CLINICAL RELEVANCE OF DRUGS AFFECTING TRYPTOPHAN TRANSPORT

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FREE AND BOUND TRYPTOPHAN IN PLASMA

Tryptophan is the only circulating amino acid highly bound to serum proteins (1). The plasma free fraction of serum tryptophan is very important for the control of the tryptophan pool in the brain (2, 2a, 3). In fact the rate of synthesis of serotonin (5-hydroxytryptamine, 5HT) in the brain is regulated principally by the availability of free L-tryptophan, whose normal concentration in the CNS is considerably below the K_m of tryptophan hydroxylase, the first enzyme in 5HT biosynthesis (3–5), which has been considered the rate-limiting step (6).

An increase of 5HT turnover—as indicated by elevated concentrations of its metabolite 5-hydroxyindoleacetic acid (5HIAA)—is frequently correlated to an increase in brain tryptophan, as observed in rats receiving drugs stimulating brain 5HT synthesis (3), submitted to prolonged fasting (7, 8) or immobilized (9).

No consistent correlations have been found, however, between brain and plasma total tryptophan concentrations. For example, food deprivation or immobilization induce a significant increase in rat brain tryptophan, in spite of the well-known fact that mammalian tissues are unable to synthesize the essential amino acid tryptophan and that therefore brain tryptophan is likely to derive from the plasma compartment. The solution comes from the observations by Gessa et al (2) and Knott & Curzon (10) indicating that *free* (ultrafilterable) tryptophan in plasma increases during food deprivation, immobilization, and other experimental conditions. Free plasma tryptophan increases when plasma free fatty acids concentrations are elevated as during catecholamine infusion (11), stresses releasing endogenous catecholamines (12), or heparin treatment (13).

A decrease of free plasma tryptophan is observed after ganglionic blockade, reducing free fatty acid levels (FFA) (11). Tryptophan and free fatty acids may compete for binding to the plasma albumin.

Free tryptophan concentrations in plasma influence brain tryptophan levels and hence 5HT synthesis and metabolism, without necessarily changing the brain 5HT

concentrations. It is also of interest that peripheral tryptophan administration decreases the firing of central 5HT neurones (14).

A recent procedure has been described for the determination of free, specifically bound and unspecifically bound tryptophan in plasma, using ultrafiltration (15). Sodium salicylate, a drug known to inhibit the binding of L-tryptophan to albumin (16), is able to release the specifically bound tryptophan only.

PLASMA-FREE TRYPTOPHAN IN MAN

The recent evidence that free tryptophan is important for tryptophan availability to the CNS and the control of 5HT turnover is well correlated with earlier investigations indicating that administration of large oral doses of tryptophan to animals induced rises in brain tryptophan and 5HT metabolites (17), and to more recent studies indicating that administration of large doses of tryptophan to depressed patients appear to be effective in relieving depressive symptoms (18).

Biochemical evidence points out that tryptophan concentrations in the cerebral spinal fluid (CSF) of both manic and depressive patients (respective means of 260 and 243 ng/ml) are markedly lower than those found in controls (488 ng/ml) and that upon recovery from depression, the concentrations rise back to normal (19). In humans, also, tryptophan availability appears to be a key factor in central 5HT biosynthesis. To exclude, in fact, an impaired activity of tryptophan hydroxylase, which could explain the low 5HIAA levels found in unipolar depressive patients, oral loads of tryptophan have been administered to control and depressed patients.

The rises of CSF tryptophan and 5HIAA are identical in both groups (20), thus suggesting that 5HT biosynthesis is normal in depression, and other factors were to be sought to explain the low CSF tryptophan levels.

In normal and depressed patients, total plasma tryptophan concentrations do not differ; there is, however, a striking difference in the *free* fraction of tryptophan, which is about 40% lower in the group of depressed patients (11.4% in normals vs 7.1 in the depressives) (21, 22). These findings prompted these and other authors to investigate the relationship between drugs known to affect tryptophan transport, the availability of tryptophan to the central stores, and the activity of drugs known to raise free tryptophan levels on depressive symptoms and/or endogenous depression.

