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STIMULATION OF PRESYNAPTIC β-ADRENOCEPTORS ENHANCES [^{3}H]-NORADRENALINE RELEASE DURING NERVE STIMULATION IN THE PERFUSED CAT SPLEEN

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1 The effects of isoprenaline, propranolol and phosphodiesterase inhibitors on ^{3}H-transmitter overflow elicited by low frequency nerve stimulation were determined in the isolated perfused spleen of the cat.

2 (−)-Isoprenaline (0.14, 1.4, and 14 nM) produced a concentration-dependent increase in [^{3}H]-transmitter overflow evoked by nerve stimulation at 1 Hz and was more effective at 1 Hz than at 2 hertz.

3 A concentration of propranolol (0.1 μM), devoid of neurone blocking activity, blocked this effect of (−)-isoprenaline. These results are compatible with the presence of β-adrenoceptors in the noradrenergic nerve endings of the cat spleen.

4 (+)-Isoprenaline (140 nM) failed to increase the release of radioactivity induced by nerve stimulation, indicating that the β-adrenoceptor mediating the facilitation of transmitter release was stereospecific.

5 The increase in ^{3}H-transmitter overflow induced by nerve stimulation during exposure to the phosphodiesterase inhibitor, papaverine (27 μM) was more pronounced than that obtained in the presence of 3-isobutyl-1-methyl xanthine (IBMX) 0.5 mM. The facilitation in transmitter release induced by papaverine was not correlated with the granular effect produced by this drug.

6 In the presence of papaverine, the concentration–effect curve for (−)-isoprenaline on transmitter release was shifted to the left and its maximum was increased. In addition, propranolol significantly reduced the enhancement in noradrenaline release obtained by exposure to papaverine under conditions in which the granular effect produced by the phosphodiesterase inhibitor was even greater than in the absence of the β-blocker.

7 It is concluded that activation of presynaptic β-adrenoceptors in the perfused cat spleen leads to an enhancement in transmitter release which appears to be linked to an increase in cyclic adenosine 3′,5′-monophosphate levels in noradrenergic nerve endings.

Introduction

The presence of presynaptic β-adrenoceptors in noradrenergic nerve endings has been recently proposed by several authors (Langer, Adler-Graschinsky & Enero, 1974; Adler-Graschinsky & Langer, 1975; Stjarne & Brundin, 1975; Dahlöf, Äblad, Borg, Ek & Waldeck, 1975). It has been suggested that activation of the presynaptic β-adrenoceptors by noradrenaline released at low frequencies of nerve stimulation triggers a positive feed-back mechanism leading to an increase in transmitter release (Langer *et al.*, 1974; Adler-Graschinsky & Langer, 1975; Langer, 1977). Evidence for the presence of presynaptic β-adrenoceptors in noradrenergic nerve endings under *in vitro* experimental conditions has been obtained in the cat nictitating membrane and aortic strips, guinea-pig atria and vasa deferentia and also in human blood vessels and oviducts (Langer *et al.*, 1974; Adler-Graschinsky & Langer, 1975; Langer, Enero, Adler-Graschinsky, Dubocovich & Celuch, 1975; Stjärne, 1975; Stjärne & Brundin, 1975; Hedqvist & Moawad, 1975). Yet, recently Endo, Starke,

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Bangerter & Taube (1977) reported that isoprenaline did not increase ^{3}H-transmitter release during nerve stimulation in isolated strips of the rabbit pulmonary artery. It therefore appears that in contrast to the inhibition mediated by presynaptic α-adrenoceptors the facilitatory mechanism mediated by presynaptic β-adrenoceptors may not be present in all noradrenergic nerve endings of the peripheral nervous system.

The cat spleen has been used extensively for studies of noradrenaline release elicited by nerve stimulation. In 1973, Kirpekar, Furchgott, Wakade & Prat reported that exposure to isoprenaline did not increase the overflow of [^{3}H]-noradrenaline from the perfused cat spleen. Since the concentrations of isoprenaline employed by these authors were rather high, it was considered of interest to re-examine the effects of β-adrenoceptor activation and blockade on [^{3}H]-noradrenaline release elicited at low frequencies of nerve stimulation in this tissue.

Methods

Cats of either sex (1.5–2.5 kg) were used. After induction of anaesthesia with ether, spinal preparations were set up as described by Burn (1952); this procedure includes destruction of the brain and the use of artificial respiration. After evisceration, the spleen was isolated and the splenic artery and vein were cannulated. A pair of fine platinum electrodes was hooked around the splenic artery in order to stimulate the post-ganglionic nerve fibres. The spleen was placed in a plethysmograph filled with liquid paraffin kept at 37°C. The tissue was perfused with Krebs solution at 37°C at a constant volume, 7.5 ml/minute. Changes in perfusion pressure, as a measure of the splenic vascular resistance, were recorded with a mercury manometer. The composition of the Krebs solution was (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. The solution was bubbled continuously with a mixture of 95% O_2 and 5% CO_2 and kept at 37°C. Stimulation was carried out with an S-44 Grass stimulator. Square pulses of 0.1 ms duration and of supramaximal voltage were applied.

Ten minutes after the supramaximal voltage was determined, an infusion of (−)-[7-^{3}H]-noradrenaline (New England Nuclear, Boston, U.S.A., sp. act. 4 to 6 Ci/mmol) was carried out for 10 min (total infused: 60 μCi). After the infusion of [^{3}H]-noradrenaline was completed, the spleen was perfused for 80 min before the periods of nerve stimulation began.

A total of 120 shocks at a frequency of 1 Hz was delivered during each of seven periods of stimulation which were applied every 29 minutes. In the control group all seven periods of stimulation were performed without the addition of drugs to the perfusion medium. In a second group of experiments, after two control periods of nerve stimulation, increasing concentrations of (−)-isoprenaline were added to the perfusion medium. Each concentration of (−)-isoprenaline was perfused for 10 min before the following period of stimulation. Similar experiments were performed with (+)-isoprenaline.

In different experimental groups after the first period of nerve stimulation, (−)-propranolol, 3-isobutyl 1-methyl-xanthine (IBMX) or papaverine was perfused for 22 min before the second period of stimulation, and the drugs were present in the perfusion medium throughout the rest of the experiment. In addition, the effects of increasing concentrations of (−)-isoprenaline was also determined in the presence of (−)-propranolol or papaverine.

