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# A COMPARATIVE STUDY OF THE FATE OF DOPAMINE-$^{14}$C IN RAT AND MOUSE

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**Abstract**—The metabolism of dopamine-$^{14}$C was investigated in selected organs of the rat and mouse, by means of a new, rapid, and quantitative assay of tissue catecholamines. Differences were found in the rate of dopamine metabolism in organs of the same species as well as between species. In each species, the rate of norepinephrine formation from dopamine was more rapid in the heart than in the spleen. Dopamine metabolism in mouse spleen was much slower than in the same organ in the rat. Pretreatment of animals with iproniazid resulted in an increased level of radioactive catecholamines in heart and spleen. This monoamine oxidase inhibitor also affected the metabolism of dopamine at early time intervals, but the differences between the control and iproniazid-treated animals greatly diminished with time.

MUCH attention has been given to the metabolism and physiologic disposition of norepinephrine,[1–3] but relatively little is known about the uptake and metabolic disposition of dopamine in tissue. Dopamine is the immediate precursor of norepinephrine, and it is readily converted to this biogenic amine[4–5] by the enzyme $\beta$-hydroxylase, which has been described by Levin *et al.*[6]

Kopin *et al.*[7] demonstrated that the main source of norepinephrine in tissue is derived by synthesis *in situ*. Since dopamine is the precursor of norepinephrine, the fate of dopamine-$^{14}$C and its rate of conversion to norepinephrine-$^{14}$C were compared in selected organs of the rat and mouse. Heart and spleen are rich in adrenergic nerve endings and contain a high concentration of catecholamines. These tissues were therefore used in our studies.

A new, rapid, experimental procedure was utilized, which greatly facilitated studies of tissue concentration and the metabolism of radioactive catecholamines.

## METHODS

Male albino mice (CF) and male albino rats (CFE), obtained from Carworth Farms, were used; average body weights were 30 and 220 g respectively. Food and tap water were allowed *ad libitum*. The animals were housed under laboratory conditions for at least 1 week prior to experiment. At the time of the experiment they were injected i.p. with 100 mg iproniazid/kg and 18 hr later with 0·53 mg per kg, of 1-$^{14}$C-labeled dopamine (New England Nuclear Corp, sp. act. 6 mc/m-mole). At selected time intervals after dopamine injection, the animals were killed by cervical dislocation. Hearts and spleens were dissected out as rapidly as possible, blotted to remove excess blood, and immediately frozen on a block of dry ice.

The frozen tissues were weighed and homogenized (glass homogenizer) in 0·01 % hydrochloric acid in ethanol (about 12 ml/g tissue). Prior to homogenization, 0·05 ml of freshly prepared ascorbic acid (10 % aqueous solution) was added to each homogenization vessel. Trichloroacetic and perchloric acids were tested for tissue homogenization but gave artifacts upon chromatography. Therefore, in all subsequent experiments, hydrochloric acid was used. The concentration of this acid in ethanol was kept at 0·01 % because a larger excess of the acid caused the breakdown of norepinephrine on paper chromatograms.

Organs from two mice were pooled, but individual rat organs were used. The vessels containing the tissue homogenates were placed in an acetone–dry ice bath for 15–20 min and the precipitated proteins removed by high-speed centrifugation for 15 min at 70,000 *g* (Spinco). Aliquots (2 × 0·1 ml samples) of the clear supernatant fraction, which contained about 96 per cent of the total radioactivity present in tissue homogenate, were counted in a Packard liquid scintillation spectrometer. The scintillation medium consisted of toluene:ethanol (10:5) containing 4 g PPO and 100 mg POPOP per liter of toluene. The remaining supernatant was concentrated to dryness at low temperature and high vacuum. The dry residue was extracted with 0·1–0·2 ml of absolute ethanol and applied on Whatman 1 paper for chromatography.

Preliminary experiments with radioactive dopamine and norepinephrine added to control tissue samples showed over 90 per cent recovery, and each compound yielded a single peak on chromatography. For purpose of identification, unlabeled dopamine, norepinephrine, and some of their known metabolites were spotted on paper simultaneously with the unknown samples. Chromatograms were developed for 20 hr by the descending method, with *n*-butanol (redistilled) saturated with 1 N HCl as the solvent system. For additional identification of catecholamines and their metabolites, another solvent system, consisting of phenol and $H_2O$ (88:12) was used on occasion. This was particularly useful in differentiation between dopamine and 3-O-methylnorepinephrine which had similar $R_f$ values in the butanol:HCl system. The air-dried chromatograms were cut into strips 1·5 in. wide and scanned in a $4\pi$ Vanguard chromatogram counter. For quantitative evaluation of results, the zones on paper strips, corresponding to radioactive peaks visible on the chart, were cut out and counted in the Packard liquid scintillation spectrometer.

