DISTRIBUTION, UPTAKE AND RELEASE OF DOPAMINE IN RABBIT ARTERIES

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Accepted March 1, 1981

Abstract—The amounts of endogenous dopamine (DA) in rabbit atria and arteries were less than 7% of total catecholamine content. Renal artery had the highest concentration of DA (0.30 μg/g), and the ratio of DA to noradrenaline (NA) was the largest among the tissues examined. After a single dose of reserpine (1 mg/kg i.p.), the levels of NA in the atria, mesenteric and renal arteries were reduced markedly, whereas the reduction of DA in the latter two arteries was not significant, suggesting that some of the DA is localized in the cytoplasm by a reserpine-resistant mechanism in these arteries. The uptake of ³H-DA or ³H-(−)-NA after incubation with 0.1 μM ³H-amine was the largest in the renal artery. After incubation with ³H-DA, the production of oxidative deaminated metabolites was 2–3-fold higher than that after with ³H-NA despite the smaller uptake of total ³H, suggesting that DA may not always be taken up by the vesicles or that this compound may be leaked from the vesicles into the cytosol more readily than NA and degraded by monoamine oxidase. Electrical transmural stimulation after incubation of renal artery with ¹⁴C-DA increased the overflow of both ¹⁴C-DA and ¹⁴C-NA, demonstrating the importance of the storage, conversion and release mechanism in this artery.

Sympathetic nerve endings in a variety of blood vessels contain appreciable concentrations of noradrenaline (NA) and take up exogenous NA by, at least, two different mechanisms (1, 2). It has been reported that the cardiovascular effects of dopamine (DA) are qualitatively different from those of NA and that such actions of DA are probably due to the effects on specific DA receptors in the renal, mesenteric, coronary and some intracerebral arteries (3–5). We carried out studies on the biochemical aspects of DA in rabbit blood vessels with special reference to comparisons of uptake, storage and release of this amine in the renal and other arteries in order to acquire data on the sympathetic nervous system in the cardiovascular system.

MATERIALS AND METHODS

1. Biochemical assay of endogenous DA and NA: Albino rabbits of both sexes weighing 2.0 kg to 2.2 kg were sacrificed by a blow on the head and the atria, the thoracic and abdominal aortae, the celiac, superior mesenteric, renal and femoral arteries were used for study. All vascular tissues were carefully cleaned of adhering tissues using scissors and a nylon blush. Some vessels were pooled for analysis, as indicated in Table 1. In one series of experiments, 1 mg/kg of reserpine was injected i.p. 20 hours before sacrifice.

DA and NA were isolated by passing the extracts (pH 4.0) through a column of
Dowex 50W × 4, 200–400 mesh (9 mm diameter, 15 mm height in water). Before the column was used it was passed through the following solutions: a) 20 ml of 2 N hydrochloric acid, b) approx. 10 ml of glass-distilled water, c) 10 ml of N acetate buffer (pH 6.0), d) 5 ml of glass-distilled water (6). Hydrochloric acid (N and 2 N) was used to elute the amines. After discarding the first 3 ml of N hydrochloric acid, NA was collected in the following five 2-ml portions of N hydrochloric acid, and DA was eluted in the next five 2-ml of 2 N hydrochloric acid. NA was estimated fluorometrically after ferricyanide oxidation (6). DA was assayed by a modification of the method of Laverty and Sharman (7). After disodium edetate (20 mg) had been added to the eluate of DA (10 ml), the solution was neutralized by the addition of approx. 1.3 g of sodium bicarbonate, then 0.75 ml of acetic anhydride was added to the solution and the preparation shaken for 15 min. One ml of a 3:2 mixture of ethylenediamine and 2 N hydrochloric acid in volume, which was freshly prepared, was added to 8.0 ml of the solution, then the mixture was heated at 65°C for 20 min in the dark. The mixture was removed from the water bath, cooled, saturated with sodium chloride and then shaken with 3.0 ml of isobutanol for 5 min. After centrifugation, the isobutanol layer was assayed fluorometrically at 513 nm after activation at 425 nm.