The influence of the frequency of nerve stimulation on the effects of (−)-isoprenaline on ^{3}H-transmitter overflow was studied in experiments in which the nerves were stimulated at 1 Hz for 300 s (S_1) and at 2 Hz for 150 s (S_2). A total of 300 shocks was delivered during each period of stimulation. The two periods of nerve stimulation at 1 Hz and at 2 Hz were repeated in the presence of (−)-isoprenaline, which was added to the perfusion medium 10 min before the periods of nerve stimulation at 1 Hz or 2 Hz were applied. In separate control experiments all four periods of stimulation were carried out without the addition of drugs to the perfusion medium.

Two 1 min samples of the splenic effluent were collected before each period of nerve stimulation. Samples were also collected for 1 min during and after stimulation until the outflow of radioactivity returned to the pre-stimulation basal levels. The venous effluent from the perfused spleen was collected and chilled in graduated centrifuge tubes. The samples were centrifuged to remove the red blood cells at approximately 1000 *g* for 10 min and 1 ml of the supernatant was assayed for total tritium by liquid scintillation spectrometry.

For determinations of total ^{3}H in the tissue at the end of each experiment, the spleen was blotted on filter paper, weighed and homogenized in 10 ml cold 0.4 M perchloric acid per g tissue, containing 1 mg EDTA and 1.25 mg sodium sulphite per ml. The homogenate was kept at 4°C for 60 min and then centrifuged at approximately 1000 *g*.

Radioactivity was measured with a mixture of toluene 600 ml, Triton X100 300 ml, absolute ethanol 100 ml, 2,5 diphenyloxazole (PPO) 5 g and 1,4 bis 2-(5-phenyl oxazolyl)benzene (POPOP) 0.1 gram.

The total overflow of radioactivity induced by nerve stimulation was calculated by subtraction of the spontaneous outflow assumed to have occurred in each sample during and after the period of nerve stimulation.

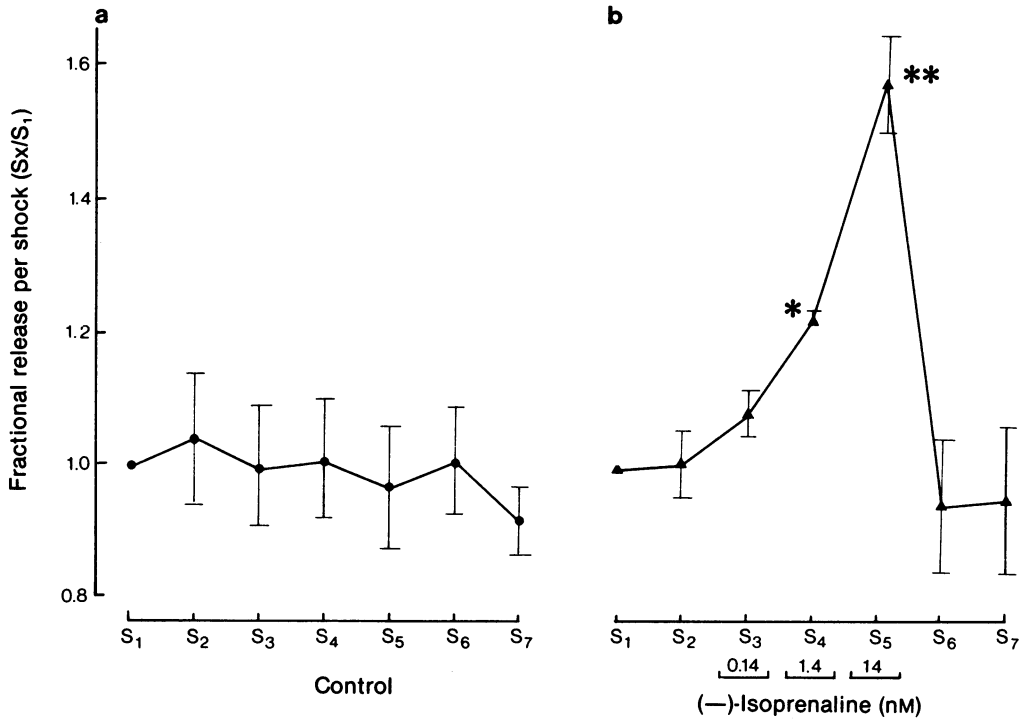

Figure 1 Effects of (−)-isoprenaline on ^{3}H-transmitter overflow elicited by nerve stimulation, in the perfused spleen of the cat. Abscissa scales: S_1 to S_7 indicate periods of nerve stimulation at 1 Hz, for 2 min (0.1 ms duration and supramaximal voltage). Ordinate scale: Ratio of fractional release per shock obtained between each period of nerve stimulation (Sx) and the corresponding control (S_1). (a) (●): Controls (S_1 to S_7); (b) (▲): (−)-isoprenaline 0.14, 1.4, and 14 nM was added to the perfusion medium 10 min before S_3, S_4 and S_5 respectively. Mean values of 4 experiments are shown. Vertical lines show s.e. means. $^*P < 0.05$; $^{**}P < 0.001$ when compared with the corresponding controls.

The value of the spontaneous outflow subtracted from the stimulation and post-stimulation samples was the basal resting release obtained in the period immediately before stimulation. The total overflow of the labelled transmitter was expressed as a fraction of the total radioactivity remaining in the tissue: total ^{3}H released per shock divided by total ^{3}H remaining in the tissue at the onset of stimulation (Langer & Enero, 1974). The total radioactivity remaining in the tissue at the start of each period of nerve stimulation was determined in every experiment by adding to the radioactivity remaining in the tissue at the end of the experiment the radioactivity collected in all the samples from that period of stimulation to the end of the sample collection.

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

The following drugs were used: (−)-isoprenaline bitartrate dihydrate, (+)-isoprenaline bitartrate; (−)-propranolol hydrochloride; papaverine hydrochloride and 3-isobutyl 1-methyl-xanthine.

