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THE EFFECTS OF CHLORPROMAZINE ON SERUM TRYPTOPHAN, BRAIN TRYPTOPHAN UPTAKE AND BRAIN SEROTONIN SYNTHESIS IN THE RAT

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Abstract—Following intraperitoneal injection of a single dose of chlorpromazine to rats, serum total tryptophan fell significantly, as did the concentration of serum total amino acids. At the same time there was a decrease in the binding of tryptophan to serum albumin, which was not attributable to any change in serum non-esterified fatty acid concentration. After repeated administration of chlorpromazine (over 10 days), serum tryptophan returned to normal within 4 days, as did the binding of tryptophan to serum albumin. However, serum total amino acid concentration remained depressed for at least 10 days, returning to the control level immediately on cessation of administration of the drug. Cessation of chlorpromazine administration had no effect on serum tryptophan concentration or binding to serum albumin. Studies on brain tryptophan uptake and serotonin accumulation showed an increase in brain serotonin pool size following single or repeated chlorpromazine administration. Although serotonin accumulation was significantly correlated with tryptophan uptake into the brain, the increase in serotonin per unit increase in brain tryptophan uptake was less after 11 days repeated chlorpromazine administration than after a single dose. This suggests that some factor other than tryptophan availability is concerned in regulation of brain serotonin synthesis; this could be feed-back inhibition by serotonin.

It is generally accepted that a major factor affecting the rate of serotonin synthesis in the central nervous system is the availability in the brain of the precursor amino acid, tryptophan [1]. Uptake of tryptophan into the brain is believed to be controlled by two factors: the blood concentrations of other large neutral amino acids (phenylalanine, tyrosine, leucine, isoleucine, valine and methionine) which compete with tryptophan for the uptake mechanism [2]; and the concentration of that small fraction of blood tryptophan that is not bound to serum albumin, but is freely diffusible [3]. Evidence of the importance of competing amino acids has been obtained *in vivo* by Gessa *et al.* [4].

Studies of serum tryptophan in chlorpromazine-treated chronic schizophrenics [5] have shown considerably lower than normal serum total tryptophan concentrations. In these patients, tryptophan binding to serum albumin was less than normal, resulting in approximately normal concentrations of freely diffusible tryptophan. On withdrawal of chlorpromazine from a similar group of patients [6] there was an increase in serum tryptophan concentration, and a considerable increase in that fraction not bound to albumin, leading to a very considerable increase in the concentration of freely diffusible tryptophan. Serum total amino acid concentrations, initially slightly lower than normal, also showed a marked increase on withdrawal of the drug. The changes in serum tryptophan appeared to follow the same relatively slow time-course as behavioural deterioration and recovery in response to chlorpromazine withdrawal and restoration, while changes in total amino acid concentration were more rapid, and appeared to correspond more or less directly with changes in drug status.

In order to attempt to differentiate between drug-induced effects and abnormalities due to the schizophrenic syndrome in these patients, the effects of chlorpromazine on serum tryptophan and brain serotonin synthesis have been assessed in rats.

MATERIALS AND METHODS

Female Courtauld Institute Wistar rats weighing between 90 and 110 g were used. They were injected intraperitoneally (i.p.) with a solution of chlorpromazine hydrochloride in 0.15 M sodium chloride, or saline alone. All animals were deprived of food, but not water, for 24 hr before killing. To minimise the effects of diurnal variation, all injections were performed between 09.00 hr and 09.30 hr, and all animals were killed between 13.00 hr and 14.00 hr. Animals were killed by decapitation; blood was collected and allowed to clot at room temperature before centrifugation at 2000 *g* to separate serum. Dialysis studies (see below) were performed within 1 hr of killing; aliquots of serum for other assays were stored at -20° until required. Brains and other tissues were dissected out rapidly and frozen in liquid nitrogen; they were stored at -20° until required.

Serum tryptophan was determined by the norharman fluorescence method [7]. Tissue tryptophan concentrations were determined by a modification of the same method; tissues were homogenised while still frozen in 100 g/l trichloroacetic acid containing 0.3 m-mole/l ferric chloride and, after centrifugation to remove denatured protein, the supernatant was used for determination of tryptophan. The extent of tryptophan binding to serum albumin was determined by small-scale equilibrium dialysis [8], serum non-esterified fatty acid concentration by the formation of solvent-extractable copper soaps [9] and serum total amino acid concentration by the formation of fluorescent zinc-stabilised pyridoxal derivatives [10].

