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MODULATION OF NORADRENERGIC TRANSMISSION IN THE RABBIT EAR ARTERY BY DOPAMINE

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1 The effects of dopamine on vasoconstrictor responses to field stimulation of sympathetic nerves and to exogenous noradrenaline were studied in the isolated ear artery of the rabbit. Responses to sympathetic nerve stimulation were depressed, initially, by infusions of dopamine (0.5 to 1 μM), but the responses partially recovered in the continued presence of dopamine. Responses to noradrenaline were unchanged at the start of the dopamine infusions but were enhanced as the infusions continued and also after cessation of the infusion.

2 Dopamine (0.5 μM) reduced the stimulation-induced efflux of tritium from segments of ear artery labelled with [^{3}H]-noradrenaline. The reduction persisted during 65 min of dopamine infusion, after which time the vasoconstrictor responses had generally recovered to 93% of control level. On ceasing the infusion, the stimulation-induced efflux and the vasoconstrictor responses were enhanced.

3 Metoclopramide, haloperidol and ergometrine, each in a concentration of 0.2 μM, prevented the inhibitory effect of 0.5 μM dopamine on the stimulation-induced tritium release, but not the inhibitory effect of 0.5 μM noradrenaline. Phenoxybenzamine (0.2 and 1 μM) and phentolamine (1 μM) prevented the inhibitory effects of both noradrenaline and dopamine on the stimulation-induced efflux, and phentolamine (0.2 μM) prevented the inhibition of the stimulation-induced release by noradrenaline but only partially prevented the inhibitory effect of dopamine on the stimulation-induced efflux.

4 A possible role for dopamine in the modulation of noradrenergic transmission is suggested.

Introduction

The concept of the control of the release of the transmitter noradrenaline by a negative feedback mechanism, which operates through α-adrenoceptors on terminal sympathetic nerves, has been based on the effects of α-adrenoceptor antagonists and agonists: the antagonists enhance and agonists inhibit stimulation-induced transmitter efflux (Rand, McCulloch & Story, 1975; Starke, 1977; Langer, 1977). However, recent studies have indicated that agonists and antagonists may have different affinities for pre- and post-junctional α-receptors (Dubocovich & Langer, 1974; Langer, 1974; Starke, Endo & Taube, 1975a, b; Starke, Montel & Endo, 1975; Drew, 1976; 1977); thus it appears that these receptors are not identical. Dopamine is far less potent as a vasoconstrictor agent than noradrenaline in the rabbit ear artery (Lazner & de la Lande, 1974); however, dopamine is equipotent with noradrenaline in producing depression of stimulation-induced efflux in the cat nictitating membrane (Langer, 1974; Enero & Langer, 1975) and the rabbit ear artery (McCulloch, Rand & Story, 1973; Hope, Law, McCulloch, Rand & Story, 1976). Furthermore, the neuroleptic drug pimozide, which has been reported to be an antagonist of dopamine receptors in the rat brain (Anden, Butcher, Corrodi, Fuxe & Ungerstedt, 1970), in a concentration of 0.2 μM, blocked the inhibitory effect on stimulation-induced efflux produced by 0.5 μM dopamine, but not that produced by 0.5 μM noradrenaline (Hope, McCulloch, Story & Rand, 1977).

Although differences in the potency of dopamine and noradrenaline in causing vasoconstriction and in reducing stimulation-induced efflux may be explained in terms of differences between pre- and post-junctional α-adrenoceptors, it is difficult to accommodate the differential effects of pimozide on the dopamine- and noradrenaline-induced depression of transmitter release in such an explanation. Indeed, the finding that pimozide can selectively prevent the pre-junctional action of dopamine indicates that, in addition to α-adrenoceptors, there may also be inhibitory dopamine receptors on the terminal sympathetic axons in the rabbit ear artery.

The present paper is concerned with a more detailed analysis of the effects of dopamine on noradrenergic transmission in the rabbit ear artery. In an attempt to differentiate between two different types of pre-junctional receptors, the effects of a number of antagonists of dopamine receptors and α-adrenoceptors on the noradrenaline-induced and dopamine-induced depressions of radiolabelled transmitter efflux were also explored.

Methods

Rabbits of either sex (2 to 4 kg) were killed by a blow to the head and 15 to 40 mm segments of the central ear arteries were isolated and cannulated at each end. Each segment was mounted vertically under a tension of about 0.5 g and perfused and superfused with Krebs-Henseleit solution at 37°C as described by Allen, Rand & Story (1973). The flow rate was maintained at 4 ml/min and the perfusion pressure monitored by a Statham P23Db pressure transducer connected to a Rikadenki potentiometric recorder.

Vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline: effects of dopamine

Vasoconstrictor responses to noradrenaline were elicited by injection of doses of 5 to 20 ng in a volume of 0.1 ml into the perfusion stream at a point just proximal to the artery. In each experiment the dose of noradrenaline was selected so as to produce approximately 50% of the maximal vasoconstrictor response. The periarterial sympathetic nerves were stimulated with monophasic square wave pulses of 1 ms duration and supramaximal voltage at a frequency of 5 Hz applied through bipolar circular platinum electrodes. Cocaine (100 μM) was present in the perfusion fluid for the duration of each experiment in order to reduce the uptake of exogenous dopamine and the subsequent displacement of noradrenaline from the artery preparations. In some experiments, 10 s periods of stimulation were given at 2 min intervals, and in others 10 s periods of electrical stimulation and doses of noradrenaline were given alternately at 2 min intervals. In other experiments, stimulation was applied for 10 s periods at 5 min intervals or for 30 s periods at 30 min intervals; with these regimes of stimulation, the injections of noradrenaline were given at random to replace electrical stimulation.

