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Synthesis of catecholamines in the locus coeruleus from ^{3}H-tyrosine *in vivo*

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HISTOCHEMICAL fluorescent methods indicate that the locus coeruleus (LC) is made up of catecholamine-containing cell bodies.[1] Indirect evidence, such as the immounhistochemical demonstration of the presence of dopamine-β-hydroxylase[2] and the use of dopamine-β-hydroxylase inhibitors to show decreased histochemical fluorescence intensity in cells in rats pretreated with these drugs,[3] suggests that these cells are noradrenergic. However, direct analysis of extracts of the LC in the rabbit and the cow by thin-layer chromatography showed that the LC contained more dopamine (DA) and its metabolite, dihydroxyphenylacetic acid, than norepinephrine (NE).[4] These latter results may suggest that the LC contains both noradrenergic and dopaminergic cells. To investigate further the biochemical nature of the LC in the rat, we have examined the synthesis of catecholamines and their metabolites in the LC after intraventricular injection of tritiated tyrosine. The methods utilized were sensitive enough to measure newly synthesized NE and DA in the locus coerulei in a single animal.

Charles River male rats (175–225 g), anesthetized with 8% chloral hydrate (0·5 ml/100 g), were injected intraventricularly with 25 μ L-tyrosine-3,5-^{3}H (44 c/m-mole, New England Nuclear Corp., Boston, Mass.). The animals were decapitated at various times after the injection and their brains rapidly removed to a dish of ice-cold saline. The locus coerulei were removed from their lateral positions in the brainstem of each animal in two small blocks of tissue (each less than 2 mg in weight) which were immediately homogenized in 1 ml of ice-cold 10% trichloroacetic acid, containng 25 μg tyrosine, DA and NE for carrier purposes. After removing insoluble material by centriifugation, radioactivity was measured in a small portion of the supernatant fluid which was designated "total tissue extract". After the pH was raised to 8·4, the total tissue extracts were passed over an alumina column to retain catechols, which were subsequently eluted from the columns with weak acid. Radioactivity was measured in a portion of the eluate which was designated "catechol fraction" and the remainder of the catechol fraction was applied to an Amberlite-CG120 column to yield three subfractions containing deaminated metabolites, DA and NE.[5] The deaminated metabolites were contained in the water washes of the Amberlite columns. DA and NE were eluted from the columns with an HCl (0·25–2 N) gradient; about twenty-five 2-ml fractions were collected and 1 ml of each fraction was counted in 20 ml of Triton X-100 phosphor (5·5 g 2,5-diphenyloxazole, 300 mg dimethyl-1,4-bis-2-(5-phenyloxazolyl), 2 l. toluene, 1 l. Triton X-100). There was no overlap of the NE and DA fractions. The levels of radioactivity in the NE fractions were at least eight times background levels. All catechol fractions were counted for a long enough period to provide a 2·5 per cent standard deviation. The recovery of total radioactivity from the Amberlite-CG120 columns was 76·4 $\pm$ 8 per cent (mean $\pm$ S.D., n = 10). The recovery of NE and DA in this procedure was at least 90 per cent. In some experiments, blocks of tissue from the brainstem central gray area that did not contain catecholamine cells and also portions of the caudate nucleus were carried through the analysis. The dissection procedure for the LC was worked out in conjunction with the use of the histochemical fluorescence technique to localize the LC.[6]

The animals were decapitated at 7·5, 15, 30 and 60 min after intraventricular injection of radioactive tyrosine. The total tritium in the tissue extracts was highest at 7·5 min, reduced by more than

50 per cent at 15 min and reduced by 50 per cent again at 30 min. At 60 min, the radioactivity in total tissue extracts was about the same as or slightly lower than that at 30 min (Table 1). The radioactivity in the catechol fraction exhibited a slightly different pattern over time. It was of intermediate level at 7·5 min, highest at 15 and 30 min and lowest at 60 min (Table 1). The rapid fall of radioactivity in the catechol fraction between the 30- and 60-min intervals may indicate a very rapid turnover of NE in these cell bodies. Only a small percentage of the radioactivity in the total tissue extract was converted to catechols (e.g. about 5 per cent at 15 min). In some experiments, blocks of tissue containing no catecholamine cells from the central gray area of the pons were carried through the analysis. In these samples there was very little or no detectable radioactivity in the catechol fraction.

