Br. J. Pharmac. (1979), **67**, 185–192

MODULATION OF SYMPATHETIC TRANSMISSION BY NEURONALLY-RELEASED DOPAMINE

W. HOPE, H. MAJEWSKI, M.W. McCULLOCH, M.J. RAND & D.F. STORY

Department of Pharmacology, University of Melbourne, Parkville, Victoria, 3052, Australia

1 When rabbits were pretreated with Fla-63, there was a marked inhibition of dopamine-β-hydroxylase such that, after incubation of the ear arteries with [^{3}H]-dopamine, 47.2% of the tritium in the tissue was retained as unchanged dopamine.

2 [^{3}H]-dopamine was released by stimulation of the sympathetic nerves in ear arteries taken from rabbits pretreated with Fla-63 and incubated with [^{3}H]-dopamine.

3 The dopamine antagonists metoclopramide (1.0 μM) and ergometrine (1.0 μM) enhanced the stimulation-induced efflux of tritium in ear arteries taken from rabbits pretreated with Fla-63 and incubated with [^{3}H]-dopamine, but not when the arteries were incubated with [^{3}H]-noradrenaline.

4 These results suggest that if dopamine is present in the transmitter stores, it can be released by stimulation of the sympathetic nerves, and if the amount is adequate, it can activate an inhibitory feedback loop where prejunctional dopamine receptors are present.

Introduction

Exogenous dopamine inhibits the stimulation-induced efflux of transmitter noradrenaline from sympathetic nerves of the cat spleen and nictitating membrane (Langer, 1973; Enero & Langer, 1975), the rabbit ear artery (Hope, Law, McCulloch, Rand & Story, 1976), human arteries and veins (Stjärne & Brundin, 1975) and dog cutaneous blood vessels (Dalemans, Janssens, Verbeuren & Vanhoutte, 1976). The evidence suggests that this effect involves prejunctional receptors that are specific for dopamine. Relatively selective antagonists of dopamine block the inhibitory effect of dopamine on transmitter release without blocking the inhibitory effect of noradrenaline which involves prejunctional α-adrenoceptors: this has been shown for chlorpromazine and pimozide in cat nictitating membrane (Enero & Langer, 1975) and for ergometrine, haloperidol, metoclopramide, and pimozide in rabbit ear arteries (Hope, McCulloch, Story & Rand, 1977; Hope, McCulloch, Rand & Story, 1978). Furthermore, the specific dopamine agonist M-7 (2-dimethylamino-5,6-dihydroxytetralin) inhibits the release of transmitter noradrenaline in cat isolated heart (Strait & Bhatnager, 1975).

Endogenous dopamine is synthesized in the cytoplasm of peripheral noradrenergic nerves and taken up into the transmitter storage vesicles where it is converted to noradrenaline by dopamine-β-hydroxylase (Rutledge & Weiner, 1967). Normally, dopamine comprises only a small proportion of the catecholamine store in peripheral noradrenergic nerves (Costa, Green, Koslow, Lefevre, Revuelta & Wang, 1972; Snider, Almgren & Carlsson, 1973; Waldeck, Snider, Brown & Carlsson, 1975); it is unlikely, therefore, that the quantity of dopamine released by exocytosis of vesicular contents is sufficient to have any marked effect on prejunctional dopamine receptors during normal sympathetic transmission. However, under certain conditions such as prolonged sympathetic nerve stimulation (Snider *et al.*, 1973) and levodopa therapy (Breese & Prange, 1971) the levels of dopamine are raised.

It was the aim of this current investigation to establish a model in which there is a raised level of dopamine in the sympathetic transmitter stores of the rabbit isolated ear artery and to determine whether under these conditions dopamine is released by sympathetic nerve stimulation and whether it would activate a dopamine-receptor mediated negative feedback mechanism thereby decreasing transmitter release. This was accomplished by preventing the conversion of dopamine to noradrenaline by pretreatment of rabbits with the dopamine-β-hydroxylase inhibitor Fla-63 (Svensson & Waldeck, 1969) and incubation of the isolated arteries with [^{3}H]-dopamine.

Methods

Pretreatment of rabbits with Fla-63

Rabbits weighing from 1.5 to 2.5 kg were given doses of Fla-63 [*bis*-(4-methyl-1-homopiperazinylthiocar-

0007-1188/79/100185-08 $01.00

bonyl)disulphide] of 40 mg/kg by intraperitoneal injection. The Fla-63 was prepared as a suspension in 0.3 ml HCl (1.0 M) and approximately 2.5 ml distilled water, by use of a mortar and pestle. The suspension was prepared freshly for each experiment just before administration of the drug.