Dopamine was infused at the rate of 0.05 ml/min by means of a Braun Unita I Slow injection apparatus connected to a polyethylene cannula inserted into the perfusion stream proximal to the artery to achieve final concentrations of 0.5 or 1 μM; in these concentrations, dopamine had no effect on resting perfusion pressure. Vasoconstrictor responses, measured as increases in perfusion pressure, were obtained to sympathetic nerve stimulation and noradrenaline before, during and after infusions of dopamine.

Determination of pA_2 values for antagonism of responses to exogenous noradrenaline

Log dose-response curves were obtained to noradrenaline before and during infusion of each antagonist drug by injection of sequential doses of noradrenaline into the perfusion solution. Each antagonist was tested in separate ear artery preparations. The pA_2 values were calculated by the method of Arunlakshana & Schild (1959).

Radiolabelling with [^{3}H]-noradrenaline and measurement of tritium effluxes

After dissection and a 30 min period of perfusion-superfusion, artery segments were incubated with [^{3}H]-(−)-noradrenaline (10 μCi/ml, 0.29 μM) for 60 min as described by Allen *et al.* (1973). After incubation, the segments were perfused and superfused with [^{3}H]-noradrenaline-free Krebs-Henseleit solution for 90 min; experiments in which the radioactivity of the perfusate was continuously monitored showed that the efflux of radioactivity had reached a steady state by 90 min. Perfusion pressure was monitored as described previously. Cocaine (100 μM) was infused 15 min before the first period of stimulation and throughout the rest of the experiment to reduce the displacement of tritiated noradrenaline by exogenous amines: the conditions were the same as those used by Hope *et al.* (1976).

The adventitial sympathetic nerves were stimulated as described above with square wave pulses of 1 ms duration and supramaximal voltage at 5 Hz for 30 s periods. The first period of stimulation was given 90 min after incubation with [^{3}H]-noradrenaline and two or three subsequent periods of stimulation were given at 30 min intervals.

Six consecutive fractions of the perfusate-superfusate were collected for 1 min periods, starting 1 min before each period of stimulation, for measurement of the efflux of radioactivity. Total radioactivity was measured since Langer (1970) points out that for the calculation of the actual output of transmitter it is important to include the metabolites and not to rely on the determination of [^{3}H]-noradrenaline alone.

The resting efflux of radioactivity was taken as the tritium content of the 1 min fraction collected immediately before stimulation. The stimulation-induced efflux was calculated by subtraction of the resting efflux from the tritium content of the fraction collected during the 1 min period in which stimulation was applied: when the tritium content of any of the four subsequent fractions exceeded the resting efflux, the difference was included as stimulation-induced efflux. The tritium content of the fractions was estimated by addition of 1 ml aliquots to 10 ml of a scintillation solution and 0.1 ml of 6 M HCl in liquid scintillation counting vials. The radioactivity was measured with a Packard Tricarb liquid scintillation counter, corrected for counting efficiency using automatic external standardization, and expressed in becqerels (Bq).

In each experiment resting and stimulation-induced effluxes in the second and subsequent periods of stimulation were calculated as ratios of the corresponding efflux during the first stimulation period. The effects of dopamine and other drugs were investigated by infusion of the drug under investigation into the perfusion cannula 15 min before the second period of stimulation, when the arteries were stimulated three times, or 10 min before the second period of stimulation, when the arteries were stimulated four times. The infusion was terminated 15 min before the last stimulation period.

In experiments to determine the ability of antagonists to reverse the inhibition of stimulation-induced efflux produced by dopamine or noradrenaline, infusion of the antagonist drug and cocaine (10 μM) was started 15 min before the first stimulation period and was maintained throughout the experiment.

The mean ratios of the resting and stimulation-induced effluxes for the second and subsequent stimulation periods to those in the first period (R_2/R_1, R_3/R_1, R_4/R_1 and S_2/S_1, S_3/S_1, S_4/S_1), for experiments in which drugs were present for the second and subsequent periods, were converted to percentages of the corresponding mean ratios from control experiments and the standard errors associated with these percentages were calculated.

Three groups of control experiments were used: (i) when the effects of dopamine on tritium efflux were studied, cocaine (100 μM) was present throughout control experiments; (ii) when the effects of antagonists on the inhibition of stimulation-induced efflux produced by noradrenaline or dopamine were studied, the concentration of cocaine was reduced to 10 μM; cocaine (10 μM) and one of the antagonists were present throughout control experiments; (iii) when the effects of the antagonists on tritium efflux were studied, no drug was present in the control experiments. In each group of control experiments, the mean (±s.e.mean) ratio of efflux during the second period of stimulation to efflux in the first period of stimulation was calculated.

Drugs and materials

Disodium edetate (0.067 mM) was added to the Krebs-Henseleit solution to retard oxidation of catecholamines. Dopamine hydrochloride (Sigma, U.S.A.) and (−)-noradrenaline hydrochloride (Sigma, U.S.A.) were dissolved with disodium edetate (0.13 mM) and ascorbic acid (0.28 mM) in distilled water. Pimozide hydrobromide (Janssen Pharmaceuticals) was dissolved in 1.7 M acetic acid to give a stock solution of 8 mM and further diluted with distilled water. Other drugs used were: phentolamine hydrochloride (Ciba); phenoxybenzamine hydrochloride (Smith, Kline & French); metoclopramide monohydrochloride (Beecham Research Laboratories); haloperidol (Searle, Australia); ergometrine maleate (Burroughs Wellcome); cocaine hydrochloride (McFarlane Smith); these were freshly prepared in distilled water. Tritiated (−)-noradrenaline (specific activity 3.7 Ci/mmol) was obtained at a radioactive concentration of 0.5 m Ci/ml in 0.2 M acetic acid from New England Nuclear Corporation, and was stored at −30°C.

