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PRELIMINARY COMMUNICATIONS

ON THE METABOLIC ORIGIN OF PLASMATIC INDOLE-3-ACETIC ACID IN THE RAT

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In a recent paper (1) we have reported that the human plasmatic concentration of indole-3-acetic acid (IAA) is very high (x30) as compared to that of 5-hydroxyindole-3-acetic acid (5HIAA), the latter being the main catabolite of serotonin (5-hydroxytryptamine, 5HT).

IAA has been shown to arise mainly from tryptamine (T) in the CNS of rats (2) and as human brain also contains T (3), the IAA found in human CSF (4) should have a similar origin. However, the physiopathological functions of T in the central and peripheral nervous system are not elucidated yet. Nevertheless, several lines of evidence indicate that it may play a substantial role in chemical neurotransmision, either by acting on specific tryptaminergic receptors (5) or by regulating serotonergic neurotransmision (6).The concentration of T in rat brain is about 1/100 to 1/1000 (7) of that of classical neurotransmitter amines, such as dopamine or serotonin (8) showing a relatively high turnover, although its metabolism seems to be quantitatively more important in the human CNS (2). Furthermore, little is known about the content (9) and function of T in the various body tissues analyzed (10) (including the peripheral nervous system) where 5HT is invariably more concentrated than T. Also, nothing is known about the renal clearance of IAA, though its mean urinary excretion level is very similar to that of 5HIAA (11). Taking these facts altogether, the relative high levels of IAA in human plasma would seem surprising, posing the question of its metabolic origin. IAA could -at least in theory- be formed from tryptophan (TP) either via indole-3-pyruvic acid (IPyA) through its transamination and decarboxylation or via TP decarboxylation to T and further oxidation of T. To the best of our knowledge, the only published data of the contribution of both pathways to the biosynthesis of IAA is a paper from Weissbach et al (12) in which the authors concluded -mainly from *in vitro* experiences- that most of the IAA in rodent tissues arises from IPyA. The purpose of this work is to clarify the metabolic origin of circulating IAA using *in vivo* pharmacological manipulations.

MATERIAL AND METHODS

Chemicals

Indole-3-acetic acid and Iproniazid were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Benserazide and Tranylcypromine were gifts of Productos Roche and Smith, Kline & French SAE

(Barcelona, Spain) respectively.

Sample preparation

Male adult Sprague-Dawley rats (weighing 180-220 g) were killed by decapitation between 1:00 and 3:00 p.m. and their blood rapidly taken up by exsanguination into polypropylene tubes containing EDTA in saline (5.0 g/L; 1 vol per 3 vol. of blood). The contents of the tubes were gently mixed by inversion and then centrifugated (1000 g, 10 min, 4°C) to obtain plasma samples. The supernates were carefully pipetted and treated as described (1). Briefly, 20 µL of 20 g/l ascorbic acid and 150 µL of cold 50 g/L of trichloroacetic acid (TCA) were added to plasma aliquots (100 µL). After protein precipitation and the subsequent centrifugation, always at 4°C, 50 µL aliquots of the supernates were directly injected into the HPLC system. Equivalent "spiked" series were assayed for quantification.

Enzyme inhibition

Enzymatic inhibition of L-aromatic aminoacid decarboxylase (L-AAD, E.C. 4.1.1.28) and monoamine oxidase (MAO, E.C. 1.41.3.4.) was performed as follows: Benserazide (inhibitor of L-AAD) was given i.p. at a dose of 100 mg/Kg 3 hours before sacrifice. Tranylcypromine (15 mg/Kg) and Iproniazid (100 mg/Kg) were given i.p. 4 hours before sacrifice. Control rats were injected i.p. with saline and killed 4 hours later.

HPLC analysis

Apparatus: The HPLC system consisted of a M 6000 solvent-delivery pump (Waters Assoc Inc, Milford, MA, USA) with an automatic sample injector (WISP 710B, also from Waters). The column was 30 cm long x 3.9 mm i.d. filled with µ-Bondapak C18 (Waters Assoc.). A guard column was attached between the injector and the chromatographic column.
Eluent: We used a equivolume mixture of 1-pentanesulfonic acid (PIC B-5 from Waters Assoc.), 5 mmol/L, pH 3.1 and methanol/water (7/3 by vol) at a flow rate of 1.0 mL/min.
Detection: Fluorimetric detection was performed with a Perkin Elmer 650-10S (Norwalk, CT, USA) fluorimeter, with excitation and emission wavelenghts set at 280 and 340 nm, respectively.(13).