Tissue levels of [^{3}H]-dopamine and [^{3}H]-noradrenaline

Untreated rabbits or rabbits treated with Fla-63 were killed by cervical dislocation. The Fla-63 was, in most cases, administered 4 h before the animals were killed but in some preliminary experiments 1, 2 and 3 h pretreatment times were used. Segments of the central artery of each ear were cannulated and set up for perfusion-superfusion at 4 ml/min with Krebs-Henseleit solution as described by Allen, Rand & Story (1973). Perfusion pressure was measured with a Statham P23Db pressure transducer and recorded on a Rikadenki potentiometric recorder. The arteries were incubated in either [^{3}H]-dopamine (10 μCi/ml; 1.3 μM) or [^{3}H]-noradrenaline (10 μCi/ml; 1.1 μM) for 1 h and then perfused-superfused with catecholamine-free Krebs-Henseleit solution for 60 min to remove loosely bound tritiated catecholamines and metabolites.

The arteries were then removed from the apparatus, blotted dry, and placed in a receptacle which contained 0.2 ml HCl (0.1 M), along with ascorbic acid (5.7 mM) and disodium edetate (1.3 mM) to prevent oxidation of the compounds to be assayed, then frozen in liquid nitrogen. They were then quickly pulverized with a pre-cooled pneumatic hammer and the resulting powder was placed in a centrifuge tube containing 0.2 ml ethanol, 0.2 ml acetone and 0.1 ml HCl (0.1 M) which contained both ascorbic acid (5.7 mM) and disodium edetate (1.3 mM). To facilitate chromatographic separation, 2 μl of a carrier solution of dopamine, noradrenaline and their metabolites was added. The aliquot of carrier solution contained the following substances, each in an amount of 30 μg: (−)-noradrenaline hydrochloride, dopamine hydrochloride, (±)-normetanephrine hydrochloride, 3,4-dihydroxyphenylglycol, 3,4-dihydroxymandelic acid, *bis*(3-methoxy-4-hydroxyphenylglycol) piperazine, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylethylamine, 3,4-dihydroxyphenylethanol, 3-methoxy-4-hydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylethanol.

The mixture was centrifuged at 1200 *g* for 10 min and the supernatant was applied 7.5 cm from the bottom of a sheet of Whatman No. 1 paper (57 cm × 3 cm) and dried under a stream of nitrogen at room temperature. The sheets were chromatographed as described by Majewski & Story (1977) in the following solvent: *n*-butanol-ethylacetate-glacial acetic acid:sulphur dioxide solution (7% w/v):formic acid (90% w/w):HCl (10 M) in the ratios of 200:170:90:140:5:10, respectively. Ascending chromatographs were run at 4°C, until the solvent had moved about 30 cm from the origin (which took 18 to 24 h). They were then allowed to dry at room temperature and divided into 3 mm horizontal strips between the origin and the solvent front. The strips were placed in liquid scintillation counting vials to which 0.2 ml HCl (6 M) was added, followed by 1 ml of distilled water. After 30 min, 10 ml of scintillation fluid was added and the vials were shaken vigorously and left to stand for 1 h at room temperature. Radioactivity was measured in a Packard 3380 Tri-Carb Liquid Scintillation counter. The locations of noradrenaline and dopamine were determined by comparing the R_F values of the peaks of radioactivity with those of authentic [^{3}H]-noradrenaline and [^{3}H]-dopamine, respectively.

Stimulation-induced efflux of [^{3}H]-dopamine and [^{3}H]-noradrenaline

Periarterial stimulation of artery preparations was delivered through circular bipolar platinum electrodes to the proximal end of the artery.

The arteries were stimulated with monophasic square wave pulses of 1 ms duration and 12 V amplitude at 5 Hz for 10 s every 2 min during a 30 min equilibration period after which they were incubated in [^{3}H]-dopamine (10 μCi/ml; 1.3 μM) for 60 min, then perfused-superfused with dopamine-free Krebs-Henseleit solution for 90 min. The arteries were stimulated twice for 60 s at 30 min intervals with monophasic square wave pulses of 1 ms duration and 12 V amplitude at a frequency of 10 Hz. For each stimulation, two samples of the perfusion solution were collected over 4 min periods, one immediately before and one commencing with stimulation. An aliquot of 0.5 ml HCl (0.1 M) containing ascorbic acid (5.7 mM) and disodium edetate (1.3 mM) was added to each sample collected for assay to prevent oxidation of catecholamines. From the final solution, a 0.5 ml aliquot was taken for the estimation of total tritium.