The composition of the Krebs-Henseleit solution (mmol/l) was as follows: NaCl 118, KCl 4.7. $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5 and D-(±)-glucose, 11.1.

The liquid scintillation solution consisted of 5.5 g of 2,5-diphenyloxazole (PPO), 0.1 g of 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (POPOP) and 333 ml of Triton-X made up to 1 litre with toluene.

Statistical analysis of results

The unpaired Student's t test was used to test for significant differences in experimental results. The tests were applied to the efflux data after converting the ratios to percentages of corresponding controls. Probability levels less than 0.05 were taken to indicate significant differences between group means.

Results

Effects of dopamine on vasoconstrictor responses to sympathetic nerve stimulation and to exogenous noradrenaline

The effects of infusions of dopamine on the vasoconstrictor responses of rabbit perfused ear artery preparations to field stimulation of intramural sympathetic nerves and to exogenous noradrenaline depended upon the concentration and the duration of the dopamine infusion. In these experiments, cocaine (100 μM) was present in the perfusate from at least 15 min

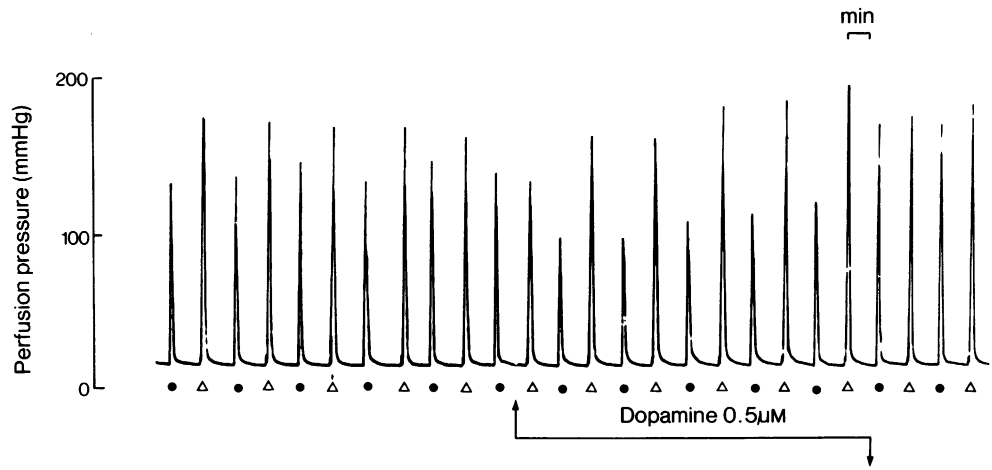


Figure 1 Effect of dopamine (0.5 μM, horizontal bar) on the vasoconstrictor responses to alternate periarterial sympathetic nerve stimulation (1 ms, 5 Hz, 10 s periods; ●) and to exogenous noradrenaline (20 ng; △) at 2 min intervals in the rabbit ear artery. The vasoconstrictor responses to sympathetic nerve stimulation were depressed initially, but during the infusion returned towards control level. Cocaine (100 μM) was present throughout.

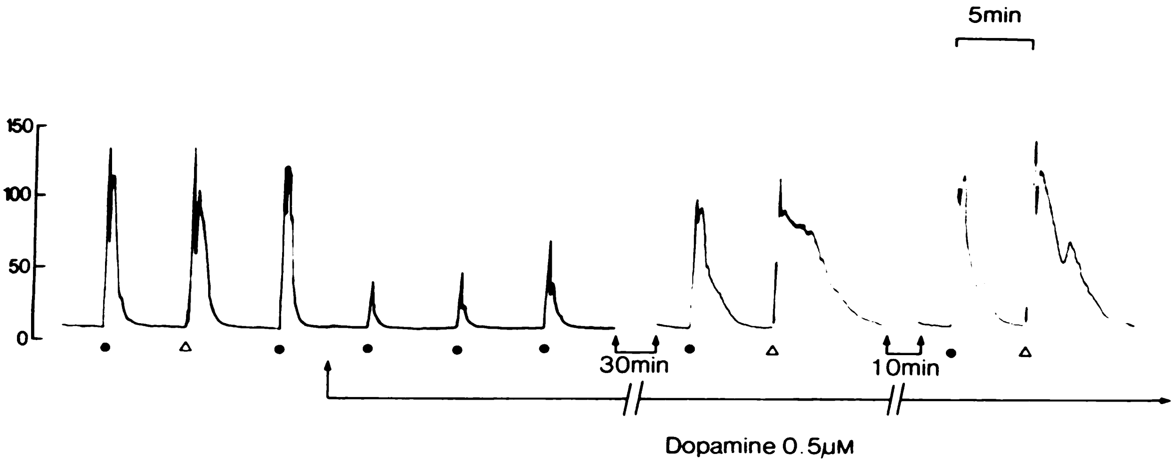
before infusion of dopamine was started and remained present throughout the period of dopamine infusion.

