Biochemical Pharmacology, Vol. 44, No. 10, pp. 2082–2085, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

Albumin binding and brain uptake of 6-fluoro-DL-tryptophan: competition with L-tryptophan

(*Received* 22 *April* 1992; *accepted* 1 *September* 1992)

Abstract—We investigated potential competition between L-tryptophan (TRP) and 6-fluoro-DL-tryptophan (6-F-TRP) for binding to albumin and for passage through the blood–brain barrier (BBB). In experiments based on equilibrium dialysis, albumin (600 μM) bound about 80% of TRP and 50% of 6-F-TRP with affinity constants (K_a) of $3.7 \pm 0.04 \times 10^4$ and $0.62 \pm 0.01 \times 10^4\ M^{-1}$, respectively. Competitive inhibition was assessed as the decrease in the apparent K_a (K'_a) of TRP in the presence of 6-F-TRP, with no modification of the N value. Competition between TRP, 6-F-TRP and L-valine (VAL) for passage across the BBB was demonstrated using two approaches. When administered concomitantly with TRP or 6-F-TRP to rats, VAL decreased brain uptake of TRP and 6-F-TRP and reversed their action on serotonin. In Oldendorf's model, 6-F-TRP and VAL decreased the brain uptake of TRP.

Among the tools used to study serotoninergic transmission, 6-fluoro-DL-tryptophan (6-F-TRP*) has been described as a specific inhibitor competing with L-tryptophan (TRP) for tryptophan hydroxylase (EC 1.14.164) *in vitro* [1], and has therefore been used *in vivo* as an inhibitor of serotonin (5-HT) synthesis [2]. However, 5-HT synthesis depends on the availability of TRP in the brain [3], which is determined by at least three factors: (1) plasma levels of free, i.e. non-albumin-bound, TRP [4]; (2) the molar plasma ratio between free TRP and other large neutral amino acids competing for brain uptake [5]; and (3) the activity of the neutral amino acid carrier in the blood–brain barrier (BBB) [6].

As 6-F-TRP is an analogue of endogenous TRP, the two compounds might compete for plasma albumin binding (total circulating TRP is 80% albumin-bound, in contrast to other amino acids [7]) and/or for brain uptake. If this is so, it would partly account for the 6-F-TRP-induced inhibition of 5-HT synthesis *in vivo*, in conjunction with competitive inhibition of tryptophan hydroxylase activity.

We studied *in vitro* and/or *in vivo* interactions between TRP and 6-F-TRP, with regard to albumin binding and brain uptake, since a better knowledge of their interaction would make 6-F-TRP a useful pharmacological tool for the study of the central serotoninergic system.

Materials and Methods

Equilibrium dialysis. Equilibrium dialysis was conducted using the Dianorm® apparatus at 37°. All the substances were dissolved in Sorensen phosphate buffer, pH 7.4. The time required to reach diffusion equilibrium for TRP and 6-F-TRP was set at 2 hr. The bovine plasma albumin (BSA, Sigma Deisenhofen, Germany) concentration was maintained at 600 μM (40 g/L). After dialysis, an aliquot (20 μL) from each compartment was directly analysed by means of liquid chromatography with electrochemical detection [8].

The binding parameters of TRP and 6-F-TRP alone were determined over the concentration range of 15–1000 μM. For the study of the competition between TRP and 6-F-TRP, the TRP concentrations ranged from 30 to 1000 μM. The displacing 6-F-TRP concentrations were 125 and 500 μM.

The mean percentage of binding to albumin was determined from the individual proportions of total and unbound TRP and 6-F-TRP in three to five experiments.

The number of binding sites (n), the association constant (K_a) and the apparent K_a (K'_a) were calculated as means ± SD using a commercially available computer program (Triomphe by P. d'Athis, Hôpital du Bocage, Dijon, France) and compared using Fisher's test. Bound (B) versus free (F) ligand plots obtained without and with different concentrations of the inhibitor were analysed altogether assuming that either N (n = [N]/[BSA]) or K_a values depended on the inhibitor concentration. The correct model was then chosen according to the best fit. In the case of a competitive binding, N is then identical and constant for all the curves.