Results

Effects of (−)-isoprenaline and (+)-isoprenaline on ^{3}H-transmitter release elicited by nerve stimulation

In the control group, the decline in overflow of total tritium, as a result of seven consecutive periods of nerve stimulation was small when these values were expressed with reference to the first period of nerve stimulation (Figure 1a). At concentrations of 1.4 and 14 nM (−)-isoprenaline in the perfusion medium, a significant increase in the total overflow of radioactivity was observed during nerve stimulation; this increase was concentration-dependent and readily reversed by washing (Figure 1b).

It is of interest to note that the concentration of (−)-isoprenaline used in these experiments do not inhibit the neuronal uptake of noradrenaline (Adler-Graschinsky & Langer, 1975).

The absolute values for the total overflow of radioactivity expressed as fractional release per shock in

Table 1 Effects of (−)-isoprenaline on ^{3}H-transmitter overflow elicited by nerve stimulation

Experimental group	n	*Fractional release per shock* ($\times 10^5$)[a]						
		S_1	S_2	S_3	S_4	S_5	S_6	S_7
Control	4	5.70 ± 1.29	5.79 ± 1.23	5.56 ± 1.19	5.58 ± 1.53	5.86 ± 1.54	5.95 ± 1.47	5.65 ± 1.53
(−)Isoprenaline				0.14 nM	1.4 nM	14 nM		
	4	4.83 ± 0.74	4.72 ± 0.63	5.24 ± 0.79	6.01 ± 0.97	7.78 ± 1.32	4.61 ± 0.76	4.57 ± 0.70

(a) Fraction of the total radioactivity in the tissue released per shock during successive periods of nerve stimulation. The nerves were stimulated at 1 Hz for 2 min, 0.1 ms duration and supramaximal voltage. (−)-Isoprenaline 0.14, 1.4, and 14 nM was added to the perfusion medium 10 min before S_3, S_4 and S_5 respectively. Mean values ± s.e. mean are shown; n = number of experiments.

these experiments are shown in Table 1. There was a considerable scatter when data from different experiments were compared. Consequently, the ratios of the fractional release per shock between each period of stimulation over the first period within the same experiment were employed (Figure 1), rather than the absolute values (Table 1).

In contrast to these results, when the nerves were stimulated in the presence of relatively high concentrations of (+)-isoprenaline (14 and 140 nM) there was no increase in the total overflow of radioactivity (Table 2).

Influence of the frequency of nerve stimulation on the effects of (−)-isoprenaline on transmitter release

The positive feed-back mechanism mediated via prejunctional β-adrenoceptors appears to be triggered by low concentrations of the transmitter (Adler-Graschinsky & Langer, 1975). Consequently, the increase in transmitter release obtained in the presence of isoprenaline would be expected to be more pronounced, the lower the frequency of nerve stimulation.

Experiments performed in order to test this possibility were carried out at 1 and 2 hertz. In the control group, the ratios between two consecutive periods of nerve stimulation were 1.19 ± 0.08 ($n = 6$) and 1.00 ± 0.05 ($n = 6$) when the nerves were stimulated at 1 and 2 Hz respectively.

When 14 nM (−)-isoprenaline was added before the second period of nerve stimulation, the ratios obtained at 1 and 2 Hz were 1.71 ± 0.17 ($n = 4$, $P < 0.05$) and 1.27 ± 0.02 ($n = 4$, $P < 0.005$) respectively. In the presence of the β-agonist, a significant increase in ^{3}H-transmitter release by nerve stimulation was observed at both frequencies. However, at 1 Hz the increase in transmitter overflow elicited by exposure to isoprenaline was more pronounced than that obtained at 2 hertz.

Prevention by (−)-propranolol of the facilitation by (−)-isoprenaline of ^{3}H-transmitter release during nerve stimulation

In the presence of the β-adrenoceptor blocking agent, (−)-propranolol (0.1 μM), there was a small but statistically significant reduction in transmitter release by nerve stimulation as judged by the ratio S_2/S_1 (Table 3). This effect of propranolol on transmitter release was obtained with a concentration that does not affect neuronal uptake of noradrenaline and that does not produce neurone blockade (Adler-Graschinsky & Langer, 1975). When individual experiments were analyzed, it was found that the decrease in ^{3}H-transmitter release obtained in the presence of propranolol was the more pronounced the higher the fractional release in the corresponding control stimulation period ($r = 0.485$, $n = 18$, $P < 0.05$).

The possibility that the increase in transmitter overflow obtained in the presence of (−)-isoprenaline was due to the activation of β-adrenoceptors was tested in experiments carried out in the presence of propranolol. As shown in Figure 2a, there were no significant differences between the ratios of transmitter overflow obtained during successive periods of nerve stimulation in the presence of propranolol. Figure 2b shows that exposure to 0.1 μM (−)-propranolol completely prevented the effects of the different concentrations of (−)-isoprenaline, on transmitter release elicited by nerve stimulation.

Effects of phosphodiesterase inhibitors on ^{3}H-transmitter release elicited by nerve stimulation

Several authors have found that an increase in noradrenaline release is obtained during exposure to phosphodiesterase inhibitors (Wooten, Thoa, Kopin & Axelrod, 1973; Langer, 1973; 1974; Cubeddu,

Table 2 ^{3}H-transmitter overflow elicited by nerve stimulation in the presence of (+)-isoprenaline

Experimental group	*Fractional release per shock Ratio Sx/S$_1$ (a)*				
	S_1	S_2	S_3	S_4	S_5
Control	1.00	1.05 ± 0.07	1.00 ± 0.07	1.04 ± 0.07	1.03 ± 0.09
(+)-Isoprenaline	1.00	0.87 ± 0.11	0.92 ± 0.14	1.06 ± 0.14	0.69 ± 0.12

(a) Ratio of fractional release per shock obtained between each period of nerve stimulation (Sx) and the corresponding control (S_1). The nerves were stimulated at 1 Hz for 2 min, 0.1 ms duration and supramaximal voltage. The values of fractional release per shock × 10^5 in the control period of nerve stimulation (S_1) were, control: 5.70 ± 1.29 and isoprenaline 4.72 ± 1.14 (mean ± s.e. mean of 4 experiments). (+)-Isoprenaline 14 nM and 140 nM was added to the perfusion medium 10 min before S_3 and S_4 respectively. Mean values ± s.e. mean of 4 experiments per group.

