Biochemical Pharmacology, Vol. 45, No. 5, pp. 1049–1057, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

METABOLISM OF 6-FLUORO-DL-TRYPTOPHAN AND ITS SPECIFIC EFFECTS ON THE RAT BRAIN SEROTONINERGIC PATHWAY

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(*Received* 15 *September* 1992; *accepted* 2 *December* 1992)

Abstract—We administered 6-fluoro-DL-tryptophan (6F-Trp) to rats (50–200 mg/kg i.p.) and evaluated its neurochemical effects on central catechole and indole compounds; we also determined the time course of its action, together with its metabolism and kinetics in four rat brain areas. Neither norepinephrine nor dopamine and its major metabolites were affected by 6F-Trp. With regard to serotonin (5-HT), 6F-Trp induced a transient depletion in all the brain areas studied, with a maximum of about 60–65% obtained between 1 and 3 hr depending on the dose administered. After 6 hr, 5-HT levels generally returned to control values. 5-Hydroxyindolacetic acid (5-HIAA) levels were also reduced 3 hr after administration (−40 to −60%). A large dose-dependent increase in tryptophan (Trp) was observed in the four brain areas, possibly because of an inhibition of Trp incorporation into protein, as suggested by experiments with mouse neuroblastoma cells. The brain elimination half-life of 6F-Trp was estimated at 0.5–1 hr. Regarding 6F-Trp metabolism, three new compounds were detected in all four brain areas after 6F-Trp administration. They were identified by means of liquid chromatography with electrochemical detection and/or radioenzymology, in comparison with fluorinated standards, or after NSD 1015 or pargyline coadministration with 6F-Trp. The first two 6F-Trp metabolites detected were probably 6-fluoro-5-hydroxytryptophan and 6-fluoro-5-HIAA. The third, identified and quantified by means of the two analytical methods, was 6-fluoro-5-HT (6F-5-HT). These findings suggest that 6F-Trp could be used as the *in vivo* precursor of 6F-5-HT with a view to tracing neuronal serotoninergic pools, as has already been done with platelets.

Among the tools used to study serotininergic transmission, 6-fluoro-DL-tryptophan (6F-Trp‡) has been described as a specific inhibitor competing with L-tryptophan (Trp) for tryptophan hydroxylase (TPH, EC 1.99.1.4) *in vitro* [1] and has therefore been used as an inhibitor of serotonin (5-HT) synthesis *in vivo* [2]. It is more potent than *p*-chlorophenylalanine (PCPA) in inhibition tests *in vitro* and as efficient as PCPA *in vivo*, but with more transient effects [3]. However, the neurochemical effects of 6F-Trp have mainly been studied on whole brains and with analytical methods which do not allow changes in central Trp metabolites to be followed in parallel to those of 6F-Trp. In addition, to the best of our knowledge, brain metabolites of 6F-Trp have been neither described nor identified.

In this study, 6F-Trp was used *in vivo* (i) to evaluate its neurochemical effects on central catechole and indole compounds, (ii) to evaluate its metabolism and kinetics simultaneously in four rat brain areas, and (iii) to identify the resulting metabolites by means of physico-chemical analysis (liquid chromatography with electrochemical detection and radioenzymology) in comparison with fluorinated standards, or by pharmacological manipulations such as the concomitant administration of an aromatic amino acid decarboxylase inhibitor or a monoamine oxidase inhibitor.

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‡ Abbreviations: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 6F-Trp, 6-fluoro-DL-tryptophan; 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; LC–ECD, liquid chromatography with electrochemical detection; 3-MT, 3-methoxytyramine; NE, norepinephrine; PCPA, *p*-chlorophenylalanine; REA, radioenzymatic assay; TPH, tryptophan hydroxylase; Trp, L-tryptophan.

MATERIALS AND METHODS

Animals and treatments

Neurochemical effects and kinetics of 6F-Trp (assay I). Ninety-six male Wistar rats (200–220 g, Iffa Credo, France) were housed at 22–23° under standard conditions with a 12-hr light–dark cycle and free access to food and water. The treatments were allocated randomly. 6F-Trp (Sigma) was dissolved in 0.5 M NaOH and the pH was adjusted to 8.5. Animals (N = 6 per dose and per time of killing) received i.p. injections (2 mL/kg) of 50, 100 or 200 mg/kg of 6F-Trp and were killed by decapitation (between 13:00 and 16:00 hr to avoid potential circadian effects) 0.5, 1, 1.5, 3 and 6 hr after administration. The whole brain was quickly removed and dissected according to the procedure of Glowinski and Iversen [4]. The hypothalamus, hippocampus, striatum and cortex were separated and stored at −70°.

