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Decarboxylation of 3, 4-dihydroxyphenylalanine in various human adult and fetal tissues

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THE DECARBOXYLATION of 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine (DOPA) by aromatic amino acid decarboxylase, a pyridoxal phosphate dependent enzyme, has been demonstrated and extensively studied in a wide variety of animal tissues.[1] In contrast, very little is known about the decarboxylation of these two amino acids in human tissues although their products, serotonin, dopamine and norepinephrine, are currently thought to act as important modulators of mood and behaviour in man and are frequently implicated in the pathogenesis of schizophrenia, affective disorders and parkinsonism.[2–5] Furthermore, DOPA is now being used in large doses in the treatment of patients suffering from parkinsonism.[6–8] Thus, it was considered important to study the decarboxylation of DOPA in various human adult and fetal tissues with and without the addition of pyridoxal phosphate.

Human tissue samples were obtained as soon as possible after death (no later than 10 hr) and stored at −73° for periods of 1–5 weeks. The gestational age of the three fetuses (entire organs were used) was approximately 3 months. The adult samples (hypothalamus–thalamus–substantia nigra area, sections of cortex of kidney, of ventricle of heart, of lung and liver) were obtained from three male patients, aged 38, 43 and 63 yr, who had died of coronary occlusion.

The decarboxylation of DOPA was determined by measuring the formation of $^{14}CO_2$ from DOPA-1-^{14}C.[9] Briefly, 0·2 ml of tissue homogenate (20% in 0·1 M phosphate buffer, pH 7·5, supernatant after centrifugation at 2000 *g* for 10 min), 1 ml of the phosphate buffer, 0·2 ml of pyridoxal phosphate (20 μg) and 0·2 ml of DOPA-1-^{14}C (0·1 μmole containing approximately 240,000 dpm) were incubated in small beakers fitted with a center well (0·2 ml of 5 N NaOH) and closed with a rubber cap at 37° for 60 min. The reaction was stopped by injecting 0·5 ml of 5 N HCl. The vessels were then shaken for another 60 min. An aliquot of the NaOH solution was counted in an automatic Packard liquid-scintillation counter. Blanks contained boiled homogenates. Since most data in the literature are based on wet weight, values in this study are reported on the same basis for purposes of control and comparison.

One rat liver homogenate was included along with the human samples in each assay performed. Values obtained for this tissue agree with those published in the literature and variations were minimal among assays performed on different days.

Since autopsy material was used in this study, the postmortem effects on the decarboxylation of DOPA were first studied in tissues obtained from rats which had been killed by cervical dislocation and then left for 2 hr at room temperature followed by 15 hr in the refrigerator. Animals sacrificed just before the assay served as controls. In agreement with previous findings,[10] no changes in decarboxylase activities were observed in brain, whereas heart and liver showed a small decrease of approximately 15 per cent. Thus, if it is assumed that the human enzyme does not differ from the animal enzyme, human tissues obtained at autopsy no longer than 10 hr previously are suited for studies on the decarboxylation of DOPA.

The results of this investigation are summarized in Table 1. Activities in the adult human samples varied greatly and were generally much weaker than those found in rat or other animal tissues.[9, 11] In some samples, the activity, if present, was below the sensitivity of the assay method. The extremely low activities of aromatic amino acid decarboxylase found in human brain samples are in agreement with previous results of this laboratory[10] and those of other investigators.[9, 12] Although these low activities could have been caused by a variety of factors such as postmortem changes, diseases or causes of death, the possibility has to be considered that aromatic amino acid decarboxylase activity in human brain tissue is indeed extremely low. A comparison of the activities of this enzyme with those of tyrosine hydroxylase (0–7 nmoles/g/hr),[10] which is the rate-limiting step in the biosynthesis of norepinephrine in animal tissues, might suggest that perhaps biogenic amine synthesis in human brain tissue is controlled by both tyrosine hydroxylase and aromatic amino decarboxylase.