The in vitro studies by McMenamy & Oncley (1), and more recently by McArthur & Dawkins (16), pointed out that tryptophan is bound to human serum albumin with a ratio of moles of protein/moles of bound tryptophan of approximately one. Acidic drugs such as salicylic acid lower the association constant K_a for tryptophan from 1 to as low as 0.0007 in the presence of high concentrations of salicylic acid. These data indicated the possibility that in some clinical conditions, salicylic acid could cause increased levels of free tryptophan in the blood; this would in turn enter tissues to a larger extent, give rise to more metabolites, and, in the long run, there would be a reduced capacity to bind tryptophan, when this was added to the circulation.

Previous clinical observations had shown that patients suffering from rheumatoid arthritis have very high plasma levels of kynurenine and 3-hydroxyanthranilic acid (23). Reexamination of these data clarified that all rheumatoid patients were on salicylate treatment and that controls also taking salicylate had a similar excretion of these tryptophan metabolites. Because tryptophan pyrrolase is induced by tryptophan (24), salicylate could also cause an increased activity of this enzyme. Tryptophan, also in humans, shares its binding sites with endogenous compounds. Lipsett et al (25) demonstrated that in physiological conditions, such as the decreased free fatty acid (FFA) levels induced by ingestion of glucose, the percentage of free tryptophan may decrease by about 35%. This is due to the occupation by tryptophan of the albumin binding sites left free by the fatty acids. In fact, in vitro addition of oleic acid to human serum considerably increases the percentage of free tryptophan. A clinical condition where this latter change occurs, i.e. an increase in FFA, is electroconvulsive therapy (ECT). In patients thus treated, Stelmasiak & Curzon (26) noted both an elevation of FFA and of free tryptophan. This change may explain the transient increase in 5HT turnover found in ECT studies in experimental animals (27).

An example of tryptophan displacement by the substrate, bilirubin, will be reported later. It is not known whether tryptophan is bound specifically and not specifically in humans, as shown in vitro by Baumann et al (15) with D- and L-typtophan.

DRUGS AND PLASMA-FREE TRYPTOPHAN

Investigations with drugs in humans, with the purpose of documenting a possible displacement of bound tryptophan, have been limited, but have provided important insights into the clinical significance of this mechanism.

Probenecid

The most widely investigated drug, as far as its relationship with tryptophan and 5HT metabolism is concerned, has been probenecid.

This organic acid markedly increases the CSF concentration of 5HIAA, as well as of homovanillic acid (HVA), indicating an effect both on 5HT and dopamine metabolism (28). In humans, the effect of acute probenecid administrations on the concentration of 5HIAA and HVA of normal subjects and patients with affective disorders has been tested. It appeared that these metabolites were raised by the treatment and that the pattern of increase was somewhat different in the same patient if the psychic conditions changed (e.g. there was less of an increase in 5HIAA when the patient changed from moderately hypomanic to severely depressed). L-dopa administration, together with a decarboxylase inhibitor, decreased the probenecid effect somewhat.

These findings, which were then attributed to a competition for the egression mechanism of 5HT metabolites from the CNS, similar to what takes place in the kidney, should probably be reexamined in the light of new findings. It has in fact been shown that probenecid (1 g bid in patients with senile psychoses) raises free

tryptophan by 52% without modification of total tryptophan concentration (29). In this study no significant difference was found in the CSF concentration of tryptophan in five patients with various illnesses before and after the end of probenecid treatment.