Metabolism of 6F-Trp (assays II and III). Forty-

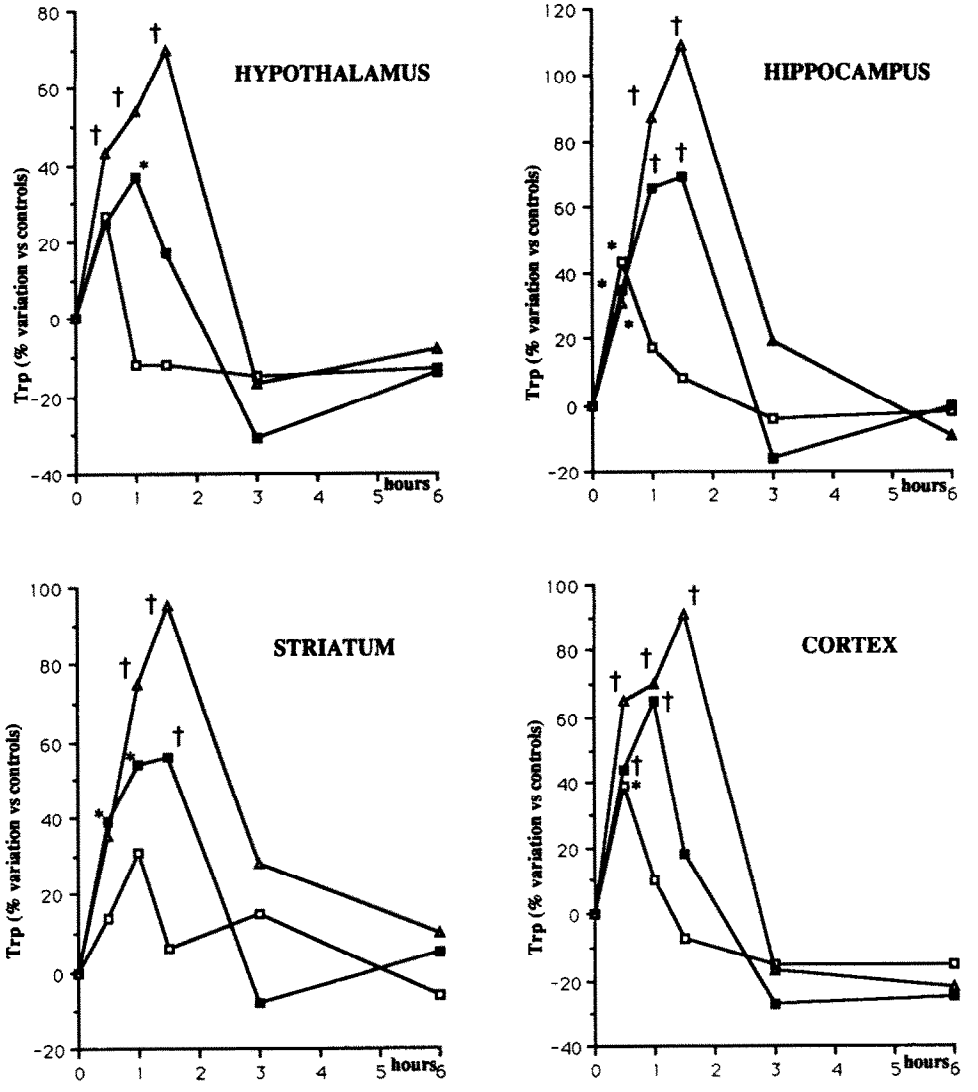

Fig. 1. Time course of the effects of 6F-Trp on Trp levels in the rat brain after i.p. administration. Control values (mean ± SEM, nmol/g wet weight tissue): hypothalamus, 21.56 ± 1.57; hippocampus, 13.24 ± 0.42; striatum, 18.59 ± 0.88; cortex, 13.90 ± 1.02. Open squares: 6F-Trp 50 mg/kg; closed squares: 6F-Trp 100 mg/kg; open triangles: 6F-Trp 200 mg/kg. Results are mean (N = 6) percentage variations versus controls. * $P < 0.05$, † $P < 0.01$ vs controls, Mann–Whitney test.

eight male Wistar rats (200–220 g, Iffa Credo, France), housed as described above, were randomly divided into six groups and received i.p. injections (2 mL/kg) of vehicle + vehicle, 6F-Trp (200 mg/kg) + vehicle, vehicle + *m*-hydroxybenzylhydrazine (NSD 1015, an amino acid decarboxylase inhibitor, Sigma, 100 mg/kg), pargyline (a monoamine oxidase inhibitor, Sigma, 75 mg/kg) + vehicle, 6F-Trp + NSD 1015, or 6F-Trp + pargyline. NSD 1015 was given 1 hr after 6F-Trp, while pargyline was concomitantly injected with 6F-Trp. Animals were killed by decapitation 1.5 hr after administration of 6F-Trp and/or pargyline, i.e. 0.5 hr after NSD 1015. The hypothalamus was isolated from the rest of the brain (without the brainstem or cerebellum) which was separated into the two hemispheres. Tissues were stored at −70°.

