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INHIBITION OF SEROTONIN BIOSYNTHESIS BY 6-HALOTRYPTOPHANS *IN VIVO*

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Abstract—The 6-halotryptophans, which had previously been shown to inhibit tryptophan hydroxylase in brain, were examined further for *in vivo* inhibition both in brain and in peripheral tissues. Serotonin, tryptophan hydroxylase and 5-hydroxyindoleacetic acid levels in brain and serotonin levels in other tissues were substantially lowered after intraperitoneal injection of DL-6-fluorotryptophan without significantly changing levels of the catecholamines or the activities of several of the enzymes involved in their biosynthesis and metabolism. The inhibitors freely entered the brain and were rapidly and completely excreted.

ALTHOUGH many inhibitors of serotonin biosynthesis are known,[1] few are specific enough in their action to substantially lower the serotonin levels without also affecting catecholamine synthesis. One group of inhibitors, the 6-halotryptophans, which had previously been shown to fulfil the specificity requirements,[2] has now been examined in detail *in vivo* in brain and other tissues.

Since the two halotryptophans studied *in vitro* did not differ significantly in their action toward tryptophan hydroxylase, only one was used for the study *in vivo*. The effect of injected DL-6-fluorotryptophan on the monoamine levels and on some of the enzymes involved in the biosynthesis and metabolism of the monoamines was studied.

MATERIALS AND METHODS

Pargyline was a gift of Abbott Laboratories and NSD 1034 was a gift of Smith & Nephew Research. Radioactive measurements were made in a Nuclear Chicago Mark 1 scintillation spectrometer. A standard Triton X/toluene counting mixture consisting of 330 ml Triton X-100, 670 ml toluene, 4 g 2,5-diphenyloxazole and 150 mg 1,4-bis-2-(4-methyl 5-phenyloxazolyl)benzene was used for the aqueous samples. Fluorometric measurements were made in an Aminco–Bowman spectrophotofluorometer.

DL-6-Fluorotryptophan (1–2 m-moles/kg) was dissolved in 0·1 N NaOH, the pH adjusted to approximately 8, and injected intraperitoneally into male or female Wistar rats weighing between 100 and 200 g. After the appropriate time interval, the animals were sacrificed by decapitation. Brain, heart, kidneys, spleen, lungs, liver and a portion of intestine of approximately 1 g weight from midway along the ileum were excised immediately. When enzyme activities were to be measured, the tissues were homogenized in 4–6 vol. of ice-cold 0·28 M sucrose and an aliquot was taken for the enzyme assays. Perchloric acid (2 M) was added to the remaining homogenate to give a final concentration of 0·3 M and the resulting mixture was left for 15 min in an ice bath.

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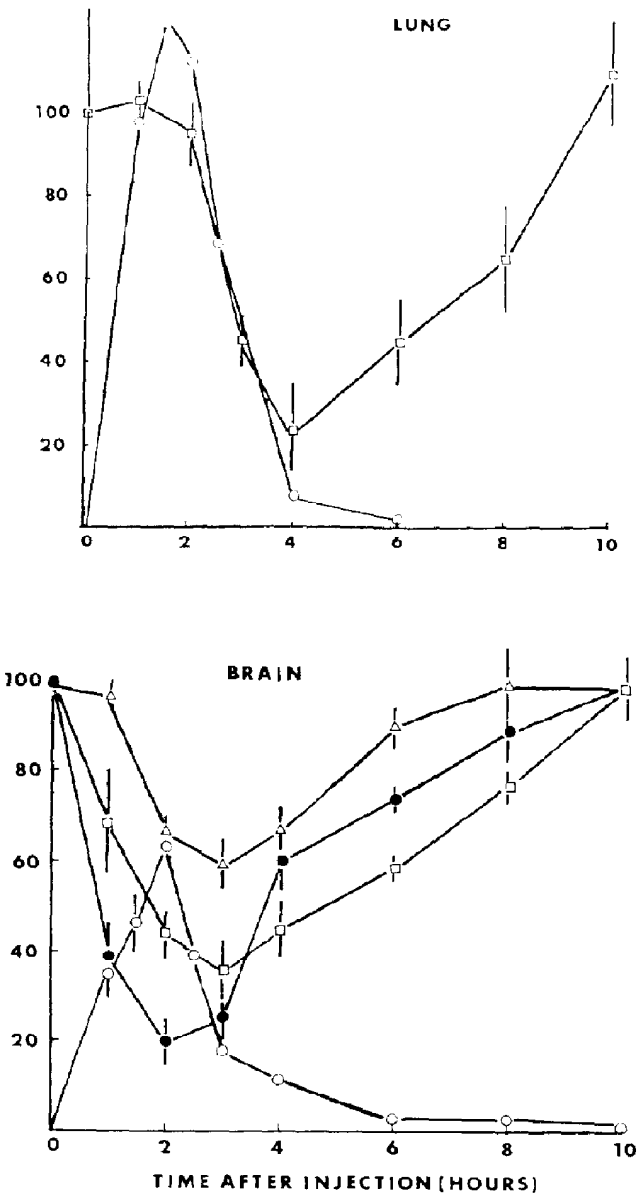
After centrifugation, the residue was washed with 6 vol. of 0·3 M perchloric acid, recentrifuged and the supernatants combined. Otherwise, the tissues were homogenized directly in 4–6 vol. of 0·3 M perchloric acid before centrifugation.