2. Separation and measurement of 3H-dopamine (3H-DA), 3H-(l)-noradrenaline (3H-NA) and these metabolites in tissues after incubation with labeled amines: Each tissue was transferred to a beaker containing 37°C Krebs-bicarbonate solution of the following composition (in mM): NaCl 118.1, KCl 4.7, CaCl₂ 1.9, KH₂PO₄ 1.18, MgSO₄ 1.18, NaHCO₃ 25.0, dextrose 5.0, EDTA-2Na 0.04, 0.1 g/l of ascorbic acid. The volume of medium was 50 ml/tissue. As shown by Hellmann et al. (8), at low perfusion concentration (0.047 × 10⁻¹⁰ mol/ml–1.3 × 10⁻¹⁰ mol/ml), the storage of the DA taken up and the deamination take place mainly within the neurons. Therefore, the tissues were incubated with 0.1 μM 3H-DA (specific activity 5.0 Ci/m mol, New England Nuclear) or 3H-NA (specific activity 3.8 Ci/m mol, New England Nuclear) for 20 min after equilibration for about 1 hour.

After incubation, the tissues were washed three times in ice-cold Krebs’ solution, weighed and homogenized in ice-cold perchloric acid (0.4 N) containing 100 mg EDTA-2Na and 30 mg of sodium metabisulfite. The homogenates were centrifuged (20,000 g), and DA, NA and the deaminated and/or O-methylated metabolites of these amines
were added to the extracts as unlabeled carriers. Radioactive DA, NA and these metabolites were separated by column chromatography, the columns being 9 mm in diameter and stoppered with glass wool. Al₂O₃ columns were used for the separation of all O-methylated metabolites from the compounds having an intact catechol structure. The columns were filled with 500 mg of alumina activated as described by Anton and Sayre (9), and passed through 10 ml of 0.2 N Tris-HCl buffer (pH 8.4). After the supernatants had been passed through alumina, the columns were washed with 10 ml of water. A sample of this effluent was used to determine the radioactivity of O-methylated and O-methylated deaminated metabolites. Elution from the alumina column was carried out with four 5-ml portions of 0.2 N acetic acid and the eluate mixed. Therefore, Dowex 50W×4 (200–400 mesh) columns were used to separate DA, NA and catechol deaminated metabolites. The columns were filled to 15 mm height with the resin at pH 7.0 and were ready for use after pretreatment according to the method of Bertler et al. (6). Alumina eluates adjusted to pH 4.0 prior to use were passed through the column. After rinsing the column with 10 ml of water and 3 ml of N HCl, elution was performed with five 2-ml portions of N HCl. ⁳H-NA was eluted in these portions, ³H-DA was eluted in successive five 2-ml portions of 2 N HCl, then each eluate was transferred to a 40 ml beaker and stirred after adding 400 mg of activated alumina at pH 8.4 for 5 min. Elution was performed by shaking the alumina with 3 ml of 0.2 N acetic acid for 15 min. A sample of the eluates was counted on a Packard Tri-Carb liquid scintillation spectrometer. All values were corrected for recoveries of 70±10% of S.E.

3. Transmural stimulation: The strip of renal artery was initially immersed in 50 ml of Krebs' solution and maintained at 37°C by circulating warm water through a glass jacket. To this medium, ¹⁴C-DA (specific activity 57.4 mCi/m mol, Radiochemical Centre) was added in a concentration of 0.1 µM. After 30 min, the organ bath was emptied and non-radioactive Krebs' solution, previously warmed to 37°C, was allowed to run over the strip at a constant rate of 3 ml/min. After preliminary washing with 10 ml of the Ringer solution, the solution was allowed to drip from the strip directly into a collecting tube which was changed regularly at 2-min intervals. A sample of the collected solution was assayed for total ¹⁴C-activity by a liquid scintillation spectrometer with Bray's solution. ¹⁴C-DA and its metabolites were separated by the method described above.

The nerve terminals in the arterial walls of these same strips were stimulated transmurally. A train of rectangular pulses delivered by an electronic stimulator (Nihon-Koden, MSE-3) was passed through electrodes, as described by Su and Bevan (10) at a frequency of 10/sec for 2 minutes and repeated at 16-min intervals, each pulse being 0.3 msec in duration and 30V in intensity.

RESULTS

1. Concentration of endogenous DA and NA: As shown in Table 1, the rabbit blood vessels examined here contained appreciable concentrations of NA. On the other hand, the amounts of DA in these tissues were less than 7% of the total catecholamine content, under untreated conditions. The thoracic aorta had the lowest concentrations of both DA and NA, 0.03 µg/g and 0.74 µg/g respectively. The concentration of DA in the mesenteric and renal arteries exceeded 0.15 µg/g tissue and was higher than levels in the other vessels examined. In the atri, thoracic and abdominal aortae, celiac and femoral arteries, the proportion of the amount of DA to NA was between 0.038 and 0.044. On the other hand, the proportion in the
renal and mesenteric arteries was 0.074 and 0.054, respectively.