When electrical stimulation was given as 10 s trains of pulses (5 Hz, 1 ms) at 2 min intervals, infusion of dopamine (0.5 or 1 μM) resulted in immediate depression of the vasoconstrictor responses to stimulation. In seven experiments with 0.5 μM dopamine, responses to electrical stimulation were reduced to a mean of 24.6 ± 4.5% of control vasoconstrictor responses; the responses were reduced to about the same extent in three experiments with 1 μM dopamine. The threshold concentration for the effect was about 0.1 μM. In most cases the responses partially recovered to the pre-infusion level during the course of a 30 min infusion. In five out of seven experiments with 0.5 μM, and in all three experiments with 1 μM dopamine, the vasoconstrictor responses had recovered to at least 75% of the pre-infusion level within 30 min of starting the dopamine infusion: in two experiments with 0.5 μM dopamine, there was no recovery of the depressed responses during 30 min of dopamine infusion.

When responses to electrical stimulation (10 s periods) and to exogenous noradrenaline (5 to 20 ng) were elicited alternately at 2 min intervals, the responses to stimulation were reduced at the onset of infusion of dopamine (0.5 μM) in six out of seven experiments. In these, the responses were 16% to 40% of control: the mean reduction for responses to electrical stimulation in the seven experiments was to 39.7 ± 11.5% of control. Dopamine (0.5 μM) did not reduce the responses of the arteries to exogenous noradrenaline. In five experiments the responses to noradrenaline were enhanced progressively during the course of the dopamine infusion: after 30 min infusion the responses ranged from 115% to 128% of control. In two experiments dopamine had no effect. The mean response to noradrenaline for all seven experiments 30 min after starting the infusion of dopamine was 115.4 ± 4.6% of control. The enhancement of the responses to noradrenaline was concurrent with the recovery of the depressed responses to electrical stimulation, as shown, for one experiment in Figure 1.

In some experiments, arteries were electrically stimulated at 5 min intervals with 10 s pulse trains, and doses of noradrenaline were injected to replace stimulation at random times. In each of five such experiments, the responses to stimulation were depressed by more than 50% when infusion of dopamine (0.5 μM) was started, but the responses returned to the pre-infusion level during the infusion. In all five experiments the responses to noradrenaline were again enhanced and prolonged by dopamine; this effect developed gradually over the period of dopamine infusion and was again concurrent with the recovery of the responses to sympathetic nerve stimulation. These effects of dopamine are shown for one experiment in Figure 2.

In another series of experiments, the effects of dopamine were investigated on vasoconstrictor responses to electrical stimulation with 30 s pulse trains at 30 min intervals; the effects on noradrenaline responses were also determined by replacing some periods of stimulation with injections of noradrenaline. After obtaining stable control responses, infusion



igure 2 The effect of dopamine (0.5 μM, horizontal bar) on the vasoconstrictor responses to periarterial ympathetic nerve stimulation at 5 min intervals (1 ms, 5 Hz, 15 s periods, ●) and to 10 ng of noradrenaline Δ). Vasoconstrictor responses to nerve stimulation were depressed and slowly recovered to control levels uring the infusion of dopamine. Vasoconstrictor responses to noradrenalilne were enhanced and prolonged. ocaine (100 μM) was present throughout.

pamine (0.5 μM) was started 10 min before a lation period. In each of four experiments, the esponse to stimulation after the start of the infu- of dopamine was depressed (to a mean of ± 3.2% of control), but the responses had re- ed by the next stimulation period (that is, within in of starting the infusion). When the infusion pamine was stopped, the responses to sympathe- rve stimulation were prolonged and enhanced, 117.3 ± 3.4% of control. In these experiments sponses to noradrenaline were either unaffected htly enhanced during and after ceasing the infu- of dopamine.

s of dopamine on resting and stimulation-induced n efflux

et al. (1976) found that dopamine, in the pres- of 100 μM cocaine, reduced the efflux of tritium onse to sympathetic nerve stimulation in rabbit teries radiolabelled with [^{3}H]-noradrenaline. In esent experiments, the effects of prolonged infu- of dopamine on stimulation-induced efflux in s which had been incubated with [^{3}H]-norad- ne were investigated. In order to reduce the dis- ment of [^{3}H]-noradrenaline from neuronal by exogenous dopamine, cocaine (100 μM) was to the perfusate before starting the stimulation e and then remained present throughout. Four periods of stimulation were delivered to the s at 30 min intervals. In four experiments when mine (0.5 μM) was infused 10 min before the second period of stimulation, the stimulation-induced efflux of tritium was significantly reduced, the mean ratio of the efflux in the second period to that in the first period (S_2/S_1) being 47.9 ± 5.6% of the ratio in corresponding control experiments (see Methods). The vasoconstrictor responses were reduced to 53.5 ± 6.4% of the initial vasoconstrictor responses. Thirty minutes later the stimulation-induced efflux for the third period of stimulation (S_3) was still depressed: the ratio of S_3/S_1 was 52.8 ± 12.9% of the ratio in corresponding control experiments, but the vasoconstrictor responses to stimulation had re- covered to 93 ± 7.7% of the control. After termina- tion of the dopamine infusion, the stimulation-induced efflux for the fourth period of stimulation (S_4) was significantly enhanced (S_4/S_1 = 154.4 ± 21.3% of the corresponding control ratio) and the vasoconstrictor responses were enhanced to 167.7 ± 15.1% of the initial vasoconstrictor responses. The resting efflux of radioactivity was not signifi- cantly different from control values for any of the four stimulation periods, and resting perfusion pres- sure was not altered. The results from one experiment in which this procedure was followed are shown in Figure 3.

