FORMATION OF ADRENALINE BY BRAIN TISSUE[1]

P. L. McGeer and E. G. McGeer

Kinsmen Laboratory of Neurological Research,
The University of British Columbia, Vancouver, Canada

Received September 8, 1964

Adrenaline is known to occur in many tissues of the body in addition to the adrenal medulla (Euler, 1956). Radioactive studies (Axelrod et al., 1959; Weil-Malherbe et al., 1961) have shown that circulating adrenaline may be picked up by a number of tissues, including the hypothalamus. This would be one way of accounting for extra-adrenal occurrence of this hormone. In humans, however, bilateral adrenalectomy does not result in the disappearance of adrenaline from the urine (Euler, 1946; Schaepdryver et al., 1961), which argues strongly in favor of production sites outside the adrenal gland.

The enzyme which synthesizes adrenaline from noradrenaline, phenylethanolamine-N-methyl transferase, has been studied by Axelrod (1962). He reported the enzyme to be highly localized in the adrenal medulla, but did note some enzyme in rabbit heart, and "minute amounts of enzyme activity" in two out of five rabbit brain stems.

Evidence for the presence of adrenaline in brain tissue is also uncertain. The bioassay studies of Euler (1946), Holtz (1950) and Vogt (1954) all suggest the presence of adrenaline in brain, but many authors have been unable to detect it chemically (Sano et al., 1960; Bertler and Rosengren, 1959). Gunne (1962) did report isolating enough adrenaline from several hundred pooled specimens of brain stem or hypothalamus for

[1] This research was supported by National Science Foundation Grant #GB-827, Canadian Federal-Provincial Mental Health Grant #609-5-146, and Medical Research Council of Canada Grant #MA-1421.

determination by chemical or bioassay methods. The amounts indicated by these various studies are of the order of 100 ng/gm of hypothalamic tissue.

In a previous study from this laboratory, the conversion of tyrosine-C^{14} to catecholamines by cat brain in vivo was reported (McGeer et al., 1963). Radioautogram of caudate tissue from this study, incubated one year, confirmed the presence of the metabolites reported. One further metabolite, which had remained unnoticed because of its close proximity to dopamine, appeared. It was identical in R_f characteristics to adrenaline, and appeared to be more heavily labelled than noradrenaline.

This paper is to report further evidence of adrenaline formation by brain tissue in vivo.

Experimental Methods and Results

DL-Noradrenaline-7-H^3 (7.5 c/mM) or L-tyrosine-U-C^{14} (360 mc/mM) were administered by injection as indicated in Table 1. Injections into

Table 1. Summary of In Vivo Experiments

Animal	Substrate	Site	Time of Injection Prior to Sacrifice and dpm Injected		% Radioactivity Recovered Total	Adrenaline
1. Monkey	NA-H^3	Brain stem	4 hrs	1.44×10^9	1.96	0.14
2. Cat	Ty.-C^{14}	Brain stem	5 hrs 1/2 hr	1.66×10^7 $+0.55 \times 10^7$	0.56	0.02
3. Cat	Ty.-C^{14}	Caudate	3 hrs	2.2×10^6	0.23	0.01
4. Cat	Ty.-C^{14}	Caudate	6 hrs	6.6×10^6 $+6.6 \times 10^6$	0.57	0.005

the brain stem were made directly in anesthetized animals in which the corpus callosum and massa intermedia had been divided. Numerous injections (ca. 5 microliters per injection) were made along either side of the third ventricle and into the rostral midbrain area. Injections into the caudate were made through permanently indwelling cannulae (McGeer et

al., 1963). After appropriate intervals the animals were given a large dose of pentothal and sacrificed by bleeding from the vena cavae. Bloodless tissues were dissected out and homogenized in 5-10 volumes of a 1:1 mixture of 0.4N perchloric acid and 0.2N acetic acid. The catecholamines in the homogenates were separated by absorption on an alumina column and elution with acetic acid (McGeer and McGeer, 1962). Control areas of brain and adrenals failed to show significant radioactivity.

In experiments 1 and 2 the acetic acid eluants were streaked on paper and chromatographed in water-saturated phenol in an atmosphere of 1N HCl with sufficient added cold carrier noradrenaline and adrenaline to allow location by spraying a thin strip with ninhydrin. The adrenaline area was eluted with 0.01N HCl, the eluant lyophilized with approximately 100 mg of additional carrier, and the mixture recrystallized as adrenaline hydrochloride from alcohol-ether. Typical results on repeated recrystallizations are shown in Table 2. All counting was done in a liquid scintillation spectrometer using an aqueous ethanol scintillation mixture.

Table 2. Activity of Material Recrystallized with Adrenaline*

	Recrystallization Number				
	2	3	4	5	6
Product Exp. 1	446	455	406	415	-
Product Exp. 2	478	553	548	539	555
NA-H^3 1.3×10^6 dpm	1080	760	430	240	0

* Figures are counts/10 minutes/mg of adrenaline HCl. In each case 100 mg of carrier adrenaline was added.

