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SHORT COMMUNICATIONS

Inhibition of brain tryptophan-5-hydroxylase by amino acids—The role of L-tryptophan uptake inhibition*

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PARA-CHLOROPHENYLALANINE (PCPA), which has been used both clinically and in research as a serotonin depleter,[1] is not specific in its action. Some inhibition of catecholamine biosynthesis occurs both *in vivo*[2] and *in vitro*.[3] Undesirable side effects of PCPA administration to both patients and volunteer subjects[1] have been reported which are not necessarily connected with its anti-serotonin action. The discovery of alternative specific serotonin depleters is therefore of importance both in the study of brain functions and in the possible clinical treatment of various types of serotonin-producing tumors.

Many substituted amino acids are known to inhibit tryptophan-5-hydroxylase,[3–6] and several of these lower brain and peripheral serotonin levels *in vivo*.[4,7,8] Few serotonin inhibitors are known which are not analogs of naturally occurring amino acids. Tryptophan-5-hydroxylase is believed to be present in brain largely in a bound form within the nerve endings which in tissue homogenates can be isolated as synaptosomes.[9] Since an active transport mechanism appears to be involved in the entry of amino acids into the synaptosomes,[10] it is possible that the amino acid analogs used in these studies compete with tryptophan for uptake into the synaptosomes, thereby inhibiting serotonin synthesis. Serotonin biosynthesis in brain is partly controlled by available tryptophan; diets deficient in tryptophan lead to reduced brain serotonin synthesis,[11,12] whereas tryptophan diet supplements increase brain serotonin levels.[13] Serotonin turnover *in vivo* could therefore possibly be inhibited by two separate mechanisms, uptake inhibition followed by enzyme inhibition, both contributing to the overall serotonin depletion.

In much of the documented work on tryptophan-5-hydroxylase, an unpurified particle-bound form from brainstem has been studied.[3,5,7,8,14] Other work using a solubilized enzyme preparation from mast cells has produced some conflicting data on inhibition by substituted amino acids. Several compounds which were moderately good inhibitors of the bound enzyme[3] had little or no effect on the soluble enzyme.[6] The difference may be due to uptake inhibition of the serotonin precursor into the synaptosomes. If uptake inhibition is a significant contributor to overall serotonin depletion, assays carried out on the solubilized enzyme might give inaccurate data on the usefulness of a new amino acid inhibitor. It is therefore of importance to investigate the relative effects of enzyme inhibition and uptake inhibition on the control of serotonin biosynthesis. The effect of several substituted tryptophans and phenylalanines known to inhibit particle-bound tryptophan hydroxylase on the uptake of tryptophan into a brain synaptosome fraction was therefore investigated. These data were then compared with the inhibition *in vitro* of both soluble and bound tryptophan-5-hydroxylase by these compounds in order to discover the relative importance of transport inhibition and enzyme inhibition in the actions of amino acid inhibitors on serotonin biosynthesis *in vivo*.

Male Wistar rats of 200–250 g weight were used in these studies. The animals were killed by decapitation and the brain stems quickly dissected out and used as a source of soluble tryptophan hydroxylase,[15] bound tryptophan hydroxylase, and a synaptosome-containing fraction. Bound tryptophan hydroxylase was assayed[16] in a particulate fraction corresponding to fraction P2 of Gray and Whittaker.[9] The same synaptosome-containing fraction was used for the uptake experiments using the procedure of Grahame-Smith and Parfitt.[10] Inhibition *in vitro* of soluble and bound tryptophan hydroxylase was studied at two or three substrate concentrations between 10^{-5} and 2×10^{-4} M and at inhibitor concentrations of from 10^{-6} to 10^{-3} M. Lineweaver–Burk plots[17] were used to calculate K_i values for the most active inhibitors. Tryptophan uptake by the synaptosome fraction was studied by the method of Grahame-Smith and Parfitt.[10] Inhibitor concentrations of 2×10^{-6} to 10^{-3} M were used at three substrate concentrations between 10^{-5} and 2×10^{-4} M.