The solvent system described by Majewski & Story (1977) for the separation of noradrenaline, dopamine and their metabolites does not allow complete resolution of dopamine and normetanephrine. Therefore, the non-catechol metabolites were removed from the catechol-containing compounds by the alumina adsorption procedure described by Anton & Sayre (1962). After adsorption of the catechols onto the alumina and their subsequent elution with acetic acid (0.2 M), 30 μl of the carrier solution previously described was added and then the eluate was quickly frozen in liquid nitrogen and freeze-dried.

Tritiated noradrenaline, dopamine and their catechol metabolites were extracted from the freeze-dried samples in a mixture of acetone:ethanol:HCl (0.1 M) in the proportion of 4:4:1, respectively. The mixture also contained ascorbic acid (0.57 mM) and disodium edetate (0.13 mM). The residue left after freeze-drying was first suspended in 0.5 ml of this solvent mixture, shaken vigorously for 5 min and then centrifuged for 5 min at 1200 *g* at 4°C and the supernatant collected. Two subsequent extractions of the residue were performed, each with 0.25 ml of the solvent mixture. The pooled supernatants were applied 7.5 cm from the bottom of Whatman No. 3 paper (57 cm × 2 cm) and dried under a stream of nitrogen at room temperature. [^{3}H]-noradrenaline and [^{3}H]-dopamine were separated by ascending chromatography as previously described, and radioactivity was measured by liquid scintillation counting.

The stimulation-induced efflux of each tritiated catecholamine was calculated by subtracting the amount of radioactivity corresponding to each catecholamine in the sample collected before stimulation from that collected during stimulation. Corrections were made for efficiency of extraction (approximately 80%).

The effects of dopamine antagonists on the stimulation-induced release of tritium

Segments of ear arteries from rabbits which had been pretreated with Fla-63 (40 mg/kg) 4 h previously, and from untreated rabbits, were set up and incubated with either [^{3}H]-dopamine (10 μCi/ml; 1.3 μM) or [^{3}H]-(−)-noradrenaline (10 μCi/ml; 1.1 μM) as described in the previous section. After the 90 min of perfusion-superfusion with catecholamine-free Krebs-Hensleit solution, the arteries were stimulated at a frequency of 10 Hz for a period of 1 min.

Consecutive samples of the solution perfusing the artery were collected over 30 s periods, two before, two during, and six after stimulation. A 1 ml aliquot of each of the samples was placed in a vial containing 0.2 ml HCl (6.0 M) and 10 ml scintillation solution, and the radioactivity was measured.

At the end of the collection of the efflux samples, the arteries were removed from the apparatus, blotted dry and placed in vials containing 0.5 ml 'Soluene-350' and placed in an oven at 65°C for 10 to 15 min until the arteries had dissolved. Then 10 ml of scintillation fluid was added to the contents, the vials were shaken vigorously and the radioactivity determined after the vials were allowed to stand at 4°C in the dark for 12 h. Corrections for counting efficiency in both the aqueous and tissue samples were made by automatic external standardization.

The total tritium content of the tissue immediately before stimulation was calculated by summing the tritium content measured in the arteries after the experiment and the total tritium present in all perfusate-superfusate samples collected during and after stimulation. Resting efflux of tritium was taken as the mean of the efflux present in the two samples collected before stimulation. The stimulation-induced efflux was calculated by subtracting the resting efflux from each of the effluxes in the samples collected during and after stimulation. Stimulation-induced efflux of radioactivity was expressed as a percentage of the total radioactivity present in the tissue at the start of the stimulation procedure. This was defined as the percentage stimulation-induced efflux.

The effects of dopamine receptor antagonists on the resting and stimulation-induced effluxes were determined by infusing solutions of the drugs, starting 15 min before the 1 min period of stimulation, into the perfusion just proximal to the artery.

Statistical analysis of results

Except where otherwise indicated, the unpaired 2 tailed Student's *t*-test was used to test for significant differences between means of experimental results. The value of *t* was calculated using the pooled variance estimate or the separate variance estimates, depending on the homogeneity of the variances. Probability levels less than 0.05 were taken to indicate statistical significance.

Materials

The Krebs-Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, $MgSO_4$ 0.45, KH_2PO_4 1.03, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.07.

The scintillation solution had the following composition: 5.5 g of 2,5-diphenyloxazol (PPO), 0.1 g of 1,3-bis-2-(5-phenyloxazolyl) benzene (POPOP) and 333 ml of Triton X-100 made up to 1 litre in toluene.

The following drugs were used: haloperidol (Searle); metoclopramide monohydrochloride (Beecham Research Laboratories); phentolamine mesylate (Ciba-Geigy); ergometrine maleate (Burroughs Wellcome) and Fla-63 (Labkemi, Sweden).