2. Effect of reserpine on the endogenous DA and NA: As shown in Fig. 1, 20 hr after a single dose, 1 mg/kg reserpine (i.p.), the level of NA in the atria and mesenteric artery was reduced to 10–20% of the control, while in the renal artery a considerable amount of NA (40–45% of untreated control) remained. As there are few data regarding changes in the level of endogenous DA in the arteries after treatment with reserpine, we studied the effect of reserpine on the level of DA in the arteries. Pretreatment with reserpine did not significantly alter but tended to lower the level of DA in the mesenteric and renal arteries, and the level of DA in the atria decreased to 57% (p<0.05) of untreated levels.

3. Uptake of 3H-DA and 3H-NA: Tables 2 and 3 show the total uptake of 3H-DA and 3H-NA and contents of their metabolites, formed during incubations with the concentration of 0.1 μM for 20 min at 37°C, in the atria and other vascular tissues. Although there were considerable variation in the capacity to accumulate 3H-DA or 3H-NA within the vascular system, the uptake of 3H-NA equivalents was larger than that of 3H-DA equivalents, in all the tissues examined. The highest uptake of 3H-NA was exhibited by the renal artery, and the uptake by the atria was approx. 15% of the former. Comparing the uptake of 3H-DA equivalents by the atria and arteries, the amounts of 3H-DA equivalents in the renal and superior mesenteric arteries were larger than those in other tissues.

Tables 2 and 3 also summarize the metabolic fate of 3H-DA and 3H-NA taken up by the isolated atria and arteries during a 20-min incubation period. When incubated with 0.1 μM DA, the formation of 3H-NA by dopamine-β-hydroxylase seemed to be a main pathway of metabolic change, and the amount of 3H-NA which exceeded 3H-DA retained in the renal artery was 46.2% of the total 3H. In the atria, however, the net change from 3H-DA to 3H-NA was small, being by 20.5% of the total 3H. On the other hand, the relative amount of deaminated catechol metabolites after incubation with 3H-DA of the renal artery was larger than that after 3H-NA incubation, being approx. 10% and 3% of total 3H, respectively. Such relatively large production
of deaminated metabolites after incubation with \(^3\)H-DA was also observed in the other vascular tissues examined. After incubation with \(^3\)H-DA, the production of radioactive O-methylated plus deaminated, O-methylated derivatives were also slightly higher than after incubation with \(^3\)H-NA, in all the tissues examined. In the renal artery, the relative amount of O-methylated plus deaminated, O-methylated derivatives to total \(^3\)H was 1.7% after incubation with \(^3\)H-NA, and the corresponding value was 7.6% after incubation with \(^3\)H-DA.

4. Release of \(^14\)C-DA and \(^14\)C-NA by transmural stimulation in renal artery: Upon electrical transmural stimulation of the renal artery preincubated with 0.1 \(\mu\)M \(^14\)C-DA for 30 min, there were elevations in the overflow of \(^14\)C-DA and \(^14\)C-NA into the superfusate, as compared with the prestimulation level (Fig. 2). During the incubation for 30 min, a portion of the \(^14\)C-DA taken up by the
artery may have been converted into $^{14}$C-NA, as during the two-minute stimulation period, the overflow of $^{14}$C-DA and $^{14}$C-NA was increased by 68% and 44% of the prestimulation level, respectively. Although there was a gradual decay of spontaneous overflow of $^{14}$C compounds, the following two-minute stimulations also increased both $^{14}$C-DA and $^{14}$C-NA overflows to double of the prestimulation levels.

**DISCUSSION**

When the concentrations of endogenous DA and NA in the rabbit atria and arteries were measured, the amount of DA in all tissues studied was found to be less than 7% of the total concentration of catecholamines, in the case of no pretreatment. However, there were considerable variations in the contents of DA and NA within the arterial system. A relatively high concentration of DA was detected in the renal and superior mesenteric arteries. DA reportedly produces dilatation by activating specific DA receptors (3, 11, 12). Comparing the proportion of DA to NA, the highest value was also shown in the renal artery, that is, about 1.5-2-fold higher than that of the other arteries. Thus, endogenous DA in the renal artery appears to play a specific role in connection with dopamine receptors. Berkowitz et al. (1) reported that the endogenous NA content in the rabbit blood vessels varied from 1.0 to 4.0 µg/g, according to the region, and that the concentrations of NA in the mesenteric and renal arteries were higher than in the atria. Furthermore, the activity of dopamine-β-hydroxylase is also high in these vascular tissues (13, 14).