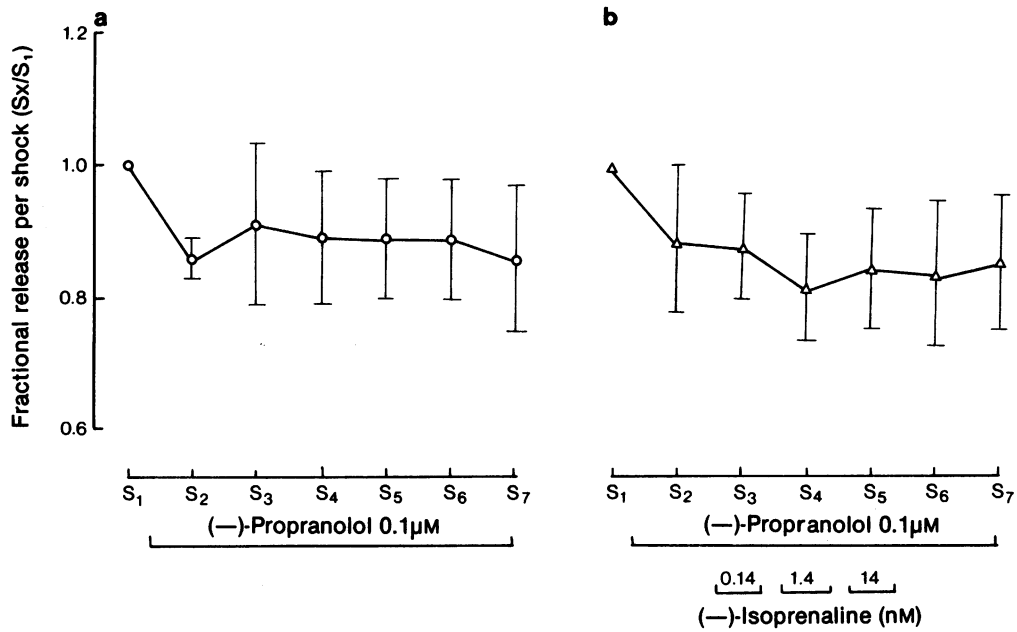

Figure 2 Prevention by (−)-propranolol of the effects of (−)-isoprenaline on ^{3}H-transmitter overflow elicited by nerve stimulation. Abscissa scales: S_1 to S_7 indicate periods of nerve stimulation (1 Hz for 2 min, 0.1 ms duration and supramaximal voltage). Ordinate scale: ratio of fractional release per shock obtained between each period of nerve stimulation (S_x) and the corresponding control (S_1). (a) (○): (−)-Propranolol 0.1 μM was present in the perfusion medium from 22 min before S_2 until the end of the experiment ($n = 4$). (b) (△): (−)-propranolol 0.1 μM was added to the perfusion medium 22 min before S_2, and it was present until the end of the experiment. (−)-Isoprenaline 0.14, 1.4 and 14 nM was added 10 min before S_3, S_4 and S_5 respectively ($n = 7$). Mean values are shown, vertical lines indicate s.e. means; n = number of experiments.

Table 3 Effects of propranolol and phosphodiesterase inhibitors on ^{3}H-transmitter release induced by nerve stimulation in the perfused cat spleen

Experimental group		n	*Fractional release per shock* × 10^5 (a) S_1	S_2	*Ratio* (b) S_2/S_1
Control		4	5.70 ± 1.29	5.79 ± 1.23	1.05 ± 0.07
(−)-Prop	0.1 μM	18	6.35 ± 0.63	5.30 ± 0.46	0.87 ± 0.04 c
IBMX	0.5 mM	7	3.94 ± 0.63	6.06 ± 0.79	1.58 ± 0.09 d
Pap	27 μM	9	4.97 ± 1.12	12.47 ± 3.23	2.49 ± 0.09 e
Pap plus (−)-Prop	27 μM 0.1 μM	7	4.70 ± 0.53	7.87 ± 0.91	1.68 ± 0.09 e, f

(a) Fraction of the total radioactivity in the tissue released per shock during nerve stimulation. The nerves were stimulated at 1 Hz for 2 min, 0.1 ms duration and supramaximal voltage. (b) Ratio of fractional release per shock between S_2 and S_1. The drugs in the concentrations indicated were present in the perfusion medium before the second period of stimulation (S_2). (−)-Prop: propranolol; IBMX: 3 isobutyl-1-methyl-xanthine; Pap: papaverine. Mean values ± s.e. mean are shown; n = number of experiments. (c) $P < 0.05$; (d) $P < 0.005$; (e), $P < 0.001$ when compared with the control. (f) $P < 0.001$ when compared with papaverine.

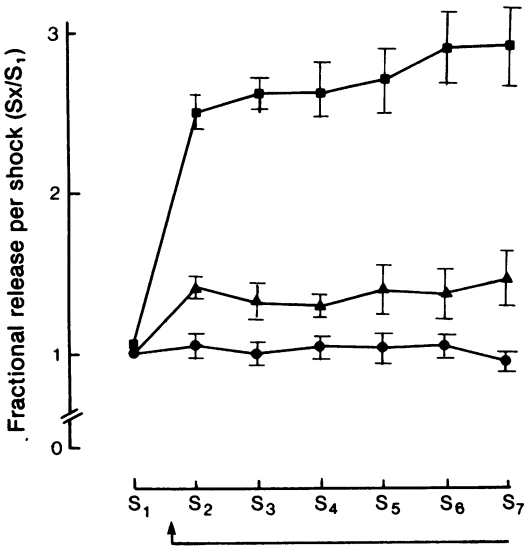

Figure 3 Effects of phosphodiesterase inhibitors on ^{3}H-transmitter overflow elicited by nerve stimulation in the perfused spleen of the cat. Ordinate scale: ratio of the fractional release per shock obtained between each period of nerve stimulation (Sx) and the corresponding control (S_1) within the same experiment. The nerves were stimulated at 1 Hz for 2 min (0.1 ms supramaximal voltage). Abscissa scale: periods of nerve stimulation (S_1 to S_7). (●): Controls ($n = 4$), $S_1 - S_7$; (▲): 3-isobutyl-1-methylxanthine, 0.5 mM, ($n = 3$), $S_2 - S_7$; (■): papaverine, 27 μM ($n = 9$), $S_2 - S_7$. The drugs were added (at arrow) 22 min before S_2 and were present throughout the rest of the experiment. Mean values are shown, vertical lines indicate s.e. mean; n = number of experiments.

