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How does displacement of albumin-bound tryptophan cause sustained increases in the free tryptophan concentration in plasma and 5-hydroxytryptamine synthesis in brain?

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Models of tryptophan catabolism and binding to serum albumin are presented to explain the observed effect of displacement of tryptophan from albumin on the concentrations of free and bound tryptophan and on the rate of 5-hydroxytryptamine (5-HT) synthesis from tryptophan in the brain. A rapid rate of dissociation of tryptophan from albumin (compared to the transit time of tryptophan through the liver) and a large fractional extraction of the free pool of tryptophan during passage through the liver are shown to be necessary factors in determining the effects observed. Because of the low fractional extraction of free tryptophan in the brain, the synthesis of 5-HT will be dependent only upon the free pool of tryptophan. Dissociation of tryptophan from albumin only causes a sustained increase in 5-HT synthesis in the brain because of the effect that this dissociation has on hepatic tryptophan catabolism and thereby on the free pool of tryptophan.

INTRODUCTION

Tryptophan is the only amino acid to bind non-covalently to serum albumin and is known to bind to the serum albumin of many species (Curzon *et al.*, 1973; Smith & Pogson, 1980). Under normal conditions tryptophan is predominantly bound, with about 10% residing in the free pool (Tagliamonte *et al.*, 1973; Spano *et al.*, 1974; Badawy, 1988). Only the L-isomer of tryptophan will bind to serum albumin and the attainment of equilibrium between the bound and free pools of tryptophan is rapid (Bender, 1982). The degree of binding of tryptophan to serum albumin has been shown to be an important factor in the unidirectional uptake of tryptophan into the brain and the concentration of brain tryptophan (Curzon, 1974; Etienne *et al.*, 1976; Yuwiler *et al.*, 1977) and in the catabolism of tryptophan involving hepatic tryptophan 2,3-dioxygenase [the first enzyme of the major catabolic route of tryptophan metabolism in the body (Young *et al.*, 1978; Salter *et al.*, 1986)] in isolated liver cells (Smith & Pogson, 1980).

The concentration of tryptophan in the blood is known to be an important determinant for the rate of synthesis of 5-HT (Curzon, 1979) and increases in the free pool of tryptophan have been shown to cause increases in brain 5-HT synthesis (Tagliamonte *et al.*, 1973; Spano *et al.*, 1974); it has been suggested that such changes in 5-HT have important behavioural effects (Young, 1986). Endogenous ligands, such as non-esterified fatty acids (Curzon & Knott, 1974; Bender *et al.*, 1975), and exogenous ligands, such as clofibrate, salicylates, benzoate and indomethacin (Smith & Lakatos, 1971; Spano *et al.*, 1974; Iwata *et al.*, 1975) displace tryptophan from serum albumin both *in vivo* and *in vitro*. It has been observed that displacement of albumin-bound tryptophan by endogenous (Badawy, 1988) and exogenous (Tagliamonte *et al.*, 1973; Spano *et al.*, 1974) ligands produces a sustained increase in the concentration of free tryptophan and thus an increase in the synthesis of 5-HT in the brain.

However, a simple displacement of tryptophan would be expected to produce a sustained increase in free tryptophan only under certain specified conditions. We have looked at four potential systems of tryptophan binding and metabolism and the results demonstrate that for there to be sustained increases in free plasma tryptophan after displacement of bound tryptophan, there must be rapid dissociation of tryptophan from albumin (compared to the transit time of tryptophan through the major catabolic organ, the liver) and there must be a significant fractional extraction of the free pool during passage through the liver. The bound pool of tryptophan is therefore important in the regulation of tryptophan catabolism in the liver but only indirectly for the synthesis of 5-HT in the brain.

MATERIALS AND METHODS

Male Wistar rats (200–250 g) were killed at approx. 12:00 h, at which time tryptophan 2,3-dioxygenase (TDO) activity is at its diurnal minimum (Salter & Pogson, 1985). TDO activity was then measured in liver homogenates by the method of Metzler *et al.* (1982).