A single dose of reserpine depletes NA in the central and peripheral tissues related to the cardiovascular system, by blocking the uptake mechanisms of storage granules (1, 15, 16). Therefore, to investigate the location of DA at the subcellular level in the peripheral sympathetic nerve terminals in the vascular wall, reserpine, 1 mg/kg i.p., was given. Reserpine caused a marked reduction of NA in the atria and superior mesenteric artery and a significant reduction in the renal artery, however, the decrease in DA was slight. These findings suggest that a portion of DA in the sympathetic nerve terminals present in the cytoplasm is not affected by reserpine, or that DA can enter the granule, even in the presence of reserpine. Almgren et al. (17) have shown in rats that the level of cardiac DA returns to normal 24 to 48 hours after the administration of 10 mg/kg of reserpine, while the level of NA remains low during this period. The slight decrease in levels of DA after the administration of reserpine may be due to an early recovery of the uptake mechanism of DA by the granules in the axon terminals (18). As shown in these studies, after reserpine treatment, the ratios of NA depletion in the tissues examined varied widely, with the renal artery being the most resistant to reserpine. Lidbrink et al. (19) suggested that the difference in sensitivity to reserpine by DA and NA storage granules might indicate a fundamental difference between the two types of granule. Thus, one explanation for the significant but
smaller decrease of NA in the renal artery, as induced by reserpine, is that DA storage granules may be intermingled with NA storage granules at the highest proportion in the tissues examined. Therefore, we assessed the uptake of exogenous amines in the arterial tissues and measured the amounts of deaminated metabolites.

We found a considerable quantitative difference of total $^3$H uptake into the atria and arteries between $^3$H-DA and $^3$H-NA. One possible explanation is that $^3$H-NA is readily taken up by the storage granules, while a part of $^3$H-DA is located and accumulated in the cytosol. In fact, Waldeck et al. (20) reported that approx. 20% of DA in the adrenal glands is localized in the supernatant fraction. Thus, it is conceivable that the site of DA-uptake in the sympathetic nerves in the blood vessels is not only the dopamine-$\beta$-hydroxylase containing granules but also the cytosol of nerve terminals. In general, the neutral metabolites of NA pass from the membranes of nerve terminals to the incubation solution, as these metabolites are uncharged (21). Therefore, it appears that some of the metabolites of DA in the cytosol may be removed from the nerve fiber terminals. Further evidence to support this view is our finding of a reduction in radioactivity in the medium following the uptake of the renal artery during incubation in 1-ml medium with $^3$H-NA. This reduction was about double that of $^3$H-DA, under the same conditions (unpublished observation).

As shown in Tables 2 and 3, most of the $^3$H-NA taken up was stored in an unchanged form, while after incubation with $^3$H-DA, the unchanged form was approx. 25$\sim$60% of the total $^3$H and the main part of metabolites was $^3$H-NA in all the tissues tested. After incubation with $^3$H-DA, the production of oxidative deaminated metabolites was 2$\sim$3-fold higher than that after with $^3$H-NA incubation, despite the smaller uptake of total $^3$H. In general, enzymatic degradation plays an important role in disposition of the amines. Brandão et al. (22) showed that the metabolism of $^3$H-amine in the spontaneous outflow from tissues labeled with tritiated compounds was mainly due to the action of the presynaptic enzymes on $^3$H-amines leaking from the vesicles into the axoplasm. Thus, it appears that a part of $^3$H-DA taken up may be present in the cytosol, or that $^3$H-DA may leak from the vesicles into the cytosol more easily than does $^3$H-NA. The amounts of the total $^3$H uptake during the incubation with $^3$H-DA or $^3$H-NA of blood vessels corresponded well with the concentration of endogenous NA, thereby suggesting the density of adrenergic innervation (23).

Excitation of the sympathetic nerves by electrical stimulation after incubation with $^3$H-NA produced a sharp increase in the output of $^3$H-NA in the isolated superfused pulmonary arteries (10). In the present experiment performed on strips of the rabbit renal artery, the elevation in $^{14}$C-DA and $^{14}$C-NA outflow was induced by electrical stimulation. These findings suggest that a portion of $^{14}$C-DA taken up by the tissue during the incubation was converted into $^{14}$C-NA and stored in the granules and that this $^{14}$C-NA was released, concomitant with $^{14}$C-DA in response to electrical stimulation. Some of the storage granules in the renal artery may be deficient in dopamine-$\beta$-hydroxylase as is the case in the dopaminergic fibers in the central nervous system, and some of the $^{14}$C-DA would remain unchanged.

