# Tryptophan availability and serotonin synthesis

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Under carefully chosen experimental conditions, the consumption of specific diets or the administration of appropriate precursor amino acids can influence the synthesis of serotonin and catecholamines in the brain (Fernstrom, 1983; Sved, 1983). Thus, if rats are starved overnight and then given a carbohydrate-rich, protein-free meal, brain serotonin (5-hydroxytryptamine, 5HT) increases; if the meal is rich in protein, brain 5HT may fall (Fernstrom & Wurtman, 1971, 1972; Leathwood, 1986). The mechanism by which diet (or tryptophan; TRP) is thought to influence 5HT synthesis involves the following sequence: the composition of food consumed changes plasma levels of the large neutral amino acids (LNAA), which affect in turn the rate of TRP transport into the brain, brain TRP levels and hence the rate of 5HT synthesis. It has been suggested that, by this mechanism, dietary interventions might influence a range of behaviours and brain functions linked to serotoninergic neurotransmission. The objective of the present review is to examine each step in the long sequence of (proposed) causality in an attempt to define the circumstances under which each step may have functional significance.

## TRP availability and brain serotonin synthesis

Serotonin in the brain is synthesized from the essential amino acid TRP. The rate-limiting step is the hydroxylation of TRP to 5-hydroxytryptophan by the enzyme tryptophan 5-monooxygenase (*EC* 1.14.16.4). The rate of this reaction is influenced by a variety of factors including TRP concentration, the firing frequency of the neuron, $Ca^{2+}$-dependent phosphorylation of the enzyme and cofactor availability (Fernstrom, 1983). End-product inhibition has been reported when brain 5HT levels are tripled or quadrupled by monoamine oxidase (*EC* 1.4.3.4) inhibitors (Macon *et al.* 1971), but his mechanism does not seem to act at physiological levels of 5HT (Fernstrom, 1983).

The Michaelis–Menten constant ($K_m$) of the enzyme for TRP is about 50 μM, both in vitro (Kaufman, 1974) and in vivo (Sved, 1983), although recent studies with isolated synaptosomes suggest that the apparent $K_m$ might be much lower (Wolf & Kuhn, 1986). Brain levels of TRP usually range between 10 and 30 μM, so that the enzyme is probably unsaturated and changes in brain TRP should change the rate of 5HT synthesis. Numerous experiments have demonstrated that this is so. Increasing brain TRP not only increases the rate of 5HT synthesis (Carlsson & Lindqvist, 1972), it increases concentrations of brain 5HT and 5-hydroxy-indoleacetic acid (5HIAA) (Hess & Doepfer, 1961; Fernstrom *et al.* 1974). In man, it increases cerebrospinal fluid (CSF) levels of 5HIAA (Eccleston *et al.* 1970;

Gillman *et al.* 1981). Lowering brain TRP decreases the rate of 5HT synthesis and lowers brain 5HT and 5HIAA concentrations (Biggio *et al.* 1974; Arimanana *et al.* 1984.

In general, it appears that a doubling of brain TRP (from about 15 to 30 μM) produces a 20–30% rise in brain 5HT and 5HIAA (Fernstrom & Wurtman, 1972; Fernstrom *et al.* 1974; Arimanana *et al.* 1984). The dose-response curve flattens off as brain TRP approaches 90 μM (the concentration at which TRP 5-monooxygenase should be saturated). The results of Young & Gauthier (1981) suggest that a similar saturation may also occur in man since a 3 g load of TRP approximately doubles CSF 5HIAA while a 6 g load produces no further increase.

### Transport of TRP into the brain

TRP is carried across the blood–brain barrier by the LNAA transport system, so it must compete with valine, leucine, isoleucine, tyrosine, phenylalanine and methionine for access to the carrier-binding site (Pardridge, 1983). The $K_m$ of the carrier for TRP in the absence of competing LNAA is approximately 0·1 mM. The apparent $K_m$ (i.e. taking into account the concentrations and binding constants of the competing LNAA) varies between 0·4 and 0·6 mM. Since total plasma TRP usually ranges between 0·04 and 0·1 mM, the binding site is unsaturated with respect to TRP, and if plasma TRP levels change while other LNAA remain constant, the rate of TRP transport into the brain will also change. Since the other LNAA compete with TRP for transport, a change in their concentrations will equally influence TRP entry into the brain, even if plasma TRP remains constant. This is illustrated in Fig. 1 where similar amounts of TRP given alone or in combination with an amino acid mix produce very different effects on brain TRP and 5HT. Under these circumstances the plasma TRP:LNAA ratio is a good predictor of brain TRP and 5HT + 5HIAA (the normal range of this ratio is shown in Fig. 1). As can be seen, a fourfold rise in the ratio doubles brain TRP which in turn produces a 20% rise in 5HT + 5HIAA.