Sample preparation and biochemical assays

We first determined (assay I) endogenous norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), homovanillic acid (HVA), endogenous tryptophan (Trp), 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA) and 6F-Trp itself, and separated new compounds in samples from treated rats by means of liquid chromatograph with electrochemical detection (LC–ECD). We then identified and quantified metabolites of 6F-Trp by means of LC–ECD in one of the two brain

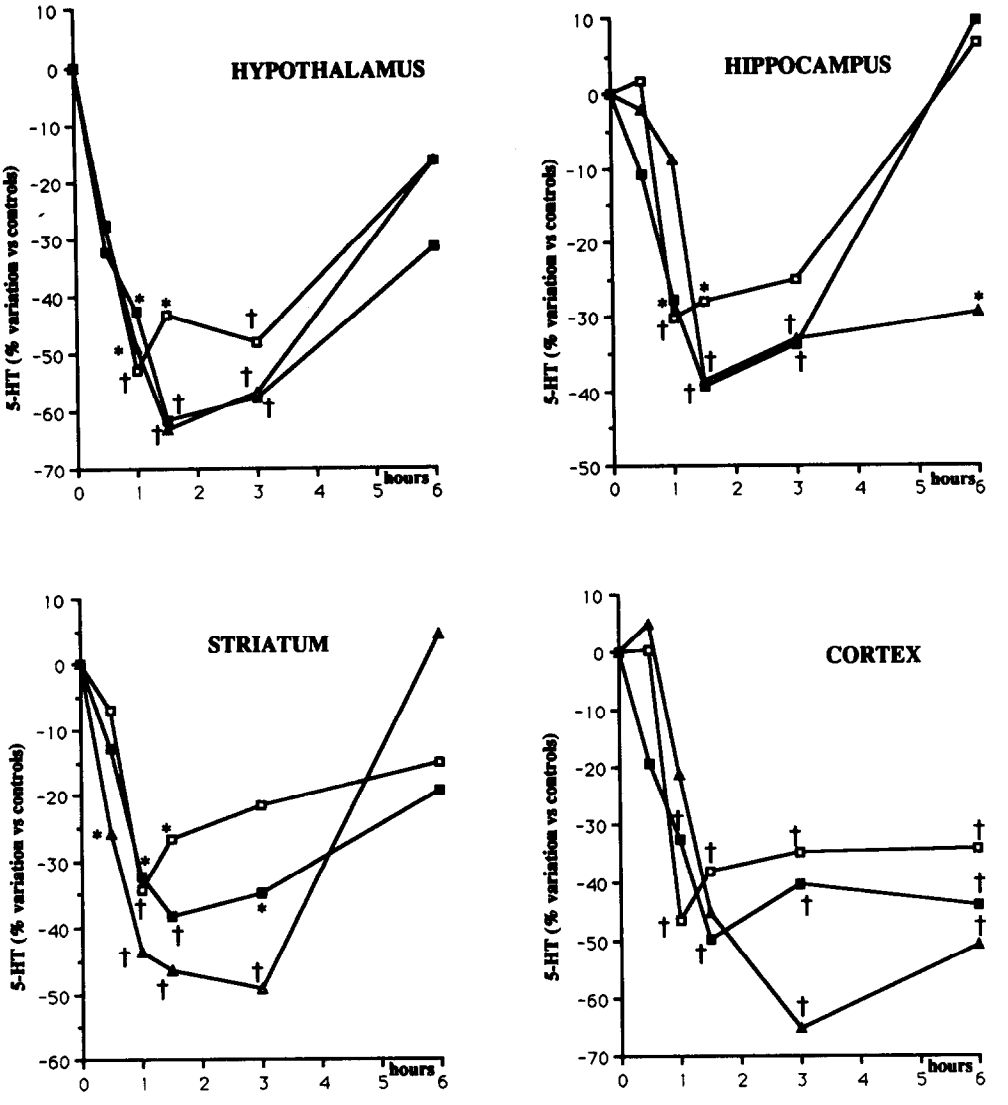

Fig. 2. Time course of the effects of 6F-Trp on 5-HT levels in the rat brain after i.p. administration. Control values (mean ± SEM, nmol/g wet weight tissue): hypothalamus, 2.08 ± 0.25; hippocampus, 0.90 ± 0.05; striatum, 1.43 ± 0.09; cortex, 1.26 ± 0.09. Symbols as in Fig. 1.

hemispheres (assay II). 5-HT and 6F-5-HT were also determined in the other hemisphere by means of radioenzymatic assay (REA) (assay III), a reference method for assaying 5-HT [5].

LC–ECD. On the day of analysis, the individual brain areas were homogenized at +4° for 30 sec in 0.2 M perchloric acid containing 0.1% EDTA, 0.1% $Na_2S_2O_5$ and 0.1% L-cysteine and then centrifuged. The clear supernatant was analysed by LC–ECD [6], using a LC9A Shimadzu pump with a Beckman Ultrasphere C18 column. The mobile phase consisted of water/methanol (95/5 v/v) containing 1 mM EDTA, 0.1 M KH_2PO_4 and 5 mM heptane sulphonic acid (Pic B7, Waters). The pH was adjusted to 3.65. Electrochemical detection was performed with a BAS LC 4B detector (Bioanalytical System, West-Lafayette, IN, U.S.A.) and the working potential was set at +0.85 V (glassy carbon electrode) with a sensitivity range of 1 nA full-scale.