Monoamine oxidase,[3] catechol-*O*-methyl transferase[4] and tryptophan hydroxylase[5] assays in brain were carried out using standard procedures. 5-Hydroxytryptophan decarboxylase was measured by the method used for tryptophan hydroxylase, except that 5-hydroxytryptophan-3-^{14}C (21·8 mc/m-mole) was used as the substrate and a



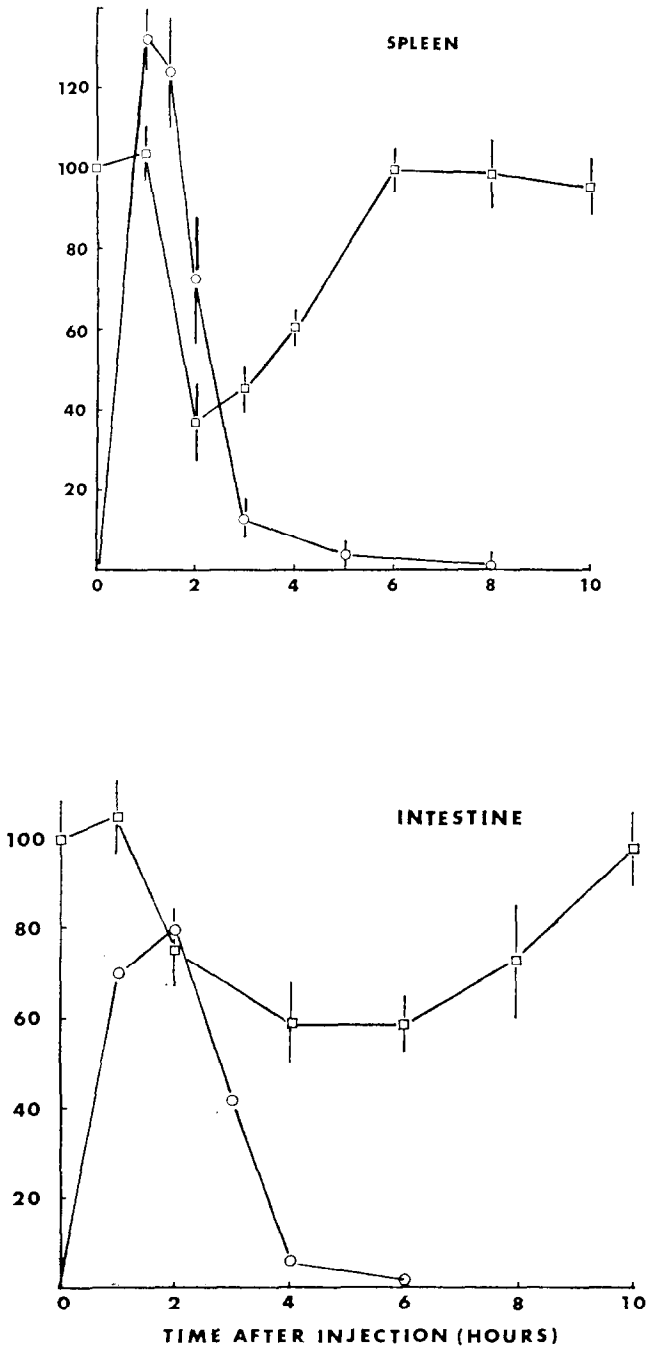


FIG. 1. Levels of 6-fluorotryptophan (○—○), tryptophan hydroxylase (●—●), serotonin (□—□) and 5-hydroxyindoleacetic acid (△—△) in rat tissues after administration of 6-fluorotryptophan, 1·5 m-moles/kg. 6-Fluorotryptophan levels are given in μg/g of tissue; other values are expressed as per cent normal. Each point represents the average $\pm$ S.E. of a minimum of five animals.

pH of 8·2 was used in the incubation. Tyrosine hydroxylase was measured both by a rapid tritium exchange method,* and the more usual ^{14}C method.[6]