The picture is further complicated by the reversible binding of TRP to albumin. Estimates of dialysable tryptophan suggest that only 15–20% of plasma TRP is 'free' but, as Pardridge (1983) has pointed out, interactions which are valid at equilibrium in vitro do not necessarily hold in vivo. During passage through the capillary bed of the brain the TRP–albumin complex disassociates and reassociates many times, while the LNAA carrier is competing for TRP. Pardridge (1983) estimates that 70–80% of total plasma TRP will be available for transport so that albumin binding should play only a minor role in determining the rate of TRP transport into the brain. Even so, measurements both in rats (Leathwood, 1986) and men (Pérez-Cruet *et al.* 1974) suggest that taking into account both albumin binding and competition with LNAA may slightly improve the predictability of brain TRP or CSF serotonin metabolites.

Until recently, there was some disagreement between different research groups as to which of plasma free TRP, plasma TRP:LNAA or plasma free TRP:LNAA best predicts brain TRP and 5HT. An experiment which helps resolve this
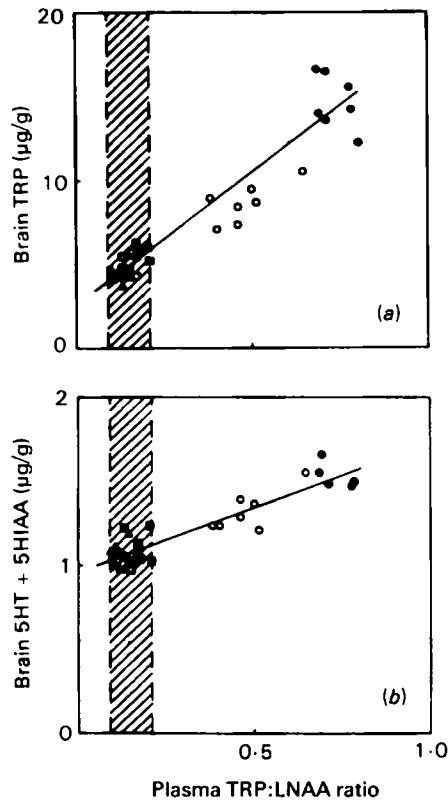
Fig. 1. Correlation between (*a*) brain tryptophan (TRP) and (*b*) brain serotonin (5-hydroxy-tryptamine, 5HT) plus 5-hydroxyindoleacetic acid (5HIAA) and the ratio of TRP:large neutral amino acids (LNAA), (LNAA comprise TRP, leucine, isoleucine, valine, phenylalanine, tyrosine and methionine).

Rats were starved for 18 h and fed nothing, a complete amino acid mix or an amino acid mix minus the LNAA which compete with TRP. Treatments were as follows: starved controls 1 h (△), 2 h (▲); complete mix 1 h (□), 2 h (■); TRP alone 1 h (○), 2 h (●). Adapted from Fernstrom & Wurtman (1972). (▨), Range of plasma TRP:LNAA ratios seen in free-feeding rats (Leathwood & Ashley, 1983*a*,*b*).