RESULTS AND DISCUSSION

There are four postulated possibilities to explain the effect of albumin-bound tryptophan on tryptophan catabolism in the liver: (A) slow rate of dissociation of tryptophan from albumin compared to the transit time through the liver and small fractional extraction of the

Abbreviations used: 5-HT, 5-hydroxytryptamine; TDO, tryptophan 2,3-dioxygenase (EC 1.13.11.11).

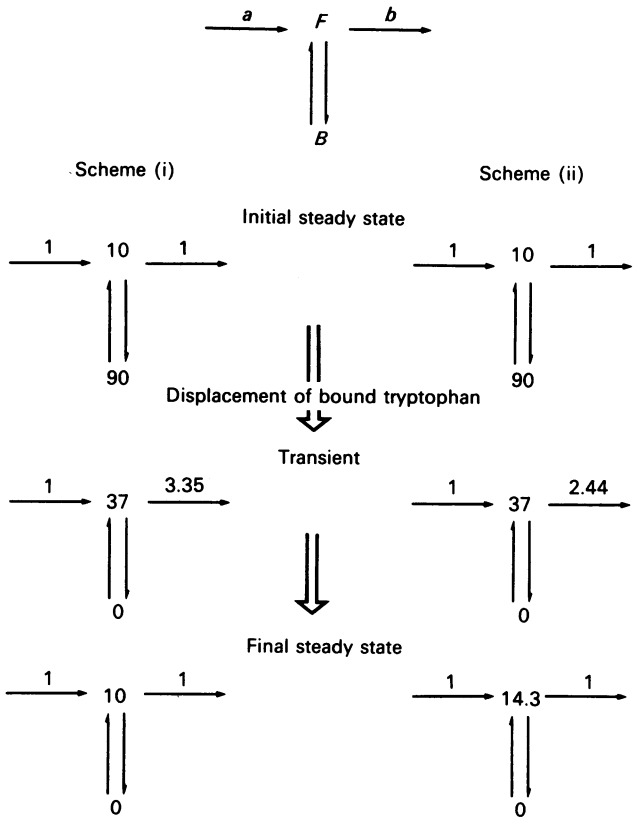

Fig. 1. Effect of displacement of albumin-bound tryptophan on the steady-state concentration of free tryptophan

Schemes (i) and (ii) represent the effects of displacement of albumin-bound tryptophan. Schemes (i) and (ii) show the consequence of displacement of tryptophan from serum albumin in A, B and C and in D respectively. *a* is the input flux (from the diet or net protein catabolism), *b* is the output flux through TDO in the liver (Young *et al.*, 1978; Salter *et al.*, 1986) and at steady state input and output fluxes are taken to be equal; both are expressed in μmol/h per rat (assuming an 8 g liver, a maximal TDO activity of 25.9 μmol/h per rat and a K_m of 249 μM for tryptophan; see the text). *F* and *B* are the concentrations (μM) of free and bound tryptophan respectively in the systemic plasma; the initial steady-state values are similar to those experimentally determined by Spano *et al.* (1974). It is assumed that the free extracellular water volume is 30% of the total water volume (Edelman *et al.*, 1952), that bound tryptophan is only present in the extracellular volume, and free tryptophan is present in the total water volume.

free pool by catabolism, (B) slow rate of dissociation and large fractional extraction, (C) rapid dissociation and small fractional extraction and (D) rapid dissociation and large fractional extraction.

Fig. 1 shows what happens to the plasma free tryptophan concentration in the four possible cases, when tryptophan is completely displaced from albumin. Under normal conditions it has been observed that approx. 90% of plasma tryptophan is bound to serum albumin. We have considered total displacement of bound tryptophan (as can occur with displacing drugs); with less than total displacement, the results obtained are qualitatively similar.