## RESULTS

### *Metabolic fate of dopamine-$^{14}$C in rat*

The results in heart and spleen, as determined by quantitative analysis of chromatographic paper strips, are presented in Table 1. Iproniazid-pretreated rats showed a progressive increase with time in tissue norepinephrine with a concurrent decrease in dopamine content. Norepinephrine formation in heart of these animals was faster than in spleen. Metabolite 3, chromatographically corresponding to authentic 3-O-methyl dopamine (in two different solvent systems) was present in the heart at a much smaller concentration than in spleen. Metabolite 1 (which has an $R_f$ value similar to homovanilic acid) and metabolite 2 (not identified) were noted only at 30 min in trace amounts.

In control animals (not pretreated with iproniazid; Table 1) the relative concentration of dopamine in heart and spleen was lower than in iproniazid-treated rats at all time intervals. In addition, a high concentration of metabolites 1 and 2 was noted.

TABLE 1: METABOLIC FATE OF DOPAMINE-$^{14}$C IN RAT HEART AND SPLEEN

| | Heart, post dopamine | | | Spleen, post dopamine | | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 120 min | 30 min | 60 min | 120 min |
| Iproniazid pretreated | | | | | | |
| *Norepinephrine* | 24·4 ± 2·19 | 45·1 ± 4·13 | 74·2 ± 3·51 | 5·4 ± 1·80 | 24·6 ± 4·54 | 42·4 ± 3·14 |
| Dopamine | 63·4 ± 1·27 | 47·5 ± 3·39 | 25·6 ± 3·52 | 44·1 ± 3·13 | 52·8 ± 3·09 | 55·4 ± 2·10 |
| Metabolite 3 | 11·4 ± 0·91 | 7·4 ± 1·03 | | 45·1 ± 3·71 | 20·6 ± 2·71 | 1·7 ± 1·72 |
| Metabolite 2 | 0·6 ± 0·61 | | | 0·4 ± 0·27 | | |
| Metabolite 1 | 0·6 ± 0·44 | | | 5·0 ± 0·86 | | |
| No. of samples analyzed | 13 | 12 | 6 | 12 | 15 | 6 |
| Control | | | | | | |
| Norepinephrine | 36·7 ± 7·55 | 39·6 ± 6·53 | 80·3 ± 2·60 | 14·0 ± 1·41 | 36·0 ± 2·59 | 60·9 ± 2·45 |
| *Dopamine* | 40·6 ± 3·62 | 38·7 ± 6·09 | 14·1 ± 1·34 | 23·9 ± 5·29 | 33·7 ± 1·30 | 31·8 ± 3·86 |
| Metabolite 3 | | 6·9 ± 6·68 | | 2·6 ± 1·41 | | |
| Metabolite 2 | 0·8 ± 0·80 | 22·8 ± 1·02 | 3·7 ± 0·32 | 12·8 ± 2·96 | 12·4 ± 2·59 | 6·5 ± 4·37 |
| Metabolite 1 | 21·9 ± 4·91 | 12·0 ± 6·09 | 1·9 ± 1·2 | 46·7 ± 5·73 | 17·9 ± 1·94 | 0·9 ± 0·83 |
| No. of samples analyzed | 4 | 4 | 4 | 4 | 3 | 4 |

Each animal received dopamine-$^{14}$C (i.p.) and was sacrificed at a specified time interval.
Results are expressed as percentage (mean value ± S.E.) of the total radioactivity found in outlined areas on chromatographic strips.

*Metabolic fate of dopamine-$^{14}$C in mouse*

The rate of dopamine-$^{14}$C metabolism in mouse spleen was entirely different from that in heart (Table 2). In heart, dopamine decreased rapidly with time, resulting in a corresponding increase in norepinephrine tissue content. In spleen, however, the ratio of dopamine to norepinephrine remained practically constant throughout the duration of experiment.

Pretreatment of mice with iproniazid (Table 2) resulted in a slower rate of dopamine metabolism in heart at initial time intervals. However, the metabolism of dopamine in spleen was hardly affected.

Figure 1 compares the metabolic fate in the mouse and rat spleen of iproniazid-pretreated animals. As shown, dopamine was metabolized quite extensively in the rat but to a very small degree in the mouse.