Inhibition of tryptophan hydroxylation and tryptophan uptake were calculated as the molar concentration of inhibitor that gave 50 per cent inhibition. The results are given in Table 1. Almost all

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TABLE 1. INHIBITION OF TRYPTOPHAN-5-HYDROXYLASE BY SUBSTITUTED AMINO ACIDS

	Soluble tryptophan-5-hydroxylase*		Bound tryptophan-5-hydroxylase inhibition†,‡,§	L-Tryptophan-^{14}C uptake inhibition†,§,‖
	Inhibition†,	K_i		
DL-5-Fluorotryptophan	$>10^{-2}$		$1{\cdot}0 \times 10^{-4}$	$9{\cdot}5 \pm 0{\cdot}6 \times 10^{-5}$
DL-5-Methyltryptophan	$>10^{-2}$		$3{\cdot}2 \times 10^{-4}$	$4{\cdot}5 \pm 0{\cdot}1 \times 10^{-5}$
DL-5-Chlorotryptophan	$>10^{-2}$		$1{\cdot}4 \times 10^{-4}$	$5{\cdot}5 \pm 0{\cdot}2 \times 10^{-5}$
DL-5-Bromotryptophan	$>10^{-2}$		$1{\cdot}4 \times 10^{-4}$	$2{\cdot}0 \pm 0{\cdot}1 \times 10^{-5}$
DL-6-Fluorotryptophan	$2{\cdot}0 \times 10^{-4}$	$2{\cdot}0 \times 10^{-4}$	$1{\cdot}2 \times 10^{-5}$	$1{\cdot}2 \pm 0{\cdot}2 \times 10^{-4}$
DL-6-Methyltryptophan	10^{-3}	$4{\cdot}8 \times 10^{-3}$	$8{\cdot}0 \times 10^{-5}$	$4{\cdot}8 \pm 1{\cdot}1 \times 10^{-4}$
DL-6-Chlorotryptophan	$2{\cdot}3 \times 10^{-4}$	$3{\cdot}2 \times 10^{-4}$	$2{\cdot}0 \times 10^{-5}$	$2{\cdot}5 \pm 0{\cdot}2 \times 10^{-4}$
DL-4-Chlorophenylalanine	$2{\cdot}7 \times 10^{-4}$	$3{\cdot}2 \times 10^{-4}$**	$2{\cdot}5 \times 10^{-5}$	$9{\cdot}0 \pm 0{\cdot}3 \times 10^{-5}$
DL-4-Fluorophenylalanine	$2{\cdot}0 \times 10^{-4}$		$1{\cdot}5 \times 10^{-5}$	$2{\cdot}2 \pm 0{\cdot}2 \times 10^{-4}$
DL-4-NO_2-phenylalanine	$3{\cdot}0 \times 10^{-3}$		$1{\cdot}0 \times 10^{-5}$	$2{\cdot}1 \pm 0{\cdot}4 \times 10^{-4}$
D-Phenylalanine	$>10^{-2}$		$1{\cdot}0 \times 10^{-4}$	$5{\cdot}0 \pm 0{\cdot}1 \times 10^{-5}$
L-Phenylalanine	$8{\cdot}0 \times 10^{-4}$		$3{\cdot}5 \times 10^{-5}$	$9{\cdot}0 \pm 0{\cdot}5 \times 10^{-4}$

* Control value: 0·20 ± 0·02 nmole tryptophan hydroxylated/mg of protein/hr.
† Molar concentration for 50 per cent inhibition.
‡ Control value: 0·040 ± 0·003 nmole tryptophan hydroxylated/mg of protein/hr.
§ Substrate concentration, 10^{-5} M.
‖ Control value: 4·40 ± 0·3 nmole/mg protein.
¶ Substrate concentration, 10^{-4} M.
** Jequier *et al.*[18] report 3×10^{-4} M.