Brain uptake. Competition with L-valine: Animals and treatments: male Wistar rats (Iffa Credo, France), weighing 200–220 g were housed at 22–23° under standard conditions. The treatments were allocated randomly. 6-F-TRP, TRP and VAL (Sigma) were dissolved in 0.5 M NaOH and the pH was adjusted to 8.5. Animals (N = 8 per treatment) received intraperitoneal injections of 2 mL/kg of vehicle + vehicle, 6-F-TRP (100 mg/kg) + vehicle, TRP (100 mg/kg) + vehicle, VAL (300 mg/kg) + vehicle, 6-F-TRP (100 mg/kg) + VAL (300 mg/kg), or TRP (100 mg/kg) + VAL (300 mg/kg). Animals were killed by decapitation 1 hr later; the hypothalamus and cortex were separated and stored at −70°.

Sample preparation and biochemical assays: the individual brain areas were homogenized at +4° for 30 sec in 0.2 M perchloric acid mixture. TRP, 6-F-TRP and 5-HT were analysed by means of liquid chromatography with electrochemical detection [8].

Oldendorf's model: Unidirectional extraction of [^{3}H]-TRP by the brain of male Wistar rats (280–320 g, Iffa Credo) was measured using the carotid artery injection technique, without impeding arterial flow [9]. The injection solution contained (10 μCi/mL of [^{3}H]TRP and 1 μCi/mL of [^{14}C]-butanol, along with 20 μM unlabelled TRP. In the competition studies, various concentrations (20–500 μM) of unlabelled 6-F-TRP, 6-F-L-TRP or VAL were injected with TRP. The animals were decapitated 15 sec after the injection, and the ipsilateral brain hemisphere was removed and processed for double-isotope counting in a scintillation spectrometer.

The brain uptake index (BUI) was computed as the brain ^{3}H dpm/^{14}C dpm ratio, divided by the corresponding ratio for the injected solution. Multiplied by 100, this yields a per cent index for brain uptake of [^{3}H]TRP relative to

* Abbreviations: BBB, blood–brain barrier; BSA, bovine serum albumin; BUI, brain uptake index; 6-F-TRP, 6-fluoro-DL-tryptophan; 6-F-L-TRP, 6-fluoro-L-tryptophan; 5-HT, serotonin; TRP, L-tryptophan; VAL, L-valine.

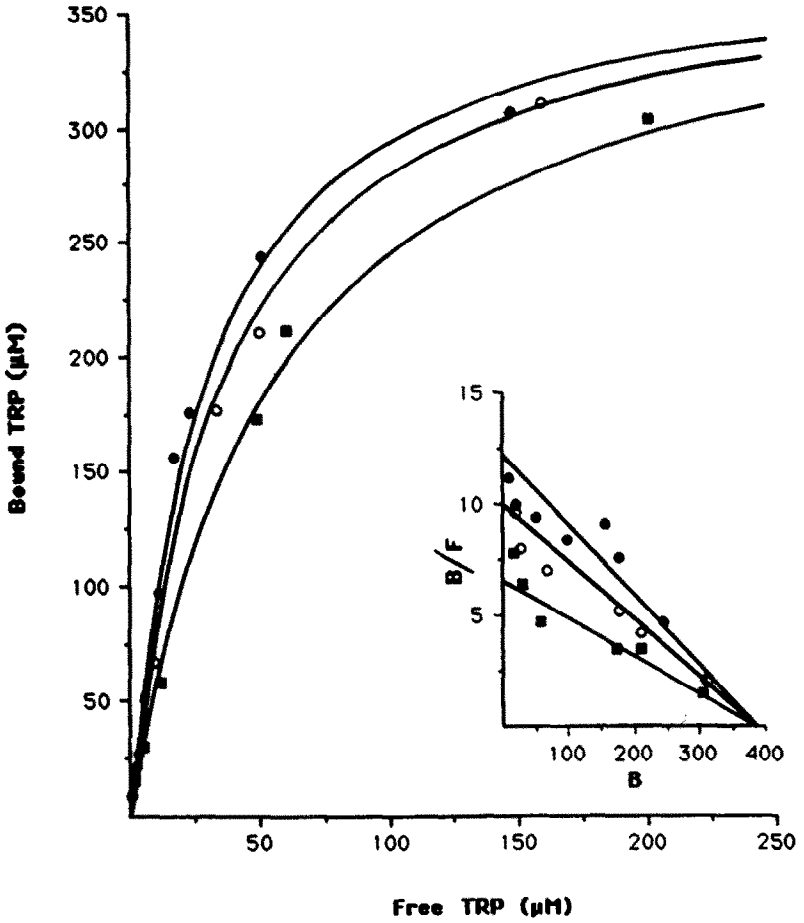

Fig. 1. TRP binding to BSA (600 μM), alone and in the presence of 6-F-TRP. (●) TRP alone; (○) TRP + 6-F-TRP 125 μM; (■) TRP + 6-F-TRP 500 μM. Inset: Scatchard plot.