question is illustrated in Figs. 2 and 3 where, in rats starved overnight and then given meals of different compositions, the TRP:LNAA and free TRP:LNAA ratios are the better predictors of brain TRP. In this particular example plasma TRP and free TRP correlate negatively with brain TRP. Fig. 3 illustrates another important point. Although the correlation between plasma TRP:LNAA and brain TRP is rather tight, the correlation with 5HT + 5HIAA is less reliable. The same conclusion can be drawn from an examination of Fig. 1. Over the full range, TRP:LNAA did predict 5HT + 5HIAA, but within the normal range the correlation is weak. This point will be taken up again during discussion of theories of serotoninergic control of protein–carbohydrate selection.
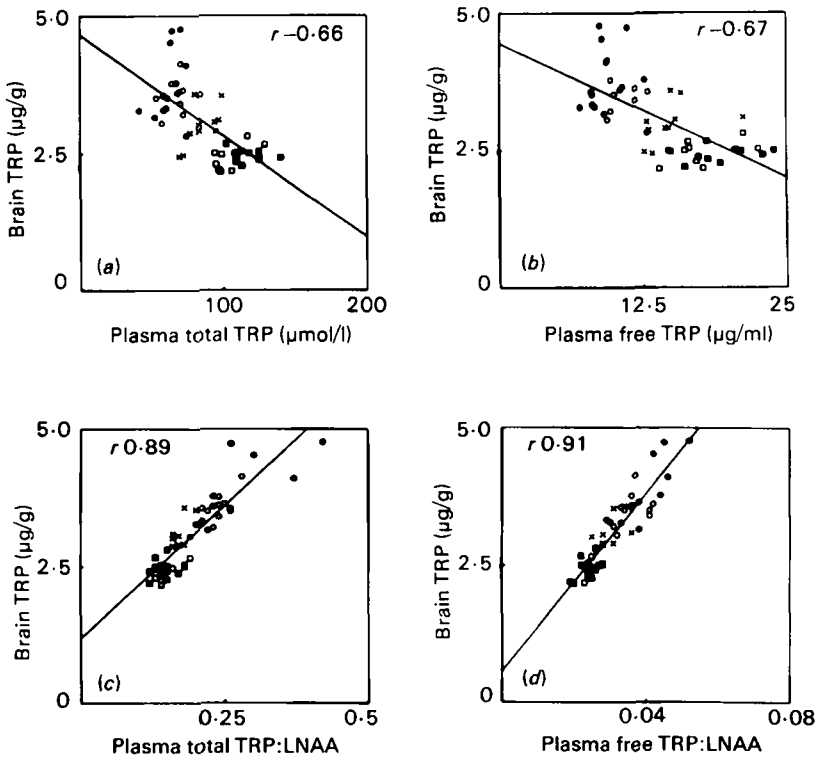
Fig. 2.   Correlations between brain tryptophan (TRP) and (*a*) plasma total TRP, (*b*) plasma free TRP, (*c*) plasma total TRP:large neutral amino acids (LNAA) ratio, (*d*) plasma free TRP:LNAA ratio in rats fed on different meals following a 15 h overnight fast. Compositions of the meals were as follows: 2·5 g (O) or 5·0 g (●) of a high-carbohydrate, protein-free diet; 2·5 g (□) or 5·0 g (■) of a 200 g protein/kg diet; fasted control (x).

## Effects of diet or TRP, or both on plasma TRP:LNAA ratio

Figs. 2 and 3 also illustrate the effects of carbohydrate- or protein-containing meals (eaten after an overnight fast) on plasma TRP:LNAA ratio and brain 5HT. The carbohydrate meal produces a sharp rise in insulin which accelerates uptake of glucose and branched-chain amino acids into muscle. Thus, although TRP levels fall slightly, the TRP:LNAA ratio rises, and brain TRP and 5HT increase. After the meal containing protein, plasma levels of all the LNAA rise. In this particular example the protein meal actually lowered the TRP:LNAA ratio (and brain TRP and 5HT) in spite of a rise in plasma TRP. The size of the meals had no significant influence on the ratio or on 5HT metabolism.

Does the same phenomenon occur in 'free-feeding' rats (or people)? In rats, after a short (3 h) fast a carbohydrate meal did produce a 50% rise in the TRP:LNAA ratio but this was insufficient to change brain TRP, 5HT or 5HIAA. A meal containing 200 g protein/kg lowered brain 5HIAA but had no effect at all on the

ratio (Ashley *et al.* 1984; Ashley & Leathwood, 1984). Carbohydrate or protein meals eaten by free-feeding rats had no effect on brain TRP or serotonin metabolism (P. D. Leathwood and L. Arimanana, unpublished results). In man, carbohydrate- or protein-containing evening meals have no effect at all on the TRP:LNAA ratio (Ashley *et al.* 1982 and Fig. 4), although addition of 400 mg TRP to the carbohydrate meal tripled the ratio. Calculated values for the ratio after combining TRP with the protein meal suggest that it would not even double (see Fig. 4).