REA. Brain hemispheres were homogenized (Potter–Elvejhem Teflon-glass homogenizer, 3 × 30 sec, 2000 rpm) in 10 vol. (w/v) of an ice-cold modified Tyrode buffer (116 mM NaCl, 4.2 mM KCl 25.0 mM Tris, 1.2 mM $MgSO_4$, 10.9 mM trisodium citrate, 1.8 mM KH_2PO_4, pH 7.4, 300 ± 10 mOsmol/L) and centrifuged at 20,000 *g* for 10 min at +4°. 5-HT and 6F-5-HT were then determined using the radioenzymatic method originally described by Saavedra *et al.* [5]. This method is based on the conversion of 5-HT to tritiated melatonin by a two step-reaction: (a) N-acetylation of 5-HT to form *N*-acetylserotonin by acetyl CoA in the presence of 5-HT-*N*-acetyltransferase partially purified from Drosophila [7], and (b) transfer of a tritiated methyl

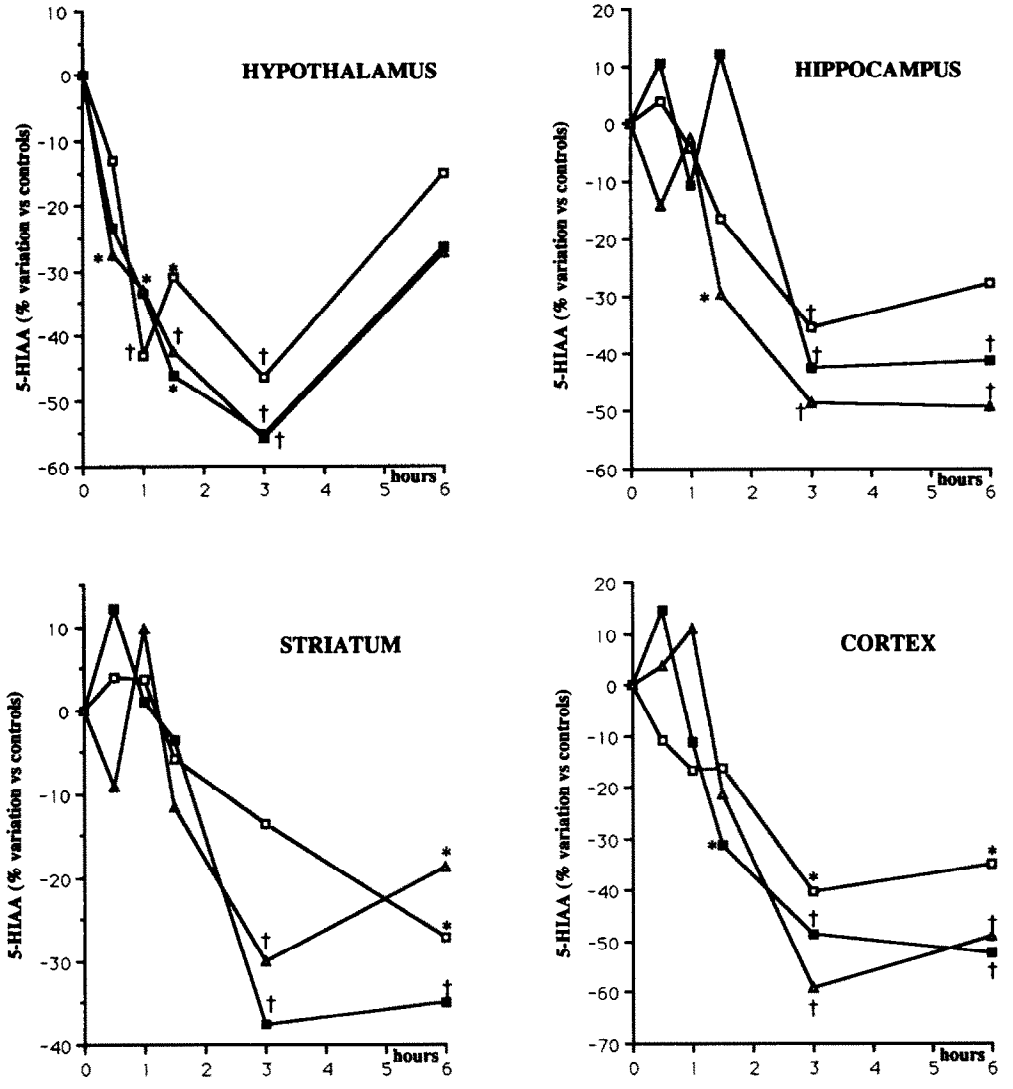

Fig. 3. Time course of the effects of 6F-Trp on 5-HIAA levels in the rat brain after i.p. administration. Control values (mean ± SEM, nmol/g wet weight tissue): hypothalamus, 6.32 ± 0.50; hippocampus, 3.00 ± 0.15; striatum, 4.55 ± 0.23; cortex, 2.04 ± 0.12. Symbols as in Fig. 1.

group from [^{3}H]SAMe (11.1 Ci/mmol, NEN, Boston, MA, U.S.A.) to the hydroxyl group of *N*-acetylserotonin by hydroxyindol-*O*-methyltransferase, semi-purified from bovine pineal gland (Collectorgane, Paris, France). After addition of carriers (50 pmol of non-radioactive melatonin and 6-fluoro-melatonin), the final chloroformic phase was evaporated to dryness under a stream of nitrogen. The residues were taken up in 50 μL of a mixture of methanol and 0.001 M HCl (1/1 v/v). Fractions (30 μL) of the supernatants were applied to TLC silicagel plates (60 F254 10 × 20, Merck) and submitted to two-dimensional chromatography [8]. After localization under UV light, the spots corresponding to melatonin and 6-fluoro-melatonin were scraped off and suspended for extraction in 0.5 mL of absolute ethanol. The extraction procedure was repeated three times until no radioactivity was detectable in the last ethanol extract. The ethanol extracts were pooled and counted by means of liquid scintillation spectrometry (Rack beta 1209, LKB Pharmacia).