Scheme (i) shows the consequences of displacement in A, B and C. In B, the large fractional extraction will mean that the effective concentration of tryptophan in the plasma within the liver will be lower than that shown for the (systemic) plasma in Scheme (i); the kinetics of TDO will therefore have to be slightly different to give the same rates as seen in A and C but this will not affect the final steady-state. Upon displacement, the tryptophan concentration rises to 37 μM (and not 100 μM) because of the approx. 3-fold greater distribution volume of free tryptophan compared with bound tryptophan. Because the K_m of TDO for tryptophan is 249 μM the increase in the concentration of free tryptophan to 37 μM, upon displacement from serum albumin, increases the flux through TDO (in this Scheme) to 3.35 μmol/h per rat assuming Michaelis–Menten enzyme kinetics. Since the output flux is now greater than the input flux, the concentration of free tryptophan will decrease until steady-state is obtained. It is clear that, in A, B and C, displacement of bound tryptophan cannot produce sustained increases in free tryptophan concentration (and hence brain 5-HT synthesis); this is because the rates of appearance and of catabolism of tryptophan at steady-state must be equal (by definition), and only the free tryptophan concentration determines the rate of catabolism under these circumstances. The net rate of disappearance of free tryptophan can be calculated using the following equation:

$$\frac{d[Trp]_{free}}{dt} = \text{rate of appearance} - \text{rate of disappearance}$$

$$= \frac{V_{max.}[Trp]_{free_i}}{K_m + [Trp]_{free_i}} - \frac{V_{max.}[Trp]_{free_t}}{K_m + [Trp]_{free_t}}$$

where $[Trp]_{free_{t \text{ and } i}}$ are the concentrations of free tryptophan, available to TDO, at times *t*, after displacement or *i*, the time before displacement. The $V_{max.}$ and K_m of TDO are 25.9 ± 0.8 μmol/h per rat (for an 8 g liver) and 249 ± 12 μM for tryptophan (mean $\pm$ S.E.M., $n = 3$) and the input rate (rate of appearance) is assumed to be equal to the output rate through TDO at $[Trp]_{free_i}$ and to be constant. To a first approximation, these data give a value of the output flux of 1 μmol/h per rat at a physiological concentration of free tryptophan in the plasma (10 μM) which under normal conditions approximates to the concentration of tryptophan in the liver cell. Under most conditions TDO 'initiates' all the net catabolic flux in the body (Young *et al.*, 1978); net uptake into other tissues, including the brain, is quantitatively insignificant (Pogson *et al.*, 1989). An equation can therefore be derived to determine the time taken for free tryptophan to return to a given concentration:

$$t = \frac{P_{ss}}{I_{ss} K_m}(C_0 - C) + (K_m + C_{ss}) \ln\left(\frac{C_0 - C_{ss}}{C - C_{ss}}\right)$$

where P_{ss} is the pool size of tryptophan (bound and free) at the final steady-state, C_0 is the concentration of tryptophan immediately upon displacement, C_{ss} is the concentration of free tryptophan at final steady-state, C is the concentration of free tryptophan at time t and I_{ss} is the rate of input.

In A, B and C, after very large displacements of tryptophan [as shown in Scheme (i)], the time taken to return to 50% of the final steady-state concentration of free tryptophan is approx. 1 h 30 min; with smaller

displacements the time taken would be longer. For example, with transient increases of free tryptophan of 30%, the time taken would be 3 h 40 min. For these calculations it is assumed that the extracellular water volume is 30% of the total water volume (Edelman *et al.*, 1952), that bound tryptophan is only present in the extracellular volume and free tryptophan is present in the total water volume. If protein degradation is decreased or protein synthesis increased by increases in free tryptophan concentration then the final steady-state will be achieved more quickly than this. It is clear therefore that A, B and C cannot account for the sustained increases in free tryptophan concentration which have been reported to occur and to remain relatively constant for several days.