### *Effects on serotoninergic neurotransmission*

Having established the conditions under which diet-induced changes in plasma amino acids can influence brain serotonin, it is appropriate to ask if there is any evidence that this 'cascade' actually effects 5HT release. One mechanism by which release might be maintained constant in the face of changing intraneuronal 5HT is that firing rates might adapt. Moderate to large (i.e. 50 mg/kg or more) doses of
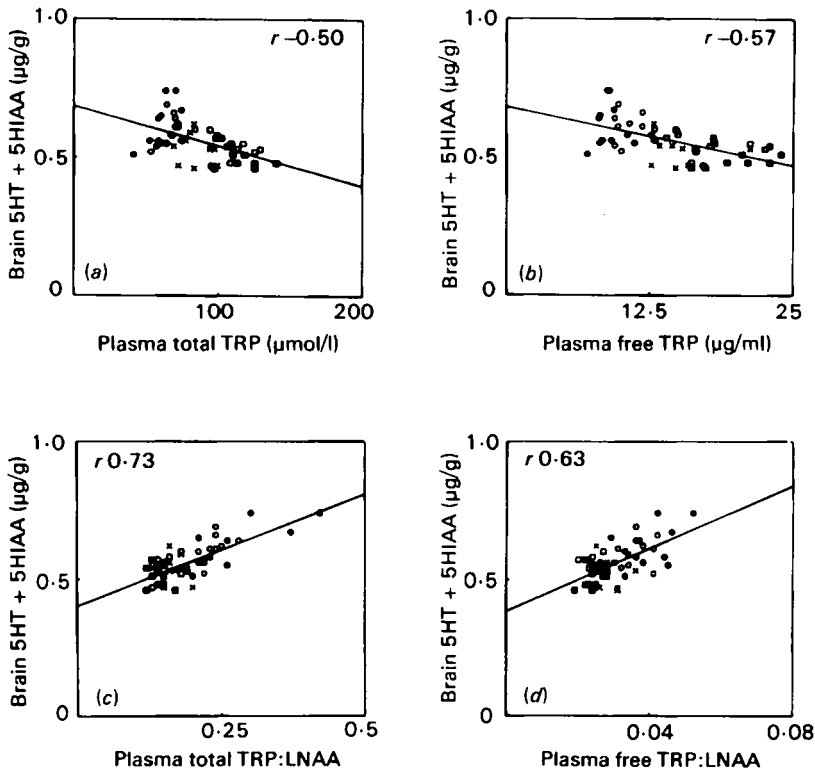


Fig. 3. Correlations between brain serotonin (5-hydroxytryptamine; 5HT) plus 5-hydroxy-indoleacetic acid (5HIAA) and (*a*) plasma total tryptophan (TRP), (*b*) plasma free TRP, (*c*) plasma total TRP:large neutral amino acids (LNAA) ratio; (*d*) plasma free TRP:LNAA ratio in rats fed on different meals following a 15 h overnight fast. Compositions of the meals were as follows: 2·5 g (○) or 5·0 g (●) of a high-carbohydrate, protein-free diet; 2·5 g (□) or 5·0 g (■) of a 200 g protein/kg diet; fasted control (x).
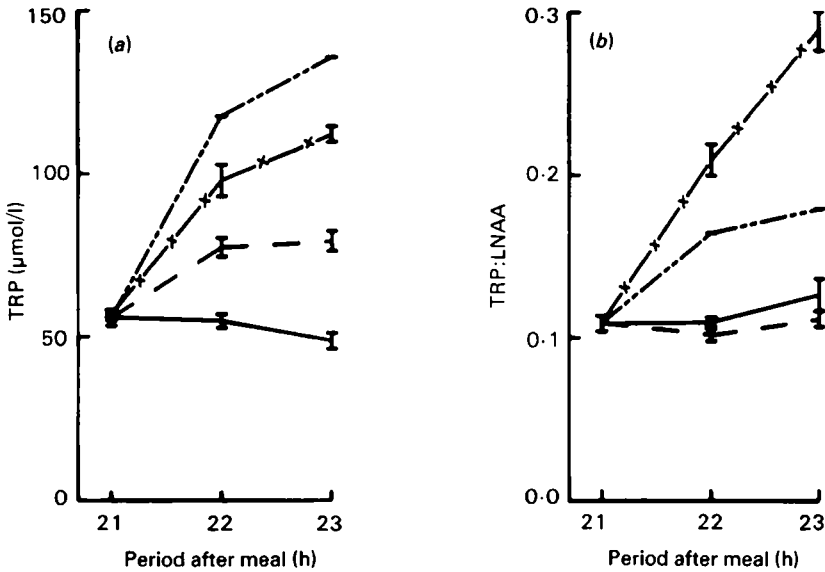
Fig. 4   (a) Plasma tryptophan (TRP) and (b) plasma TRP:large neutral amino acids (LNAA) ratio in healthy men given a carbohydrate-rich soup containing 70 g carbohydrate, 25 g fat and 1·6 g protein (————); a protein-rich soup containing 50 g carbohydrate, 25 g fat and 22 g protein (— — —); a carbohydrate-rich soup plus 400 mg TRP (—x—x—). Values for the protein-rich soup plus 400 mg tryptophan (—·—) were calculated. Values are means, with their standard errors represented by vertical bars. Adapted from Ashley *et al.* (1982).