The uptake of tryptophan into the brain and its conversion to serotonin were assessed by a modification of the method of Hyyppä *et al.* [11]. Six min before killing, animals were injected i.p. with 0.1 ml of a solution of [G-^{3}H]-L-tryptophan in 0.15 M sodium chloride (6.4 Ci/m-mole, 0.17 μCi/ml). Brains were dissected out and frozen in liquid nitrogen, then stored at $-20°$ until required. They were homogenised while still frozen in 7 ml of 0.4 M perchloric acid containing 10 g/l ascorbic acid. After centrifugation to remove denatured protein (20 min at 2000 *g*) the supernatant was adjusted to pH 2.0 by addition of 0.4 M sodium hydroxide solution. It was then poured over 2-cm long columns of Dowex 50 X-8 ion exchange resin (acid form, 350 mg dry wt of resin per column). The columns were then washed with 6-ml of water. Tryptophan was eluted with 15 ml of 0.1 M sodium acetate adjusted to pH 6.0, and after washing the columns with a further 6 ml of water, serotonin was eluted with 4 or 6 ml of 0.5 M trisodium phosphate. Elution with 4 ml gave slightly lower recovery of serotonin, but increased the concentration, thus rendering subsequent fluorimetric assay more reliable. Aliquots of these column eluates were used for measurement of radioactivity, using a water miscible scintillant (5 g PPO, 0.5 g POPOP, 250 ml Triton X-100 per litre of toluene) in a Packard liquid scintillation spectrometer. With up to 1 ml of eluate in 15 ml of scintillant, ^{3}H counting efficiency was routinely between 35 and 45 per cent. Similar aliquots of the eluates were used for assay of tryptophan (by a modification of the norharman fluorescence method [7] and serotonin by the following modification of the *o*-phthaldialdehyde fluorescence method [12]. 1 ml of the sample was mixed with 1 ml of a solution of 100 mg/l *o*-phthaldialdehyde and 100 mg/l L-cysteine hydrochloride in 12 M hydrochloric acid, heated at 105° for 15 min and cooled in tap water before fluorimetry (excitation 360 nm, fluorescence 480 nm, uncorrected wavelengths). Recovery of tryptophan and serotonin through this procedure was assessed using ^{14}C-labelled material added to the brain supernatants at pH 2.0; recovery of tryptophan was 70–75 per cent and of serotonin 75–80 per cent.

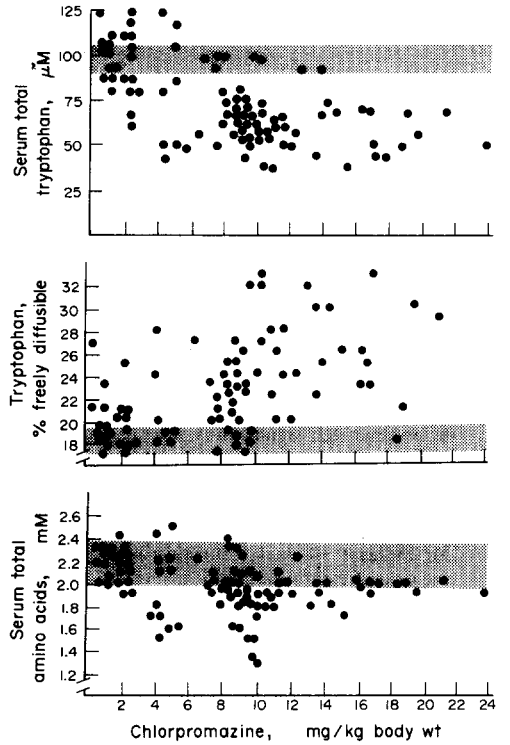

Fig. 1. Responses of serum total tryptophan, albumin binding of tryptophan and serum total amino acid concentration to a single dose of chlorpromazine hydrochloride. The shaded areas represent mean ± S.D. for 25 saline-injected control animals.

RESULTS

The effects of a single administration of chlorpromazine on serum tryptophan, albumin binding of tryptophan and serum total amino acid concentration are shown in Fig. 1. Four hr after administration of chlorpromazine at 10 mg/kg body wt and above, there was a significant reduction in serum tryptophan and total amino acid concentrations compared with saline-injected control rats. The percentage of tryptophan freely diffusible (i.e. not bound to serum albumin) was significantly elevated at the same dose of the drug, although the concentration of non-esterified fatty acids (known to displace tryptophan from albumin binding [8, 13] was not affected, even at higher doses of the drug. In all subsequent experiments described here, chlorpromazine was given at 10 mg/kg body wt.