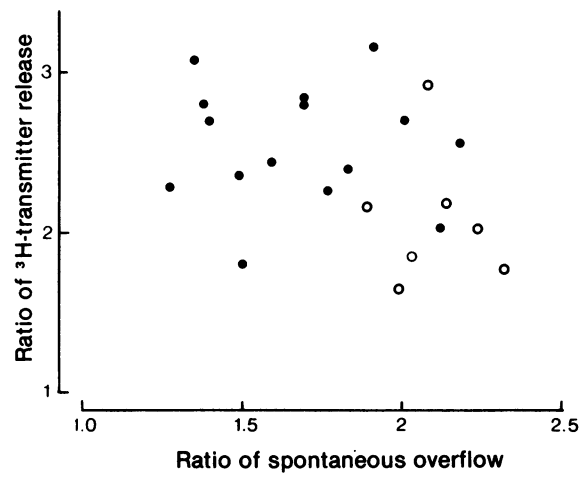

Figure 4 Lack of correlation between the granular effect produced by papaverine and the enhancement in ^{3}H-transmitter overflow obtained during nerve stimulation. Abscissa scale: granular effect of the drug determined as the increase in the spontaneous outflow of radioactivity and expressed as the ratio Papaverine (Pap) or Pap + propranolol (Prop) over the corresponding control. Ordinate scale: increase in ^{3}H-transmitter overflow during nerve stimulation expressed as the ratio Pap or Pap + Prop over the corresponding control (Cont). (●): (Pap/Cont), $n = 15$; (○): (Pap + Prop)/(Prop), $n = 7$. Correlation coefficient (r): −0.348; $P > 0.05$, NS. Papaverine concentration 27 μM; propranolol, 0.1 μM.

Barnes & Weiner, 1974; 1975). Since many effects induced by β-adrenoceptor activation are mediated through an increase in the tissue levels of cyclic adenosine 3'5'-monophosphate (cyclic AMP), it was considered of interest to examine the effects of (−)-isoprenaline and of (−)-propranolol on transmitter release in the presence of a phosphodiesterase inhibitor.

As shown in Figure 3 and Table 3, exposure to 3-isobutyl-1-methyl-xanthine (IBMX) 0.5 mM or to papaverine 27 μM produced an increase in ^{3}H-transmitter overflow elicited by nerve stimulation. While papaverine produced a nearly 3-fold increase in transmitter release the effects obtained with IBMX were rather small. Cubeddu *et al.* (1974) attributed the increase in transmitter release obtained with papaverine to the granular (reserpine-like) effect of the drug. Yet we failed to observe a positive correlation between the increase in spontaneous outflow of radioactivity induced by papaverine and the enhancement in ^{3}H-transmitter release obtained during nerve stimulation (Figure 4).

In the presence of papaverine, it was found that (−)-isoprenaline increased even further the overflow of ^{3}H-transmitter (Figure 5b). This effect of (−)-isoprenaline was also concentration-dependent and readily reversed by washing. Comparison of Figure 5a and 5b shows that (−)-isoprenaline produced a larger increase in ^{3}H-transmitter overflow in the presence of papaverine when compared against the control, obtained in the absence of the phosphodiesterase inhibitor.

When the absolute values of the increase in ^{3}H-transmitter overflow induced by (−)-isoprenaline were taken into account it was found that exposure to papaverine produced a shift to the left and an increase in the maximum in the concentration–effect curve for the β-adrenoceptor agonist on transmitter release (Figure 6).

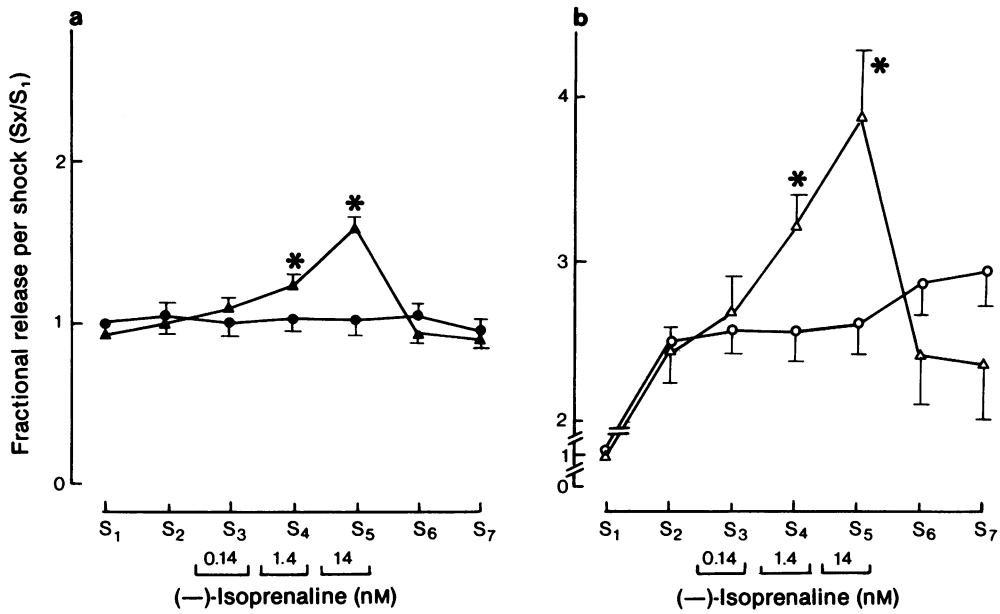

Figure 5 Effect of different concentrations of (−)-isoprenaline on ^{3}H-transmitter overflow induced by nerve stimulation in the controls and in the presence of papaverine. Ordinate scale: ratio of the fractional release per shock obtained between each period of nerve stimulation (Sx) and the corresponding control (S_1) within the same experiment. The nerves were stimulated at 1 Hz for 2 min (0.1 ms, supramaximal voltage). Abscissa scale: periods of nerve stimulation (S_1 to S_7). (a) (●): Controls ($n = 4$) $S_1 - S_7$; (▲): controls S_1, S_2; (−)-Iso S_3, S_4, S_5; after washout of (−)-Iso S_6, S_7 ($n = 4$). (b) (○): Control, S_1; Pap, $S_2 - S_7$ ($n = 9$); (△): Control, S_1; Pap, S_2; Pap + Iso, S_3, S_4, S_5; Pap after washout of Iso S_6, S_7 ($n = 5$). Iso: (−)-isoprenaline 0.14, 1.4 and 14 nM was added 10 min before S_3, S_4 and S_5 respectively. Pap: papaverine, 27 μM was added 22 min before S_2 and was present throughout the rest of the experiment. Mean values are shown, vertical lines indicate s.e. means; n = number of experiments.