The supernatants were each divided into two portions: the first was used for the estimation of serotonin, noradrenaline and dopamine;[7] and the remainder for 5-hydroxyindoleacetic acid and 6-fluorotryptophan. Serotonin,[8] 5-hydroxyindoleacetic acid,[9] noradrenaline[10] and dopamine[10] were determined fluorometrically on the extracts. Approximate 6-fluorotryptophan levels in tissues were determined from the fluorescence, measured at 285 mμ activation, 360 mμ fluorescence, of the crude supernatants at pH 11 by the method used for trytophan.[11] The final extract used for the 5-hydroxyindoleacetic acid measurement was found to contain a small but consistent portion (approximately 30 per cent) of the 6-fluorotryptophan together with a similar proportion of the free tryptophan. Measurement of the fluorescence as before gave a more reproducible assay of the tissue inhibitor level and this measurement was used as an additional check. Since this method of analysis did not distinguish between 6-fluorotryptophan and endogenous tryptophan, inhibitor levels could only be calculated by assuming tryptophan levels to remain unchanged. This assumption has been shown to be correct when using labeled 6-chlorotryptophan (D. A. V. Peters, unpublished observations) and is probably correct for the 6-fluoro analog also, particularly as the tissue levels found were considerably higher than the endogenous tryptophan levels.

The presence of tryptophan hydroxylase in normal mammalian tissues other than brain and mast cells has been a matter of dispute.[12–15] However, recent reports suggest that a tryptophan-hydroxylating enzyme is present in liver,[16,17] kidney[17] and intestine,[17,18] although with low activities *in vitro*. No activity was detected in either heart or spleen.[17] Attempts by us to measure tryptophan hydroxylase in spleen, lung and intestine by the method used for brain were unsuccessful. Although there was some evidence of hydroxylation by intestine homogenates, none of the tissues studied showed levels of activity that could be measured with any degree of reliability either with or without the addition of a pteridine cofactor. Consequently, for tissues other than brain the amine levels alone were measured. 5-Hydroxyindoleacetic acid levels in peripheral tissues were also usually too low for accurate measurement.

RESULTS

Effect on serotonin biosynthesis

The effect of 6-fluorotryptophan on tryptophan hydroxylase and brain serotonin levels previously briefly reported[2] was confirmed by this study. A corresponding decrease in the levels of the serotonin metabolite, 5-hydroxyindoleacetic acid, was also found. Brain activities of L-amino acid decarboxylase and monoamine oxidase, the other enzymes involved in the synthesis and breakdown of serotonin in brain, were not significantly changed even at near lethal doses of the inhibitor (2·0 m-moles/kg). A small and transient drop in activity of both of these enzymes of less than 20 per cent approximately 2 hr after injection was not considered significant with regard to changes in serotonin levels, since both enzymes have much higher activities than tryptophan hydroxylase, the apparent rate-controlling step in serotonin biosynthesis in brain.[19] In addition, serotonin levels followed closely the activity of tryptophan hydroxylase

* D. A. V. Peters, manuscript in preparation.

TABLE 1. LEVELS OF 6-FLUOROTRYPTOPHAN, SEROTONIN, NORADRENALINE AND DOPAMINE AFTER ADMINISTRATION OF THREE CONSECUTIVE DOSES OF DL-6-FLUOROTRYPTOPHAN (1·5 m-moles/kg)

	6-Fluorotryptophan	Serotonin		Noradrenaline		Dopamine	
Tissue	(μg/g)	(μg/g)	(% normal)	(μg/g)	(% normal)	(μg/g)	(% normal)
Brain	29 ± 4	0·14 ± 0·02	28 ± 3	0·51 ± 0·02	90 ± 5	0·55 ± 0·09	98 ± 2
Spleen	66 ± 7*	2·5 ± 0·3*	62 ± 8	0·64 ± 0·04*	105 ± 6	0·14 ± 0·01*	105 ± 8
Lung	104 ± 11	†	†	0·15 ± 0·02	94 ± 10	0·18 ± 0·01	115 ± 7
Small intestine	86 ± 10	2·6 ± 0·2	53 ± 7	0·51 ± 0·04	108 ± 12	0·11 ± 0·01	114 ± 11
Heart	42 ± 5			1·60 ± 0·06	113 ± 5	0·14 ± 0·02	112 ± 11
Adrenal	176 ± 29			587 ± 73‡	89 ± 11	40 ± 3	91 ± 7
Kidney	21 ± 3						
Liver	8·7 ± 0·7						

* Micrograms per total organ (see text).
† Values too inconsistent to be accurately calculated.
‡ Noradrenaline + adrenaline.

and not the fall in either of the other enzymes. In most experiments serotonin levels were noticeably depressed before any effect on monoamine oxidase or L-amino acid decarboxylase was evident and were still depressed several hours after the enzyme activities had returned to normal. For tissues other than brain, similar decreases of serotonin were found, although in some cases a longer time interval before levels began to fall was noted and the time for maximum depression was later. Graphs of serotonin, 5-hydroxyindoleacetic acid and tryptophan hydroxylase correlated with inhibitor levels are shown in Fig. 1.

