Shimane J. Med. Sci. Vol. 4, pp. 31—35, 1980

Dopamine Uptake by Rabbit Atria and Peripheral Arteries and Effects of Haloperidol

(dopamine/arteries/haloperidol)

EMIKO NAKASHIMA and KEISUKE HATTORI

Department of Pharmacology, Shimane Medical University, Izumo 693, Japan

(Received December 28, 1979)

The uptake and metabolism of dopamine by isolated rabbit atria, aortae and arteries were measured after a 20 min incubation with ^{14}C-dopamine (10^{-7}M). The total uptake of ^{14}C-dopamine equivalents in the tissues was the highest in the renal arteries, and about one third of this uptake proved to be noradrenaline, the most important metabolite of dopamine. The uptake of ^{14}C-dopamine was markedly reduced in all the vascular tissues preincubated with 10^{-5}M haloperidol for 30 min. Thus, dopamine as well as noradrenaline may be taken up and stored in the same sites of adrenergic nerve terminals.

Haloperidol, a butyrophenone, is a neuroleptic used in the treatment of psychiatric disorders (1—3).

In an earlier study on the central nervous system (1, 4, 5), it was suggested that haloperidol inhibits the transport of catecholamines through the neuronal cell membrane and into the small transmitter pool, and also inhibits the activation of the receptor by the transmitter.

Parallel studies have demonstrated that dopamine exerted unusual renal vasodilatation which was due in part to an action on dopamine receptors, and haloperidol attenuated the vasodilatation (6—8). However, regional differences in the action and mechanism of action of haloperidol on vascular systems have not been systematically analyzed.

We compared the uptake of exogenous dopamine by isolated rabbit atria and peripheral arteries and the effect of haloperidol on this uptake was compared.

MATERIALS AND METHODS

Albino rabbits of either sex weighing 1.7 to 2.5 kg were anesthetized with 50 mg/kg of sodium pentobarbital i. v., and exsanguinated from the common carotid arteries. The cardiovascular tissues analyzed included atria, thoracic and abdominal aortae, and common carotid, celiac, superior mesenteric, renal and femoral arteries. These tissues were transferred to dishes containing warm Krebs' Ringer solution, previously saturated with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37°C. The composition

of the Krebs' Ringer solution was (millimolar concentration) : NaCl, 118.1 ; KCl, 4.7 ; $CaCl_2$, 1.9 ; K_2HPO_4, 1.18 ; $NaHCO_3$, 25.0 ; glucose, 5.0 ; Na_2-EDTA, 0.04 ; ascorbic acid, 0.568. The preparations were allowed to equilibrate for 60 min in control medium.

Incubation with ^{14}C-dopamine

Each tissue was incubated in a beaker containing haloperidol (10^{-5}M) for 30 min, then 10^{-7}M ^{14}C-dopamine (specific activity 60 mCi/mmol, Amersham Radiochemical Centre) was added to the incubation medium, and incubation extended for a further 20 min. As control, some of tissues were incubated without haloperidol. After the incubation, the tissues were quickly removed, rinsed with ice-cold Krebs' Ringer solution, blotted, weighed and homogenized in ice-cold perchloric acid (0.4 N) containing 100 mg Na_2-EDTA and 30 mg sodium metabisulfite.

Determination of ^{14}C-dopamine and Its Metabolites in Tissues

The homogenates were centrifuged (20,000g) and 0.2 ml of unlabelled carriers (mixtures of dopamine and its metabolites) was added to the supernatants. Radioactive dopamine and its metabolites were separated by column chromatography, the columns being 9 mm in diameter and stoppered with glass wool. Al_2O_3 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 by 10 ml of 0.2 N Tris-HCl buffer (pH 8.4). After the supernatants have passed through the 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 was mixed. Thereafter, Dowex 50 W $\times$4 (200—400 mesh) columns were used to separate dopamine, noradrenaline and catechol deaminated metabolites. The columns were filled to 15 mm height with the resin at pH 7.0, and were ready for use after the pretreatment according to the method of Bertler *et al.* (10). Alumina eluates adjusted to pH 4.0 prior to use were passed through the columns. After rinsing the column with 10 ml of water and 3 ml of N HCl, elution was performed with five 2 ml portions of N HCl. ^{14}C-noradrenaline was eluted in these portions. ^{14}C-dopamine was eluted in successive five 2 ml portions of 2 N HCl, then each eluate was transferred to a 40 ml beaker, and stirred together with 400 mg of activated alumina at pH 8.4 for 5 min. Elution was performed by shaking the alumina with 3 ml of 0.2 N acetic acid for 15 min. A sample of the eluates was counted on a Packard Tri Carb liquid scintillation spectrometer. All values were corrected for recoveries of 70$\pm$10 % S. E.. Student's *t*-test was used to evaluate data.

