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STUDIES OF BRAIN AMINOTRANSFERASE AND PSYCHOTROPIC AGENTS—I

INTERACTION OF β-β′-IMINODIPROPIONITRILE AND RAT BRAIN AROMATIC AMINOTRANSFERASES

SABIT GABAY and KUEI-HUANG HUANG*

Biochemical Research Laboratory, Veterans Administration Hospital, Brockton, Mass. and Department of Biochemistry, Boston University School of Medicine, Boston, Mass., U.S.A.

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Abstract—Rat cerebral phenyl (tyrosine and phenylalanine) and indolyl (tryptophan and 5-hydroxytryptophan) aminotransferases used in this study were separated and partially purified from acetone precipitation extracts on hydroxyapatite chromatography and Sephadex G-200 gel filtration columns eluted with phosphate buffers of increasing molarity. The substrate specificity and kinetics of these aromatic aminotransferases have been determined. The effect of various concentrations of pyridoxal phosphate on both the enzymic activity and the stability were investigated. The administration *in vivo* of β-β′-iminodipropionitrile (IDPN) was found to markedly decrease the specific activity of L-5-hydroxytryptophan:2-oxoglutarate aminotransferase, whereas phenylalanine, tyrosine and tryptophan aminotransferases remained unaltered. Studies *in vitro* showed that IDPN inhibits the aminotransferases in the following order—5-HTP-T ⩾ Tyr-T > Phe-T ⩾ Tryp-T. On the basis of kinetic studies, IDPN was found to inhibit the Tyr-T and 5-HTP-T in competition with its substrate, the K_i for these reactions being $4{\cdot}3 \times 10^{-3}$M (Tyr at pH 8·35) and $3{\cdot}8 \times 10^{-3}$M (5-HTP at pH 8·30). The possibility of IDPN competing with the amino group acceptors (2-oxoglutarate or oxalacetate) or with its coenzyme, pyridoxal phosphate, has been eliminated. In addition, it was shown that IDPN produced a greater inhibitory effect on these aminotransferases than a structurally similar compound, β-aminopropionitrile (BAPN). It is suggested therefore that this observation may account for their dissimilar physiological role; IDPN is a psychotropic agent, whereas BAPN is an osteolathyrogen.

THE PRESENT study is part of a series of investigations concerning the interactions of brain aminotransferases with certain psychotropic and neurologic agents. The choice of beginning with β-β′-iminodipropionitrile† (IDPN) and aromatic aminotransferases‡ was dictated by the available information and experience on this compound and enzyme(s) both in terms of a postulated metabolic pathway in IDPN[1] and the purification and substrate specificity of rat brain aromatic aminotransferases.[2]

* Fellow of the China Institute of America, New York, N.Y. Present address: University of Illinois, Department of Microbiology, Urbana, Ill. 61803.

† β-β′-Iminodipropionitrile (IDPN), *bis*-β-aminopropionitrile and amino-β-β′-dipropionitrile, with a chemical formula on NH = $(CH_2.\ CH_2.\ CN)_2$, are synonymous.

‡ Recommended trivial names by the IUB Enzyme Commission. The systematic name and number, if known, will be incorporated in the text followed by a parenthetically defined abbreviation to be used throughout; thus, an abbreviation followed by "-T" signifies aminotransferase. Abbreviations used are: Phe (phenylalanine), Tyr (tyrosine), Tryp (tryptophan), 5-HTP (5-hydroxytryptophan).