In experiments 3 and 4 the eluants from the alumina column were acetylated after the addition of 25 micrograms of cold carrier adrenaline, and the derivatives isolated and chromatographed according to the procedure of Goldstein et al. (1959). Trials of this procedure with

labelled dopamine and noradrenaline had shown the radioactivity to be highly localized to the expected areas, and with good separation of these metabolites from adrenaline. After chromatography, the chromatograms were cut and counted in a liquid scintillation spectrometer (Wang and Jones, 1959).

In each experiment, three radioactive peaks were found (Table 3). Although R_f's cannot be obtained in this system because the solvent travels beyond the end of the paper, the areas occupied by the peaks corresponded to those occupied by acetylated standards of noradrenaline, adrenaline and dopamine on parallel chromatograms. These derivatives travelled approximately 1.5, 4.3 and 8 inches respectively in 24 hours.

Table 3. Radioactivity in Acetylated Derivatives of Catecholamines as Per Cent of Total on Chromatographic Strip

	Experiment 3	Experiment 4
Peak A (dopamine)	77	75
Peak B (adrenaline)	17	18
Peak C (noradrenaline)	06	07

As shown in Table 1, the amounts of adrenaline indicated to be formed in each of these experiments is small, but is in keeping with the low amounts reported to be found in brain by other workers (Vogt, 1954; Gunne, 1962).

Further evidence of phenylethanolamine-N-methyl transferase activity in brain tissue was sought using the *in vitro* system of Axelrod (1962) whereby normetanephrine is converted to metanephrine in the presence of S-adenosyl-L-methionine-methyl-C^{14}. The radioactivity extractable into isoamyl alcohol-toluene in the test, compared with a blank incubation in which normetanephrine is left out, is taken as a measure of the metanephrine formed during the incubation.

Incubations of rabbit tissue were carried out exactly as described by Axelrod with two exceptions: S-adenosyl-L-methionine-methyl-C^{14} of much higher specific activity was used (39.4 mc/mM), and, because higher conversions were obtained, homogenates were spun at 15,000 G for 15 minutes instead of 78,000 G for one hour.

In confirmation of the results of Axelrod, the adrenal test had much more extractable radioactivity than the adrenal blank (Table 4). In addition, differences were consistently found for the cerebral cortex, hypothalamus and caudate nucleus.

Table 4. Ratio of Cpm's Extracted into Isoamyl Alcohol/ Toluene Mixture in Test as Compared to Blank.

	Adrenal	Brain stem	Cortex	Caudate
Average	3.8 ± 1.14	1.75 ± 0.45	1.85 ± 0.45	1.7 ± 0.39
Range	2.1 - 5.7	1.3 - 2.4	1.15 - 2.5	1.4 - 2.1
No. of Expts.	9	9	4	5

Not all the extractable radioactivity was due to metanephrine. Products from adrenal and brain incubations, with their blanks, were chromatographed in isopropanol/ammonia/water (8:1:1). The high R_f areas were eluted and then re-chromatographed bidimensionally in methanol/ butanol/benzene/water (4:3:2:1) followed by n-butanol/acetic acid/water (4:1:1). Appropriate areas were cut from the papers and counted directly in a liquid scintillation spectrometer. In addition to a compound indistinguishable in chromatographic characteristics from metanephrine in these solvent systems, two other radioactive areas appeared in the test incubations but not the blanks. These other compounds, whose presence was confirmed by radioautography, contributed substantially to the extractable activity in brain but not adrenal. Their identification is currently being investigated.

The results of this study provide further evidence that brain has the capacity to synthesize adrenaline. Taken with the reports of other

workers, they suggest that there may be physiological significance to this capacity, but different kinds of experiments would be needed to establish this point.

We wish to express thanks to Drs. J. Wada and G. Ling for assistance in preparing the animals.

References

Axelrod, J., Weil-Malherbe, H., and Tomchik, R.J. J. Pharmacol. Exp. Therap. 127, 251 (1959).
Axelrod, J. J. Biol. Chem. 237, 1657 (1962).
Bertler, A., and Rosengren, E. Acta Physiol. Scand. 47, 350 (1959).
Euler, U.S. von Acta Physiol. Scand. 12, 73 (1946).
Euler, U.S. von In: Noradrenaline, Charles C. Thomas, Springfield, Ill. p. 143 (1956).
Goldstein, M., Friedhoff, A.J., and Simmons, C. Experientia 15, 80 (1959).
Gunne, L. Acta Physiol. Scand. 56, 324 (1962).
Holtz, P. Acta Physiol. Scand. 20, 354 (1950).
McGeer, E.G., Ling, G., and McGeer, P.L. Biochem. Biophys. Res. Comm. 13, 291 (1963).
McGeer, P.L., and McGeer, E.G. Can. J. Biochem. 40, 1141 (1962).
Sano, I., Taniguchi, K., Gamo, T., Takesada, M., and Kakimoto, Y. Klin. Wschr. 38, 57 (1960).
Schaepdryver, A.F. de, and Smets, W. Arch. Intern. Pharmocodyn. 131, 467 (1961).
Vogt, M. J. Physiol. 123, 451 (1954).
Wang, C.H., and Jones, D.E. Biochem. Biophys. Res. Comm. 1, 203 (1959).
Weil-Malherbe, H., Whitby, L.G., and Axelrod, J. In: Regional Neurochemistry (Eds. S.S. Kety and J. Elkes), Pergamon Press, p. 284 (1961).