Somewhat contradictory findings were reported by Korf & Van Praag (30). These authors previously designed a probenecid loading test, combining an oral dose of 4 g of the drug with an intravenous infusion of 1 g, to allow a biochemical recognition of depressed patients; these have, in fact, a lesser rise in 5HIAA in the CSF as compared with controls following probenecid (31, 32). Using the probenecid loading test in patients with different psychiatric conditions, these authors could show a 50% fall of total plasma tryptophan. Experimental observations in similarly treated rats indicated that brain tryptophan content was also increased about 25%; according to these authors, there was not, however, an increase in 5HT synthesis, because 5HT accumulation following pargyline administration did not differ in controls and probenecid-loaded rats. No data were reported on brain tryptophan following pargyline alone, which might itself increase brain tryptophan, thus saturating tryptophan hydroxylase and masking any further stimulation of 5HT synthesis caused by probenecid.

In conclusion, probenecid increases acid metabolites of 5HT in the CSF, because it competes for their carrier sites from the CSF to blood (33), and also probably because more free tryptophan is made available for 5HT synthesis. Although a definite increase in central 5HT synthesis has not been shown after probenecid, several of the data previously reported in depressed patients following probenecid treatment can now be explained by a displacement of the bound plasma tryptophan in these subjects allowing more of the amino acid to enter the CSF.

Anti-Rheumatic Drugs

Anti-rheumatic drugs other than acetylsalicylic acid, whose effect was confirmed in vivo in healthy volunteers have also been shown to displace tryptophan. McArthur et al (34) demonstrated that patients with rheumatoid arthritis (RA) chronically treated with any of the commonly used drugs have total tryptophan levels which may be as low as one fifth of those normally found, and the percentage of free tryptophan may be over 30% of the total. Of the drugs tested in vitro, indomethacin and phenylbutazone were the most potent in causing displacement, followed by chloroquine, whereas aurothiomalate and prednisolone were relatively weak displacers. Following a longer incubation, however, aurothiomalate also appeared to be of the same order of potency as the first two agents.

In a subsequent study Smith et al (35) compared two newer anti-rheumatic agents, flufenamic and mefenamic acid, with a variety of drugs that are known to have a high degree of protein binding: the two anti-inflammatory agents displaced tryptophan similarly to salicylate and indomethacin, whereas cloxa- and dicloxacillin, ampicillin, and paracetamol did not displace the amino acid. It appeared thus that this feature was not common to all highly protein-bound drugs. Of particular

interest is the case of paracetamol, which, while being widely used for RA has not anti-inflammatory, but only analgesic activity.

McArthur et al (36) have postulated that a decrease of total tryptophan and an increase in the free fraction may be part of the mechanism of action of drugs effective in RA. They could in fact confirm the previous findings in a large series of RA patients and noted a similar rise of free tryptophan in two other clinical conditions: jaundice (where free tryptophan rises from 8 to 18%) and pregnancy (where the rise is small although statistically significant). Both jaundice and pregnancy are known to induce improvements in RA symptoms (37). A dilute solution of tryptophan can inhibit leucocyte infiltration into an inflammatory area in the rat (38), with a potency of about one tenth of that of cortisone acetate. Denko et al (39) have documented alterations in the amino acid composition of albumin in patients with RA. Because it is possible that this albumin has affinity for tryptophan or other small peptides, which is different from normal, anti-rheumatic agents could act by restoring a normal equilibrium between free and bound tryptophan and other peptides (40).

From the point of view of the behavioral effect of the anti-rheumatic treatment, recent data by Aylward & Maddock (41) indicate that there was an improvement in the Hamilton Depression Scale Score in RA patients after anti-rheumatic treatment. Moreover, there was a significant correlation between the Hamilton scores and the mean free tryptophan concentrations achieved after treatment, amelioration being maximal in patients having free tryptophan levels over 1.4 μg/ml.

Clofibrate

A hypolipidemic drug, which is not known to possess anti-inflammatory properties, clofibrate (*p*-chlorphenoxyisobutyrate) displaces tryptophan from plasma albumin binding both in the experimental animals (42) and in humans (43). In normal volunteers, some of whom were hospitalized, and some leading a normal life, a common therapeutic dose of clofibrate (1 g bid) induces a 50–70% decrease of total tryptophan, with a corresponding increase in the free fraction (Figure 1, 2).