The following compounds were obtained from Sigma, U.S.A.: (−)-noradrenaline hydrochloride, (±)-normetanephrine hydrochloride, 3,4-dihydroxyphenylglycol, 3,4-dihydroxymandelic acid, (±)-3-methoxy-4-hydroxymandelic acid, *bis*-(3-methoxy-4-hydroxyphenylglycol) piperazine, 3,4-dihydroxyphenylacetic acid and dopamine hydrochloride. The 3-methoxy-4-hydroxyphenylethanol was obtained from Calbiochem, U.S.A. and 3-methoxy-4-hydroxyphenylethylamine was obtained from K & K labs, U.S.A. All reagents used were analytical grade. Tritiated noradrenaline and dopamine were obtained

from New England Nuclear Corporation, U.S.A.: (−)-[7-^{3}H]-noradrenaline had a specific activity of 9.1 Ci/mmol; [ethyl-2-^{3}H]-dopamine had a specific activity of 7.5 Ci/mmol. Both were stored at 4°C and used without further dilution.

Results

Tissue levels of [^{3}H]-dopamine and [^{3}H]-noradrenaline

When ear arteries from normal rabbits were incubated in either [^{3}H]-dopamine or [^{3}H]-noradrenaline, almost all of the tritium retained was present as [^{3}H]-noradrenaline (Table 1). [^{3}H]-normetanephrine, as a possible interfering substance in the estimation of [^{3}H]-dopamine, was not detectable when arteries were incubated in [^{3}H]-noradrenaline and thus was unlikely to be a source of error.

When arteries from rabbits which had been pretreated with Fla-63 were incubated with [^{3}H]-dopamine, considerable amounts of [^{3}H]-dopamine were retained as such by the tissue. Preliminary experiments in which Fla-63 was administered 1, 2, 3 and 4 h before the animals were killed indicated that retention of [^{3}H]-dopamine by the arteries increased with pretreated time up to 2 h and then remained approximately constant. In each case there was a proportional decrease in the amounts of [^{3}H]-noradrenaline formed from the [^{3}H]-dopamine. The mean levels of [^{3}H]-dopamine and [^{3}H]-noradrenaline present in arteries from rabbits which had been pretreated with Fla-63 4 h previously and then incubated in [^{3}H]-dopamine are given in Table 1. In all subsequent experiments where Fla-63 was administered, it was given 4 h before taking the ear arteries for study. In arteries from Fla-63 pretreated rabbits incubated in [^{3}H]-noradrenaline, almost all of the tritium was retained as [^{3}H]-noradrenaline (Table 1). As in the untreated rabbits, [^{3}H]-normetanephrine was not detectable in arteries from Fla-63 pretreated rabbits after [^{3}H]-noradrenaline incubation. These findings show that the dopamine-β-hydroxylase inhibitor Fla-63 reduced the conversion of [^{3}H]-dopamine into [^{3}H]-noradrenaline, and that [^{3}H]-dopamine was retained as such in the tissue.

Stimulation-induced efflux of [^{3}H]-dopamine and [^{3}H]-noradrenaline after Fla-63 pretreatment

Measurements were made of the amounts of [^{3}H]-noradrenaline and [^{3}H]-dopamine in samples of perfusion-superfusion fluid from ear arteries taken from rabbits treated with Fla-63 (40 mg/kg) and incubated in [^{3}H]-dopamine. No [^{3}H]-dopamine or [^{3}H]-noradrenaline was detectable in samples collected before stimulation. However, in samples collected during the first period of stimulation, 40.8% (s.e. mean = 1.7, n = 3) of the tritium present was as [^{3}H]-noradrenaline and 35.9% (s.e. mean = 1.0, n = 3) was as [^{3}H]-dopamine. During the second period of stimulation, 32.2% (s.e. mean = 5.8, n = 3) of the tritium was identified as [^{3}H]-noradrenaline and 10.2% (s.e. mean = 9.6, n = 3) was [^{3}H]-dopamine.

Effect of dopamine antagonists on the stimulation-induced efflux of tritium

Under the experimental conditions used, there was no significant difference in the ability of arteries from Fla-63 pretreated rabbits to accumulate tritium during incubation with [^{3}H]-dopamine and [^{3}H]-noradrenaline. Further, the addition of phentolamine (0.5 μM), haloperidol (1.0 μM), ergometrine (1.0 μM) and metoclopramide (1.0 μM) after incubation of arteries in [^{3}H]-noradrenaline or [^{3}H]-dopamine did not significantly alter the mean tissue tritium accumulation. Therefore any change in the stimulation-induced efflux of tritium expressed as a percentage of tissue tritium content (percentage stimulation-induced efflux) can be attributed to changes in the stimulation-induced efflux of tritium.