IDPN induces a psychiatric syndrome of a maniacal agitation and neurological disorders.[3–6] It was referred to as an "ECC-syndrome," that is, excitement, chorea and circling.[7] In the absence of gross neurologic lesions,[8] Gabay reported that the psychomotor manifestations of animals in which the ECC-syndrome is induced may represent a useful "functional model" to elucidate neurobiochemical alterations at the molecular level.[9]

The investigations to be reported herein deal with the study of brain aromatic aminotransferases for the following reasons: (1) The abundance of glutamic acid in the nervous system is unique, since approximately 75 per cent of the free amino acids present in the nerve centers are represented by glutamic acid and the "glutamate group" (glutamine, γ-aminobutyric acid, aspartic acid). (2) Many of the major peculiarities concerning the aminotransferases can be inferred from a close analysis of the glutamic acid metabolism in brain. (3) Glutamic acid can be considered as a point of departure as well as arrival, since its enzymic transformation by aminotransferases is reversible. (4) IDPN has been shown to be metabolized through monoamine oxidase to cyanoacetic acid. Although this postulated metabolic pathway, for which evidence has been reported,[1] proceeds through an oxidative deamination, it is also conceivable that the amino group is transferred to a number of already present keto acids which could serve as acceptors. (5) The metabolism of aromatic amino acids in the brain is of great interest in view of their role as precursors of biologically active catecholamines and indole amines.

MATERIALS AND METHODS

Reagents

All reagents were prepared in doubly glass-distilled water or in appropriate buffer and tested as stated below.

Aminonitriles. IDPN, generously supplied as IDPN-succinate by Dr. W. E. Bruce of Wyeth Research Laboratories, Radnor, Pa., was prepared as follows. To 188 g of freshly distilled iminodipropionitrile, boiling from 195 to 200° at 15 min, in 500 ml isopropanol was added 90 g succinic acid and the mixture was warmed on a steam bath until solution was complete. Upon cooling this solution to 45–50° and scratching it with a stirring rod, a white crystalline solid separated, together with a small amount of oil. The product, filtered, pressed down well, and washed with isopropanol, melted at 69–70° and weighed 182 g. It was recrystallized from isopropanol when it melted at 77–78° and weighed 160 g.

Anal. Calc. for $C_{10}H_{15}N_3O_4$, the acid salt $(CNCH_2CH_2)_2$ NH.$HOOCCH_2CH_2$-COOH: C, 49·78; H, 6·27; N, 17·42. Found: C, 50·05; H, 6·03; N, 17·49. Parenthetically, it was noted that the tartrate failed to crystallize, but the acetate and benzoate did crystallize, although not as promptly as the succinate. β-Aminopropionitrile (BAPN), a fumarate salt, was a gift from Abbott Laboratories, North Chicago, Ill.

Miscellaneous. Phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid were obtained from Sigma Chemical Co., St. Louis, Mo., and Sephadex G-200 was obtained from Pharmacia Fine Chemicals, New Market, N.J. Hydroxylapatite was prepared according to the method of Tiselius *et al.*[10] as described by Levin.[11] In some experiments, commercial hydroxylapatite from Bio-Rad Laboratories, Richmond, Calif. was used. All remaining compounds used were of the highest purity commercially obtainable. The amino acids used, with the exception of DL-5-HTP, were all of the L-configuration.

IDPN administration in vivo

Female rats weighing 150 ± 10 g (Carworth Farms, Inc., New City, N.Y.) were subcutaneously injected with freshly neutralized IDPN solutions (300 mg of the base/kg body weight). The optimum dose of IDPN was determined after numerous toxicity studies. With this dose and by this route of administration, the ECC-syndrome never fails to appear on the seventh day. Assessment of the animal reaction was made by a swimming test (*cf.* reference 9). Control animals were maintained under identical conditions, but received injections of pyrogen-free water only. In all of the experiments *in vivo*, brains were removed in a standard, reproducible way and the tissues were washed *in situ* with the appropriate cold buffer. Brains were quickly dried by touching them to a clean piece of filter paper and were weighed on a Roller–Smith balance, transferred to a Potter–Elvehjem glass homogenizer in ice, and homogenized with a motor-driven Teflon pestle for 2 min in a small volume of 0·02 M phosphate buffer, pH 7·4, kept at 4°. The volume was then adjusted to 20% (w/v) homogenate and centrifuged for 40 min at 70,000 g_{max} (Spinco L-2). The supernatant thus obtained was designated as "brain extract". The "partially purified" enzyme preparations were obtained when "brain extract" was subjected to acetone precipitation. The fraction precipitating between 25 and 60% acetone was collected by centrifugation and dissolved in a small volume of phosphate buffer. The supernatant obtained by centrifugation (30 min at 100,000 g_{max}) represented a partially purified and stable enzymic preparation and it was used throughout.