[^{14}C]butanol. The extraction of [^{14}C]butanol being 100%, the brain extraction of [^{3}H]TRP (E) is E ([^{14}C]butanol) × BUI, i.e. E = BUI.

Statistical analysis. Data from each experiment were analysed using a global one-way ANOVA followed, when significant ($P \leq 0.05$), by individual comparisons against control values using Dunnett's t-test.

Results and Discussion

Equilibrium dialysis. TRP binding to albumin (600 μM) was about 80% (in agreement with previous reports [5, 7]), whereas under the same experimental conditions 6-F-TRP binding was about 50%. For both compounds, binding site saturation occurred at concentrations higher than 300 μM. The affinity constants (K_a) of TRP and 6-F-TRP were $3.70 \pm 0.04 + 10^4$ and $0.62 \pm 0.01 \times 10^4\ M^{-1}$, respectively. The affinity of 6-F-TRP for BSA was thus nearly six times lower than that of TRP.

In the presence of 6-F-TRP (125 and 500 μM), TRP binding fell progressively (Fig. 1). However, the binding of a physiological concentration of TRP (around 100 μM in normal rats under standard feeding conditions [5]) was not significantly modified by a pharmacological concentration (125 μM) of 6-F-TRP (the mean peak of 6-F-TRP concentration after oral administration of 100 mg/kg, was 145 μM [10]). The apparent K_a (K'_a) of TRP was significantly reduced in the presence of increasing 6-F-TRP concentrations (2.26 ± 0.01 and $1.65 \pm 0.02 \times 10^4\ M^{-1}$, $P \leq 0.01$, with 125 and 500 μM of 6-F-TRP, respectively), although the number of binding sites was unaffected ($N = 389 \pm 17.9$ μM). Competitive inhibition between TRP and 6-F-TRP thus occurred. However, TRP binding appeared to be only slightly altered by 6-F-TRP under physiological/pharmacological conditions.

Brain uptake. Concomitant administration of L-valine: The administration of VAL (300 mg/kg), which uses the neutral amino acid carrier [11, 12], had no significant effect on *TRP* levels in either brain region (even though a slight decrease was observed), whereas the administration of exogenous TRP (100 mg/kg) induced sharp increases (+520% and +700% in the hypothalamus and the cortex, respectively). TRP + VAL also led to a significant increase in TRP levels in both regions but it was about 2-fold lower than that observed after administration of TRP alone (+200% and +280% in the hypothalamus and the cortex, respectively) (Table 1). The administration of 6-F-TRP alone (100 mg/kg) or in combination with VAL (300 mg/kg) had no significant effect on brain TRP levels. The concentrations of 6-*F-TRP* in the hypothalamus and in the cortex were 70% and 35% lower, respectively, after the administration of VAL + 6-F-TRP (Table 1). The administration of VAL alone weakly decreased 5-*HT* levels in the hypothalamus (−28%) but not in the cortex, whereas TRP induced a significant increase in both regions (+33% and +72% in the hypothalamus and the cortex, respectively) (Table 1). In contrast, TRP + VAL had no significant

Table 1. Effects of concomitant administration of VAL with TRP or 6-F-TRP on the levels of TRP, 5-HT and 6-F-TRP in the rat hypothalamus and cortex

Treatment	TRP		5-HT		6-F-TRP	
	Hypothalamus	Cortex	Hypothalamus	Cortex	Hypothalamus	Cortex
Vehicle (V) + V	3372 ± 365	2405 ± 212	255 ± 21	180 ± 11	—	—
VAL + V (300 mg/kg)	2147 ± 264	1541 ± 164	184 ± 16*	156 ± 13	—	—
TRP + V (100 mg/kg)	21021 ± 3244‡	19342 ± 966‡	338 ± 31*	310 ± 32‡	—	—
6-F-TRP + V (100 mg/kg)	4564 ± 672	3486 ± 468	154 ± 9†	112 ± 9†	9436 ± 2447	3873 ± 740
TRP + VAL	10730 ± 622†	9172 ± 1429‡	258 ± 33	217 ± 24	—	—
6-F-TRP + VAL	3139 ± 517	2951 ± 385	186 ± 22*	187 ± 20	2788 ± 1253	2522 ± 1363

Each value is the mean ± SEM (ng/g wet tissue, N = 7 or 8).
*P ≤ 0.05; †P ≤ 0.01; ‡P ≤ 0.001 vs control, Dunnett's *t*-test.

effect on 5-HT levels in either region, indicating that VAL reversed the effect of TRP. The administration of 6-F-TRP alone (100 mg/kg) induced a 40% decrease in 5-HT levels in the hypothalamus and in the cortex. 6-F-TRP + VAL slightly decreased 5-HT levels in the hypothalamus (−27%) but had no effect in the cortex indicating that VAL reversed the action of 6-F-TRP.