TRP do slow the firing frequency of raphé neurons (Gallager & Aghajanian, 1976). On the other hand, small, diet-induced changes in 5HT (+12 to −26%) did not change firing frequencies (Trulson, 1985) and so presumably did influence serotoninergic neurotransmission.

A second mechanism which might limit functional effects is that the 'extra' 5HT synthesized might be metabolized intracellularly without entering the functional pool. Wolf & Kuhn (1986) have recently shown in synaptosomes that raising TRP increases 5HT synthesis but the 5HT is immediately metabolized to 5HIAA without being released. Extracellular 5HT is also rapidly taken up and metabolized to 5HIAA. However, if the synaptosomes are depolarized, increasing TRP availability does increase 5HT release.

In summary, the evidence for or against TRP-induced increases in neurotransmission is inconclusive. Measurements of extracellular or CSF 5HIAA tell us little because it is impossible to know how much of the 5HIAA comes from the 'functional' pool and how much comes directly from 5HT that has not been released. An optimistic interpretation suggests that changes in intraneuronal 5HT might influence release when firing rates are high.

## The role of serotonin in the brain

The majority of serotoninergic neurons in the mammalian central nervous system are located in the raphé nuclei of the brain stem. From these cell bodies, fine, slowly conducting fibres project throughout the brain (Moore, 1981). Most of the terminals seem to discharge into the extracellular fluid without making clear synaptic connections with other neurons. This anatomical lack of specificity has led to speculation that 5HT acts as a 'modulator', having broad tonic effects rather than operating in a 'stimulus-response' mode (Bloom, 1981). This idea finds circumstantial support from analysis of the effects of 5HT on the brain. The most consistent change following treatments which could be expected to produce a small increase in 5HT availability is an increase in sleepiness or sedation (Garattini & Valzelli, 1965; Wojcik *et al.* 1980; Young, 1985). Lowering 5HT produces insomnia or sustained arousal (Jouvet, 1973). In addition, 5HT has been reported to influence arousal, temperature regulation, pain sensitivity, sexual behaviour and aggression (Garattini & Valzelli, 1965; Hoebel, 1977; Fernstrom, 1983; Jouvet, 1983; Young, 1985), with a general tendency to be associated with a tonic suppression of these functions (for review, see Stein & Wise, 1974).
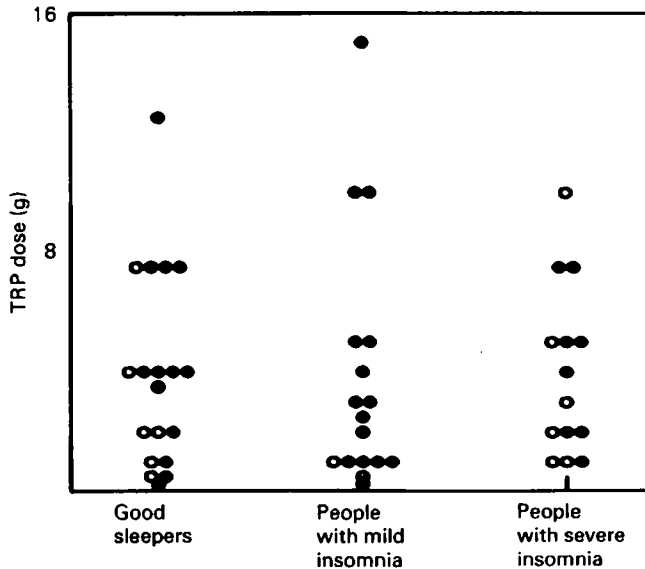
Fig. 5. A summary of the results of nearly forty different studies on the effect of tryptophan (TRP) on sleep in man comparing the effects of different doses of TRP on three types of subjects: (1) good sleepers, (2) people with mild insomnia or long sleep latency, (3) people suffering from chronic or severe insomnia. Points represent individual studies in which the indicated dose of TRP was with (●) or without (○) a sedative effect. From Hartman & Greenwald (1984).

## TRP and sleep

Serotonin seems to play a role in the triggering of the onset of sleep but the mechanism involved is still not clearly understood (Jouvet, 1983; Koella, 1984). On the one hand, lowering brain 5HT by pharmacological or surgical means produces insomnia. Re-establishing 5HT levels leads, after a fixed delay of about 45 min, to sleep onset. On the other hand, 5HT release and neuronal activity are maximal during arousal. A plausible explanation for this apparent contradiction is that 5HT release during arousal has a cumulative effect, gradually increasing the propensity to fall asleep (see Jouvet, 1983).

