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Differential Uptake, Metabolism and Behavioral Effects of the D and L Isomers of 5-Hydroxytryptophan[1]

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PENN, P. E., W. J. MCBRIDE, J. N. HINGTGEN AND M. H. APRISON. *Differential uptake, metabolism and behavioral effects of the D and L isomers of 5-hydroxytryptophan.* PHARMAC. BIOCHEM. BEHAV. 7(6) 515–518, 1977. – The relative contribution of the D and L isomers of 5-hydroxytryptophan (5–HTP) to the uptake and metabolism of 5–HTP and their associated behavioral effects were investigated. For the metabolic study, an injection of 25 mg/kg of D or L–5–HTP was administered IP and the rats killed 15, 30, 45 or 60 min later. Endogenous levels of 5–HTP, serotonin (5–HT) and 5-hydroxyindoleacetic acid (5–HIAA) were measured in the telencephalon following D– or L–5–HTP administration. Levels of 5–HTP, 5–HT and 5–HIAA were also measured in the brain stem (including diencephalon) following L–5–HTP administration. In the behavioral study, the effects of IP injections of D–5–HTP, L–5–HTP and D,L–5–HTP upon operant responding on a VI 1 schedule were investigated. Compared to vehicle controls, L–5–HTP significantly increased the levels of 5–HTP, 5–HT and 5–HIAA in the telencephalon and brain stem at all time points investigated. Behaviorally, 25 mg/kg of L–5–HTP and 50 mg/kg of D,L–5–HTP produced similar changes. Following the injection of either compound there was a large decrease in response rate with a duration of about 1 hr which paralleled the neurochemical changes. Injections of D–5–HTP produced an increase in the levels of 5–HTP and 5–HIAA in the telencephalon at 15 min but no change in the level of 5–HT was observed. In the operant situation, following D–5–HTP injections, a brief decrease in responding occurred in some animals which did not correlate with the neurochemical data. It was concluded that the L isomer is mainly responsible for the neurochemical and behavioral effects seen when D,L–5–HTP is administered.

D–5–Hydroxytryptophan L–5–Hydroxytryptophan Behavior Metabolism Serotonin

THERE have been numerous reports demonstrating that injections of D,L–5–HTP produce an increase in brain levels of serotonin [9–12, 14, 19]. Studies from our laboratory have indicated that injections of D,L–5–HTP into rats and pigeons increased the levels of 5–HT in the CNS and that this increase in 5–HT was temporally correlated with certain behavioral changes [3–8]. In all the biochemical and behavioral studies, D,L–5–HTP was used because it was the only form of 5–HTP commercially available at that time. Recently, both the D and L forms of 5–HTP (non-radioactive) have become available. Therefore, we undertook studies to determine the relative contributions of the D and L forms of 5–HTP (a) to the formation of 5–HT in the CNS and (b) to the behavioral effects observed when the racemic mixture of 5–HTP is injected.

METHOD

Neurochemical

Adult male Wistar rats (250–300 g) on food and water ad lib were handled and adapted to the apparatus used for killing. D– or L–5–HTP (25 mg/kg; Sigma) was injected intraperitoneally (IP) 15, 30, 45, or 60 min prior to killing by the near-freezing method [18]. A saline control group received injections 15 min prior to killing. Five rats were used for each time point for each compound injected. The telencephalon and the brain stem (including diencephalon) were immediately dissected from the brain at –4°C; the tissue was frozen in liquid nitrogen and then stored at –70°C until extraction and assay. The brain parts were individually pulverized in liquid N_2 and the compounds of interest were extracted with formic acid/acetone and assayed as previously described [17].

Behavioral

Six adult, male Wistar rats were trained to bar press for sweetened condensed milk reward on a Variable Interval 1 (VI 1) schedule as previously described [13]. The animals were also adapted to saline injections until no effect upon responding was found following injections. After establishment of a stable baseline rate of responding during daily VI

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sessions (30 min duration), injections were made and response rates were monitored until responding returned to baseline levels. Half of the rats were first injected IP with 25 mg/kg D–5–HTP while the other half received L–5–HTP in a counterbalanced design. Injections of D,L–5–HTP (50 mg/kg) were also tested in these animals. At least two weeks elapsed between injections.

RESULTS

Compared to saline controls, IP injections of L–5–HTP (25 mg/kg) significantly ($p<0.05$) increased the levels of 5–HTP, 5–HT and 5–HIAA in both the telencephalon (Fig. 1) and brain stem (Fig. 2) at all time points measured. However, by 60 min the levels of 5–HT are almost back to normal.

Injections of D–5–HTP (25 mg/kg) caused a transient increase in the levels of 5–HTP and 5–HIAA in the telencephalon 15 min later (Fig. 3). The levels of 5–HT were not changed following D–5–HTP injection at any of the time points measured.