After the catechol fractions were resolved into subfractions of deaminated metabolites, NE and DA, the bulk of the radioactivity was found in the NE fraction at all times studied (Table 2). At 7·5, 15 and 30 min, about 70 per cent of the Amberlite-CG120 eluate was NE, 20 per cent was deaminated metabolites and 10 per cent was DA. At 60 min, there were no detectable levels of deaminated metabolites

TABLE 1. RADIOACTIVITY IN TOTAL TISSUE EXTRACT AND CATECHOL FRACTION*

Time (min)	Total tissue extract (dis./min)	Catechol fraction (dis./min)
7·5	223,000 ± 51,00 (4)	3755 ± 458 (4)
15	104,000 ± 19,000 (4)	4880 ± 285 (4)
30	49,000 ± 7500 (5)	5056 ± 510 (5)
60	42,000 ± 4400 (5)	1868 ± 291 (5)

* After animals were injected intraventricularly with a tracer dose of ^{3}H-tyrosine, they were decapitated at the designated time after injection and their locus coerulei were removed and homogenized. Radioactivity in "total tissue extract" indicates the total disintegrations per minute in supernatant fluid from homogenate. "Catechol fraction" was that part of the total extract retained on an alumina column at pH 8·4 and subsequently eluted with weak acid. Results are mean ± S.E. (n). See Methods for details.

or DA. In some experiments, slices of the caudate nucleus, a brain region with very high levels of DA and very low levels of NE, were carried through the analysis. In these samples (dissected 15 min after intraventricular injection), about 75 per cent of the radioactivity in the catechol fraction was DA and the remaining 25 per cent was deaminated metabolites. There were only traces of NE in these samples.

These experiments indicate that, at the time points investigated, the cells of the LC have much higher levels of radioactive NE than DA (at least 70 per cent vs. 10 per cent). The presence of radioactive DA at earlier times would be expected, since it is the immediate precursor of NE. A similar

TABLE 2. PER CENT OF TOTAL RADIOACTIVITY IN ELUATE AMBERLITE COLUMNS RECOVERED IN THE DEAMINATED METABOLITE, NOREPINEPHRINE AND DOPAMINE SUBFRACTIONS*

Time (min)	Deaminated metabolite	Norepinephrine	Dopamine
7·5	25 ± 6 (5)	64 ± 9 (3)	10 ± 2 (3)
15	19 ± 4 (4)	71 ± 6 (4)	10 ± 4 (4)
30	20 ± 4 (4)	68 ± 10 (5)	12 ± 5 (5)
60	ND	100 ± 14 (5)	ND

* The catechol fractions were further resolved on Amberlite columns into deaminated metabolites, norepinephrine and dopamine subfractions. Results are mean ± S.E. (n). ND = none detected.

pattern of metabolism has been found in peripheral nerve tissues (e.g. bovine splenic nerve,[5] guinea pig was deferens[7] and guinea pig atria[8]) in which NE is the sympathetic neurotransmitter. On the other hand, radioactive DA was the only measurable catecholamine that accumulated in tissue in which DA rather than NE is thought to be the major neurotransmitter (e.g. the caudate nucleus and molluscan ganglia[9-11]). Thus, the present results are in accordance with the notion that NE is the neurotransmitter in the cells of the LC in the rat, although this may not be the case in other species.[4]

Departments of Psychiatry and Pharmacology, MICHAEL J. KUHAR
Yale University School of Medicine and Connecticut Mental Health Center, ROBERT H. ROTH
New Haven, Connecticut, U.S.A. GEORGE K. AGHAJANIAN

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Effect of *para*-chlorophenylalanine on catecholamine synthesis in rat brain, heart and adrenals

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PARA-CHLOROPHENYLALANINE (PCPA) has been used extensively as a serotonin depleter during investigations of the involvement of brain serotonin in temperature control,[1] seizures,[2-5] sleep,[6,7] mental disorders,[8] sexual activity[8,9] and other functions. However, PCPA also has actions on catecholamine biosynthesis. Several authors have reported significant lowering of brain catecholamines following PCPA treatment[10-12] and in mice depletion of noradrenaline was as pronounced as that of serotonin.[10] *In vitro*, PCPA inhibits tyrosine hydroxylase to a slight extent[11] and is a potent inhibitor of phenylalanine hydroxylase.[12] Date on the role of serotonin in seizures obtained by the use of PCPA have been questioned.[13,14] These observations suggest that the effect of PCPA may not be as specific as is generally accepted. Since changes in biogenic amine turnover rates tend to be more relevant to changed neuronal activity than changes in content,[15,16] we investigated the effect of PCPA on the turnover rates of the catecholamines in several tissues including brain.

The catecholamine synthesis rate was measured by a slight modification of the method of Neff *et al.*[17] Catecholamine content was assayed by the method of McGeer *et al.*[18] Male Sprague–Dawley rats, 250–270 g, were injected with PCPA methyl ester (CalBiochem) in aqueous propylene glycol[19] at a dose of 300 mg/kg intraperitoneally. Control animals received only the injection vehicle. The catecholamine synthesis rates and noradrenaline and adrenaline content were measured in brain, heart and adrenals at 2 and 24 hr after the PCPA injection. L-tyrosine-3,5-H^3 58 c/mM, 200 μc was infused into the tail veins of restrained rats over a period of 1 hr. At the end of the infusion, the