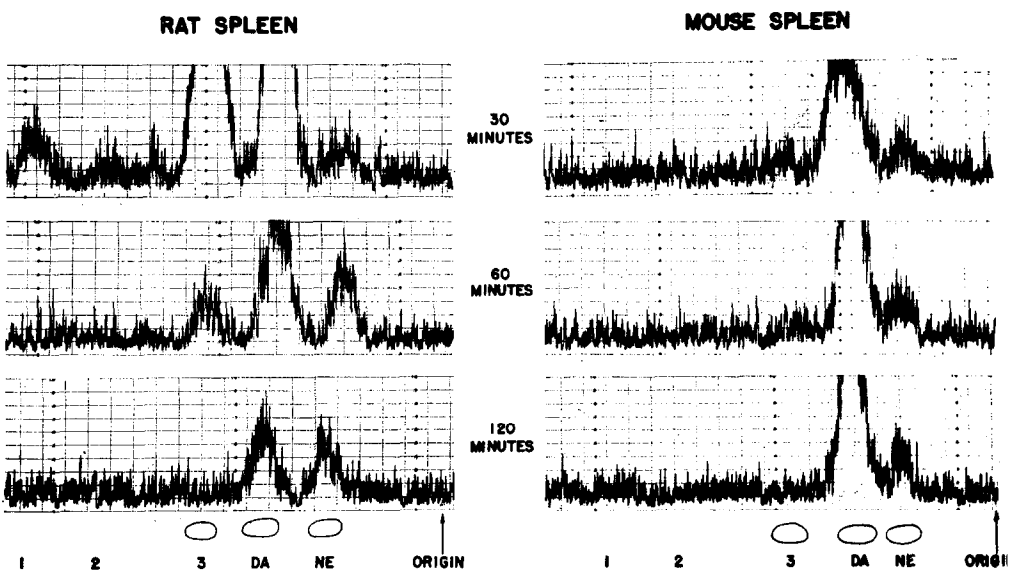


FIG. 1. Chromatographic pattern of dopamine-$^{14}$C metabolism in rat and mouse spleen. The position of authentic dopamine (DA), norepinephrine (NE), and 3-O-methyl-dopamine (3), are indicated at the bottom of the paper strips. Radioactive peaks of metabolites 1 ($R_f$ similar to homovanilic acid) and 2 (not identified) are also indicated.

*Tissue levels of radioactivity in control and iproniazid-pretreated animals*

The concentration of radioactivity in heart and spleen after administration of dopamine-$^{14}$C is given in Table 3. Pretreatment with iproniazid resulted in higher concentration of radioactivity in the heart of both species and in rat spleen. Its effect on mouse spleen could not be positively demonstrated. The results in Table 3 also indicate that in iproniazid-pretreated animals, the radioactivity disappeared faster from rat than from mouse tissue.

## DISCUSSION

The conversion of dopamine-$^{14}$C to norepinephrine was studied by a new procedure which permitted a rapid and quantitative evaluation of tissue radioactivity after

TABLE 2. METABOLIC FATE OF DOPAMINE-$^{14}$C IN MOUSE HEART AND SPLEEN

| | Heart, post dopamine | | | Spleen, post dopamine | | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 120 min | 30 min | 60 min | 120 min |
| Iproniazid pretreated | | | | | | |
| Norepinephrine | 34·6 ± 2·56 | 69·8 ± 3·99 | 86·1 ± 2·04 | 10·7 ± 1·79 | 12·3 ± 1·19 | 9·6 ± 1·20 |
| Dopamine | 52·1 ± 1·63 | 28·8 ± 3·81 | 13·8 ± 2·07 | 82·2 ± 1·22 | 85·7 ± 1·16 | 89·2 ± 0·43 |
| Metabolite 3 | 6·5 ± 0·47 | 0·3 ± 0·26 | | 6·6 ± 1·58 | 1·9 ± 1·19 | 1·1 ± 1·16 |
| Metabolite 2 | 6·9 ± 1·05 | 1·1 ± 1·06 | | 0·5 ± 0·48 | | |
| No. of samples analyzed | 5 | 5 | 5 | 6 | 5 | 3 |
| Control | | | | | | |
| Norepinephrine | 43·7 ± 1·64 | 80·7 ± 0·32 | 86·8 ± 1·36 | 6·1 ± 1·90 | 10·0 ± 0·05 | 12·7 ± 1·60 |
| Dopamine | 38·0 ± 2·84 | 19·3 ± 0·32 | 12·4 ± 1·83 | 85·6 ± 10·2 | 89·9 ± 0·05 | 87·3 ± 1·60 |
| Metabolite 3 | | | 1·0 ± 1·00 | 1·4 ± 1·90 | | |
| Metabolite 2 | 18·3 ± 2·05 | | | 6·9 ± 6·90 | | |
| No. of samples analyzed | 3 | 3 | 3 | 2 | 2 | 3 |

Conditions as in Table 1.