Incorporation of 6F-Trp into proteins

The incorporation of Trp and 6F-Trp into proteins was measured according to Eckel *et al.* [9]. Mouse neuroblastoma cells (NIE 115) were incubated at 37° in a culture medium (RPMI + 10% foetal calf serum) containing 1 μCi/mL of [^{14}C]Trp (56 mCi/mmol, NEN, Boston, MA, U.S.A.), in the absence or in the presence of 6F-Trp at concentrations chosen on the basis of the maximum brain concentrations observed after administration of 50, 100 and 200 mg/kg (i.e. 30, 110 and 250 μM). Cells were incubated for different times (from 0 to 24 hr) and washed twice with 1 mL HEPES buffer at +4°. After

precipitation of proteins with 10% trichloroacetic acid (v/v) for 1 hr at +4°, residues were scraped and washed twice with 10% trichloroacetic acid at +4°. Proteins were resuspended in 0.3 mL of 1N NaOH for at least 3 hr at 37°. The amount of protein was determined by the Bradford method [10]. Aliquots of 100 μL of radiolabelled cell suspensions were counted by liquid scintillation spectrometry.

Statistical analysis

Data from each experiment were analysed using a global Kruskal–Wallis analysis, followed, when significant ($P < 0.05$), by individual comparisons with the non-parametric Mann–Whitney test.

RESULTS

Neurochemical effects of 6F-Trp

As circadian variations were minimal (no statistical difference at any time of killing in the control groups), a mean (N = 12) control value was calculated for each compound in each brain area. Regardless of the dose of 6F-Trp and the time of killing, no significant variations were observed in the concentrations of NE or DA and its metabolites (DOPAC, 3-MT and HVA) in any of the structures studied (data not shown).

The time courses of Trp, 5-HT and 5-HIAA variations in the four brain areas are shown in Figs 1–3. All the values are expressed as percentage variations relative to controls. The lowest dose (50 mg/kg) of 6F-Trp had no significant effect on Trp levels in any brain area. In contrast, 0.5–1 hr after 6F-Trp administration, Trp levels increased (from +37% in the hypothalamus to +66% in the hippocampus at 1 hr) with the 100 mg/kg dose and after 1–1.5 hr with the highest dose (200 mg/kg) (+70% in the hypothalamus to +110% in the