Table 1 Effect of Fla-63 treatment on the proportions of [^{3}H]-dopamine and [^{3}H]-noradrenaline present in rabbit ear arteries after incubation with [^{3}H]-dopamine or [^{3}H]-noradrenaline

Incubation medium	n	*Pretreated with Fla-63*	*Proportion (%) of tritium in tissue as:* [^{3}H]-*dopamine*	[^{3}H]-*noradrenaline*
[^{3}H]-noradrenaline	3	No	Undetectable	93.0 ± 1.2%
[^{3}H]-dopamine	3	No	4.0 ± 0.1%	94.3 ± 0.4%
[^{3}H]-noradrenaline	3	Yes	Undetectable	95.0 ± 0.9%
[^{3}H]-dopamine	3	Yes	47.2 ± 2.9%	56.2 ± 3.9%

The Fla-63 (40 mg/kg) was given 4 h before the tissues were removed for study. The values are means ± s.e. means.

The percentage stimulation-induced efflux of tritium was the same for control arteries after incubation with [^{3}H]-dopamine or [^{3}H]-noradrenaline. Similarly there was no difference in the percentage stimulation-induced tritium efflux after incubation with either catecholamine when the arteries were from rabbits pretreated with Fla-63 although here the efflux was significantly greater than in the controls (Table 2).

In arteries from unpretreated rabbits incubated in [^{3}H]-dopamine, metoclopramide (1.0 μM) did not significantly alter the percentage stimulation-induced efflux of tritium compared to control. The percentage stimulation-induced efflux of tritium in the presence of metoclopramide (1.0 μM) was 2.9% (s.e. mean = 0.4, $n = 4$).

In arteries taken from rabbits treated 4 h previously with Fla-63 (40 mg/kg) and incubated with [^{3}H]-noradrenaline, the α-adrenoceptor blocking drug phentolamine (0.5 μM) enhanced the percentage stimulation-induced efflux of tritium. However, the dopamine antagonists metoclopramide (1.0 μM), and ergometrine (1.0 μM) were without effect, and haloperidol (1.0 μM) reduced the percentage stimulation-induced efflux of tritium. When the arteries taken from pretreated rabbits were incubated with [^{3}H]-dopamine instead of [^{3}H]-noradrenaline, phentolamine (0.5 μM), ergometrine (1.0 μM) and metoclopramide (1.0 μM) enhanced the percentage stimulation-induced efflux of tritium, whereas haloperidol (1.0 μM) was without effect (Figure 1). Neither phentolamine nor the dopamine antagonists altered the resting efflux of tritium from arteries incubated in either [^{3}H]-dopamine or [^{3}H]-noradrenaline.

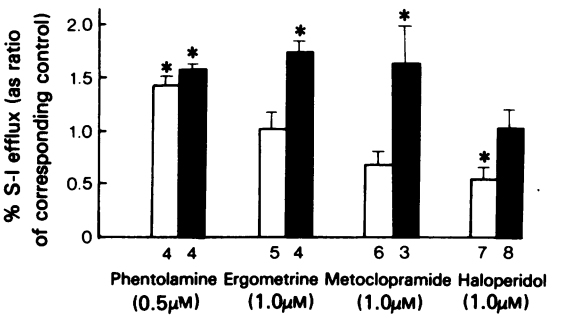

Figure 1 The effects of phentolamine, and dopamine antagonists on the percentage stimulation-induced (S-I) efflux of tritium from rabbit ear arteries taken from rabbits pretreated with Fla-63 (40 mg/kg, i.p.) 4 h previously. Open columns = incubated in [^{3}H]-noradrenaline; solid columns = incubated in [^{3}H]-dopamine. Vertical bars represent s.e. means. Numbers below the histograms refer to the number of experiments performed. * Significantly different from the corresponding control.

Discussion

This study has shown that [^{3}H]-dopamine is taken up and almost completely converted to [^{3}H]-noradrenaline in the rabbit ear artery, presumably by dopamine-β-hydroxylase in transmitter storage vesicles. Fla-63, a potent dopamine-β-hydroxylase inhibitor (Svensson & Waldeck, 1969), was used to prevent this conversion. Ear arteries from rabbits pretreated with Fla-63 showed an increase in the proportion of tritium retained as [^{3}H]-dopamine and a concomitant decrease in the proportion of tritium retained as [^{3}H]-noradrenaline in the tissue after incubation with [^{3}H]-dopamine. Further, in arteries from rabbits pretreated with Fla-63 and incubated with [^{3}H]- dopamine, [^{3}H]-dopamine and [^{3}H]-noradrenaline were released upon intramural sympathetic nerve stimulation. The release of dopamine after inhibition of dopamine-β-hydroxylase has also been reported by Thoenen, Haefely, Gey & Huerlimann (1967) in cat spleen.