The addition of pyridoxal phosphate increased DOPA decarboxylation. The relative increase

TABLE 1. DECARBOXYLATION OF DOPA IN VARIOUS HUMAN ADULT AND FETAL TISSUES*

Organ	Adult tissue		Fetal tissue	
	$+B_6$	$-B_6$	$+B_6$	$-B_6$
	(nmoles CO_2 formed/g/hr)			
Liver	32, 225, 400	20, 65, 120	1400, 1875, 2000	1200, 1425, 1800
Heart	8, 10, 28	<4, <4, 4	14, 16 , 20	4, 6 , 16
Lung	14, 36, 48	4, 9, 20	350, 370 , 400	40, 55 , 85
Kidney	60, 65, 80	12, 12, 24	1450, 2020, 2060	480, 570 , 1235
Brain	<4, 11, 20	<4, 6, 6	8, 25 , 40	4, 9 , 12

* Average activities (nmoles CO_2 formed/g/hr) for rat tissues were found to be: liver, 8000; kidney, 6600; lung, 1000; brain, 800; and heart, 800.

varied among the tissues, being greatest in fetal lung and adult heart and smallest in fetal liver tissue. In general, increases were larger than those observed with animal tissue; this, however, could be due to a postmorten reduction of pyridoxal phosphate in some of the human tissues.

A comparison between fetal and adult tissues shows that fetal activities were usually higher than those found in adult tissues, except in heart and brain where they did not differ markedly. This seems to be in contrast with the development of the activity of aromatic amino acid decarboxylase in animal tissues. For instance, during fetal and postnatal development of rats and other animals, the activity of this enzyme was found to increase in the brain and in kidney, liver and lung.[13, 14]*

In summary, decarboxylation of DOPA has been demonstrated in various human tissues obtained at autopsy. Activities observed were low and in some human brain samples activities (if present) were below the sensitivity of the method used. Addition of pyridoxal phosphate to the incubation medium markedly increased DOPA decarboxylation in some but not all tissues. In contrast to observations in animal tissues, DOPA decarboxylation was generally higher in fetal tissues than in adult tissues.

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Long-term variation in basal and phenobarbital-stimulated oxidative drug metabolism in the rat

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SEVERAL studies have shown that responses to drugs may vary as a function of time in a rhythmic manner. We have demonstrated an endocrine-dependent circadian rhythm in oxidative drug metabolism in the rat and mouse;[1] this has recently been confirmed by Vesell[2] and by Colas *et al.*[3] Similar rhythms in drug responsiveness or toxicity or in both have been shown for morphine,[4] pentobarbital,[5] nikethamide[6] and lidocaine.[7] Longer term fluctuations, known as seasonal or circannual rhythms and having a 12-month base, are present in a variety of physiologic and biochemical functions. Ankier *et al.*[8, 9] report the presence of a seasonal dependence in the rat to the anaphylactic properties of dextrans and other antigens with a winter peak in mortality. Nayler[10] has demonstrated a seasonal rhythm in cardiac phosphorylase activity in the toad with summer values of about 60 per cent of the winter value, while Kennedy and Nayler[11] have shown that summer levels of magnesium-dependent, sodium- and potassium-activated ATPase in toad cardiac muscle are about 30 per cent of winter values. A striking example of seasonal dependence in a response of pharmacologic interest is afforded by the studies of Fearn *et al.*,[12] who examined the blood pressure response of the rat to histamine at different times of the year. During the period September–January, 10–50 μg/kg doses of histamine evoked an average depressor response of 40 mm Hg; from February to April, histamine at 100–500 μg/kg elicited an average depressor response of 20 mm Hg, whereas during the period May–August doses of histamine from 1 to 5 mg/kg failed to modify blood pressure. In a study employing 28,000 mice over a 3½-yr period, Sterne and Hirsch[13] have demonstrated a marked reduction in the lethality of dimethylguanylguanidine, a non metabolized hypoglycemic agent, during the summer months, which appears to relate to a seasonal susceptibility to the effects of hypoglycemia. Kalser and Kunig[14] have recently suggested the presence of a seasonal dependence in the response of Wistar rats to hexobarbital hypnosis. In our own laboratory,* an apparent seasonal variation in the lethality of drugs such as morphine and insulin in the rat has been noted.

Observations of this type suggest that in studies of long duration drug responses may be measured against a constantly changing baseline and that significant qualitative and quantitative alterations may be expected. The specific objectives of the preliminary experiments to be reported were: (1) to determine if seasonal rhythmicity in oxidative drug metabolism is evident in the rat; (2) to determine if rhythmicity is present in the response of this species to enzyme-stimulating drugs such as phenobarbital; and (3) to attempt a correlation of long-term alterations in drug metabolism with drug response measured under *in vivo* conditions.

Male, Holtzman rats, weighing between 135 and 150 g at the time of use, were employed as experimental animals. Animals were maintained on wire-mesh flooring in the laboratory for at least 5 days after receipt from the supplier prior to use and had free access to water and commercial chow.

*D. E. Blake, D. R. Haubrich and J. E. Thornburg, unpublished observations.