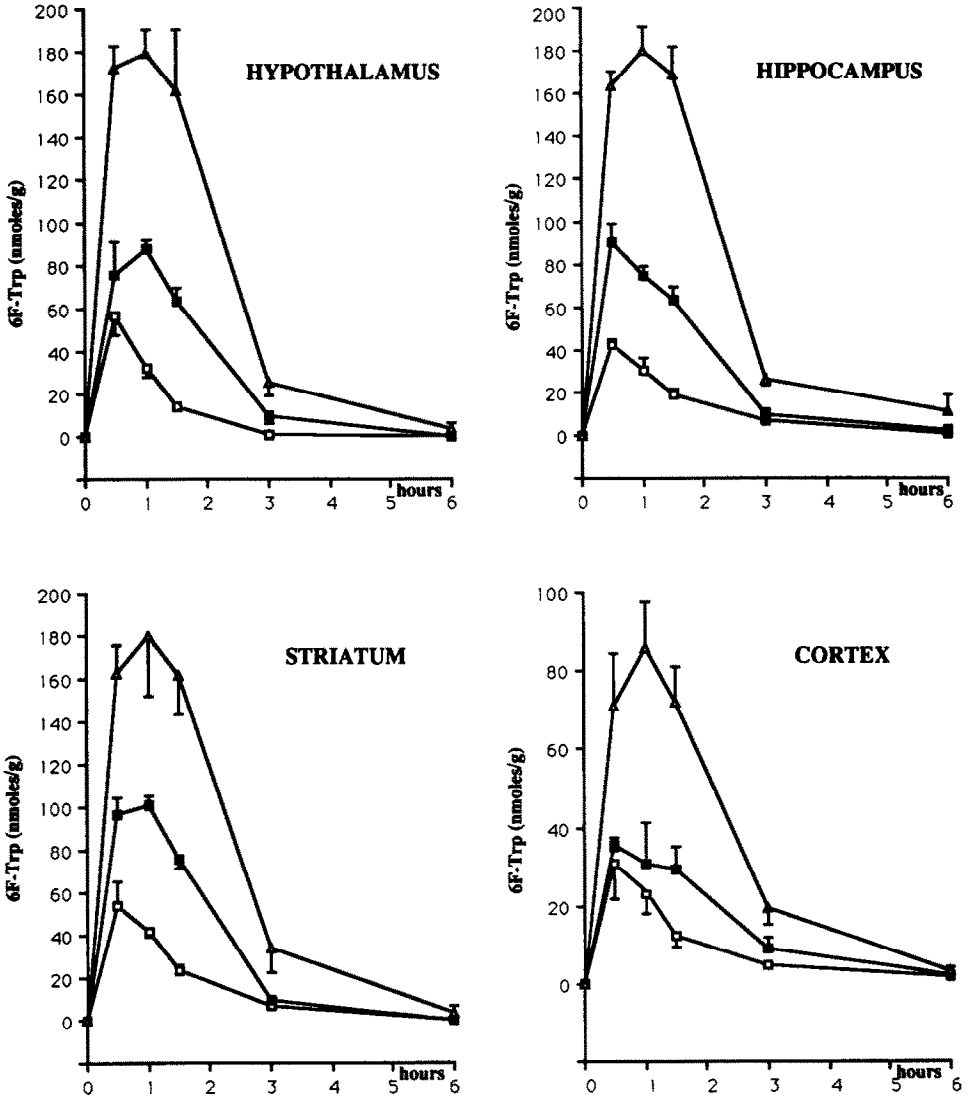

Fig. 4. Time course of 6F-Trp concentrations in four rat brain areas after i.p. administration. Open squares: 6F-Trp 50 mg/kg; closed squares: 6F-Trp 100 mg/kg; open triangles: 6F-Trp 200 mg/kg. Results are means ± SEM (nmol/g wet weight tissue) of six determinations.

hippocampus at 1.5 hr). Levels returned to control values after as little as 3 hr after administration in the four brain areas, regardless of the dose administered (Fig. 1).

5-HT levels fell rapidly in the four brain areas (Fig. 2). The largest depletion occurred between 1 and 3 hr after 6F-Trp administration according to the dose and structure. With the lowest dose (50 mg/kg), depletion was maximal at 1 hr (−30% in the hippocampus, −53% in the hypothalamus). With the 100 mg/kg dose, depletion was maximal at 0.5 or 1 hr (−38% in the striatum, −62% in the hypothalamus). With the highest dose (200 mg/kg), the maximum effect was observed between 1.5 and 3 hr (−39% in the hippocampus, −63% in the hypothalamus, −65% in the cortex). 5-HT levels returned to control values after 6 hr except in the cortex (all doses) and hippocampus (200 mg/kg).

At the first two time points studied, 5-HIAA concentrations (Fig. 3) were not significantly altered; a marked decrease occurred 1.5 and 3 hr after 6F-Trp administration, with a maximal depletion generally obtained 3 hr after the injection (−27% in the striatum to −47% in the hypothalamus with the lowest dose, and −30% in the striatum to −59% in the cortex with the highest dose). In most cases, 5-HIAA levels remained significantly lower than control values 6 hr after 6F-Trp injection.

The time course of 6F-Trp concentrations in the four brain structures is shown in Fig. 4. Regardless of the structure and dose, 6F-Trp concentrations were maximal between 0.5 and 1 hr. 6F-Trp concentrations fell at a similar rate in the four structures, becoming practically undetectable 6 hr after the injection. An elimination half-life of 0.5 to 1 hr, depending on the structure, was calculated from the linear regression of the terminal logarithm of brain concentration versus time slope (at least 4 points).

Metabolism of 6F-Trp

LC–ECD procedure. After 6F-Trp treatment, three new compounds, X1, X2 and X3, were detected in all the studied brain areas of treated rats, whatever the dose administered. When the solvent front was narrow, the three peaks were detectable in the hypothalamus, whereas only peaks X2 and X3 were easily detectable in the other structures. These peaks were probably due to the treatment with 6F-Trp, since their decline was quite similar to that of 6F-Trp (data not shown); moreover, they were not present in chromatograms of control samples, nor after the administration of pargyline or NSD 1015 alone. Chromatograms of hypothalamus samples obtained after treatment with 6F-Trp alone (Chromatogram 1), as well as 6F-Trp combined with NSD 1015 (Chromatogram 2) and pargyline (Chromatogram 3) are shown in Fig. 5. Given the respective pattern of change in X1, X2 and X3 after treatment of rats with NSD 1015 or pargyline, i.e. (i) in the presence of NSD 1015, an increase in X1 and a decrease in X2, with no change in X3 and (ii) in the presence of pargyline, no change in X1, the virtual disappearance of X2 and an increase in X3, the three metabolites X1, X2 and X3 may be the 6-fluoro-analogues of 5-HTP, 5-HIAA and 5-HT, respectively. Modification of the eluent pH and the potential of the electrochemical detector, respectively, induced similar variations in the retention time and the signal response of X1, X2 and X3 relative to 5-HTP, 5-HIAA and 5-HT standards (data not shown), suggesting a high degree of similarity in the chemical structure of these pairs of compounds. Furthermore, X1, X2 and X3 were eluted after 5-HTP, 5-HIAA and 5-HT, respectively, and 6F-Trp was eluted after Trp. Finally, the retention time of X3 was identical to that of 6F-5-HT.