Because a variation in the degree of binding of any drug or physiological substrate can induce an alteration of the rate of metabolism or excretion, the disappearance rate of exogenously administered labeled tryptophan in normal subjects and depressed patients, before and after a week of clofibrate administration has been tested in our laboratory (44). We assumed that the higher protein binding in depression would prolong the permanence of the drug in plasma, and this would be shortened by clofibrate.

^{3}H-tryptophan was injected iv and plasma radioactivity was monitored at intervals for 6 hr. Samples were also taken at intervals to measure specific activities of both free and total tryptophan. In the normal subjects disappearance of the labeled tryptophan was rapid and further accelerated by clofibrate treatment. In the severely depressed patients, the rate of disappearance was very slow and did not change after clofibrate treatment.

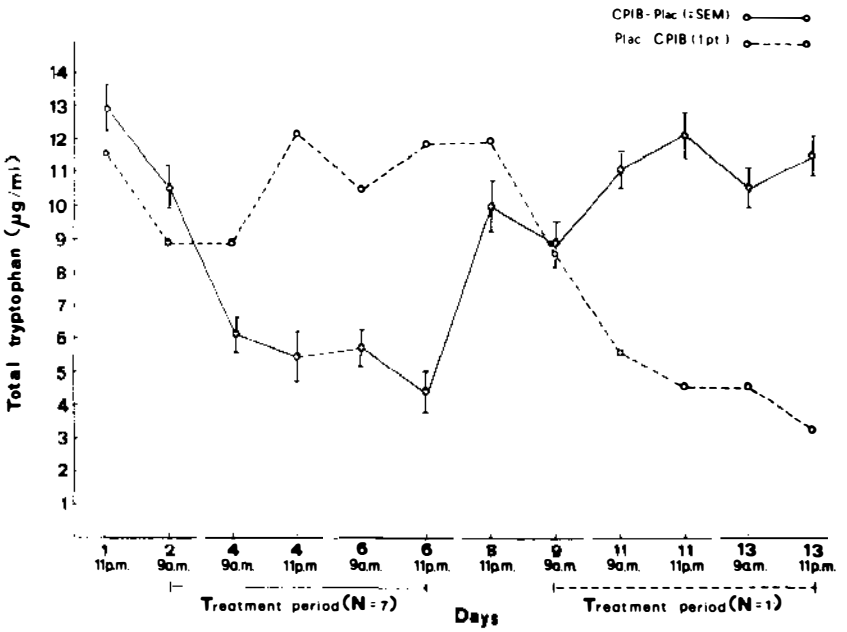

Figure 1 Total plasma tryptophan levels in normal human subjects during clofibrate and placebo treatment (43).

An experiment of a similar type, in manic-depressive patients, has been tried by Takahashi et al (45). These authors, giving oral loads of tyrosine, and, in a later experiment, of tryptophan (46) to a group of patients and to healthy volunteers, demonstrated a decreased tolerance for both amino acids in the depressives. An improvement was shown after clinical recovery.

Our experiments, as well as those of Takahashi et al (45) allow us to conclude that plasma of depressed patients has a high affinity for tryptophan and that apparently a drug such as clofibrate, which is very effective in displacing tryptophan in normal individuals, does not behave similarly in the depressed.

Displacement of tryptophan from plasma binding sites is probably not the only explanation for the clinical improvement of depressed patients. Coppen et al (47) have reported observations on large series of female patients with depression. These patients were tested before and after recovery, some while being off drugs, some others, who had also recovered, were kept on prophylactic lithium. Free tryptophan had risen after recovery a mean of 6.32 to 10.58% in all patients. In lithium-treated patients, total tryptophan was about 10% less than in the other recovered depressives. Lithium has been reported to markedly increase tryptophan levels and the synthesis of 5HT in the rat brain, while only moderately affecting plasma concentration (8). Its effects on tryptophan binding are not known. However, the increase in free tryptophan after recovery is not influenced by lithium administration (47).

Oral Contraceptives

A critical view on the problem of tryptophan transport and clinical depression has been raised by a study by Coppen et al (48) on oral contraceptives.