REFERENCES

1) Berkowitz, B.A., Tarver, J.H. and Spector, S.: Norepinephrine in blood vessels: Concentration, binding, uptake and depletion. J. Pharmacol. exp. Ther. 177, 119$\sim$126 (1971)

2) Iversen, L.L.: The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: A novel catecholamine uptake
3) Goldberg, L.I.: Cardiovascular and renal actions of dopamine: Potential clinical applications. Pharmacol. Rev. 24, 1–29 (1972)

4) Goldberg, L.I., Volkman, P.H. and Kohli, J.D.: A comparison of the vascular dopamine receptor with other receptors. Ann. Rev. Pharmacol. Toxicol. 18, 57–79 (1978)

5) Brodde, O.E. and Schemuth, W.: Specific antagonism by metoclopramide of dopamine-induced relaxation on isolated rabbit mesenteric arteries contracted with prostaglandin F2α. Life Sci. 25, 23–30 (1979)

6) Bertler, A., Carlsson, A. and Rosengren, E.: A method for the fluorometric determination of adrenaline and noradrenaline in tissues. Acta physiol. scand. 44, 273–292 (1958)

7) Laverty, R. and Sharman, D.F.: The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. Brit. J. Pharmacol. 24, 538–548 (1965)

8) Hellmann, G., Hertting, G. and Peakar, B.: Uptake kinetics and metabolism of 7-3H dopamine in the isolated perfused rat heart. Brit. J. Pharmacol. 41, 256–269 (1971)

9) Anton, A.H. and Sayre, D.F.: A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. exp. Ther. 138, 360–375 (1962)

10) Su, C. and Bevan, J.A.: The release of H3-noradrenaline in arterial strips studied by the technique of superfusion and transmural stimulation. J. Pharmacol. exp. Ther. 172, 62–68 (1970)

11) Goldberg, L.I. and Toda, N.: Dopamine-induced relaxation of isolated canine renal, mesenteric, and femoral arteries contracted with prostaglandin F2α. Circulation Res. 36 and 37 Supp. 1–97 (1975)

12) Toda, N. and Hatano, Y.: Antagonism by droperidol of dopamine-induced relaxation in isolated dog arteries. Europ. J. Pharmacol. 57, 231–238 (1979)

13) Hartman, B.K. and Udenfriend, S.: Immuno-fluorescent localization of dopamine-β-hydroxylase in tissues. Mol. Pharmacol. 6, 85–91 (1970)

14) Spector, S., Tarver, J.H. and Berkowitz, B.A.: Proc. Symp. Physiol. Pharmacol. Vascular Neuroeffector Systems. Interlaken, p. 111–118, Karger, Basel (1971)

15) Fuxe, K. and Saddall, G.: Histochemical and biochemical observations of the effect of reserpine on noradrenaline storage in vasoconstrictor nerves. Acta physiol. scand. 61, 121–129 (1964)

16) Burn, J.H. and Rand, M.J.: Norepinephrine in arterial walls and its disposal by reserpine. Brit. med. J. 1, 903–968 (1968)

17) Almgren, O., Snider, S.R. and Carlsson, A.: Recovery of dopamine in peripheral adrenergic nerves after reserpine treatment. Naunyn-Schmiedeberg’s Arch. Pharmacol. 292, 133–136 (1976)

18) Haggendal, J. and Dahlström, A.: The recovery of the capacity for uptake-retention of 3H noradrenaline in rat adrenergic nerves after reserpine. J. Pharm. Pharmacol. 24, 565–574 (1972)

19) Lidbrink, P., Jonsson, G. and Fuxe, K.: Selective reserpine-resistant accumulation of catecholamines in central dopamine neurones after dopa administration. Brain Research 67, 439–456 (1974)

20) Waldeck, B., Snider, S.R., Brown, R. and Carlsson, A.: Studies on the synthesis and subcellular distribution of dopamine in the rat adrenal medulla. Naunyn-Schmiedeberg’s Arch. Pharmacol. 287, 1–10 (1975)

21) Levin, J.A.: The uptake and metabolism of 3H-1- and 3H-dl-norepinephrine by intact rabbit aorta and by isolated adventitia and media. J. Pharmacol. exp. Ther. 190, 210–226 (1974)

22) Brandão, F., Paiva, M.O. and Guimarães, S.: The role of neuronal and extraneuronal systems in the metabolism of adrenaline and noradrenaline released from nerve terminals by electrical stimulation. Naunyn-Schmiedeberg’s Arch. Pharmacol. 311, 1–7 (1980)

23) Falck, B.: Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta physiol. scand. 56, Supp. 197, 1–24 (1962)