The metabolites of [^{3}H]-dopamine or [^{3}H]-noradrenaline were not detected in the ear arteries after incubation in either [^{3}H]-noradrenaline or [^{3}H]-

Table 2 The percentage stimulation-induced (S-I) efflux of tritium from arteries incubated in either [^{3}H]-dopamine or [^{3}H]-noradrenaline

Incubation medium	n	*Pretreated with Fla-63*	*% S-I efflux of tritium*
[^{3}H]-noradrenaline	4	No	2.2 ± 0.4
[^{3}H]-dopamine	4	No	2.3 ± 0.1
[^{3}H]-noradrenaline	4	Yes	3.4 ± 0.4*
[^{3}H]-dopamine	5	Yes	3.9 ± 0.5*

Values are means ± s.e. means.
* Pretreatment with Fla-63 significantly increased % S-I efflux (2-way analysis of variance, $P < 0.01$).

dopamine whether they were taken from rabbits pretreated with Fla-63 or untreated rabbits. However, metabolites were detected in the stimulation-induced tritium effluxes suggesting that the metabolism of these catecholamines occurred subsequent to release; thus, stimulation-induced efflux of tritium can be taken as an index of tritiated dopamine and noradrenaline released from sympathetic nerve terminals.

There were no significant differences in the percentage stimulation-induced effluxes of tritium from ear arteries taken from rabbits pretreated with Fla-63 whether they were incubated with [^{3}H]-dopamine (when both [^{3}H]-dopamine and [^{3}H]-noradrenaline are released), or incubated with [^{3}H]-noradrenaline (when only [^{3}H]-noradrenaline is released), suggesting that both catecholamines were released equally as well and probably form part of the same transmitter storage pool. Further, there was no significant difference in the percentage stimulation-induced efflux of tritium from arteries from untreated rabbits whether they were incubated in [^{3}H]-dopamine or [^{3}H]-noradrenaline, suggesting that newly synthesized [^{3}H]-noradrenaline is released equally as well as [^{3}H]-noradrenaline taken up as such by the tissue, and is therefore probably part of the same transmitter storage pool. This is contrary to the findings of Kopin, Breese, Krauss & Weise (1968) who found that newly synthesized noradrenaline was preferentially released in cat spleen. The discrepancy may have occurred because they used radiolabelled tyrosine as the precursor which may be subsequently converted to noradrenaline at different sites within the neurone from that of exogenously administered [^{3}H]-dopamine.

Transmitter noradrenaline can modulate its own release from sympathetic nerves by activation of an inhibitory feedback mechanism mediated by prejunctional α-adrenoceptors (see review by Starke, 1977). Phentolamine (0.5 μM), an α-adrenoceptor blocking drug, enhanced the percentage stimulation-induced efflux of tritium in arteries from rabbits pretreated with Fla-63 whether they were incubated in [^{3}H]-dopamine or [^{3}H]-noradrenaline. Under the conditions of these experiments noradrenaline is released by stimulation in both cases and phentolamine probably enhances transmitter release by blockade of noradrenaline activation of the α-adrenoceptor mediated inhibitory feedback loop.

Dopamine comprises only a small proportion of catecholamines in peripheral sympathetic nerves (Costa *et al.*, 1972; Snider *et al.*, 1973; Waldeck *et al.*, 1975). Enero & Langer (1975) have shown that pimozide and chlorpromazine, in concentrations that abolish the inhibitory effects of dopamine on transmitter release, do not by themselves enhance stimulation-induced [^{3}H]-noradrenaline release. These results suggest that dopamine, released from nerves, does not normally activate an inhibitory feedback mechanism through dopamine receptors. This is in contrast to findings in rabbit ear artery where several dopamine receptor antagonists (pimozide, metoclopramide, ergometrine and haloperidol) have been shown to enhance stimulation-induced [^{3}H]-noradrenaline release (Hope *et al.*, 1978). However, the effect with metoclopramide was only observed in concentrations greater than 1.0 μM whereas pimozide and haloperidol depressed stimulation-induced efflux in concentrations greater than 0.1 and 1.0 μM respectively. It is possible that the enhancement of stimulation-induced efflux observed by Hope *et al.* (1978) may be explained, at least in part, by blockade of prejunctional α-adrenoceptors or by other non-specific effects of the drugs which make it difficult to draw conclusions about the mechanism of action.