Effects of antagonists on the inhibition of stimulation-induced efflux produced by noradrenaline and dopamine

Dopamine and noradrenaline, infused 15 min before the second period of stimulation, inhibited the stimu- lation-induced efflux of tritium from artery segments

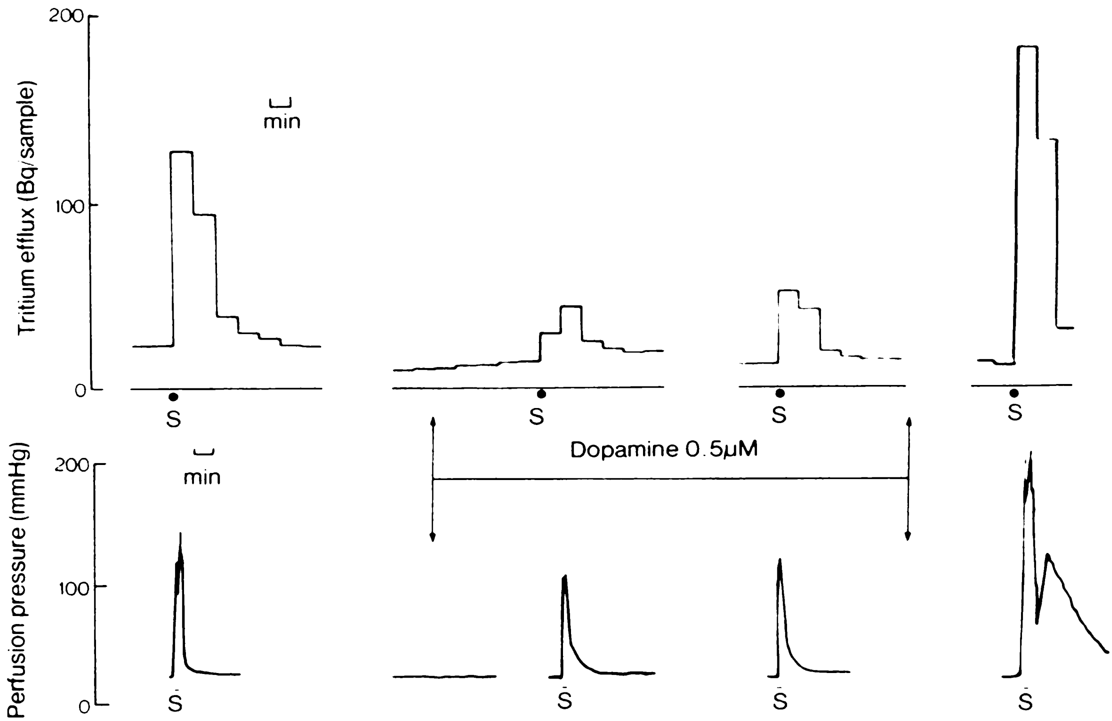


Figure 3 The effect of dopamine (0.5 μM, horizontal bar) on the efflux of tritium from the rabbit ear artery previously labelled with [^{3}H]-noradrenaline during the vasoconstrictor responses to periarterial sym-pathetic nerve stimulation (1 ms, 5 Hz, 30 s periods, S) applied at 30 min intervals. Dopamine was infused 10 min before the second period of stimulation. The vasoconstrictor responses were depressed and the stimulation-induced efflux inhibited during the second period of stimulation. In the third stimulation period, 35 min after the infusion of dopamine was started, the vasoconstrictor responses partially recovered towards control levels while the stimulation-induced efflux remained depressed. The infusion was terminated 15 min before the fourth stimulation period and a rebound effect on both vasoconstrictor responses and stimulation-induced efflux was noted. Cocaine (100 μM) was present throughout.

which had been previously incubated with [^{3}H]-noradrenaline (Hope *et al.*, 1976). In the presence, throughout, of 100 μM cocaine, the mean ratio of the stimulation-induced efflux in the second period of stimulation to that in the first period was reduced by dopamine (0.5 μM) to 54.0 ± 14.6% (n = 18) and by noradrenaline (0.5 μM) to 55.2 ± 15.0% (n = 15) of the corresponding mean ratio in control experiments (Hope *et al.*, 1976). Furthermore, the inhibitory effect of dopamine but not that of noradrenaline, on stimulation-induced efflux, was prevented in the presence of 0.2 μM pimozide, but higher concentrations of pimozide were not selective for dopamine (Hope *et al.*, 1977). In the present study, the effects on the dopamine- and noradrenaline-induced depression of stimulation-induced efflux of a range of dopamine receptor and α-adrenoceptor antagonists were determined. Infusions of the antagonists and of 10 μM cocaine were started 15 min before the first period of stimulation and continued throughout the experiment. Dopamine or noradrenaline (0.5 μM) was in-fused 15 min before the second period of stimulat[ion]. The results of these experiments are summarized [in] Table 1.

The inhibitory effects of both dopamine and nor[ad]renaline were prevented by phenoxybenzamine (0.[…] 1 μM) and by phentolamine (1 μM). Phentolamine [...] μM abolished the inhibition of stimulation-induced efflux by noradrenaline, but not by dopamine. Me[to]clopramide, haloperidol and ergometrine (all 0.2 [μM]) prevented the inhibitory effect of dopamine but [not] of noradrenaline on stimulation-induced efflux.