In conclusion, VAL reduced exogenous TRP and 6-F-TRP levels and their respective effects on 5-HT, indicating competition at the neutral amino acid carrier level for these drugs at doses classically used in pharmacological studies [10–13].

BUI: The BUI of TRP (20 μM) was approximately 63% (mean ± SEM: 66.7 ± 3.1% in the study with 6-F-L-TRP and 6-F-TRP; 59.7 ± 9.0% in the study with VAL) (Fig. 2). This value was higher than that reported previously [11, 12, 14] when tritiated water as reference rather than [^{14}C]butanol was used. 6-F-TRP, at 20 and 50 μM, did not significantly affect the BUI of TRP (20 μM) whereas at 100 and 500 μM, it induced a significant decrease (−21% and −26%, respectively) (Fig. 2). 6-F-L-TRP induced a greater decrease in the TRP BUI (−20% at 20 μM, −52% at 50 μM and −55% at 500 μM), confirming the stereospecificity of the carrier [15]. In the same way, VAL induced a significant decrease in the TRP BUI (−30% at 20 μM, −31% at 500 μM) (Fig. 2).

In conclusion, we demonstrate that 6-F-TRP competitively inhibits TRP binding to BSA and that 6-F-TRP and TRP share the same carrier at the BBB level. However, exogenous 6-F-TRP, at pharmacological concentrations, alters only slightly the availability of TRP for albumin binding and BBB passage, suggesting that these peripheral interactions between TRP and 6-F-TRP contribute little to the central mechanism of action of 6-F-TRP, i.e. the inhibition of 5-HT synthesis. The main mechanism of action of 6-F-TRP thus seems to be the direct competitive inhibition of brain tryptophan hydroxylase activity [1]. We have found previously that 6-fluoro-5HT is formed in the rat brain after peripheral administration of 6-F-TRP (unpublished data); this fluorinated amine has been used as a tracer of serotonin-ergic pools in platelets [16]. 6-F-TRP could therefore serve as a precursor of 6-F-5-HT in the brain following systemic administration, allowing studies of neuronal serotoninergic transmission.

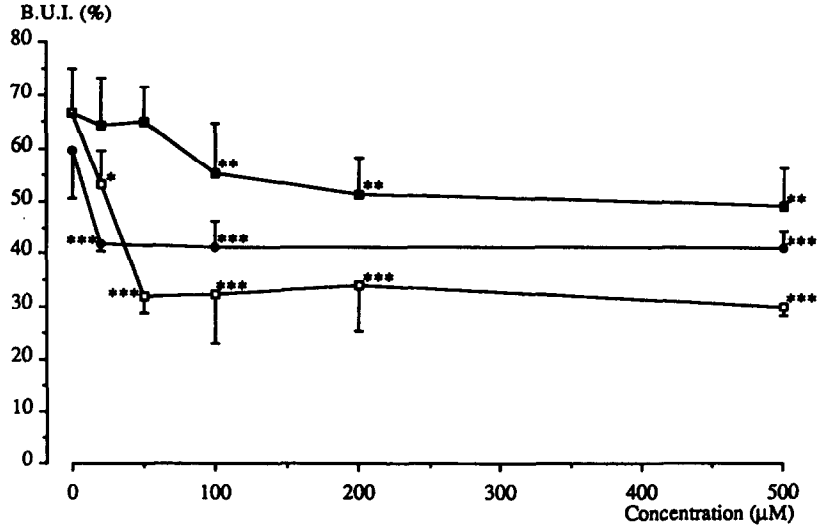

Fig. 2. Effect of 6-F-TRP, 6-F-L-TRP and VAL on the brain uptake index of TRP (20 μM). (■) 6-F-TRP; (□) 6-F-L-TRP; (●) VAL. Each value is the mean ± SD of four determinations. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 vs control, Dunnett's *t*-test.

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