Partial purification of the enzymes used in studies in vitro

Aromatic aminotransferases were prepared by a modification of the procedure of Tangen *et al.*[12]

Step 1. *Preparation of cell-free extract.* Rat cerebra, excised immediately after decapitation, were placed in a precooled Potter–Elvehjem tube and homogenized for 2 min with 4 vol. of cold 0·02 M potassium phosphate buffer (pH 7·4). The homogenate was centrifuged for 40 min at 70,000 g_{max}.

Step 2. *Preparation of acetone powder.* The clear supernatant solution was brought to 25% of volume (4°) by gradual addition of cold acetone (− 15°); after standing for 10 min, the precipitate was removed by centrifugation. The acetone concentration of the supernatant solution was then increased by adding cold acetone to 60% volume; after standing for 10 min, the precipitate was collected by centrifugation and the supernatant was discarded. The precipitate was dissolved in 2 ml of 0·02 M potassium phosphate buffer (pH 7·4). After clarification of this solution by centrifugation, cysteine, α-ketoglutarate and pyridoxal phosphate were added to give final concentrations of 0·02, 0·01 and 0·01% respectively; acetone was added to 60% by volume. After standing for 10 min, the precipitate was collected by centrifugation and dissolved in an amount of 0·05 M potassium phosphate buffer (pH 7·0) equivalent to one-seventh of the volume of the original brain extract. The solution was then clarified by centrifugation for 30 min at 100,000 g_{max}.

Step 3. *Calcium phosphate column.* The clear supernatant was then added to the top of a calcium phosphate column (7 × 1·9 cm) previously equilibrated with 0·05 M potassium phosphate buffer (pH 7·0). Most preferable conditions were obtained by using 10 mg protein/ml of column volume. The protein was eluted stepwise with 80-ml portions of the following solutions: 0·05 M, 0·08 M, 0·15 M and 0·20 M potassium

phosphate buffers (pH 7·0). Fractions of approximately 5 ml were collected. The indolyl aminotransferases (Tryp-T and 5-HTP-T) were eluted with the 0·08 M buffer, whereas the phenyl aminotransferases (Tyr-T and Phe-T) were eluted with 0·2 M buffer. Fractions containing the respective enzymes were pooled and concentrated to nearly 5-fold in an ultrafiltration cell equipped with a membrane having a 40,000 mol. wt. solute cutoff.

Step 4. *Sephadex G-200 column.* The concentrated enzyme solutions obtained from step 3 were dissolved in a minimum amount of doubly glass-distilled cold water and added to the top of a Sephadex G-200 column (9 × 0·95 cm) previously equilibrated with 0·05 M potassium phosphate buffer (pH 7·0). This column was eluted with the same buffer and fractions of approximately 2 ml were collected. The tubes containing most of the activity were pooled, concentrated as above, and frozen.