In a separate group of experiments (−)-propranolol was added to the perfusion medium before papaverine. In these experiments, the inhibitor of phosphodiesterase elicited a significantly smaller increase in ^{3}H-transmitter overflow than that obtained in the absence of (−)-propranolol (Figure 7, Table 3). It is of interest to note that the granular effect produced by papaverine (determined as the increase in the spontaneous outflow of radioactivity above the basal levels) was even more pronounced in the presence of propranolol than in the absence of the β-blocking agent: 1.69 ± 0.07 fold in the controls ($n = 15$) and 2.18 ± 0.11 fold in the presence of propranolol ($n = 7$; $P < 0.005$).

Potentiation by papaverine of the enhancement in ^{3}H-transmitter release induced by (−)-isoprenaline (Figures 5 and 6) and the reduction by (−)-propranolol of the facilitation in ^{3}H-transmitter overflow obtained in the presence of papaverine (Figure 7) are compatible with the view that neuronal cyclic AMP accumulation might be involved in the facilitation of ^{3}H-transmitter release induced during exposure to (−)-isoprenaline.

Discussion

The concentration-dependent increase in ^{3}H-transmitter overflow obtained during exposure to low concentrations of (−)-isoprenaline appears to be mediated through β-adrenoceptors since it was entirely blocked by a concentration of (−)-propranolol devoid of neurone blocking activity (Adler-Graschinsky & Langer, 1975). The failure of Kirpekar *et al.* (1973) to show an increase in [^{3}H]-noradrenaline release by isoprenaline was probably due to the fact that these authors used a rather high concentration of the β-agonist and these concentrations of isoprenaline already stimulate α-adrenoceptors in the cat spleen (Granata & Langer, 1973). In addition, in the

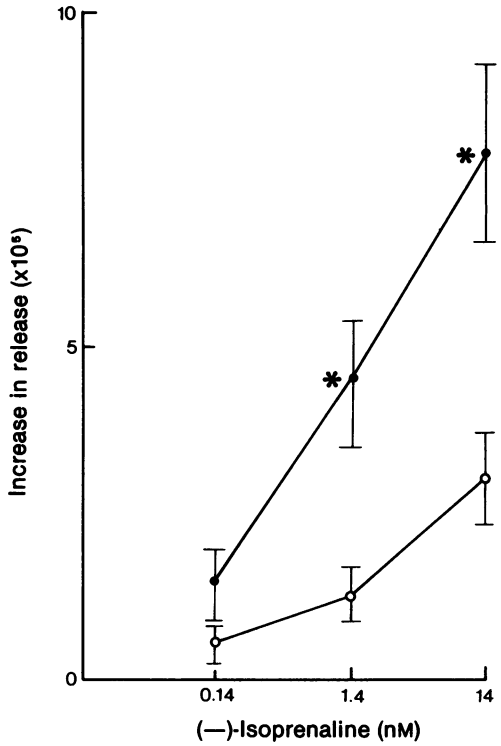

Figure 6 Potentiation by papaverine of the increase in ^{3}H-transmitter overflow obtained in the presence of isoprenaline. Ordinate scale: absolute increase in ^{3}H-transmitter overflow above the corresponding controls expressed as fractional release per shock, $\times 10^5$. The nerves were stimulated at 1 Hz for 2 min (0.1 ms, supramaximal voltage). Abscissa scale: concentration of (−)-isoprenaline (nM). Each concentration of isoprenaline was added 10 min before the corresponding stimulation. (○): (−)-Isoprenaline ($n = 4$); (●): (−)-isoprenaline in the presence of papaverine 27 μM ($n = 5$). Mean values are shown, vertical lines show s.e. means; n = number of experiments. $^*P < 0.05$ when compared with isoprenaline alone.

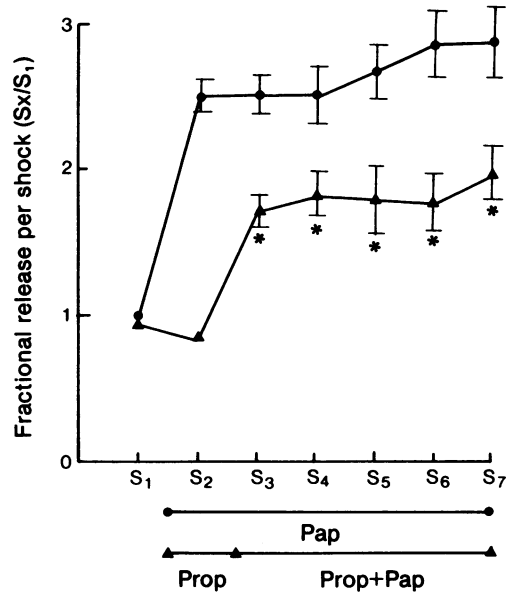

Figure 7 Reduction by (−)-propranolol of the effects of papaverine on ^{3}H transmitter overflow during nerve stimulation. Ordinate scale: ratio of the fractional release per shock obtained between each period of nerve stimulation (Sx) and the corresponding control (S_1) within the same experiment. The nerves were stimulated at 1 Hz for 2 min (0.1 ms, supramaximal voltage). Abscissa scale: periods of nerve stimulation ($S_1 - S_7$). (●): Control, S_1 Pap = papaverine, 27 μM, $S_2 - S_7$, ($n = 9$); (▲): control, S_1; Prop = propranolol, 0.1 μM, S_2; Prop + Pap, $S_3 - S_7$ ($n = 7$). The drugs were added 22 min before the corresponding period of nerve stimulation and were present throughout the rest of the experiment. Mean values are shown, vertical lines show s.e. means; $^*P < 0.05$ when compared with the corresponding values obtained with papaverine.

experiments by Kirpekar *et al.* (1973), the nerves were stimulated at 10 Hz and the facilitation by isoprenaline of the release of noradrenaline is more pronounced at low frequencies of nerve stimulation. In support of this view, we found that the increase in ^{3}H-transmitter release obtained in the presence of isoprenaline was more pronounced when stimulation was carried out at 1 Hz than at 2 hertz.