As TRP can increase brain 5HT levels, there have been many clinical studies on its effectiveness as a sedative. Fig. 5 (adapted from Hartman & Greenwald, 1984) summarizes the results of nearly forty different experiments. It shows that TRP is relatively ineffective in normal 'good sleepers' and in patients with severe insomnia. However, in doses of 1 g or more it consistently reduces sleep latency in people with mild insomnia or long sleep latencies. In two recent studies (Leathwood & Pollet, 1983, 1984) on over 100 volunteers, we have shown that 500 mg TRP combined with a carbohydrate load is also an effective sedative. This dose should be enough to produce a 10–20% rise in brain 5HT.

## TRP and mood

The observations that 5HT tends to produce tonic suppression in a variety of brain functions and behaviours provides the theoretical framework for the use of TRP in treating mania, chronic pain, pathological aggression and hypersexuality. For some of these problems, large doses of TRP have been used with success (for review, see Young, 1985) but results have been inconsistent. Although much of this variability is probably linked to the biochemical heterogeneity of the syndromes being treated, it is appropriate to look at the studies from a nutritional viewpoint to see if the composition of meals eaten with the TRP load might influence its availability to the brain. This is important because the validating studies demonstrating, in man, that TRP increases CSF and brain 5HT metabolites (Eccleston et al. 1970; Gillman et al. 1981; Young & Gauthier, 1981) have used patients fasted overnight and given TRP alone. In clinical practice, TRP is usually given in doses of about 2 g, in combination with a meal (Young, 1985). Table 1 shows how the composition of meals eaten concomitantly with the TRP load would be expected to influence brain 5HT if all the assumptions in the hypothesis outlined above are correct. The changes in plasma TRP after the TRP load have been calculated from the values of Eccleston et al. (1970); the effects of different meals on plasma amino acids from Fernstrom et al. (1979), and the extrapolation to changes in brain 5HT and 5HIAA from the correlation in Fernstrom & Wurtman (1972). It can be seen that, according to the composition of the meal, the same 2 g load of TRP may produce anything from a 7% to a 50% rise in brain 5HT and 5HIAA. Thus it should hardly be surprising that clinical studies have, so far, produced inconsistent results.

Table 1. *Estimated changes in plasma tryptophan (TRP) and large neutral amino acids (LNAA) after consuming 2 g TRP with different midday meals in man*

| Measurements | Protein content of meal (g) | | | References |
|---|---|---|---|---|
| | 50 | 25 | 0 | |
| Increase in plasma TRP 2 h after a 2 g TRP load (mM) | 150 | 150 | 150 | Values extrapolated from Eccleston *et al.* (1970) |
| Plasma TRP 2 h after meal (mM) | 80 | 60 | 40 | Values from Fernstrom *et al.* (1979) |
| Total LNAA 2 h after meal (mM) | 1140 | 630 | 270 | Values from Fernstrom *et al.* (1979) |
| TRP:LNAA ratio | 0·20 | 0·33 | 0·77 | |
| Expected change in brain 5HT (%) | +7 | +14 | +50 | Values extrapolated from Fernstrom & Wurtman (1972) |

5-HT, 5-hydroxytryptamine.

Several studies have set out to examine the effects of manipulating precursor availability in normal subjects. With large doses of TRP the most commonly reported effect is a mixture of drowsiness and euphoria, but with dizziness, headache and nausea as side effects (Smith & Prockop, 1962; Greenwood *et al.* 1975). Lowering the plasma TRP:LNAA value by giving large (100 g) doses of a mixture of LNAA produces a lowering of mood (Young, 1985).

In a double-blind, placebo controlled trial, Leathwood & Pollet (1983) examined the effects of 500 mg TRP combined with a 50 g carbohydrate load on mood, sleepiness and appetite in sixty volunteers. The TRP–carbohydrate mixture led to feelings of lethargy and sleepiness, and was perceived as sedating. Hunger and protein–carbohydrate preferences were unaffected and there were no untoward side effects. The results also suggested that there was a subgroup of 'responders', i.e. people who consistently felt sedated after TRP–carbohydrate.