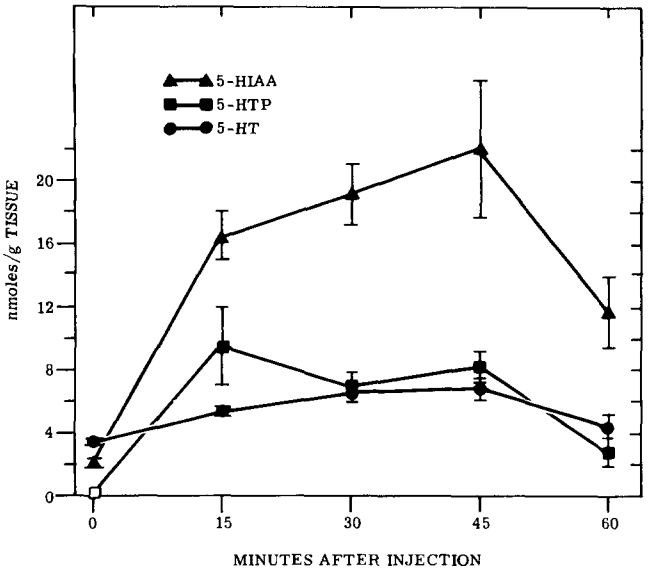

FIG. 1. Levels of 5–HTP, 5–HT and 5–HIAA in the telencephalon following an IP injection of 25 mg/kg L–5–HTP.

The effect of injections of D–5–HTP, L–5–HTP and D,L–5–HTP upon operant responding of rats on a VI 1 schedule were determined (Fig. 4; Table 1). The effects of L–5–HTP and D,L–5–HTP on the duration and response rates were very similar (Fig. 4; Table 1). Within 10 min of administration, L–5–HTP (25 mg/kg) produced a large mean decrease in responding (17% of control) which lasted about 1 hr. In contrast, injections of D–5–HTP (25 mg/kg) produced a rapid decrease in responding (41% of control) in 4 of 6 rats tested but within 10 min the response rate returned to normal levels. Two rats did not show any behavioral alterations after an injection of 25 mg/kg D–5–HTP.

DISCUSSION

The data indicate that L–5–HTP given IP in the dose tested is taken up into the telencephalon and brain stem

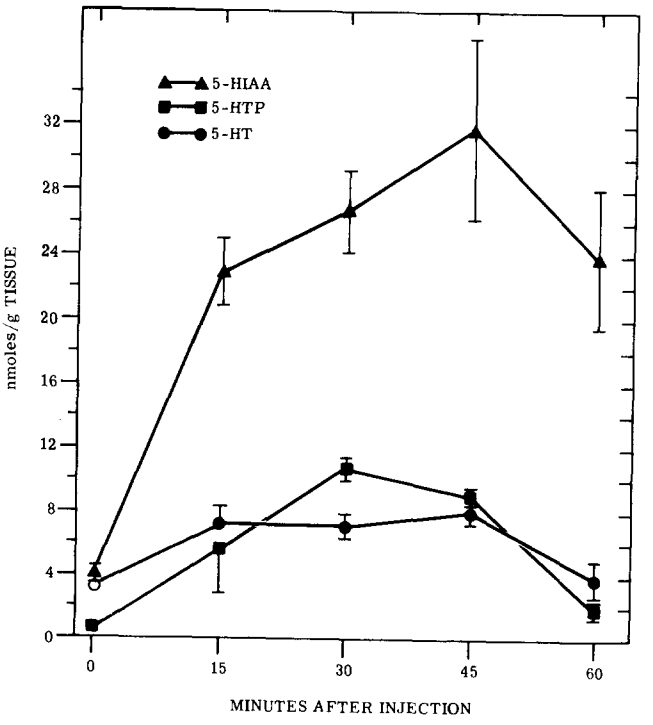

FIG. 2. Levels of 5–HTP, 5–HT and 5–HIAA in the brain stem following an IP injection of 25 mg/kg L–5–HTP.

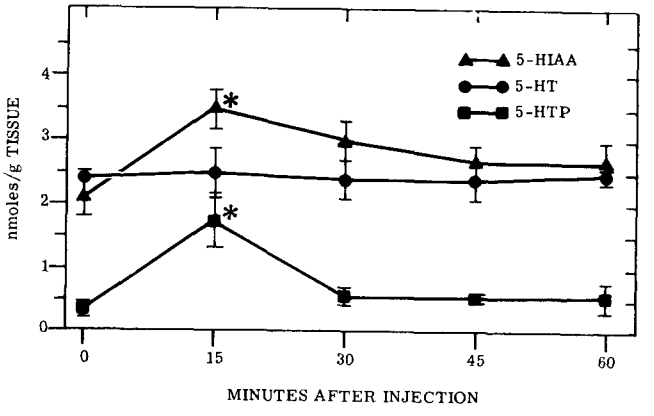

FIG. 3. Levels of 5–HTP, 5–HT and 5–HIAA in the telencephalon following an IP injection of 25 mg/kg of D–5–HTP. Values significantly ($p<0.05$) different than control levels are indicated by an asterisk.

and is metabolized to 5–HT and 5–HIAA (Figs. 1 and 2). This finding is in agreement with that found following an injection of D,L–5–HTP (50 mg/kg) in brain parts of rats and pigeons [3, 4, 8] as well as into nerve terminals isolated from these regions [15]. Parallel to the neurochemical changes, a decrease in responding on a VI 1 schedule was found following D,L (50 mg/kg) or L (25 mg/kg) 5–HTP administration (Fig. 4; Table 1). This was also previously observed following IP injections of D,L–5–HTP (50 mg/kg) in pigeons and rats on different operant schedules [1, 2, 4–7].