The β-adrenoceptor mediating the facilitation in transmitter release was shown to be stereospecific since (+)-isoprenaline even in higher concentrations than the laevo-isomer failed to increase the release of [^{3}H]-noradrenaline. In isolated atria of the rat the potency differences between the enantiomers of isoprenaline is 3 log units both for rate as well as for force (Birnbaum, Abel & Buckner, 1973). The stereospecificity for noradrenaline has also been demonstrated for the presynaptic α-adrenoceptor which inhibits transmitter release (Stjärne, 1974). Consequently, both the presynaptic α- and β-adrenoceptors appear to be stereo-selective.

The reduction in ^{3}H-transmitter overflow observed in the presence of 0.1 μM (−)-propranolol was rather small although it reached statistical significance when a large group of experiments was analyzed. It is of interest to note that propranolol was more effective in reducing [^{3}H]-noradrenaline release in those experiments in which the output of the transmitter was

highest. It is possible that blockade of presynaptic β-adrenoceptors is most effective in reducing noradrenaline release when the presynaptic facilitatory β-adrenoceptors are activated either by the transmitter or by exogenous isoprenaline.

A decrease in responses to adrenergic nerve stimulation has been reported after the administration of propranolol in the guinea-pig isolated vas deferens (Mylecharane & Raper, 1970; 1973) and in the cat nictitating membrane under *in vivo* conditions (Eliash & Weinstock, 1971). These effects can be attributed to a guanethidine-like neurone blocking effect of propranolol;(Eliash & Weinstock, 1971; 1972; Mylecharane & Raper, 1973) or to a local anaesthetic effect of the β-blocking agent (Barret & Nunn, 1970; Mylecharane & Raper, 1973). However, with the concentration of propranolol employed in the present study neither a local anaesthetic nor a neurone blocking action of propranolol appears to be involved (Barret & Cullum, 1968; Barret & Nunn, 1970; Del Rio & Ceballos, 1975; Adler-Graschinsky & Langer, 1975; Hughes & Kneen, 1976). Since the concentration of propranolol employed in this study prevented the increase in [^{3}H]-noradrenaline release induced by (−)-isoprenaline, it follows that the presynaptic effects of propranolol are probably due to the blockade of prejunctional β-adrenoceptors.

The presence of presynaptic β-adrenoceptors in noradrenergic nerve endings has been described in several tissues of different species, independently of the α- or β-nature of the postsynaptic receptors which mediate the response of the effector organ. The presynaptic facilitatory β-adrenoceptors have been found in the perfused cat spleen (present study), cat aortic strips and nictitating membrane (Langer *et al.*, 1975), human blood vessels (Stjärne & Brundin, 1975), guinea-pig isolated atria (Langer *et al.*, 1974; Adler-Graschinsky & Langer, 1975), human oviduct (Hedqvist & Moawad, 1975), guinea-pig vas deferens (Stjärne, 1975), cat's hind limb (Dahlöf *et al.*, 1975) and rat pineal gland (Pelayo, Dubocovich & Langer, unpublished observations). On the other hand, Endo *et al.* (1977) found recently that isoprenaline does not increase [^{3}H]-noradrenaline release from isolated strips of the rabbit pulmonary artery. It therefore appears that the presynaptic β-receptors may not be present in all noradrenergic nerve endings. However, since the report by Endo *et al.* (1977) represents the only exception so far, it is still too early to assess the importance of the tissue and species differences with regard to the presynaptic β-adrenoceptors.

In the tissues in which the presynaptic β-adrenoceptors have been described, propranolol blocks the increase in transmitter release induced by isoprenaline. Yet, the β-blocking agent does not *per se* decrease the stimulation-induced release of noradrenaline in several of these tissues (Stjärne & Brundin, 1975; Hedqvist & Moawad, 1975; Langer *et al.*, 1975). The ability of higher concentrations of propranolol to inhibit neuronal uptake of noradrenaline and thus to increase transmitter release may be responsible for these findings (Werner, Wagner & Schümann, 1971; Starke & Schümann, 1972). Alternatively, it is possible that in some tissues the presynaptic β-adrenoceptors can be demonstrated only when release of noradrenaline is increased by a β-receptor agonist. A decrease in noradrenaline release was observed with propranolol in guinea-pig atria (Adler-Graschinsky & Langer, 1975) and in the cat's hind limb after α-blockade with phenoxybenzamine (Dahlöf *et al.*, 1975), although these effects were rather small (approximately a 40% reduction). It is possible that long term β-receptor blockade may be more effective than acute administration of antagonist in reducing the amount of transmitter released per impulse from noradrenergic nerves. Ljung, Äblad, Dahlöf, 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.

It is of interest to note that Chubb & Raine (1976) demonstrated that long term treatment with propranolol reduced tyrosine hydroxylase activity in the superior cervical ganglia of the rabbit. These authors attributed their results to a decreased release of noradrenaline in response to chronic β-blockade. More recently, it was shown that long term treatment with propranolol leads to a reduction in adrenal tyrosine hydroxylase activity in spontaneously hypertensive rats (Äblad, Almgreen, Carlsson, Henning, Jonasson & Ljung, 1977).