Determination of enzyme activity

During the course of purification and to carry out kinetic studies, enzyme activity was followed by determining the rate of formation of the enol tautomers of the aromatic keto acids from the aromatic amino acids essentially as described by Fonnum *et al.*[13] The reaction mixtures contained phenyl- or indolylamino acid (11·40 μmoles), α-keto acid (α-ketoglutarate for phenylamino acids and oxaloacetate for indolylamino acids; 13 μmoles), pyridoxal phosphate (160 μg), sodium borate buffer (325 μmoles, pH 8·9, for Tyr and 650 μmoles, pH 8·1, for other amino acids), and enzyme in a final volume of 1·3 ml. The reaction was started by the addition of the α-keto acid after a 5-min, 37° preincubation. When inhibitors were used, a 15-min, 37° preincubation took place prior to starting the reaction. To make sure that difficulties on this score were avoided, each rate measurement was made with two different levels of enzyme. The course of the reaction was followed by measuring the increase in absorbancy at either 320 mμ (phenylamino acids) or 328 mμ (indolylamino acids) in a Beckman DU spectrophotometer equipped with thermospacers and maintained at 37° with a Haake water-circulating pump. The absorbancy changes were measured at 5-min intervals for 1 hr during which time the reaction was always linear. Controls (zero time and without α-keto acid) were included in each set of determinations. Protein was determined by the method of Lowry *et al.*[14] with crystalline bovine serum albumin as standard. A unit of enzyme activity is defined as that amount of enzyme which catalyzes the transamination of 1 μmole substrate/hr under the above conditions. Specific activity is defined as the units of enzyme/mg protein.

Determination of molar extinction coefficient

Molar extinction coefficients of aromatic α-keto $\rightleftarrows$ enol acid equilibrium mixtures were determined under the above conditions with the exception that the normal substrate (aromatic amino acid plus its aliphatic α-keto acid) was replaced by the corresponding aromatic α-keto acid and pyridoxal phosphate was omitted from the reaction mixture.

RESULTS

Effect of administration in vivo *of IDPN*

The data summarized in Table 1 show that 5-HTP-T (5-hydroxytryptophan: oxaloacetate aminotransferase) was markedly inhibited by subcutaneous injections of

TABLE 1. EFFECT OF IDPN ON ACTIVITY OF RAT BRAIN AROMATIC AMINOTRANSFERASES*

Enzyme	Activity expressed as Normal	Δo.d./mg protein/hr† IDPN-treated
I. "Brain extract"		
Tyr-T‡	0·301 ± 0·028 (4)	0·304 ± 0·018 (5)
Phe-T‡	0·055 ± 0·020 (3)	0·055 ± 0·003 (3)
Tryp-T§	0·274 ± 0·026 (4)	0·279 ± 0·014 (5)
5-HTP-T§	0·268 ± 0·024 (5)	0·171 ± 0·010 (5)
II. "Partially purified"		
Tyr-T‡	0·577 ± 0·053 (4)	0·592 ± 0·073 (6)
Phe-T‡	0·120 ± 0·008 (4)	0·116 ± 0·028 (6)
Tryp-T§	0·922 ± 0·055 (4)	0·956 ± 0·034 (6)
5-HTP-T§	0·817 ± 0·033 (4)	0·691 ± 0·039 (6)

* For the components of the reaction mixture, see "Determination of enzyme activity" (Methods). 1·5–2 mg of "brain extract" or 0·3–0·6 mg of "purified enzyme preparation" on the basis of active protein was used in a total volume of 1·3 ml borate buffer (pH 8·1), except in the case of Tyr where buffer was used at pH 8·9. The enzyme activity is expressed as change in optical density at a wavelength 320 mμ with phenylamino acids and at 328 mμ with indolylamino acids, respectively, using a Beckman DU spectrophotometer equipped with a thermospacer at a uniform temperature of 37°.

† Mean value ± S.D.; figures in parentheses indicate number of animals used in each experiment.

‡ α-Ketoglutarate used as amino acceptor.

§ Oxaloacetate used as amino acceptor.

IDPN. Conversely, this psychotropic agent had no effect on the aminotransferase activity when Tyr, Phe or Tryp was used as substrate.

Purification and general catalytic properties of the enzyme

The results of a typical purification sequence of the enzymes used in the kinetic studies described below are summarized in Table 2.