The effect of repeated daily administration of chlorpromazine at this dose is shown in Fig. 2. After about 4 days, serum tryptophan concentration returned to the control level; at the same time the percentage

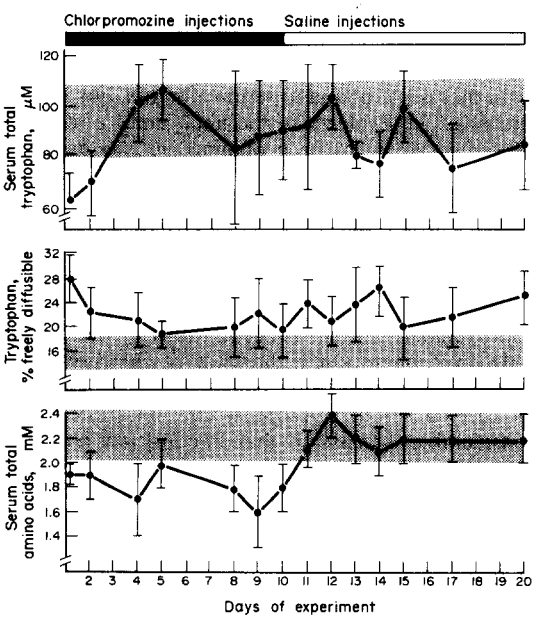

Fig. 2. Responses of serum total tryptophan, albumin binding of tryptophan and serum total amino acid concentration to repeated administration of chlorpromazine and following withdrawal. Points represent mean ± S.D. for 5 animals per group. The shaded areas represent mean ± S.D. for 25 saline-injected control animals. Chloropromazine hydrochloride (10 mg/kg body wt) given i.p. for up to 10 days, as shown by the solid bar.

Table 1. Tissue trichloroacetic acid soluble tryptophan following administration of a single dose of chlorpromazine (10 mg/kg body wt) to female rats

	Control	Chlorpromazine
	(nmole tryptophan/g wet wt tissue)	
Liver	5.2 ± 0.7	13.1 ± 3.7*
Spleen	1.2 ± 0.5	4.7 ± 0.6*
Pancreas	11.8 ± 3.3	20.1 ± 5.4*
Heart	9.7 ± 1.5	7.9 ± 1.3
Lung	4.2 ± 2.1	6.4 ± 4.3
Kidney	9.5 ± 2.3	11.9 ± 2.7

* Significantly different from control, $P < 0.02$ (t-test). (Figures show mean ± S.D., 5 animals in each group).

of tryptophan freely diffusible fell towards the control level, although it remained slightly (but not statistically significantly) above the control level throughout the remainder of this experiment. The concentration of serum total amino acids remained significantly lower than in control animals throughout the 10 days of the experiment. On withdrawal of chlorpromazine after 10 days repeated administration, the serum total amino acid concentration returned to the control level; there was no evidence of any compensatory rise in serum amino acids as seen in schizophrenic patients on withdrawal of chlorpromazine [6]. The concentration of serum tryptophan was unaffected by cessation of chlorpromazine administration, as was binding to albumin.

The concentration of trichloroacetic acid soluble tryptophan (i.e. tryptophan not incorporated into proteins) was measured in various tissues from animals treated with a single dose of chlorpromazine and in control (saline injected) animals. As can be seen from Table 1, there was a significant increase in trichloroacetic acid soluble tryptophan in liver, spleen and pancreas; because of its small size (about 500 mg), the contribution of pancreatic tryptophan uptake to whole body tryptophan metabolism is unlikely to be important. There was also a slight (non-significant) increase in trichloroacetic acid soluble tryptophan in lung and kidney following administration of a single dose of chlorpromazine, and a slight fall in the heart. Following repeated administration of the drug for 10 days, the concentrations of trichloroacetic acid soluble tryptophan in these tissues returned to the control levels.

In order to assess the relative rates of tryptophan uptake into the brain, and its conversion to serotonin, rats were injected 6 min before killing with a tracer amount of [^{3}H]tryptophan, and radioactive tryptophan and serotonin were then determined in the brains of saline-injected animals and animals treated with a single dose of 10 mg chlorpromazine/kg body wt, or the same dose repeated for 11 days. The results obtained are shown in Table 2. There was a non-significant increase in the brain tryptophan concentration of chronically chlorpromazine-treated animals, and the uptake of radioactive tryptophan was slightly increased following single or repeated administration of the drug. There was a significant increase in the size of the brain serotonin pool following a single dose of chlorpromazine, and a further significant increase following repeated doses of the drug. Although in animals given repeated doses of the drug there was a slight increase in the amount of radioactive serotonin accumulated during the 6 min between injection of radioactive tryptophan and killing, there was no change in animals given a single dose of the drug.