TABLE 3. RADIOACTIVITY TISSUE CONTENT IN RAT AND MOUSE

| Time post dopamine (min) | Rat | | | | | | Mouse | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Heart | | | Spleen | | | Heart | | | Spleen | | |
| | Ipron.* | Control | *P* | Ipron. | Control | *P* | Ipron. | Control | *P* | Ipron. | Control | *P* |
| 30 | 9·7 ±0·4 | 3·8 ±0·3 | <0·01 | 8·3 ±0·4 | 4·0 ±0·6 | <0·01 | 7·3 ±0·2 | 6·1 ±0·8 | >0·20 | 8·4 ±1·9 | 6·2 ±0·4 | >0·20 |
| 60 | 6·1 ±0·1 | 4·7 ±0·9 | >0·20 | 4·9 ±0·1 | 2·7 ±0·4 | <0·01 | 6·9 ±0·9 | 3·4 ±0·4 | <0·05 | 8·2 ±1·2 | 4·9 ±1·4 | >0·10 |
| 120 | 5·5 ±1·1 | 2·7 ±0·2 | <0·05 | 3·5 ±0·6 | 1·6 ±0·1 | <0·05 | 5·2 ±0·2 | 2·4 ±0·3 | <0·01 | 5·8 ±0·2 | 3·7 ±0·8 | >0·10 |

The concentration of the $^{14}$C-label in heart and spleen was measured at different time intervals after administration of radioactive dopamine. Results are expressed as counts/min/g tissue × $10^3$. Each value represents mean (±S.E.) of three to four samples.
* Iproniazid-pretreated animals.

administration of dopamine-$^{14}$C. The recovery of tissue radioactivity was greater than 90 per cent. This method can be used effectively *in vivo* and *in vitro* in studies with drugs on the fate of catecholamines and its metabolites.

The comparative study of the fate of dopamine-$^{14}$C in rat and mouse revealed a difference in the rate of dopamine metabolism between individual organs of the same species as well as a difference between species. In the two species analyzed, the conversion of dopamine to norepinephrine was faster in the heart than in the spleen. Mouse heart synthesized norepinephrine at a faster rate than rat heart. Particularly striking in our data, however, was the extremely slow rate of dopamine metabolism in mouse spleen as compared with the same organ of the rat. The formation of labeled norepinephrine in mouse spleen was low and remained practically constant over the entire period investigated.

Burack and Draskoczy[8] reported that within 2 hr after i.v. injection of dopa-$^{3}$H, practically all the catecholamines present in mouse heart were $\beta$-hydroxylated, while in the spleen, a large portion of radioactivity was in the form of dopamine. This, the authors pointed out, may be due to the ability of the spleen to trap platelets which could take up and store catecholamines. Our data, however, would not favor this explanation as the reason for the low rate of dopamine metabolism in mouse spleen. As shown in Table 3, the concentration of the total radioactivity in mouse spleen changed with time in a fashion similar to that observed in the heart, but the metabolic rate in the two organs varied widely. If the catecholamines were "trapped" by the platelets in the spleen, one would expect that radioactivity in this organ to be retained longer than in heart. Also it should be noted that only the mouse but not the rat spleen exhibited this very slow rate of dopamine-$^{14}$C metabolism.

Dopamine is a better substrate for monoamine oxidase (MAO) than norepinephrine and is subject to intensive degradation by this enzyme. It was necessary, therefore, to pretreat the animals with iproniazid in order to prevent excessive degradation of dopamine by MAO, and thus to make more of the administered dopamine available to the tissues.[5] Because of iproniazid pretreatment, it seemed advisiable to determine the effect of this MAO inhibitor on the fate of dopamine-$^{14}$C. As shown, pretreatment with iproniazid resulted in increased levels of radioactive catecholamines in the heart of both species and in rat spleen. The effect of iproniazid on heart catecholamine in the mouse was not previously demonstrated.[9]

Iproniazid also affected the metabolism of dopamine to an appreciable degree at early time intervals. This effect was reflected particularly in the distribution of radioactivity between metabolites. However, as time progressed, the differences in dopamine metabolism in normal and iproniazid-treated animals diminished. The metabolism of dopamine in mouse spleen remained an exception in that its fate was not affected by iproniazid treatment at any time period.

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