TABLE 2. SUMMARY OF A TYPICAL PURIFICATION OF INDOLYL AMINOTRANSFERASES AND PHENYL AMINOTRANSFERASE

Step	Fraction	Protein (mg)	Activity		Yield	Purification
			Total (units)	Specific (units/mg)		
(A) Indolyl aminotransferases*						
1.	Cell-free extract	1500	303	0·202	(100)	(1·0)
2.	Acetone powder	333	188	0·563	62	2·8
3.	$Ca_3(PO_4)_2$ eluate	16·5	64	3·88	21	19·2
(B) Phenyl aminotransferase†						
1.	Cell-free extract	5600	151	0·027	(100)	(1·0)
2.	Acetone powder	338	68	0·202	45	7·5
3.	$Ca_3(PO_4)_2$ eluate	42·2	20	0·474	13·3	17·6
4.	Sephadex G-200 eluate	3·5	2·0	0·560	1·3	20·7

* Fractions emerging at 0·08 M buffer (see Methods).

† Fractions emerging at 0·2 M buffer (see Methods).

The time course of α-keto acid formation was linear for at least 60 min after an initial lag of 10 min for the phenyl and 20 min for the indolyl aminotransferase reactions under the conditions of assay described in Methods. Maximal activity was observed in the pH range of 8–9 for both enzymes. The K_m values for Phe, Tryp and 5-HTP were, respectively, 4×10^{-1}M, $1{\cdot}2 \times 10^{-1}$ M and $8{\cdot}5 \times 10^{-3}$ M) (Table 3).

TABLE 3. KINETIC DATA FOR RAT BRAIN AROMATIC AMINOTRANSFERASES

Enzyme*	V_{max}†	K_m‡
Phe-T	$5{\cdot}0 \times 10^{-7}$	$4{\cdot}0 \times 10^{-1}$
Trypt-T	$4{\cdot}0 \times 10^{-6}$	$1{\cdot}2 \times 10^{-1}$
5-HTP-T	$8{\cdot}5 \times 10^{-8}$	$8{\cdot}5 \times 10^{-3}$
Tyr-T		$2{\cdot}5 \times 10^{-2}$

* Enzymatic activity determined as a function of its substrate concentration with all other reactants kept constant.

† V_{max} expressed as moles of substrate "transaminated"/mg protein/hr.

‡ K_m expressed in terms of moles/liter.

The K_m of Tyr could not be graphically derived from the classical plots. This was due to the difficulty in controlling the pH of the reaction mixture of 8·9 (optimal pH for Tyr-T). Thus, the maximum concentration of Tyr (dissolved in sodium hydroxide) which could possibly be used was $1{\cdot}31 \times 10^{-2}$ M or 17·10 μmole. Furthermore, the poor buffering capacity of the borate buffer at this pH precluded studies under this condition. The calculated K_m for Tyr, from the inhibitor constant studies (see Fig. 2a), was found to be $2{\cdot}5 \times 10^{-2}$ M.

The molar extinction coefficients for equilibrium mixtures of the aromatic α-keto acid and borate were determined under the assay conditions already described (*cf.* Methods). The molar extinction coefficient of *p*-hydroxyphenylpyruvic acid at 320 mμ, pH 8·35, was 5030, whereas a value of 4880 was found at pH 8·8. The value obtained for phenylpyruvic acid at 320 mμ, pH 8·3, was 2070. In the case of indolylpyruvic acid a value of 14,000 was used at 328 mμ, pH 8·3, as reported by Lin *et al.* under similar conditions.[15] Knox and Pitt have shown that the tautomerization of *p*-hydroxyphenylpyruvic acid, in the absence of enzyme, is base catalyzed.[16] At pH 7·7, the reaction rate of the tautomerase-catalyzed reaction and that of the spontaneous reaction were the same. Since the pH of the assay system used in the present study was 8·3 or higher, neither in the enzymatic activity measurement nor in the molar extinction coefficient determinations was tautomerase used.