We then attempted to quantify these metabolites. X3 was quantified using 6F-5-HT as standard, and the results were therefore expressed in nmoles per gram of wet tissue. Since other suitable fluorinated standards were not available, X1 and X2 were quantified using 5-HTP and 5-HIAA, respectively,

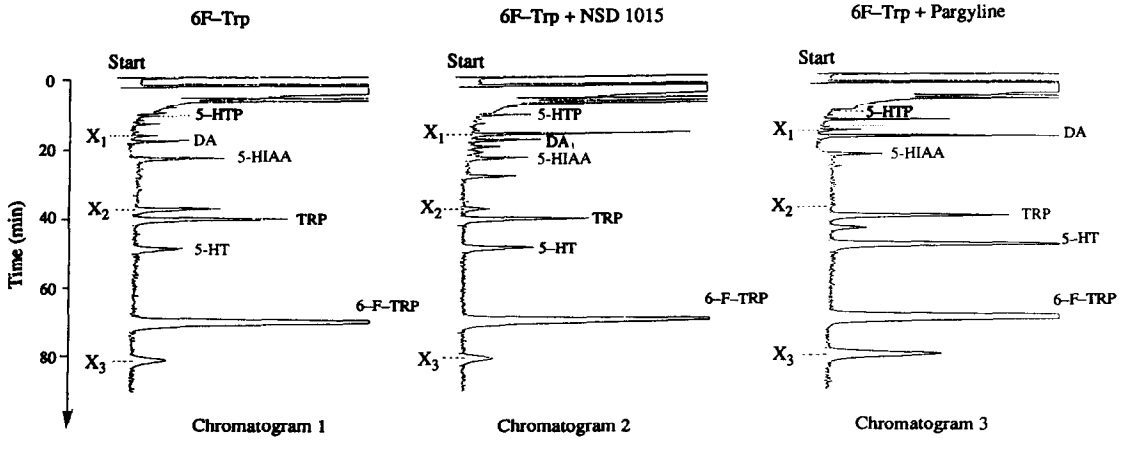

Fig. 5. Chromatograms of rat hypothalamus after 6F-Trp treatment i.p., alone (1) and combined with NSD 1015 (2) or pargyline (3).

as references, and their concentrations were thus expressed as the equivalent nmoles per gram of wet tissue. The numerical values (Table 1) confirmed the qualitative results: (i) the concentration of the first 6F-Trp metabolite, X1, increased, like 5-HTP, after concomitant treatment with NSD 1015: in all likelihood, X1 was thus 6F-5-HTP. (ii) The concentration of X2 fell, like that of 5-HIAA, after concomitant treatment with NSD 1015, and fell in the presence of pargyline: X2 was thus likely to be 6F-5-HIAA. (iii) Like 5-HT, the X3 concentration was not significantly affected by NSD 1015 treatment. In contrast, its concentration increased markedly, like that of 5-HT, after coadministration of pargyline; X3 was thus probably 6F-5-HT.

REA procedure. The values of 5-HT and 6F-5-HT obtained with the REA procedure were very similar to those obtained with the LC–ECD procedure. A positive correlation between the two sets of values was obtained, with correlation coefficients (r values) of 0.994 and 0.992 ($P < 0.001$) for 5-HT and 6F-5-HT, respectively.

DISCUSSION

No significant changes in catechole compound levels were observed in the rat hypothalamus, hippocampus, striatum or cortex after treatment with various doses of 6F-Trp i.p, corroborating the specificity of 6F-Trp-induced TPH inhibition with regard to NE and DA [1, 2]. In addition, DA metabolite (HVA, 3-MT and DOPAC) levels were unaffected by 6F-Trp treatment.

In accordance with previous reports [1, 2, 11], 6F-Trp behaved as a short-acting inhibitor of serotonin synthesis *in vivo*. This study provides further data on the dose-dependent effect of 6F-Trp on endogenous compounds in four rat brain areas, together with the time course of its action during the 6 hr following its administration. The strongest depletion of 5-HT (about 60–65%, irrespective of the brain area) was obtained between 1 and 3 hr depending on the dose administered. The effect was reversible: after 6 hr 6F-Trp was practically undetectable, but 5-HT levels started to return to control values, indicating that its synthesis was no longer inhibited. Miwa *et al.* [12] reported no change in 5-HIAA levels 1 hr after 6F-Trp administration (200 mg/kg, i.p.). We obtained similar results at this time, but we also observed a fall in 5-HIAA levels 3 hr after administration, contrary to the observation by Miwa *et al.* that 6F-Trp did not modify 5-HIAA: their conclusion that 6F-Trp had no releasing effect would thus be reviewed.

No data has been reported previously on the effect of 6F-Trp on endogenous Trp levels. As only a small percentage of brain Trp is converted to 5-HT [13, 14], no variation in Trp levels was expected after 6F-Trp administration. However, we observed a concomitant increase in Trp (Fig. 1) and 6F-Trp (Fig. 4) levels in all four brain areas. The first explanation could be a displacement of Trp bound to circulating serum albumin by 6F-Trp. Previous results showed that 6F-Trp had little effect on Trp albumin binding and thus cannot account for the increase in brain Trp levels observed after 6F-Trp administration [15].