Effects of α-adrenoceptor and dopamine receptor an[tag]onists on resting and stimulation-induced tri[tium] effluxes

Cocaine was not present in these experiments. Nei[ther] the resting nor the stimulation-induced efflux of [tri]tium from radio-labelled ear artery segments cted by low concentrations (0.01, 0.1 or 1.0 μM) etoclopramide. A significant increase in both the ing (R_2/R_1 was increased to 136.8 ± 5.9%, $n = 4$ he corresponding control ratio) and stimulation-ced efflux (S_2/S_1 was enhanced to 243.3 ± 73.1%, 4 of the control ratio) was observed with 10 μM oclopromide. Neither the vasoconstrictor re-ses to periarterial nerve stimulation nor the rest-erfusion pressure was altered by metoclopramide ny of the concentrations tested (Figure 4).

aloperidol (0.01 and 0.1 μM) has no significant t on resting efflux, but in concentrations of 1 and M, enhanced resting tritium efflux (R_2/R_1) respect-y to 157.4 ± 7.9% ($n = 3$) and 325.6 ± 28.4% 3) of control. Stimulation-induced efflux was in-ed by haloperidol in concentrations of 0.1 μM 1 μM, the mean ratios for S_2/S_1 being, respect-, 150.0 ± 25.2% ($n = 4$) and 137.5 ± 19.8% 3) of the control ratios. In a higher concentration μM), haloperidol decreased the stimulation-ced efflux ($S_2/S_1 = 48.8 \pm 9.0\%$, $n = 4$ of con-

gometrine (0.01 to 10 μM) did not alter resting but enhanced stimulation-induced efflux in con-ations of 1 and 10 μM (the mean ratio of S_2/S_1 142.9 ± 15.8%, $n = 3$, and 179.4 ± 13.7%, $n = 3$ ntrol, respectively).

The α-adrenoceptor antagonist drugs phenoxyben-zamine and phentolamine slightly increased the resting efflux of tritium, but only in a concentration of 10 μM: R_2/R_1 was increased to 146.7 ± 5.8% of the cor-responding control ratio by phenoxybenzamine and to 137.4 ± 6.7% of control by phentolamine ($n = 4$). Phenoxybenzamine and phentolamine increased sti-mulation-induced efflux of tritium in a concentration-dependent manner. The maximum effects with both drugs occurred at concentrations of 10 μM, the ratios for S_2/S_1 being increased to 260.3 ± 24.9% (phenoxy-benzamine) and to 202.2 ± 19.7% (phentolamine) of the corresponding control ($n = 4$). At this concen-tration, vasoconstrictor responses to sympathetic nerve stimulation were reduced markedly by both drugs.

The effects of pimozide on tritium effluxes in rabbit ear arteries have been previously reported (Hope *et al.*, 1977): resting efflux was enhanced in concen-trations of 0.1 μM and 1 μM, the maximum effect being at 1 μM.

Stimulation-induced efflux was also enhanced by pimozide in a concentration of 0.01 μM, the mean ratio S_2/S_1 being increased to 133.0 ± 18.0% of con-trol ($n = 4$). In a concentration of 1 μM, pimozide had a depressant effect on both stimulation-induced efflux and vasoconstrictor responses.

able 1 Effect of dopamine and α-adrenoceptor antagonists on the inhibition of the stimulation (S-I)-fflux produced by noradrenaline and dopamine (0.5 μM)

	Agonist			
Antagonist (μM)	*Noradrenaline* S-I efflux	n	*Dopamine* S-I efflux	n
None	55.6* ± 15.0	36	54.0* ± 14.6	36
Phenoxybenzamine (1)	105.9 ± 8.4	4	112.6 ± 11.6	4
Phenoxybenzamine (0.2)	108.0 ± 11.6	4	99.9 ± 9.3	4
Phentolamine (1)	102.6 ± 10.0	4	103.2 ± 8.6	4
Phentolamine (0.2)	97.9 ± 8.8	4	77.1* ± 11.5	4
†Pimozide (1)	95.4 ± 8.5	4	101.3 ± 10.9	4
†Pimozide (0.2)	60.2* ± 14.3	4	100.4 ± 9.3	4
Metoclopramide (0.2)	51.6* ± 15.6	4	101.3 ± 9.1	4
Haloperidol (0.2)	51.3* ± 18.3	4	106.4 ± 10.3	4
Ergometrine (0.2)	63.0* ± 14.6	4	94.0 ± 10.0	4

oradrenaline and dopamine were infused for the second period of stimulation only and antagonists were fused before the first period of stimulation and throughout the experiment. Cocaine (10 μM) was also fused throughout each experiment. The data represent the mean ratios of the stimulation-induced tritium flux in the second period of stimulation to that in the first period (S_2/S_1), expressed as a % of the rresponding control. The mean ratio of S_2/S_1 for experiments in which only cocaine (10 μM) was esent throughout was 0.834 (s.e.mean = 0.065, $n = 6$); the corresponding mean ratios for S_2/S_1 r each control series of experiments with antagonists plus cocaine present throughout ($n = 4$) were ot significantly different from this ratio ($P < 0.05$). The asterisks indicate when a value differs significantly om its control.

†The data for pimozide have been published elsewhere (Hope *et al.*, 1977).