Cofactor effect

The activities of the enzymes toward Tyr and 5-HTP were determined as a function of added pyridoxal phosphate concentration (Fig. 1). The increase of the specific activity of the enzyme was found to run parallel to its dependence on pyridoxal phosphate. This effect could be attributed to the depletion of the cofactor from the enzyme preparation during the process of purification. It is particularly worth noting that, with the preparations possessing the highest specific activity, 5-HTP-T went through a maximum and then decreased with increasing concentrations of pyridoxal

phosphate. This effect was not immediately evident for Tyr-T (Fig. 1). Thus, 160 μg pyridoxal phosphate was used throughout the kinetic studies. Similar observations have been reported for γ-aminobutyrate-α-ketoglutarate transaminase of beef brain.[17]

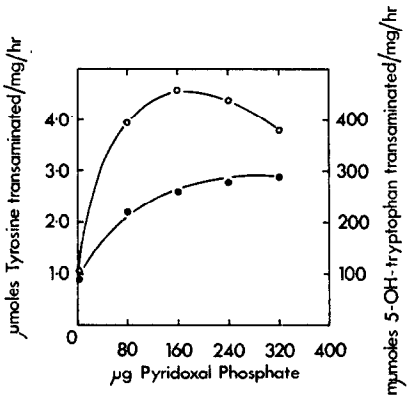

FIG. 1. Effect of pyridoxal phosphate on tyrosine and 5-hydroxytryptophan aminotransferases activity. The reaction mixture contained sodium borate buffer, 325 μmoles, pH 8·9 (Tyr), or 650 μmoles, pH 8·1 (5-HTP), 11·40 μmoles substrate, 13 μmoles α-keto acid (α-ketoglutarate for Tyr or oxaloacetate for 5-HTP), 68 or 136 μg enzyme, and pyridoxal phosphate (added always last) as indicated in a total volume of 1·3 ml. See Methods for activity measurement and its definition.

Effect in vitro *of IDPN (Kinetic studies)*

5-HTP-T was completely inhibited by 17·5 mM IDPN, whereas the degree of inhibition observed with Tyr-T (L-tyrosine: 2-oxoglutarate aminotransferase; EC 2.6.1.5.), Tryp-T (L-tryptophan:oxaloacetate aminotransferase) and Phe-T (L-phenylalanine:2-oxoglutarate aminotransferase) was 50, 18 and 12 per cent respectively. IDPN was found to be a competitive inhibitor for both Tyr and 5-HTP, with the K_i for Tyr and 5-HTP being $4{\cdot}3 \times 10^{-3}$ M and $3{\cdot}8 \times 10^{-3}$ M respectively (Fig. 2, a and b).

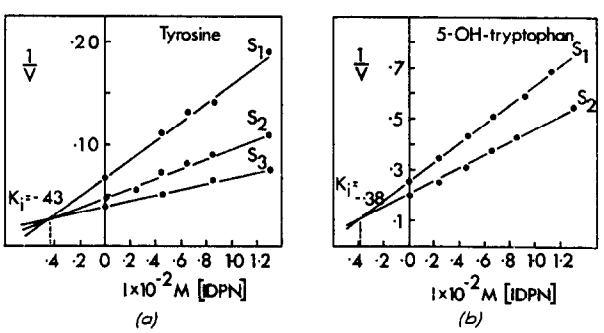

FIG. 2. Substrate-competitive inhibition of aminotransferases by IDPN. The reaction mixture and conditions, with the exception of substrate and coenzyme concentrations, were identical with those in Fig. 1.

(a) Tyrosine aminotransferase (Tyr-T): $K_i = 4{\cdot}3 \times 10^{-3}$M. Final concentrations of substrate: S_1, 5·7 μmoles; S_2, 11·40 μmoles; S_3, 17·10 μmoles. Pyridoxal phosphate, 160μg.

(b) 5-Hydroxytryptophan aminotransferase (5-HTP-T): $K_i = 3{\cdot}8 \times 10^{-3}$M. Final concentrations of substrate: S_1, 11·40 μmoles; S_2, 17·10 μmoles. Pyridoxal phosphate, 160 μg.