Table 1. Brain levels of endogenous tryptophan metabolites and 6F-Trp metabolites after i.p. administration of 6F-Trp (200 mg/kg), alone and with NSD 1015 (100 mg/kg) or pargyline (75 mg/kg)

Treatment	5-HTP (nmol/g)		5-HIAA (nmol/g)		X2*		5-HT (nmol/g)		X3 (nmol/g)	
	Hypothalamus	X1*	Hypothalamus	Rest of brain	Hypothalamus	Rest of brain	Hypothalamus	Rest of brain	Hypothalamus	Rest of brain
V + V	0.19 ± 0.03	—	3.17 ± 0.20	1.59 ± 0.08	—	—	3.04 ± 0.17	1.83 ± 0.12	—	—
V + NSD 1015	2.56 ± 0.80‡	—	1.00 ± 0.29‡	1.15 ± 0.14†	—	—	3.22 ± 0.54	1.99 ± 0.14	—	—
Pargyline + V	0.19 ± 0.02	—	1.06 ± 0.22‡	0.64 ± 0.07‡	—	—	8.97 ± 0.52‡	2.99 ± 0.16‡	—	—
6F-Trp + V	0.17 ± 0.03	0.28 ± 0.05	2.03 ± 0.19‡	0.95 ± 0.08‡	1.70 ± 0.26	0.57 ± 0.05	1.59 ± 0.10‡	0.80 ± 0.05‡	1.52 ± 0.13	0.56 ± 0.03
6F-Trp + NSD 1015	0.40 ± 0.12	1.27 ± 0.51	1.25 ± 0.13‖	0.95 ± 0.09	0.75 ± 0.16‖	0.42 ± 0.08	1.80 ± 0.22	1.04 ± 0.11	1.43 ± 0.1	0.49 ± 0.03
6F-Trp + Pargyline	0.10 ± 0.02	0.32 ± 0.05	0.91 ± 0.29§	0.50 ± 0.15§	0.34 ± 0.23‖	0.15 ± 0.09§	6.70 ± 1.77¶	1.63 ± 0.21¶	3.78 ± 0.43¶	1.24 ± 0.08¶

—, No peaks were observed at the corresponding retention times; V, vehicle.
Values obtained by means of LC–ECD. Values are means ± SEM (N = 6–8).
* X1 and X2 were quantified using the 5-HTP and 5-HIAA response factors, respectively (see text), and their concentrations are thus expressed in equivalent nmol/g wet weight tissue.
† $P < 0.05$, ‡ $P < 0.001$ vs controls; § $P < 0.05$, ‖ $P < 0.01$, ¶ $P < 0.001$ vs 6F-Trp + V, Mann–Whitney test.

Secondly, we measured the displacement of Trp by 6F-Trp in cell protein using a mouse neuroblastoma cell line. We observed a dose-dependent displacement and then replacement of [^{14}C]Trp (after 24 hr, at a dose equivalent to 50 mg/kg i.p., 6F-Trp displaced 38.0 ± 5.1% of Trp and replacement by 6F-Trp reached 27.2 ± 4.6%; these values were respectively 56.6 ± 6.8% and 52.2 ± 7.00% at a dose equivalent to 100 mg/kg i.p. and 68.1 ± 5.1 and 61.2 ± 5.8 at a dose equivalent to 200 mg/kg i.p.). This concorded with the 6F-Trp-induced inhibition of Trp incorporation into proteins reported by Brown *et al.* [16] and Pratt and Ho [17] who used an *Escherichia coli* model. The inhibition probably accounted for the increase in Trp levels observed *in vivo* after 6F-Trp treatment.

Our data thus provide further information on the mechanism of action of 6F-Trp as an inhibitor of 5-HT synthesis. 6F-Trp may prove to be a valuable reagent for depleting brain 5-HT: indeed, (i) 6F-Trp competes with Trp for TPH soon after administration and in a reversible manner [1, 2], (ii) PCPA, the widely used, long-acting 5-HT synthesis inhibitor, is incorporated into TPH at or near its active site and has an irreversible action [3, 18], and (iii) α-methyltryptophan induces prolonged stimulation of hepatic tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), leading to decreased Trp availability for the brain and a subsequent fall in 5-HT formation [19, 20].

Three 6F-Trp metabolites, X1, X2 and X3, were detected and identified tentatively. The results we obtained pointed to the formation of fluorinated indole compounds through the endogenous serotoninergic pathway. Indeed, the chromatographic and electrochemical behaviour of the metabolites was comparable to that of 5-HTP, 5-HIAA and 5-HT. X1, X2 and X3 are therefore very likely to be 6F-5-HTP, 6F-5-HIAA and 6F-5-HT, respectively. This result was confirmed by the changes in X1, X2 and X3 brain levels after concomitant treatment with NSD 1015 and pargyline, the variations being parallel to those of 5-HTP, 5-HIAA and 5-HT. Finally, 6F-5-HT (X3) was unequivocally identified and quantified by means of two different analytical methods, LC–ECD and REA, the latter being considered as a reference method for 5-HT determination [5].

The identification of potentially active 6F-Trp metabolites, particularly 6F-5-HT, can be useful for understanding 6F-Trp-induced behavioural effects, which could be due to either 6F-Trp itself or its metabolites (e.g. 6F-Trp-induced effects on sleep [11, 21]). Since 6F-5-HT is formed in the brain after i.p. administration of 6F-Trp, the latter could serve as a systemic precursor of 6F-5-HT, which, in turn, could be used as a tracer of serotoninergic pools, permitting detailed studies of neuronal serotoninergic compartmentation, as with human platelets [22, 23].

Acknowledgements—We are grateful to K. L. Kirk, Laboratory of Chemistry, NIH, Bethesda, Maryland 20205, U.S.A. for his generous gifts of 6-fluoro-serotonin and 6-fluoro-melatonin.

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