This paper as well as the other reports discussed above demonstrated the presence of presynaptic facilitatory β-adrenoceptors under *in vitro* experimental conditions. The presence of this positive feed-back mechanism for noradrenaline release *in vivo* was recently demonstrated by Yamaguchi, De Champlain & Nadeau (1977). These authors measured noradrenaline release into the coronary sinus blood during cardioaccelerator nerve stimulation in anaesthetized dogs. The overflow of noradrenaline elicited by low frequency nerve stimulation (1 to 5 Hz) was greatly enhanced by isoprenaline (400% increase at 3 Hz). On the other hand, after the administration of sotalol they found an 80% reduction in noradrenaline release at 1 Hz and a nearly 50% decrease at 5 hertz. These results obtained in the dog *in vivo* support the view that the presynaptic β-adrenoceptors play a physiological role in the regulation of noradrenaline release during nerve stimulation. Earlier, Äblad, Ek, Johansson & Waldeck (1970) had reported that (±)-propranolol reduced the vasoconstrictor responses to low

frequency lumbar sympathetic stimulation in the cat. They also showed that (+)-propranolol, which lacks β-receptor blocking activity did not reduce the vasoconstrictor response to sympathetic nerve stimulation and suggested that (±)-propranolol reduced noradrenaline output through an effect related to β-receptor blockade.

β-Adrenoceptor blocking agents are becoming increasingly important in antihypertensive therapy. It is possible that a reduction of noradrenaline release through the blockade of presynaptic β-adrenoceptors in the central and the peripheral nervous system contributes to the antihypertensive activity of these drugs (Adler-Graschinsky & Langer, 1975; Langer *et al.*, 1975; Ljung *et al.*, 1975; Lewis & Haeusler, 1975; Langer, 1976; Yamaguchi *et al.*, 1977).

The view that the β-adrenoceptor-mediated facilitation of noradrenaline release involves an increase in intraneuronal cyclic AMP was proposed by Adler-Graschinsky & Langer (1975) and Langer *et al.* (1975). In common with other authors (Wooten *et al.*, 1973; Langer, 1973; 1974; Cubeddu *et al.*, 1974; 1975), we found that phosphodiesterase inhibitors increased the stimulation-evoked overflow of ^{3}H-transmitter. While the increase in transmitter release induced by papaverine was more pronounced than that of IBMX, we did not find a positive correlation between the granular effect produced by papaverine and the increase in transmitter release induced by the drug (Figure 4). Our results do not represent a conflict of evidence with the findings reported by Cubeddu *et al.* (1974) because they obtained the positive correlation by pooling data from different concentrations of papaverine, and such a regression may simply reflect the concentration–effect relationship for the phosphodiesterase inhibitor. In our experiments, we explored the correlation for one concentration of papaverine which represents a more sensitive test for the possible causal relationship between the granular effect produced by the drug and the facilitation of transmitter release.

The hypothesis that presynaptic β-adrenoceptor activation leads to the accumulation of neuronal cyclic AMP was tested in experiments in which the interactions between papaverine–isoprenaline and papaverine–propranolol were examined. In the presence of the phosphodiesterase inhibitor, the increase in noradrenaline release induced by isoprenaline was potentiated: the concentration-effect curve for the β-adrenoceptor agonist was shifted to the left and the maximum was increased. In addition, propranolol significantly reduced the enhancement in noradrenaline release elicited by papaverine, even though the granular effect induced by the phosphodiesterase inhibitor in the presence of propranolol was more pronounced than that observed in the absence of the β-blocking agent. Yet, this concentration of propranolol, which completely abolished the effects of isoprenaline only reduced partially the effects of papaverine. These results can be interpreted as follows. Noradrenaline released by low frequency nerve stimulation activates presynaptic β-adrenoceptors leading to an increase in cyclic AMP formation in the nerve terminals and to an enhancement in transmitter release. Phosphodiesterase inhibition increases noradrenaline release because (a) it potentiates the effects of low concentrations of noradrenaline on the presynaptic β-adrenoceptors by preventing degradation of cyclic AMP and (b) it increases endogenous cyclic AMP levels by inhibition of phosphodiesterase even in the absence of activation of presynaptic β-adrenoceptors; while (a) should be propranolol-sensitive, (b) should be propranolol-resistant. Consequently, the increase in transmitter release obtained in the presence of papaverine should be only partially reduced in the presence of propranolol.

Our results are therefore compatible with the view that cyclic AMP accumulation in noradrenergic nerve endings may be involved in the facilitation of transmitter release induced by activation of presynaptic β-adrenoceptors. It is of interest to note that cyclic AMP and cyclic nucleotide analogues induce catecholamine release from the adrenal medulla (Serk-Hanssen, 1974) and the guinea-pig vas deferens (Wooten *et al.*, 1973) in the absence of extracellular calcium. It is possible that cyclic AMP may facilitate noradrenaline release by mobilizing intracellular bound calcium and thus increasing the availability of calcium for the stimulation secretion coupling. In addition, cyclic nucleotides facilitate the influx of sodium and calcium into cells (Rasmussen, 1970) and this can carry depolarizing currents and trigger the release of transmitter. Recently, it was shown that intraneuronal cyclic AMP plays a role in neuromuscular transmission by promoting the influx of calcium into the motor nerve terminal (Dretchen, Standaert, Skirboll & Morgenroth, 1976; Skirboll, Baizer & Dretchen, 1977). It appears that a similar phenomenon occurs in noradrenergic nerve endings. In a recent report by Roth, Morgenroth & Salzman (1975) a presynaptic increase in cyclic AMP levels was postulated to mediate the activation of tyrosine hydroxylase which occurs in noradrenergic nerve endings as a result of nerve stimulation. Evidence for the presynaptic location of adenylate cyclase and of the cyclic AMP-stimulated protein kinase was recently reported by Weller (1977).

It is of interest to note that in the perfused cat spleen, monobutyryl cyclic AMP and 8-methylthiocyclic AMP produce a concentration-dependent increase in the release of noradrenaline, [^{3}H]-noradrenaline and dopamine-β-hydroxylase during nerve stimulation at 5 Hz (Cubeddu *et al.*, 1975). It is possible that the effects of cyclic nucleotides on transmit-

ter release in the perfused spleen are related to the facilitatory mechanism mediated by presynaptic β-adrenoceptors.

In conclusion, the present results support the view that presynaptic β-adrenoceptors are present in the noradrenergic nerve endings of the cat spleen. Activation of these presynaptic β-adrenoceptors leads to an enhancement in transmitter release which appears to be linked to an increase in cyclic AMP levels in noradrenergic nerve endings.

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