These authors failed to note any difference in total and free tryptophan in users of the pill as compared to controls. Depression scores were also practically negative in the former. Briggs (49) questioned the significance of these findings because contraceptives reduce plasma albumin as well as the FFA pool size, so that an imbalance between these two changes would make an alteration of tryptophan transport very likely. On the other hand, disturbances in pteridine coenzymes and pyridoxal phosphate metabolism induced by contraceptive steroids are also likely to influence tryptophan metabolism.

CONCLUSIONS

Experimental and clinical data indicate that free tryptophan in plasma plays an important role in controlling the action of a variety of therapeutic agents and that possibly it is also a significant factor in the control of central serotonin turnover in normal and pathological conditions.

This mechanism may also explain some side effects of drugs able to displace bound tryptophan, such as weight gain and antidepressant activity and the low withdrawal rate of patient during long-time therapy with such drugs (salicylates, clofibrate).

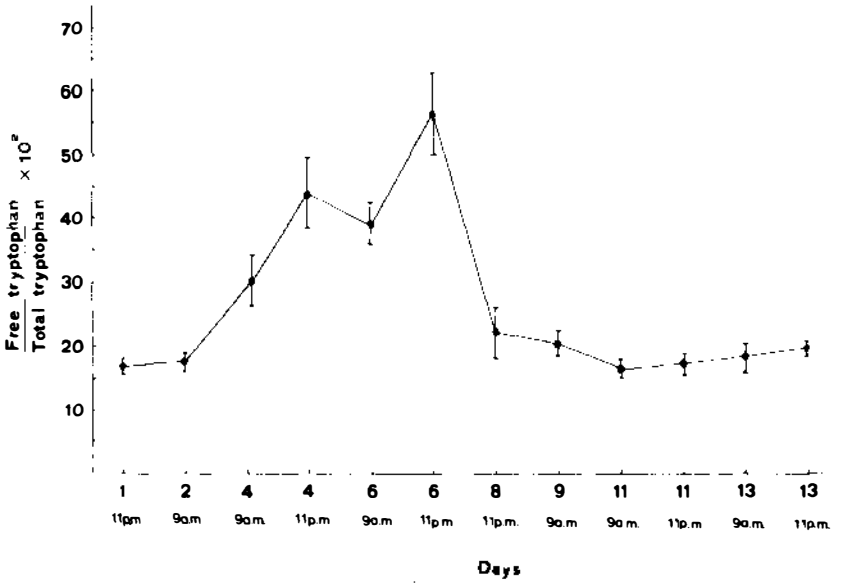

Figure 2 Percentage of free over total tryptophan during clofibrate and placebo treatments in normal human subjects (43). Days 1–6, clofibrate treatment; days 8–13, placebo.

Other factors, however, must be taken into account before assessing the significance of tryptophan displacement for the central regulation of the serotoninergic mechanisms. First, an egression mechanism from the CSF is continuously operant for tryptophan. Similarly to all highly protein-bound agents, tryptophan concentrations in plasma and CSF are in equilibrium, and any increase in CSF tryptophan might be concealed by a simultaneous removal of tryptophan from the CSF. This can explain the failure of probenecid to induce a significant increase of free tryptophan levels in human subjects.

The other observation is that there is no definite proof that an increase in 5HT synthesis is necessary to relieve depression. Treatment with tricyclic antidepressants lowers 5HIAA in the CSF (50, 51) and this effect is more clearly observed following probenecid administration (because of blockade of 5HIAA egress or increased availability of free tryptophan). Bowers (52) has recently shown that large doses of tryptophan administered to depressed patients failed to increase 5HIAA in the CSF when amitriptyline was given concomitantly. Tryptophan concentrations in the CSF were not changed following amitriptyline. Because it is not known whether amitriptyline can inhibit tryptophan hydroxylase, it can only be concluded at this moment that an increase in 5HT synthesis does not appear to be a requirement for the correction of clinical depression.

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