TABLE 1

BEHAVIORAL EFFECTS FOLLOWING ADMINISTRATION OF SALINE, D-5-HTP, L-5-HTP OR D,L-5-HTP TO RATS RESPONDING ON A FOOD-REINFORCED VARIABLE INTERVAL 1 (MIN) SCHEDULE*

Treatment	Duration (min) of decreased responding†	Responses/min after saline or during period of decreased responding after 5-HTP	Latency (min) of behavioral effect
Saline	0	16.7 ± 2.5	—
D-5-HTP (25 mg/kg)	4.8 ± 2.2‡	6.8 ± 1.9	1.9 ± 1.2
L-5-HTP (25 mg/kg)	59.2 ± 12.4	2.8 ± 0.9	4.3 ± 1.2
D,L-5-HTP (50 mg/kg)	60.1 ± 10.3	0.9 ± 0.2	6.6 ± 4.1

*Means ± S.E.M. following IP injections; N = 6.

†Decreased responding was defined here as a response rate at least 50% less than baseline response rates.

‡Two rats did not display any decreased responding after D-5-HTP.

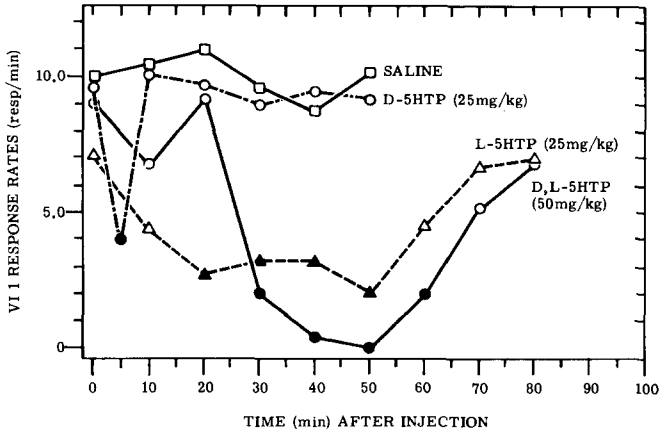

FIG. 4. Effects of IP injections of D–5–HTP, L–5–HTP and D,L–5–HTP upon the rate of operant responding on a VI 1 schedule in a representative rat (E 612). Closed symbols indicate a response rate of less than 50% of control levels.

Injection (IP) of D–5–HTP (25 mg/kg) did produce some transient increases in the levels of 5–HTP and 5–HIAA in the telencephalon (Fig. 3). However, these changes were small (3-fold increase vs. 27-fold increase) in comparison to those produced by the same dose of L–5–HTP (Fig. 1). Furthermore, injections of D–5–HTP did not produce an increase in the level of 5–HT. There are several possible explanations for these data. First, some D–5–HTP could be taken up and not metabolized to 5–HT but could be shunted via 5–hydroxyindolepyruvic acid to 5–HIAA; there is evidence which supports the existence of such a by-pass [16]. Second, D–5–HTP could be taken up in liver or brain and converted to L–5–HTP by a reversible transamination reaction. The metabolism, but not the levels of 5–HT, may then be altered by the small increase in 5–HTP availability, as the increase in 5–HIAA may reflect increased 5–HT metabolism. Third, the purity of the D–5–HTP used has not been tested and it is possible that it may be contaminated with some L–5–HTP.

The brief behavioral effects (Fig. 4; Table 1) observed following injection of D–5–HTP were too brief (5 min vs. 59 min for L–5–HTP) and not consistent enough (occurring in 4 of 6 animals) to make any conclusive statements. Therefore, if the neurochemical changes seen after D–5–HTP injection are really the result of L–5–HTP (e.g., formed via a reversible transamination reaction) then the resulting small increase in 5–HT metabolism is not significant enough to alter the behavior tested.

In conclusion, the L isomer of 5–HTP is clearly the major contributor to the increase in 5–HTP, 5–HT and 5–HIAA levels and to the associated behavioral effects previously observed following injections of D,L–5–HTP [7]. However, if D–5–HTP is taken up into the CNS at approximately 16% of the rate of L–5–HTP (compare data for 15 min time point in Figs. 1 and 3) and is metabolized to 5–HIAA via 5-hydroxyindolepyruvic acid [16] then studies in which radioactive D,L–5–HTP is injected may be difficult to interpret since the specific radioactivities of 5–HIAA to 5–HT and of 5–HTP to 5–HT may not show the usual product-precursor relationship.

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