When a group of male Wistar rats, 200–220 g, were injected with three consecutive doses of 6-fluorotryptophan, 1·5 m-moles/kg, at 2-hr intervals and sacrificed 2 hr after the final injection, little if any potentiation of inhibition was obtained. Tissue concentrations of 6-fluorotryptophan, serotonin, noradrenaline and dopamine were comparable to those found 2 hr after a single injection of the same dose of inhibitor. The

TABLE 2. EFFECT OF DOSE LEVEL OF 6-FLUOROTRYPTOPHAN ON SEROTONIN AND TRYPTOPHAN HYDROXYLASE IN RAT BRAIN*

Dose (m-moles/kg)	6-Fluorotryptophan (μg/g)	Tryptophan hydroxylase (% normal)	Serotonin (% normal)
0·50	16 ± 3 (5)	41 ± 7 (5)	70 ± 5 (5)
1·00	40 ± 9 (6)	23 ± 4 (6)	44 ± 7 (6)
1·50	69 ± 11 (3)	25 ± 5 (8)	35 ± 6 (8)

* Figures in parentheses refer to the number of animals.

results are summarized in Table 1. Values for serotonin, noradrenaline and dopamine are expressed as the percentage of those obtained from an identical group of rats receiving only the injection medium. Since spleen weights varied greatly without significant change in the total amine content, the figures for this tissue are expressed on the basis of total weight. All other values are expressed as weight per gram of tissue.

When different dose levels were used, it was found that higher doses did not result in correspondingly greater inhibition of tryptophan hydroxylase or lower serotonin levels. The results are given in Table 2.

Effect on catecholamine biosynthesis

Injection of large doses of 6-fluorotryptophan (up to 1·5 m-moles/kg) caused negligible changes in brain tyrosine hydroxylase or catechol-*O*-methyl transferase. No significant changes in noradrenaline or dopamine were found in any of the tissues studied. Absolute values of the catecholamines[20–22] and their associated enzymes were within the normal ranges.

DISCUSSION

p-Chlorophenylalanine, probably the most extensively used inhibitor of serotonin biosynthesis to date, also inhibits phenylalanine hydroxylase[23] and lowers brain noradrenaline levels.[24] Although it is a competitive inhibitor of tryptophan hydroxylase *in vitro, in vivo* it acts as an irreversible inhibitor, and a more complex mechanism of inhibition may be operating.[25,26]

In contrast, this study has shown that the 6-halotryptophans, as represented by the fluoro compound, apparently act as simple tryptophan hydroxylase inhibitors. There was no effect on catecholamine levels either by enzyme inhibition or by catecholamine release. Lowering of serotonin levels in brain follow tryptophan hydroxylase activities and inhibition is not prolonged even at maximum dose levels. At low dose levels, injected inhibitor, tissue inhibitor concentrations, serotonin levels and tryptophan hydroxylase inhibition showed an approximately linear relationship, but at higher dose levels the enzyme activities did not decrease further and serotonin levels fell only slightly. A similar result was obtained with repeated doses; serotonin levels and enzyme activities were not markedly different from those obtained after a single injection. However, the inhibitor concentrations calculated for whole brain give no information on the amount of inhibitor within the synaptic vesicles where most of the tryptophan hydroxylase in brain is believed to be located.[27] The failure of increased or prolonged doses to increase enzyme inhibition or to appreciably lower serotonin levels may therefore be due to a factor limiting the buildup of inhibitor within the synaptic vesicles.

A report that soluble tryptophan hydroxylase is not significantly inhibited *in vitro* by 5-bromotryptophan (10 per cent at 10^{-3} M)[28] contrasts markedly with data obtained using the particle-bound enzyme which was moderately inhibited (45 per cent at 10^{-4} M).[2] In addition, *p*-chlorophenylalanine showed different inhibitory properties *in vitro* toward the soluble and bound enzyme.[25] The possibility that two pools of tryptophan hydroxylase exist in brain, one inhibited by 6-fluorotryptophan and one not affected, cannot therefore be excluded. Such a condition could also explain the inhibition pattern noted here.

In brain uptake of inhibitor, inhibition of tryptophan hydroxylase and depletion of serotonin occurred more or less simultaneously. However, in other tissues there was a significant delay after tissue concentrations of inhibitor reached a maximum before serotonin levels fell to their lowest levels. These data are in agreement with reports that the turnover rate of serotonin in several tissues is much less than that in brain.[29,30] Brain serotonin with a half-life of around 30 min is rapidly depleted, whereas intestinal serotonin with a half-life of 6–8 hr is only slowly depleted.

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