There have also been reports that fasted volunteers feel more sleepy after a carbohydrate meal compared with a protein meal and that, in children, a carbohydrate-rich drink lowers spontaneous activity relative to an artificially sweetened drink (Spring *et al.* 1983; Lieberman, 1986). These changes are in the direction predicted by the expected effects of protein and carbohydrate (consumed after a fast) on brain 5HT, but we are still a long way from demonstrating that this really is the mechanism involved.

### 5HT and protein–carbohydrate selection

If rats are starved overnight and then fed on a carbohydrate meal, the plasma TRP:LNAA ratio and brain TRP and 5HT concentrations increase. A protein meal can have the opposite effect (Fernstrom & Wurtman, 1974; Figs. 2 and 3). Curiously, these observations have been extrapolated to the generalization that any carbohydrate meal will, by the same mechanism, increase brain 5HT and any
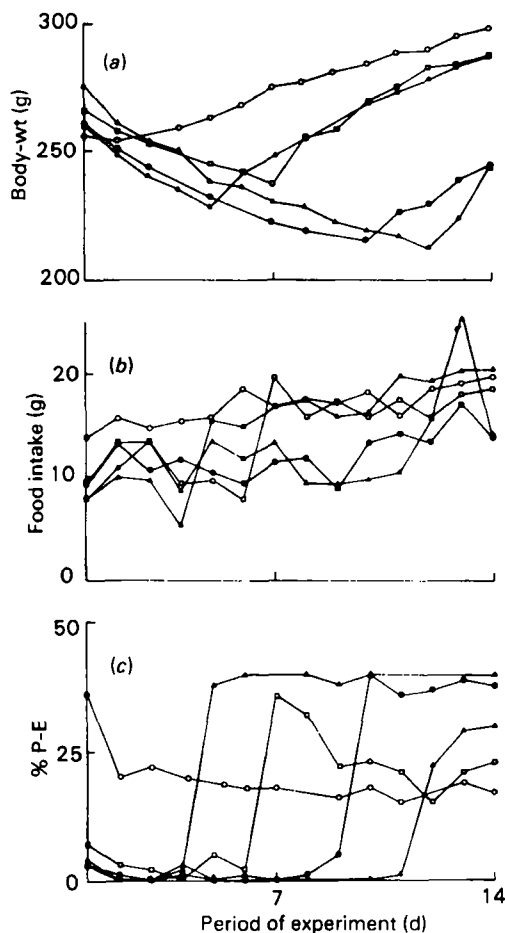
Fig. 6.  (a) Body-weights, (b) food intake and (c) percentage of energy as protein (% P-E) selected by five adult male rats offered a choice of 0 and 400 g casein/kg diets during 2 weeks. Most animals initially selected inadequate amounts of protein. They lost weight and ate less food. After anything from 4 to 11 d they abruptly began to eat the 400 g casein/kg diet. Food intake immediately increased and body-weight began to rise.

protein meal will lower it (for example, see Wurtman, 1973; Fernstrom & Wurtman, 1974; Fernstrom, 1983; Harper & Peters, 1983; Spring et al. 1983; Blundell, 1984; Lieberman, 1986). In turn, this has led to the hypothesis that 5HT might operate as a 'variable ratio sensor' detecting and determining the proportions of energy selected as protein and carbohydrate in the diet.

First, is protein–carbohydrate selection regulated? In the paradigm most often used to examine this question, animals are allowed to select from two diets, one low in protein and high in carbohydrate, the other high in protein and with little carbohydrate. For convenience, the selection pattern is usually expressed in

percentage of energy selected in the form of protein (% P-E). Although this technique appears simple and straightforward, it is beset with methodological difficulties (Blundell, 1983) and results have been contradictory. Musten *et al.* (1974) reported that, over a range of diet pairs, rats appeared to select about 30% P-E. (This is about double the requirement for adequate growth.) Unfortunately, others have not been able to confirm this tight pattern of selection. Leathwood & Ashley (1983a) noted that rats offered a choice of low- and high-protein diets may take up to 10 d to select adequate and stable levels of protein intake (Fig. 6). Even then, the range is extremely wide (e.g. 15–62% P-E) and selection can be influenced by the composition, taste and texture of the diets as well as by the age of the animals (Bise *et al.* 1973; Leathwood & Ashley, 1983a,b; Leathwood & Arimanana, 1984). Furthermore, if the diet pairs are changed, % P-E selected also changes (Fig. 7). This suggests that, while stable selection patterns can occur and animals tend to avoid extremely high- or low-protein intakes, there is not much evidence for a mechanism tightly regulating protein–carbohydrate selection.