The remaining possibility (D) does however, support such observations, as shown by Scheme (ii). In D, there is a large fractional extraction of the free pool of tryptophan during passage through the liver, and tryptophan from both the free and bound pools is oxidized by TDO during transit through the liver. This is because of the rapid dissociation of tryptophan from albumin in comparison with the transit time. In Scheme (ii) we have assumed that the high fractional extraction causes a 30% decrease in the mean effective concentration of free tryptophan in the plasma within the liver (in the absence of albumin-bound tryptophan); this value of 30% for the fractional extraction of the free pool is consistent with the measured rates of hepatic blood flow and tryptophan catabolism (see below). In order to calculate rates of catabolism through TDO, following displacement of tryptophan from serum albumin, we have therefore assumed that the effective concentration of tryptophan available for catabolism is 70% of the free tryptophan concentration in the systemic plasma. Thus, in the transient stage, the 37 μM-tryptophan in the plasma is equivalent to an 'effective' concentration of 26 μM and, given the kinetics of TDO, will give a rate of catabolism of 2.44 μmol/h per rat. Similarly, in the final steady-state, a concentration of 14.3 μM-tryptophan will give an effective concentration of 10 μM and therefore a catabolic rate of 1.0 μmol/h per rat, as shown in Scheme (ii). After displacement of bound tryptophan in this model, the concentration of free tryptophan must remain elevated (compared to its pre-displacement concentration) to overcome the loss of supply from the bound tryptophan. The degree to which the concentration of free tryptophan will remain elevated upon returning to steady-state will depend upon the proportion of catabolism previously contributed by the bound tryptophan, as a consequence of its buffering of the pool of free tryptophan and thereby minimizing the degree in free tryptophan during passage through the liver. With lower fractional extractions of tryptophan in D, the free tryptophan concentrations at final steady-state will still be increased but to a lesser degree.

There has been much speculation about the importance of bound tryptophan for the supply of this amino acid to the brain. It is clear from our conclusions above that the bound tryptophan is an important factor in determining the rate of catabolism in the liver; a decrease in the bound pool must, therefore, be accompanied by a concomitant increase in the free tryptophan concentration if the same rate of catabolism, and thus the steady-state within the body, are to be maintained. Observations have shown that, after sustained displacement of bound tryptophan and consequent elevation of free tryptophan, there is an elevation of 5-HT synthesis in the brain (Tagliamonte *et al.*, 1973; Spano *et al.*, 1974). Thus, although the rate of catabolism in the liver will be the same as before displacement, the rate of 5-HT synthesis in the brain will be increased as a consequence of the elevated concentration of free tryptophan. The bound tryptophan seems therefore to be of less direct importance for supply of tryptophan to the brain than it is for the liver. This is not surprising, because the brain is likely to extract only a small proportion of the free tryptophan so that a depletion of the bound tryptophan will have less effect on brain than on liver metabolism. It can be calculated that complete removal of the bound tryptophan will have only very small direct effects on tryptophan concentrations within the brain and thus on 5-HT synthesis. If the blood flow through the brain is 1.3 ml/min per g (Fish *et al.*, 1986), the rate of metabolism of tryptophan in the brain, both through indoleamine 2,3-dioxygenase and to 5-HT, is approx. 70 pmol/min per g (Carlsson *et al.*, 1972; Gal & Sherman, 1980; Tappaz & Pujol, 1980; Long *et al.*, 1982), and if 40% of the blood volume is taken up by red blood cells [from which the net efflux of tryptophan will be very slow compared to the rate of blood flow across the brain (Rosenberg *et al.*, 1980) such that this tryptophan will be essentially unavailable for metabolism], then only 0.9% of the free tryptophan will be utilized. Buffering of this free pool by the bound pool would therefore have little effect upon the rate of tryptophan metabolism by the brain.

The situation in the liver, however, is different. If the rate of blood flow through the liver is 1.6 ml/min per g (Fish *et al.*, 1986), the minimum rate of catabolism through TDO in the liver, at a time when TDO activity would be at its minimum in its diurnal cycle (Salter & Pogson, 1985), is 2.1 nmol/min per g at 10 μM-tryptophan (calculated from the $V_{max.}$ and K_m values given above), and if 40% of blood volume is taken up by red blood cells as before, then over 20% of the free pool will be utilized; induction of TDO, during its diurnal cycle, will lead to an even greater fractional extraction. However, the rapid dissociation of bound tryptophan from albumin will minimize the decrease in the free tryptophan concentration and will thereby contribute to the catabolism of this amino acid.

In conclusion, the experimental observations of sustained increases in tryptophan concentration when bound tryptophan is displaced are explicable only when there is both a large fractional extraction of free tryptophan (during passage through the liver) *and* a rapid dissociation of tryptophan from serum albumin. In contrast, in tissues such as the brain, in which there is a low fractional extraction of free tryptophan, the albumin-bound pool of tryptophan will not be a significant factor in determining the rate of tryptophan metabolism.

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