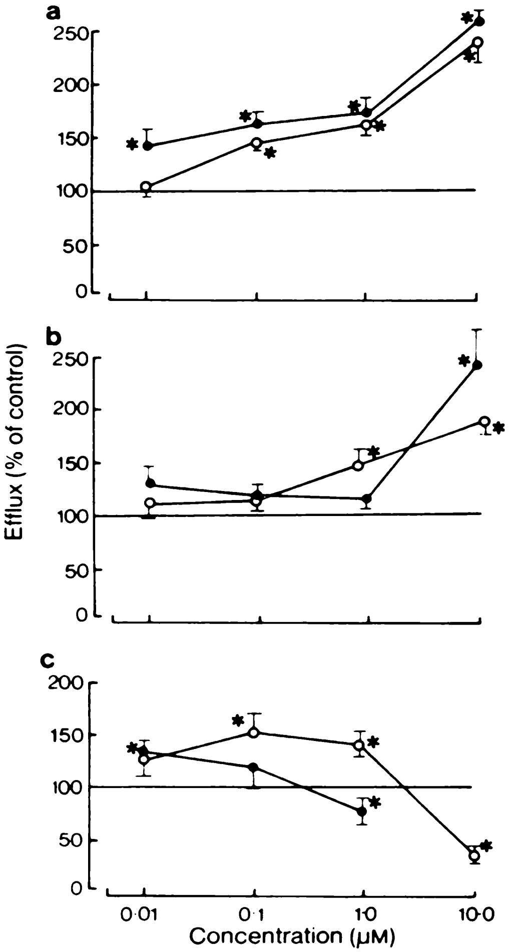


Figure 4 The effects of α-adrenoceptor and dopamine receptor antagonists on the efflux of tritium from the rabbit ear artery induced by nerve stimulation. Infusion of the antagonist was started 15 min before the second, and terminated 15 min before the third stimulation period. Cocaine was not present in these experiments. (a) (●) Phenoxybenzamine; (○) phentolamine; (b) (●) metoclopramide; (○) ergometrine; (c) (●) pimozide; (○) haloperidol.

Antagonism of vasoconstrictor responses to noradrenaline by α-adrenoceptor and dopamine receptor antagonists

The values for the antagonism in terms of pA_2 values of noradrenaline-induced vasoconstriction in ear artery segments by the various drugs are summarized in Table 2. Phenoxybenzamine and phentolamine were the most potent, pimozide and haloperidol were less so; ergometrine and metoclopramide were virtually devoid of α-adrenoceptor antagonistic activity.

Discussion

Low concentrations of dopamine lower blood sure in the anaesthetized cat, rabbit, guinea-pig & Rand, 1958), and dog (McDonald & Gol 1963; Sampson, Scroop & Louis, 1974; Bois Belliard & Haepille, 1975) and in unanaesth man (Horwitz, Fox & Goldberg, 1962; McD Goldberg, McNay & Tuttle, 1964). Furthe hypotension of a short duration and orthostatic tension are common features of levodopa-trea of parkinsonism in man (Godwin-Austen, Toml Frears & Kok, 1969). The hypotensive effect of mine is of particular interest as dopamine is a sympathomimetic agent (Sheys & Green, Lazner & de la Lande, 1974) and various hypot have been proposed to explain it. Burn & Rand (1 suggested that dopamine has a partial agonist on post-junctional α-receptors; the possibility specific dopamine receptors which mediated pheral vasodilatation was suggested by Eble (1 McNay & Goldberg (1966) and Hamilton (1 Boismare *et al.* (1975) suggested that a central ac of dopamine was responsible for its hypotensive e

In the rabbit ear artery, dopamine depressed v constrictor responses to sympathetic nerve st lation in a concentration which caused neither va latation nor vasoconstriction. The depre occurred immediately on infusion of 0.5 μM d mine, but responses generally returned towards trol levels during the continued infusion of the d The depression of vasoconstrictor responses appe to be a pre-junctional effect of dopamine, as respo to exogenous noradrenaline were not depressed. vasoconstrictor responses to sympathetic nerve sti lation returned towards their control level, respo to exogenous noradrenaline were usually enhan and prolonged.

Table 2 Values of pA_2 for antagonism of the vaso constrictor responses to noradrenaline in the rabb ear artery, calculated by the method of Arunlaksha & Schild (1959)

Drug used	*pA_2 value*
Phenoxybenzamine	8.9 ± 0.7
Phentolamine	8.0 ± 0.8
Pimozide†	7.5 ± 0.6
Haloperidol	7.0 ± 0.7
Ergometrine	< 5
Metoclopramide	< 5

† the pA_2 for pimozide is taken from Hope *et* (1977).

Dopamine interferes with transmission in peripheral structures innervated by adrenergic nerves (Farmer, 1965; Whitsett, Halushka & Goldberg, 1970; Ilhan & Long, 1975). Both Farmer (1965) and Ilhan & Long (1975) suggested that dopamine inhibited the release of the transmitter noradrenaline. Dopamine reduces stimulation-induced efflux of noradrenaline from guinea-pig hypothalamus (Bryant, McCulloch, Rand & Story, 1975), human arteries and veins (Stjärne & Brundin, 1975) and cat spleen and nictitating membrane (Langer, 1973; Enero & Langer, 1975). The concentrations of dopamine used in these experiments were all in the range 0.2 μM to 5 μM which, in rabbit ear artery, is not high enough to produce a vasoconstrictor effect (Hope, unpublished observations). Our experiments with radiolabelled transmitter support these findings. Dopamine was found to be a potent inhibitor of stimulation-induced transmitter release from sympathetic nerves in rabbit ear arteries which had been previously incubated in tritiated noradrenaline; it was equipotent with noradrenaline in this respect (Hope *et al.*, 1976). However, in some tissues, dopamine is ineffective in reducing stimulation-induced efflux of noradrenaline e.g. in the rabbit pulmonary artery (Endo, Starke, Bangeter & Taube, 1977), rat cerebral cortex (Farnebo & Hamberger, 1973; Starke & Montel, 1973), guinea-pig atria (McCulloch, Rand & Story, 1974) and guinea-pig vas deferens (Stjärne, 1975).

