CONVERSION OF TYROSINE TO CATECHOLAMINES BY CAT BRAIN IN VIVO[1]

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It has now been established that dopamine and noradrenaline are widely distributed in brain and that they are contained in nervous rather than vascular tissue. They are undoubtedly manufactured in brain tissue, but whether the initial conversion of tyrosine to dihydroxyphenylalanine (dopa) takes place in brain or in some remote organ is still not known. A number of *in vitro* and *in vivo* studies have demonstrated that this step occurs enzymically in both adrenal medulla and heart, although the enzyme responsible has never been isolated (Kirschner, 1959; Spector *et al.*, 1963). There are two reports on the conversion of tyrosine to dopa by brain slices (Masuoka *et al.*, 1961; Iyer *et al.*, 1963) which provide strong evidence for enzymic conversion, but these studies do not completely rule out the possibility of nonenzymic hydroxylation under the *in vitro* conditions of the experiment. This report describes the conversion of L-tyrosine-C^{14} to catecholamines by cat brain *in vivo*.

Methods: 1.2-2.4 µg. of radioactive L-tyrosine-UL-C^{14} (300 µc/µmole) in 20-40 µl. of 0.01N HCl were introduced into discrete brain areas through indwelling cannulae. By methods previously described (Yamaguchi *et al.*, 1963), cannulae were inserted under stereotaxic guidance into one or more of the following brain areas of cats anesthetized with sodium pentobarbital (35 mg/kg): cerebral cortex, caudate, hypo-

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thalamus, brain stem reticular formation. In 3 cases, the radioactive tyrosine was introduced into freely moving, conscious animals after recovery from the pentobarbital; in other cases the tyrosine was introduced into anesthetized animals immediately after cannulae implantation. The experimental results were similar, but there was less technical difficulty in injecting animals who had previously recovered from the implantation.

After periods varying from one-quarter to 3 hours following the injection, the animals were anesthetized with sodium pentobarbital, the carotid arteries isolated, and the animals sacrificed by perfusing the brains with approximately 400 ml. of saline. The bloodless brains were removed, checked for cannulae placement, and dissected. Tissues were immediately frozen.

Each frozen sample was homogenized in 0.4N $HClO_4$ and the perchlorate-free extract passed through an alumina column as previously described for catecholamine separation (McGeer and McGeer, 1962). The total radioactivities of eluant and effluant from the column were determined by counting aliquots in a liquid scintillation counter. Other aliquots were used for two-dimensional paper chromatography in the solvent systems described in Table 1 in order to separate the various radioactive metabolites. In each case duplicate chromatograms with appropriate cold carriers were prepared and examined under ultraviolet light. One chromatogram of each pair was sprayed with diazotized sulfanilic acid for spot location, while the other was cut and the individual sections counted directly in a liquid scintillation counter (Wang and Jones, 1959). Correspondence between location of spots on one chromatogram and the areas cut in the duplicate were assured not only by the correspondence of ultraviolet spots between duplicate pairs but also by the excellent agreement found between many such pairs when both chromatograms were sprayed. The quantitative distribution of metabolites obtained from chromatograms run in one solvent pair agreed closely with data obtained from chromatograms run in the second solvent pair.

TABLE 1.

R_f(s) of Some Standard Compounds in Solvent Systems Used.*

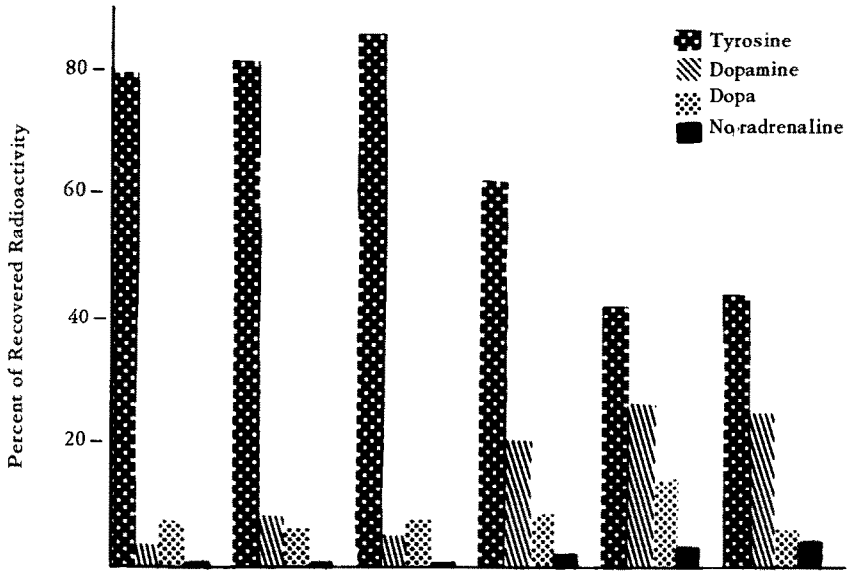
	A	B	C	D
Tyrosine	0.49	0.35	0.45	0.38
Tyrosine-O-sulfate	0.15	0.05	0.10	0.06
p-Hydroxyphenylacetic acid	0.79	0.90	0.64	0.84
Dopa	0.37	0.21	0.21	0.19
3,4-Dihydroxyphenylacetic acid	0.68	0.80	0.54	0.67
Dopamine	0.61	0.42	0.51	0.22
Noradrenaline	0.55	0.30	0.42	0.13
3-Methoxytyramine	0.70	0.58	0.61	0.33
Homovanillic acid	0.91	0.84	0.67	0.61
3-Methoxytyrosine	0.63	0.37	0.53	0.26