RESULTS AND DISCUSSION

Figure 1 corresponds to a HPLC profile of the plasma of a control rat. The concentration of IAA found in rat plasma (n=5) is 191 ± 29 ng/mL ($\bar{x}$ ± S.E.M.). To the best of our knowledge, the concentration of IAA in rat plasma had only been described once before, also using HPLC coupled to fluorimetric detection (14). These authors gave values of 29 ng/ml (using ethanol for plasma deproteneization), which are far below the concentration found in this study. We have checked both deproteinizating agents (50 g/L TCA and ethanol) to see whether the use of different plasma treatments could account for the observed differences. The replicate analyses (n=5) of a pool of rat plasma gave concentrations for both methods which were not statiscally different (Student's t- test). The coefficient of variation for both of the deproteinizing treatments were 8% (TCA method) and 18% (Ethanol method). Moreover, the same authors had reported occasional levels of IAA in rat plasma of the order of 100 ng/mL (15). These differences could probably be explained on the basis of different TP content in the diets given to the animals. Figure 1B shows the percentage of control IAA values found in rats treated either with benserazide (inhibitor of L-AAD), iproniazid and tranylcypromine (both inhibitors of MAO). The percentages of the control value of plasmatic IAA found for each treatment were ($\bar{x}$ ± S.E.M.) (n=5), benserazide:31 ± 6, iproniazid:43 ± 7, tranylcypromine:27 ± 6. Thus, the mean decreases in the plasmatic IAA content produced by these treatments were 69%, 57% and 73% for benserazide, iproniazid and tranylcypromine, respectively. All decreases were found to be significative (Student's t- test). In contrast with the conclusions of Weisbach et al, these results indicate that the TP ⟶ T ⟶ IAA is the main biosynthetic

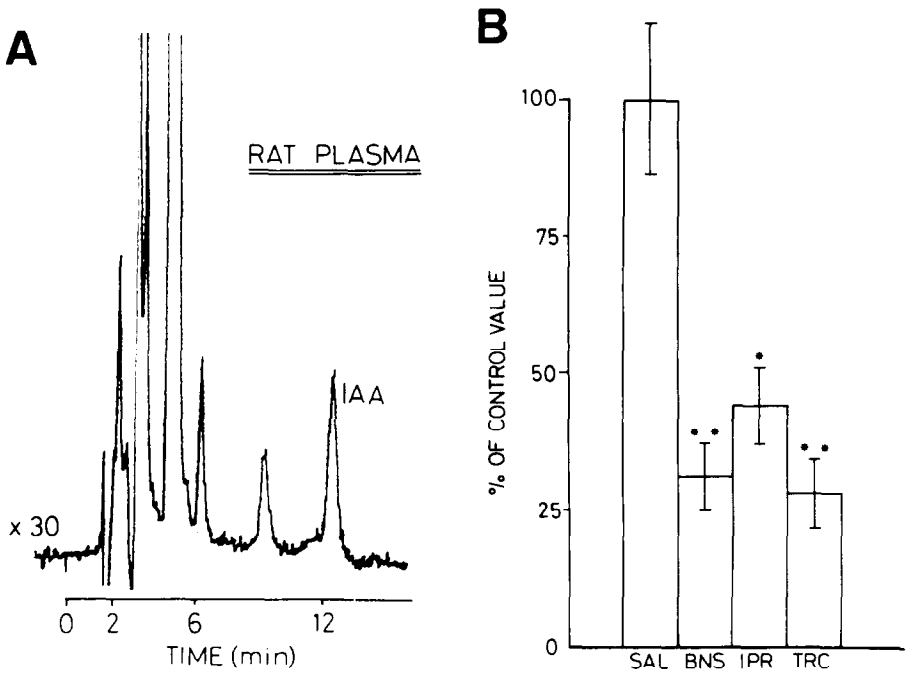

Figure 1. (A) HPLC profile of a sample of rat plasma. Fluorimetric detection at 280 and 340 nm for the excitation and emission wavelenghts, respectively. (B) Percentages of control value of plasma IAA of rats treated either with benserazide (BNS), iproniazid (IPR), tranylcypromine (TRC). Values significantly lower than control values (SAL), as determined by the Student's t- test are indicated as: * $P < 0.05$; ** $P < 0.005$. n=5 for each treatment. Vertical bars represent S.E.M.

pathway of IAA in rat tissues.

Although the use of iproniazid by these authors (12) also led to a decrease of the synthesis of IAA, they concluded instead that this drug might show some kind of inhibitory properties of TP-aminotransferase. In view of the results shown herein, it would certainly be unreasonable to assume that benserazide, as well as tranylcypromine, could also act as inhibitors at the TP-transaminating step.

In support of our observations, Stanley et al (16) have found that the rat liver TP-aminotransferase activity is very low (6% of that of tyrosine-aminotransferase) and that it is inhibited to a 34% of the original value by an antiserum to tyrosine-aminotransferase, indicating that TP-aminotransferase activity is mainly due to unespecificity of Tyr-aminotransferase. Overall, these results provide further evidence against the possibility that transamination of TP could account to a large extent for the high levels of IAA found in rat and human plasma.

The full confirmation of our results would require evaluating the extent in which the TP ⟶ IPyA ⟶ IAA pathway contributes to the plasma concentration of IAA in normal as well as in pharmacologically altered conditions. However, in a literature survey we have not found analytical methods suitable for the analysis of IPyA in mammalian tissues and biological fluids.

Thus, since it has been proven that inhibition of the enzymes involved in the IAA synthesis via T decreases substantially the levels of plasma IAA, it appears quite reasonable to as-

sume -as a preliminary conclusion- that most of the IAA found in the rat plasma arises from tryptamine.

The concentrations of T and IAA in adult rat brain are both very low (< 0,5 ng/g and 13.1 ng/g, respectively) (7), thus placing T in the class of the "trace amines". However, the relative higher levels of its IAA metabolite in human (269 ng/ml) (1) and rat (191 ng/mL) (this work) plasma indicate that, -whatever the functions of T in mammalian tissues -its peripheral metabolism is quantitatively more important than in CNS. Work is progress to develop a suitable method for the determination of IPyA in order to determine the exact relative contributions of both metabolic pathways to the biosynthesis of IAA.

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