TABLE. *The Effect of Haloperidol on the Uptake of ^{14}C-dopamine in Isolated Rabbit Atria, Aortae and Arteries*

		Atria	Thoracic aorta	Abdominal aorta	Common carotid arteries	Celiac artery	Superior mesenteric artery	Renal arteries	Femoral arteries
D A	C	29±12	149±54	101±25	116±58	106±41	189±44	257±161	157±77
	H	25±10	18±5	9±2	28±13	37±22	24±11	27±7	13±2
N A	C	25±12	207±68	237±51	111±31	280±78	301±19	401±158	262±88
	H	24±12	37±14	21±14	30±17	32±6	37±18	55±3	42±14
DEAM	C	14±5	69±17	79±18	106±30	180±48	151±38	242±86	94±59
	H	11±3	15±5	24±16	37±12	74±19	92±24	78±19	22±12
OME + OMEDEAM	C	23±6	237±108	199±25	314±102	300±26	253±33	328±32	296±90
	H	32±4	102±14	96±6	121±16	188±24	145±25	128±60	125±51
Total	C	91±32	662±239	616±100	646±150	866±153	895±70	1228±313	809±80
	H	92±18	173±14	150±25*	216±55	331±28*	296±18**	287±78*	201±49*

Each value is a mean (3 to 5 experiments) ± S. E. M., and expressed as pmole/g. C : control, H : haloperidol-preincubated, DA : dopamine, NA : noradrenaline, DEAM : deaminated metabolites, OME : O-methylated metabolites, OMEDEAM : O-methylated and deaminated metabolites. Significantly different from control, *$P<0.05$, **$P<0.005$.

RESULTS AND DISCUSSION

Uptake of ^{14}C-dopamine

The table shows the uptake of ^{14}C-dopamine equivalents in the tissues during incubation with concentration of 10^{-7}M ^{14}C-dopamine for 20 min. In each pair of values, the upper one (C in Table) represents the uptake of ^{14}C-dopamine without haloperidol treatment. At the incubation concentration used, there was a considerable variation in the capacity of arteries to accumulate ^{14}C-dopamine. The highest neuronal uptake of ^{14}C-dopamine was seen in the renal arteries, 1228±313 pmole/g, while the atria had the lowest concentration of ^{14}C-dopamine of any blood vessel examined, 91±32 pmole/g. All the arteries exceeded 500 pmole/g and hence had higher concentrations than the atria.

Metabolism of ^{14}C-dopamine

The metabolic fate of ^{14}C-dopamine taken up at an incubation concentration of 10^{-7}M is also shown in the table. The most important metabolite was ^{14}C-noradrenaline. As much as 25%−38% of the total radioactivity was represented by ^{14}C-noradrenaline after incubation for 20 min. Although ^{14}C-dopamine and ^{14}C-noradrenaline were the main components of the total uptake, the percentage of metabolites exceeded 50% in the renal, celiac and common carotid arteries.

Effects of Haloperidol on the Uptake of ^{14}C-dopamine

The tissues were incubated in Krebs' Ringer solution containing 10^{-5}M

haloperidol for 30 min, then 10^{-7}M ^{14}C-dopamine was added. Effect of haloperidol is represented by the lower value (H in Table) in each pair of values in the table. Haloperidol caused a marked reduction of ^{14}C-dopamine uptake in all the vascular tissues but did not affect the uptake by the atria. The renal arteries were more sensitive to haloperidol reduction than the atria and the other arteries studied.

