoxybenzamine and phentolamine on the arterio-venous difference for [^{3}H]-noradrenaline in the perfused heart of cat. Ordinates: net removal of [^{3}H]-noradrenaline (the arterio-venous difference as a percentage of the arterial concentration). Abscissae: time of perfusion (min). C: control values.

(a) Phenoxybenzamine (PB): 8.7×10^{-7} M, 20 min, followed by 165 min of perfusion with drug-free medium (W).
(b) Phentolamine (P) 3.2×10^{-5} M, 30 min, followed by 155 min of perfusion with drug-free medium (W).
(c) Phentolamine (P) 3.2×10^{-5} M, 10 min, followed by phentolamine plus phenoxybenzamine (PB) 8.7×10^{-7} M for 20 min and then by 155 min of perfusion with drug-free medium (W).
Each point is the mean of four experiments. Vertical bars indicate s.e. mean.

Effects of cocaine on transmitter release and on neuronal uptake of [^{3}H]-noradrenaline in the perfused cat heart

In the group in which phenoxybenzamine was perfused and subsequently washed out a small inhibition in neuronal uptake of [^{3}H]-noradrenaline was still found at the time of the third period of nerve stimulation (in other experiments). Since such a residual effect might be responsible for the increase in transmitter overflow observed in this group during nerve stimulation it was of interest to perform comparable experiments with a concentration of cocaine that produced a similar degree of inhibition of neuronal uptake of noradrenaline. With cocaine (3.4×10^{-7}M) the reduction in uptake achieved was approximately 30% and this was associated with a nearly 2-fold increase in the overflow of the ^{3}H-transmitter during nerve stimulation (Table 2).

The inhibition in neuronal uptake of [^{3}H]-noradrenaline obtained during exposure to cocaine did not differ significantly from that observed after perfusion with phenoxybenzamine. Nevertheless, cocaine was considerably less effective than phenoxybenzamine in increasing transmitter overflow (Table 2).

Discussion

Under our experimental conditions, when phentolamine was added to the perfusion medium neuronal uptake of [^{3}H]-noradrenaline was inhibited. Similar results were reported by Starke *et al.* (1971b) for perfused rabbit heart and by Cubeddu *et al.* (1974a) for perfused cat spleen. In cat heart the recovery of neuronal uptake of [^{3}H]-noradrenaline after exposure to phentolamine was complete 2.5 h after the drug was removed from the perfusion medium. In similar experiments the rate of recovery from exposure to phenoxybenzamine was slower and it is possible that this is due to the non-competitive nature of the inhibition of neuronal uptake of noradrenaline by phenoxybenzamine (Iversen & Langer, 1969). When phentolamine was perfused before and during the exposure to phenoxybenzamine the rate of recovery from the inhibition of neuronal uptake of [^{3}H]-noradrenaline was faster than that observed for phenoxybenzamine alone. These results are compatible with the view that both α-receptor blocking agents act on the same sites to inhibit neuronal uptake of noradrenaline.

Exposure to phentolamine increases noradrenaline release by nerve stimulation irrespective of whether the response of the effector organ is mediated by α- or β-adrenoceptors (Langer *et al.*

1971; Starke *et al.*, 1971b; Farnebo & Hamberger, 1971; Adler-Graschinsky & Langer, 1974; Cubeddu, Barnes, Langer & Weiner, 1974b). With the experimental design employed, 2.5 h after the exposure to phentolamine, there was no increase in transmitter overflow elicited by nerve stimulation. These results are in keeping with the reversible nature of the blockade produced by phentolamine. In contrast the effects of the irreversible α-adrenoceptor blocking agent, phenoxybenzamine, on transmitter overflow persisted in spite of the 2.5 h of washing. The marked increase in transmitter overflow observed appears to be due to an actual increase in transmitter release. The residual inhibition of neuronal uptake obtained in the group exposed to phenoxybenzamine was too small to account for the 8-fold increase in transmitter overflow observed. When a similar degree of inhibition of neuronal uptake was obtained with cocaine (3.4×10^{-7} M), the increase in transmitter overflow during nerve stimulation was much smaller.

It has been reported recently that phenoxybenzamine enters adrenergic nerve endings and releases endogenous noradrenaline through an effect on granules, resembling that of reserpine-like drugs (Adler-Graschinsky, Langer & Rubio, 1972; Graefe *et al.*, 1973; Cubeddu *et al.*, 1974a). Moreover, the reserpine-like agent Ro 4 −1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7,-hexahydro-11b-*H*-benzo [a] quinolizine) increased the overflow of [^{3}H]-noradrenaline and dopamine-β-hydroxylase elicited by nerve stimulation (Cubeddu, Weiner & Langer, unpublished observations). Consequently, it could be argued that phenoxybenzamine increases transmitter release by nerve stimulation as a result of an effect on granules in adrenergic nerve endings. This is excluded as a possible explanation of our results because (a), inhibition of neuronal uptake by exposure to cocaine does not prevent access of phenoxybenzamine to adrenergic nerve endings or its ability to release endogenous noradrenaline (Chang, 1968) and (b), the concentration of phenoxybenzamine required to increase the spontaneous outflow of the labelled transmitter in several adrenergically innervated organs is higher than the one employed in the present studies (Adler-Graschinsky *et al.*, 1972; Cubeddu *et al.*, 1974a).

Since the perfusion with phentolamine before and during the exposure to phenoxybenzamine effectively prevented the effects of the irreversible blocking agent on transmitter release it can be concluded that both drugs may increase transmitter release by acting on the same receptor sites.

In separate experiments with phentolamine at the lower concentration of 1.0×10^{-6} M, transmitter overflow during nerve stimulation in the perfused cat heart was increased 2-fold without inhibiting neuronal uptake of noradrenaline (Farah & Langer, unpublished observations). Consequently, it is likely that both agents, phentolamine and phenoxybenzamine, block the presynaptic α-adrenoceptors that regulate transmitter release through a negative feed-back mechanism. In the present experiments the persistent increase in transmitter release caused by phenoxybenzamine seems to be due overwhelmingly to blockade of these receptors.

The authors are indebted to Dr R.A. McLean of Smith, Kline & French Laboratories (Philadelphia, U.S.A.) for the supply of phenoxybenzamine and to Dr H. Brunner of Ciba-Geigy Laboratories (Basel, Switzerland) for the supply of phentolamine.

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(Revised April 25, 1974)