In the present experiments metoclopramide, haloperidol and ergometrine, each in a concentration of 1.0 μM, did not enhance the percentage stimulation-induced efflux of tritium from arteries taken from rabbits pretreated with Fla-63 and incubated with [^{3}H]-noradrenaline (Figure 1). Thus, even if the conversion of dopamine to noradrenaline is inhibited, the amount of endogenous dopamine released does not appear to be sufficient to activate the negative feedback loop mediated through dopamine receptors. In these experiments and in others (Enero & Langer, 1975; Hope *et al.*, 1978), the tissues were incubated in [^{3}H]-noradrenaline and thus the endogenous transmitter stores may be partly replaced by [^{3}H]-noradrenaline to the extent that the amount of endogenous dopamine in the stores and released is less than that under normal conditions. However, in the present study when ear arteries from untreated rabbits were incubated in [^{3}H]-dopamine, although [^{3}H]-dopamine was largely metabolized to [^{3}H]-noradrenaline, about 4% of the tritium was retained as [^{3}H]-dopamine. In this situation, the levels of dopamine may more closely resemble a normal state. Nevertheless, metoclopramide (1.0 μM) failed to enhance the percentage stimulation-induced efflux of tritium, indicating that even under these conditions dopamine is not released in sufficient amounts to activate the inhibitory feedback loop mediated through dopamine receptors.

If arteries from rabbits pretreated with Fla-63 were incubated in [^{3}H]-dopamine, so that a greater proportion of dopamine was released by sympathetic nerve stimulation, metoclopramide (1.0 μM) and ergometrine (1.0 μM) enhanced the percentage stimulation-induced efflux of tritium (Figure 1). This may be due to blockade of the activation by dopamine of the inhibitory feedback mechanism mediated through prejuctional dopamine receptors, thus increasing transmitter release. When arteries from pretreated rabbits were incubated in [^{3}H]-noradrenaline (conditions

under which no [^{3}H]-dopamine is released), metoclopramide (1.0 μM) and ergometrine (1.0 μM) failed to enhance release; thus, the facilitatory effects of metoclopramide and ergometrine are dependent on transmitter dopamine release. The effects of haloperidol (1.0 μM) are harder to interpret since its specificity for dopamine receptors is not marked (Goldberg, Volkman & Kohli, 1978). When the transmitter stores in arteries from pretreated rabbits contained [^{3}H]-noradrenaline, haloperidol (1.0 μM) decreased the stimulation-induced release of tritium. However, in arteries from pretreated rabbits which had been incubated in [^{3}H]-dopamine, both [^{3}H]-noradrenaline and [^{3}H]-dopamine are released by nerve stimulation and haloperidol (1.0 μM) had no significant effect on tritium release (Figure 1). It is possible that an increase in tritium release due to blockade by haloperidol of the inhibitory feedback mechanism mediated through dopamine receptors is balanced by a depressant effect of haloperidol on transmitter release, resulting in no overall effect.

These results suggest that dopamine released by sympathetic nerve stimulation can activate an inhibitory feedback loop mediated through specific dopamine receptors if the amount of dopamine in the nerve terminal is raised. Such conditions may occur during levodopa therapy when the proportion of dopamine in sympathetically innervated tissues is raised (Breese & Prange, 1971). Indeed, orthostatic hypotension is a common side-effect of levodopa (Barbeau, 1969; Goldberg, 1972), suggesting impairment of the vascular control exerted by the sympathetic nervous system. Increased levels of dopamine are also found in the rat salivary gland after prolonged periods of stimulation (Snider *et al.*, 1973) and in the rat adrenal gland after neurogenic stimulation (Snider & Carlsson, 1973). This may occur because of an increase in tyrosine hydroxylase activity during nerve stimulation which is not matched with a concomitant increase in dopamine-β-hydroxylase activity, so that dopamine-β-hydroxylase becomes rate-limiting and the levels of dopamine increase (Snider *et al.*, 1973). Thus, the possibility exists that transmitter dopamine activation of the inhibitory feedback loop mediated through dopamine receptors may be important in conserving transmitter release during prolonged periods of stimulation, as initially suggested by McCulloch, Rand & Story (1973).

This work was supported by grants from the National Health and Medical Research Council and the National Heart Foundation of Australia.

References

ALLEN, G.S., RAND, M.J. & STORY, D.F. (1973). Techniques for studying adrenergic transmitter release in an isolated perfused artery, *Cardiov. Res.*, **7**, 423–428.

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

BARBEAU, A. (1969). l-Dopa therapy in Parkinson's disease: a critical review of nine years experience. *Can. med. Ass. J.*, **101**, 791–800.

BREESE, G.R. & PRANGE, A.J. (1971). Chronic dopa treatment: effect on the concentration of norepinephrine in the heart and brain of rats. *Eur. J. Pharmac.*, **13**, 259–301.

COSTA, E., GREEN, A.R., KOSLOW, S. H., LEFEVRE, H.F., REVUELTA, A.V. & WANG, C. (1972). Dopamine and norepinephrine in noradrenergic axons: a study *in vivo* of their precursor product relationship by mass fragmentography and radiochemistry. *Pharmac. Rev.*, **24**, 167–190.