The inhibition *in vitro* of these aromatic aminotransferases by BAPN was similarly investigated. BAPN was less inhibitory than IDPN. The aminotransferase activity of 5-HTP-T was only partially inhibited (22 per cent) by 17·5 mM BAPN and that of Tyr-T only very slightly (7 per cent) by 13·1 mM BAPN.

DISCUSSION

Studies *in vivo* showed that in brain extracts from IDPN-treated animals no changes in the activity of aromatic aminotransferases could be detected, with the exception of 5-HTP-T which was found to be significantly inhibited. Such an inhibition of 5-HTP-T was more marked with the partially purified preparations. Parenthetically, no Dopa-T activity could be detected in the brain extract or partially purified enzyme. Since 5-HTP-T is a precursor for the biologically important 5-hydroxytryptamine (5-HT), this marked specific effect of IDPN (or of psychotropic and neurotropic agents producing a similar effect) might have a regulatory role in the 5-HT synthesis in the brain. The necessary enzyme being present in the brain, agents capable of reducing 5-HTP-T activity would influence the subsequent step (decarboxylation) in the reaction sequence. The observation that 5-HTP-T was inhibited by IDPN and that Tryp-T remained unaltered led us to conclude that the indolylaminotransferases have a different affinity for this agent. Attempts to separate Tryp-T and 5-HTP-T by other investigators have been unsuccessful.[12] However, other authors have provided evidence that Tryp-T and 5-HTP-T are two separate enzymes, since additive rates of glutamic acid formation were observed when Tryp and 5-HTP were used as substrate.[18]

On the other hand, studies *in vitro* revealed that IDPN, when used at a final concentration ($1{\cdot}7 \times 10^{-2}$ M) equimolar to the substrate concentration, inhibits the aminotransferases in the following order: 5-HTP-T $\gg$ Tyr-T $>$ Phe-T $\geqslant$ Tryp-T. It should be noted that, since IDPN was used as a succinate salt, it was found necessary to see the effect of succinate on the aminotransferases activity. Succinate at a concentration of $1{\cdot}75 \times 10^{-2}$ M was found to have no effect on the enzyme activity. In addition, no significant influence on the tautomerization of the *p*-hydroxyphenylpyruvic acid by IDPN could be observed. The inhibitor constants (K_i) for only Tyr-T and 5-HTP-T were determined, since no appreciable inhibition was observed with Phe and Tryp. On the basis of the high K_i values, one might question whether or not concentrations of the inhibitors capable of causing enzymic blockade can occur in brain *in vivo*. Our lack of knowledge as to intracellular conditions and the behaviour of inhibitors within cells precludes any adequate explanation. However, since similar high specificity has been achieved *in vivo* (see Table 1), it might be predicted that relatively lower concentrations would be needed to achieve such an effect. The greater sensitivity of Tyr-T than of Phe-T toward IDPN would cause an overproduction of phenylpyruvic acid due to the inhibition of the hydroxylation process of Tyr. Furthermore, experiments *in vitro* have firmly established that the phenylpyruvic acid and its metabolites, phenylactic acid and phenylacetic acid, strongly inhibited the decarboxylation of 3,4-dihydroxyphenylalanine,[19] 5-HTP[20] and glutamic acid,[21] which ultimately would be responsible for the lower levels of adrenaline, noradrenaline, 5-HT and γ-aminobutyric acid found in phenylpyruvic oligophrenia patients. Inhibition of Tyr-T by IDPN as observed in studies *in vitro* could produce similar accumulation of

phenylpyruvic acid and ultimately a lowering of the levels of various biologically active amines.

Finally, the observation that IDPN produced a greater inhibitory effect than BAPN on aromatic aminotransferases may provide a plausible explanation for their different physiologic action, for IDPN is a psychotropic (neurolathyrogenic) agent whereas BAPN is an osteolathyrism inducing agent.[22]

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