Second, if 5HT is part of a feedback link detecting and then influencing choice of protein and carbohydrate, the proportions of protein and carbohydrate eaten should influence brain 5HT. As pointed out earlier, this mechanism does seem to function after a period of starvation, but after short fasts or during free-feeding the
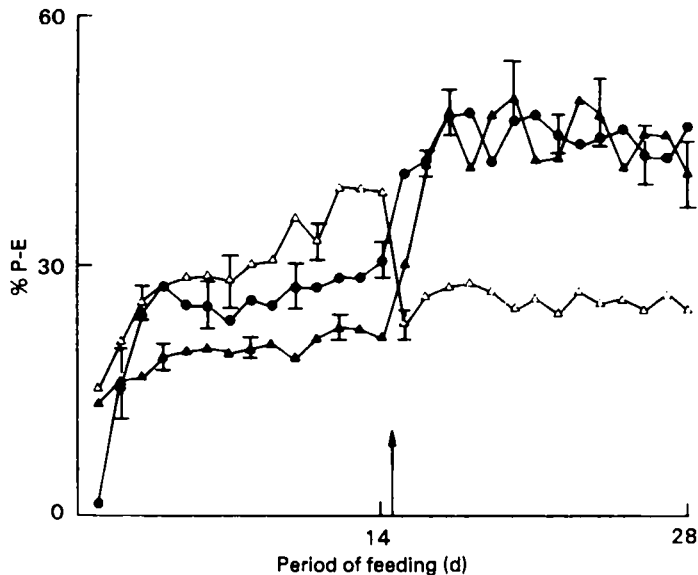


Fig. 7. Mean percentage energy as protein (% P-E) selection by three groups of weanling rats offered the following amounts of casein. Group 1 (——●——): period 1 (days 1–14) 0 and 400 g casein/kg diets; period 2 (days 15–28), 200 and 600 g/kg. Group 2 (——△——): period 1, 0 and 600 g/kg; period 2, 100 and 400 g/kg. Group 3 (——▲——): period 1, 100 and 400 g/kg; period 2, 0 and 600 g casein/kg diets. ↑, Change in diets offered. Points are means with their standard errors represented by vertical bars.

composition of the last meal either does not affect the plasma TRP:LNAA ratio or the change is so small that brain TRP and 5HT concentrations do not change (Ashley *et al.* 1982, 1984). If the composition of individual meals is unlikely to influence brain 5HT, perhaps long-term eating patterns will. Several groups have reported weak correlations in the expected direction between % P-E (selected or imposed) and plasma TRP:LNAA, but no detectable changes in brain 5HT or 5HIAA (Harper & Peters, 1983; Leathwood & Ashley, 1983*a,b*; Fernstrom *et al.* 1985; Peters & Harper, 1985).

Third, if the hypothesis is to have any value, changes in brain 5HT availability should have predictable effects on consumption of carbohydrate and protein. If rats are fasted, given an oral dose of TRP and then offered a choice of high- and low-protein diets, they do select a significantly higher % P-E than expected (Li & Anderson, 1982; Arimanana *et al.* 1984). Similarly, if rats are fasted for 16 h and then given a small dose of fenfluramine (a 5HT re-uptake blocker), they select proportionately more protein. In free-feeding rats, however, neither fenfluramine nor TRP has any effect on selection (Harper & Peters, 1983; Blundell, 1984). In man, amounts of TRP which might be expected to influence 5HT synthesis do not seem to change protein–carbohydrate preference or % P-E selected, nor do they influence food intake in people who are 'carbohydrate cravers' (Leathwood & Pollet, 1983; Hrboticky *et al.* 1985; Strain *et al.* 1985).

In summary, the current evidence for a feed-back control of protein–carbo-hydrate selection via plasma TRP:LNAA-induced changes in brain 5HT synthesis is very weak. In the special case of eating after a fast, brain 5HT availability may induce small changes in selection, but the ecological significance of such an effect is obscure.

## Conclusions

Consumption of TRP can increase the rate of 5HT synthesis in the brain. A similar effect can be seen when a carbohydrate meal is eaten following a prolonged fast, and carbohydrate can potentiate the effect of a TRP load. Direct evidence for a change in serotoninergic neurotransmission is still inconclusive, but indirect clinical and pharmacological observations suggest that serotoninergic function can be changed. TRP combined with carbohydrate does seem to have useful sedative properties, and may also find applications in the treatment of mania and aggression once an effective method of administration is achieved. On the other hand, the evidence for a feed-back mechanism influencing selection of protein and carbohydrate is still far from convincing.

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