DALEMANS, P., JANSSENS, W., VERBEUREN, T. & VANHOUTTE, P.M. (1976). Effects of naturally occurring catecholamines on adrenergic neuroeffector interactions in isolated cutaneous veins. *Archs int. Pharmacodyn. Thér.*, **220**, 330–334.

ENERO, M.A. & LANGER, S.Z. (1975). Inhibition by dopamine of [^{3}H]-noradrenaline release elicited by nerve stimulation in isolated cat's nictitating membrane. *Naunyn-Schmiedebergs Arch. Pharmac.*, **289**, 189–203.

GOLDBERG, L.I. (1972). Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmac. Rev.*, **24**, 1–29.

GOLDBERG, L.I., VOLKMAN, P.H. & KOHLI, J.D. (1978). A comparison of the vascular dopamine receptor and other dopamine receptors. *A. Rev. Pharmac. Tox.*, **18**, 57–79.

HOPE, W., LAW, M., MCCULLOCH, M.W., RAND, M.J. & STORY, D.J. (1976). Effects of some catecholamines on noradrenergic transmission in the rabbit ear artery. *Clin. exp. Pharmac. Physiol.*, **3**, 15–28.

HOPE, W., MCCULLOCH, M.W., RAND, M.J. & STORY, D.F. (1978). Modulation of noradrenergic transmission in the rabbit ear artery by dopamine. *Br. J. Pharmac.*, **64**, 527–537.

HOPE, W., MCCULLOCH, M.W., STORY, D.F. & RAND, M.J. (1977). Effect of pimozide on noradrenergic transmission in the rabbit ear artery. *Eur. J. Pharmac.*, **46**, 101–111.

KOPIN, I.J., BREESE, G.R., KRAUSS, K.R. & WEISE, V.K. (1968). Selective release of newly synthesized norepinephrine from cat spleen during sympathetic nerve stimulation. *J. Pharmac. exp. Ther.*, **161**, 271–278.

LANGER, S.Z. (1973). The regulation of transmitter release elicited by nerve stimulation through a presynaptic feedback mechanism. In *Frontiers in Catecholamine Research*. ed. Usdin, E. & Snyder, S.H., pp. 542–549. New York: Pergamon Press.

MAJEWSKI, H. & STORY, D.F. (1977). Paper chromato-

graphic separation of noradrenaline and its major metabolites. *J. Chromat.*, **139**, 218–222.

McCULLOCH, M.W., RAND, M.J. & STORY, D.F. (1973). Evidence for a dopaminergic mechanism for modulation of adrenergic transmission in the rabbit ear artery. *Br. J. Pharmac.*, **49**, 141–142P.

RUTLEDGE, C.O. & WEINER, N. (1967). The effect of reserpine on the synthesis of norepinephrine in the isolated rabbit heart. *J. Pharmac. exp. Ther.*, **157**, 290–302.

SNIDER, S.R., ALMGREN, O. & CARLSSON, A. (1973). The occurrence and functional significance of dopamine in some peripheral adrenergic nerves of the rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **273**, 1–12.

SNIDER, S.R. & CARLSSON, A. (1973). The adrenal dopamine as an indicator of adrenomedullary hormone biosynthesis. *Naunyn-Schmiedebergs Arch. Pharmac.*, **275**, 347–357.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

STJÄRNE, L. & BRUNDIN, J. (1975). Affinity of noradrenaline and dopamine for neural α-receptors mediating negative feedback control of noradrenaline secretion in human vasoconstrictor nerves. *Acta physiol. scand.*, **97**, 88–93.

STRAIT, M.R. & BHATNAGER, R.K. (1975). Inhibition of norepinephrine release elicited by nerve stimulation by an analog of apomorphine. *Fedn Proc.*, **34**, 740.

SVENSSON, T.H. & WALDECK, B. (1969). On the significance of central noradrenaline for motor activity: experiments with a new dopamine-β-hydroxylase inhibitor. *Eur. J. Pharmac.*, **1**, 278–282.

THOENEN, H., HAEFELY, W., GEY, K.F. & HUERLIMANN, A. (1967). Diminished effects of sympathetic nerve stimulation in cats pretreated with disulfiram. *J. Pharmac. exp. Ther.* **156**, 246–251.

WALDECK, B., SNIDER, S.R., BROWN, R. & CARLSSON, A. (1975). Studies on the synthesis and subcellular distribution of dopamine in the rat adrenal medulla. *Naunyn-Schmiedebergs Arch. Pharmac.*, **287**, 1–10.

(*Received November 9, 1978.*
Revised February 23, 1979.)