The inhibition of transmitter release in the presence of α-adrenoceptor agonists may be due to an activation of an inhibitory pre-junctional α-adrenoceptor (see reviews by Rand *et al.*, 1975; Langer, 1977; Starke, 1977). In the rabbit ear artery, dopamine has only about 2% of the activity of noradrenaline on post-junctional α-adrenoceptors (Lazner & de la Lande, 1974), yet it is equipotent in inhibiting the stimulation-induced efflux of noradrenaline from sympathetic nerves in the rabbit ear artery. If the inhibitory effect of dopamine on transmitter release is due to an action on pre-junctional α-adrenoceptors, then these receptors differ considerably in their agonist specificity from post-junctional α-adrenoreceptors. An alternative suggestion (Enero & Langer, 1975) is that the pre-junctional membrane has two populations of receptors, one the classic α-adrenoceptor and the other more responsive to dopamine.

In this series of experiments it was possible to antagonize selectively the inhibition of stimulation-induced efflux produced by dopamine and noradrenaline. Metoclopramide, a specific dopamine receptor antagonist (Dougan, Mearrick & Wade, 1974), ergometrine, and haloperidol, in concentrations of 0.2 μM, blocked the inhibition of stimulation-induced efflux caused by dopamine, but not that caused by noradrenaline. In the rabbit ear artery, a low concentration of the specific dopamine receptor antagonist (Andén *et al.*, 1970), pimozide (0.2 μM) blocked only the inhibition of stimulation-induced efflux of transmitter caused by dopamine; however, in a higher concentration (1 μM), a presynaptic α-adrenoceptor antagonist action was also noted (Hope *et al.*, 1977). Phenoxybenzamine (0.2 and 1 μM) and phentolamine (1 μM) blocked the inhibition of stimulation-induced efflux produced by both dopamine and noradrenaline, and phentolamine (0.2 μM) prevented the inhibition of stimulation-induced release by noradrenaline, but only partially prevented the inhibitory effect of dopamine on stimulation-induced efflux. A comparison of the potency of these six drugs on post-junctional α-adrenoceptors showed that metoclopramide and ergometrine had little or no α-adrenoceptor activity.

These findings points to the possibility of two different types of prejunctional inhibitory receptors. This explanation is supported by the fact that the preferred conformation of the dopamine molecule is quite different from that of the noradrenaline molecule (Kier & Truitt, 1970).

Although neither chlorpromazine nor pimozide were effective in the cat nictitating membrane (Enero & Langer, 1975), stimulation-induced efflux of transmitter from the rabbit ear artery was increased both by dopamine antagonists and by α-adrenoreceptor antagonists. In the rabbit ear artery, sufficient dopamine may be released during normal sympathetic nerve transmission to activate pre-junctional inhibitory dopamine receptors: alternatively, the dopamine antagonists in high concentrations may block pre-junctional α-adrenoreceptors.

The findings allow speculation on a possible physiological role for dopamine in inhibiting transmitter release from sympathetic nerves. Costa, Green, Koslow, LeFevre, Revuelta & Wang (1972) have shown that dopamine comprises up to 12% of catecholamines in vesicles present in sympathetic nerves, transmitter release from which occurs by exocytosis of vesicular contents (Geffen, Livett & Rush, 1969). When dopamine-β-hydroxylase has been inhibited in rabbit ear artery preparations and the transmitter stores are loaded with [^{3}H]-dopamine, dopamine is released by sympathetic nerve stimulation (Hope, Majewski, McCulloch, Rand & Story, 1978). It is possible, therefore, that dopamine is released along with noradrenaline during normal transmission. It seems unlikely that the small amount of dopamine normally present would have much effect on transmitter release when compared to the effects of noradrenaline, which modulates its own release by feedback inhibition (for reviews, see Rand *et al.*, 1975; Langer, 1977; Starke, 1977). However, under conditions of rapid stimulation, the conversion of dopamine to noradrenaline by dopamine-β-hydroxylase becomes rate-limiting and the proportion of dopamine in the transmitter vesicles increases (Kopin, Breese, Krauss &

Weiss, 1968). When sufficient dopamine is released, it might inhibit further release of the transmitter noradrenaline by acting on pre-junctional dopamine receptors. Thus, as suggested by McCulloch *et al.* (1973), inhibition of transmitter release by dopamine would enable the synthetic mechanism to make good the deficit of transmitter noradrenaline. At the same time, the released dopamine could act post-junctionally to increase the sensitivity of the effector cells. Thus, despite a reduction in transmitter release, there is a compensatory effect which in the long term tends to maintain transmission.

From the results obtained, it may be suggested that a presynaptic inhibitory system for sympathetic transmitter release, mediated by receptors which are sensitive to dopamine, is present in the rabbit ear artery. It is apparent, from a survey of the literature, that this system is not present in all tissues with adrenergic innervation, and as yet no physiological significance has been demonstrated for pre-junctional dopamine receptors in tissues where a dopamine-sensitive negative feedback mechanism exists.

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