* Used: A (MeOH-n-BuOH-C_6H_6-H_2O, 2:1:1:1), followed by
B (n-BuOH-AcOH-H_2O, 4:1:1), or
C (MeOH-n-BuOH-C_6H_6-H_2O, 4:3:2:1), followed by
D (n-BuOH satd. with 1N HCl)

Identification of the catechol derivatives (dopa, dopamine, noradrenaline and dopac) is based on their absorbability on alumina and chromatographic identity with standards in the four solvents used. Further evidence for dopa, dopamine and noradrenaline was obtained by elution from chromatograms prepared with and without added carriers, oxidation to fluorescent derivatives (McGeer and McGeer, 1962), and comparison of the fluorescent spectra with those obtained by the oxidation of standards. Finally, several extracts were combined, and tyrosine, dopa, dopamine and 3-methoxytyramine separated by paper chromatography. Appropriate cold carrier was added, and each of these substances was recrystallized as the hydrochloride to constant specific activity. Identification of other metabolites is tentative and based only upon chromatographic identity with authentic standards in the two bidimensional solvent systems used.

Results and Discussion. Results obtained for the caudate nucleus in young to middle-aged cats are illustrated in Figure 1. With the exception of one cat where radioactive material was found along the cannula track,

4-5.6% of the injected radioactivity was recovered in the caudate. Most of this was still in tyrosine with much less distributed amongst various catechol compounds and other metabolites. No significant amount of radioactivity was ever recovered in contralateral control areas, ruling out the possibility that radioactive products formed in remote tissues could have been transported back to the brain.



Sex and Wt.:	♀, 2.5 kg.	♂, 3 kg.		♂, 2.3 kg.	♂, 3 kg.	♂, 2.6
Elapsed time:	30 mins.	55 mins	1¼ hr.	1¾ hrs.	2 hrs.	2 hrs.
wt. of caudate:	223 mgs.	159 mgs.	145 mgs.	214 mgs.	210 mgs.	226 mgs.
% of Injected radioactivity recovered:	1.4%*	5.1%	5.6%	4.8%	4.3%	4.0%

* Another 0.9% was recovered from the cortex along the needle track.

Fig. 1. Percent of recovered radioactivity in tyrosine and the catecholamines.

The greater part of the radioactivity found in the column eluants was chromatographically localized in the area occupied by dopamine, while almost all of the radioactivity in the effluant was localized in the spot occupied by tyrosine, with lesser amounts in the spot occupied by tyrosine-O-sulfate. Some radioactivity was found in the dopa spots in both eluant and effluant; this was not surprising in view of the generally poorer recoveries of dopa than of dopamine in the alumina procedure

(McGeer and McGeer, 1962). Some radioactivity was always found in the area corresponding to noradrenaline in chromatograms of column eluants, but this was greater than 1% of the total radioactivity recovered only in those cats showing more than 20% of the recovered radioactivity in dopamine. The proportion of the radioactivity found in dopamine and noradrenaline appeared to increase with elapsed time while the proportion in tyrosine decreased.

The proportion of recovered radioactivity found in spots corresponding to some minor metabolites were: p-hydroxyphenylacetic acid (0.8-6%), 3,4-dihydroxyphenylacetic acid (0-4.6%), and 3-methoxytyramine (0-2.1%).

Table 2 shows a comparison of the conversion of tyrosine to catecholamines in caudate versus cortex, hypothalamus or brain stem. These preliminary data suggest that the distribution of metabolites varies from area to area, and that the net conversion to dopamine is considerably greater in the caudate than in the other areas examined. This is in accord with the known distribution of catecholamines. It may, of course, reflect more efficient storage or less efficient conversion of dopamine in the caudate than in other brain areas, rather than faster synthesis.

TABLE 2.

Distribution of Radioactivity Among the Major Metabolites in Various Brain Areas

		Percent of Recovered Radioactivity in			
		Tyrosine	Dopamine	Dopa	Noradrenaline
♀, 2.5 kg.	(Caudate	79%	3.4%	7.3%	0.8%
	(Cortex	86%	0.6%	5.4%	nil
♂, 4.4 kg.	(Caudate	76%	2%	5.4%	0.14%
	(Hypothalamus	58%	0.4%	17%	0.3%
♂, 2.6 kg.	(Caudate	45%	25%	5.4%	4.6%
	(Hypothalamus	72%	3%	8.3%	4.8%
♀, 4 kg.	(Caudate	59%	4.95%	12.5%	1.2%
	(Brain stem	68%	0.7%	11%	2.6%

The results of this study demonstrate that cat brain has the capacity to convert tyrosine to dopa and the catecholamines. Since the presence of dopa in the blood stream under normal conditions has never been demonstrated, these results argue in favor of brain obtaining its catecholamines by a synthetic pathway starting from tyrosine. The techniques described may be useful in the comparison of the metabolic activity of different brain areas and in studying the effect of various pharmacological agents on the metabolism in defined locations. A more extensive report on these and related studies will be published elsewhere.

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