The uptake of ^{14}C-dopamine in the isolated rabbit atria, aortae and arteries, and the metabolites of dopamine in the tissues for 20 min incubation were measured by assaying ^{14}C radioactivity. At the concentration (10^{-7}M) of the amine used in this experiment, there is practically no extraneuronal uptake by the sympathetically innervated tissues (11). In the experiment with the low dopamine incubated concentration, the concentration of radioactive substances in the aortae and arteries but not atria exceeded the concentration of dopamine in the incubation medium. Moreover, as shown in the table, more than 40 % of dopamine taken up into the tissues was converted into the deaminated and/or O-methylated metabolites for 20 min incubation. However, the main metabolite was noradrenaline. These findings suggest that not only noradrenaline but also dopamine may be taken up and stored in the same sites of adrenergic nerves, presumably, in part, in the intraneuronal dopamine β-hydroxylase containing granules.

There was a considerable quantitative difference in the uptake of exogenous dopamine by the tissues during the incubation. When the tissues were incubated with 10^{-7}M dopamine for 20 min, the renal arteries took up and stored 1230 pmole/g whereas the aortae and atria retained less than 700 p mole/g. The mesenteric, celiac and femoral arteries took up and stored less than three fourths as much ^{14}C-dopamine as the renal arteries. Since the renal and mesenteric arteries are well innervated (12), our results may support the findings of Kopin *et al.* (13) that uptake by the tissues parallels their density of sympathetic nerves.

It is of interest that the uptake of exogenous dopamine by the renal arteries was the highest among the tissues tested. The renal arteries contain specific dopamine receptors (6), so it is possible that there may be a specific mechanism for dopamine uptake and storage in the presynaptic sites in the sympathetic nerve terminals corresponding to the presence of the dopamine receptors. This is in agreement with previous findings of the highest concentration of endogenous dopamine in rabbit renal arteries (14).

REFERENCES

1) Janssen, P. A. J. (1967a) The pharmacology of haloperidol. *Int. J. Neuropsychiatr.* **3**, 510–518
2) Janssen, P. A. J. (1967b) Haloperidol and related butyrophenones. In : Psychopharmacological Agents, 4, (Gordon, M., ed.) Academic Press, New York
3) Ban, T. A. (1969) In : Psychopharmacology, Williams & Wilkins, Baltimore
4) Glowinski, J. and Axelrod, J. (1966) Effects of drugs on the disposition of ^{3}H-norepinephrine in the rat brain. *Pharmacol. Rev.* **18**, 775–785
5) Schildkraut, J. J., Schawberg, S. M., Breese, G. R., and Kopin, I. J. (1967) Norepinephrine

metabolites and drugs used in the affective disorders : a possible mechanism of action. *Am. J. Psychiatry.* **124**, 600–608

6) Goldberg, L. I. (1972) Cardiovascular and renal actions of dopamine : potential clinical applications. *Pharmacol. Rev.* **24**, 1–29
7) Toda, N. and Goldberg, L. I. (1973) Dopamine-induced relaxation of isolated arterial strips. *J. Pharm. Pharmacol.* **25**, 587–589
8) Yeh, B. K., McNay, J. L., and Goldberg, L. I. (1969) Attenuation of dopamine renal and mesenteric vasodilatation by haloperidol : evidence for a specific dopamine receptor. *J. Pharmacol. Exp. Ther.* **168**, 303–309
9) Anton, A. H. and Sayre, D. F. (1962) A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.* **138**, 360–375
10) Bertler, Å., Carlsson, A., and Rosengren, E. (1958) A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta Physiol. Scand.* **44**, 273–292
11) Iversen, L. L. (1965) The uptake of catecholamines at high perfusion concentrations in the rat isolated heart : a novel catecholamine uptake process. *Br. J. Pharmacol.* **25**, 18–33
12) Falck, B. (1962) Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta Physiol. Scand.* **56**, (suppl. 197) 1–24
13) Kopin, I. J., Gordon, E. K., and Horst, W. D. (1965) Studies on the uptake of l-norepinephrine-^{14}C. *Biochem. Pharmacol.* **14**, 753–759
14) Shibata, S., Kuchii, M., Hattori, K., and Fujiwara, M. (1974) The effect of cocaine on the ^{3}H-norepinephrine uptake by cold stored aorta from rabbit. *Jpn. J. Pharmacol.* **24**, 151–162