the amino acids studied inhibit the bound enzyme appreciably, whereas only a few were active against the soluble enzyme. The 5-substituted tryptophans were completely inactive against soluble tryptophan hydroxylase, but moderately inhibited the particle-bound enzyme. Since this group were the most potent competitive inhibitors of tryptophan uptake, it is suggested that their action on the bound enzyme consists predominantly of uptake inhibition. 5-Bromotryptophan was not effective in lowering brain serotonin content *in vivo*.[19] The 6-halo tryptophans and 4-halo phenylalanines, several of which are known to lower brain serotonin content significantly[8,20] acted primarily as enzyme inhibitors. Each was a potent inhibitor of tryptophan-5-hydroxylase, but none were effective uptake inhibitors. Uptake inhibition alone does not appear to lower brain serotonin synthesis, appreciably since 5-bromotryptophan, previously shown to be inactive as a serotonin depleter *in vivo*,[19] was the most potent uptake inhibitor. No compound was discovered which was a potent inhibitor of both tryptophan uptake and tryptophan hydroxylation. However, the discovery of such a compound might still provide the best possibility of an effective replacement for *p*-chlorophenylalanine.

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Increased plasma enzyme activity induced by 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A)

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Considerable interest has been focused on the effects of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) as an inhibitor of drug metabolism[1] and thereby as a modifier of chemically induced hepatotoxicity. Pretreatment of rats with SKF 525-A protects against the hepatotoxicity of carbon tetrachloride assessed histologically[2] and by measurement of serum enzyme activity and liver triglycerides.[3] Although this effect has been attributed to the inhibitory action of SKF 525-A on microsomal enzymes, the responsible mechanism has not been established.[3] During the course of studies in this laboratory on the toxicity of halogenated hydrocarbons, treatment with SKF 525-A *per se* was found to produce significant elevations of plasma levels of glutamic oxaloacetic transaminase (GOT), resembling that observed in hepatic cell necrosis. Marchand *et al.*[3] recently noted elevation of serum glutamic pyruvic transaminase (GPT) in rats treated with SKF 525-A. The present report extends these observations and indicates that the pattern of increased plasma enzyme activity induced by SKF 525-A is consistent with altered permeability of muscle and, to a lesser extent, hepatocyte membranes, resulting in leakage of intracellular enzymes.

Female, Sprague–Dawley rats weighing 180–200 g were fasted but allowed access to water for 2–4 hr prior to and after the intraperitoneal (i.p.) injection of isotonic saline or SKF 525-A, dissolved in isotonic saline (pH of injected solutions ranged between 4·4 and 5·6). Doses of SKF 525-A administered are indicated in Table 1. One hr after injection, the rats were sacrificed by bleeding from the abdominal aorta. Plasma was assayed for GOT, GPT[4] and creatine phosphokinase (CPK)[5] by standard spectrophotometric methods. Liver and skeletal and cardiac muscle tissue fixed in buffered formalin was stained with hematoxylin and eosin for light microscopic examination. As shown in Table 1, elevated levels of plasma GOT and CPK were noted in rats treated with as little as 5 mg/kg of SKF 525-A, and higher enzyme levels were found with increasing dosage. Increased GPT activity was observed at the highest dose employed, 100 mg/kg.

In a second series of experiments, SKF 525-A was administered in a fixed dose of either 100 or 40 mg/kg by i.p. injection, and groups of rats were sacrificed at the intervals indicated in Tables 2 and 3 respectively. Peak plasma enzyme activity was observed at 1 hr after the larger dose and at approximately 8 hr after the lower dose. Plasma GPT and CPK levels had returned to normal at 24 hr after the 100 mg/kg dose and at 48 hr after the 40 mg/kg dose. GOT was still moderately elevated at 48 hr after the i.p. injection at each dose level. Light microscopic examination of sections of liver and cardiac and skeletal muscle, obtained in both sets of experiments, failed to show evidence of hepatic or muscle necrosis or inflammation. Liver triglycerides, measured by standard chemical techniques,[6] were similar in control and SKF 525-A-treated animals (100 mg/kg) at 24 and 48 hr after i.p. injection.