DISCUSSION

There is a considerable amount of evidence that a major factor regulating the rate of serotonin synthesis in the central nervous system is the availability of tryptophan. *In vitro* determination of the K_m of the first enzyme of the pathway, tryptophan hydroxylase (L-tryptophan monooxygenase, EC 1.14.16.4) has suggested that the enzyme normally functions at less than a saturating substrate concentration, even in the presence of what is assumed to be the natural cofactor, tetrahydrobiopterin [14]. This implies that an increase in the concentration of tryptophan available to the enzyme would be reflected in an increase in the synthesis of 5-hydroxytryptophan. Since there is no evidence that the decarboxylation of 5-hydroxytryptophan to serotonin is rate limiting, and there is normally a considerably greater activity of 5-hydroxytryptophan decarboxylase (L-aromatic amino acid carboxy-lyase, EC 2.6.1.26) than of tryptophan hydroxylase in brain tissue [15], this would lead to an increase in the synthesis of serontonin. *In vivo* studies support this view; an increase in cerebral tryptophan concentration has been shown to lead to an increase in the rate of serotonin synthesis [16].

In view of this evidence, it would be expected that in the present work, where an increase in brain tryptophan concentration has been observed, there would also be an increase in the accumulation of radioactive

Table 2. Brain tryptophan and serotonin in chlorpromazine-treated rats following i.p. administration of [^{3}H]tryptophan

	Saline-injected controls (n = 11)	Chlorpromazine injected animals: Single dose (n = 11)	Chlorpromazine injected animals: 11 days treated (n = 18)
	Concentration, nmole/g wet wt tissue		
Tryptophan	91.9 ± 12.4	100.0 ± 14.1	155.3 ± 33.7
Serotonin	2.4 ± 0.1	2.8 ± 0.3*	3.4 ± 0.4*†
	Radioactivity, nCi/g wet wt tissue		
Tryptophan	9.9 ± 1.6	12.5 ± 4.2	16.1 ± 4.4
Serotonin	5.7 ± 0.9	5.7 ± 2.3	6.6 ± 1.8

* Significantly different from control animals $P < 0.001$ (t-test).
† Significantly different from single dose group $P < 0.001$ (t-test).
(Figures show mean ± S.D., numbers of animals as shown).

serotonin. In Fig. 3, the uptake of radioactive tryptophan into the brain (nCi accumulated per g of tissue in 6 min) has been plotted against the accumulation of radioactive serotonin during the same time. Considering all points together, there is a significant correlation ($r = 0.59$, $n = 39$), thus providing support for the hypothesis that increased brain tryptophan uptake leads to increased serotonin synthesis. However, if the two groups of chlorpromazine-treated animals are considered separately it can be seen that different regression lines can be drawn through the points representing acutely-treated animals (gradient = 0.446) and those treated with the drug for 11 days (gradient = 0.299). These two gradients (computed by unweighted least squares procedure) are significantly different ($P < 0.001$). The relative increase in serotonin synthesis per unit increase in brain tryptophan accumulation was greater in animals given a single dose of chlorpromazine than in animals treated with the drug for 11 days. This suggests that some factor other than tryptophan availability also acts to regulate serotonin synthesis from tryptophan in the brain.

It is possible that the degradation of serotonin by monoamine oxidase (monoamine oxygenase, EC 1.4.3.4) is reduced after prolonged chlorpromazine therapy. While this would lead to an increase in the size of the brain serotonin pool, as observed here, without requiring an increase in the rate of synthesis, it would not account for the observed lower rate of serotonin synthesis with increased tryptophan accumulation observed in the chronically-treated animals compared with animals treated with a single dose of the drug.

There is some evidence of feed-back inhibition of serotonin synthesis *in vivo*, possibly acting by means of serotoninergic overstimulation, and thus by a neuronal rather than enzymic mechanism [16, 17]. Although there seems to be little unequivocal evidence of inhibition of tryptophan hydroxylase by serotonin [14], there is clear evidence that 5-hydroxytryptophan decarboxylase is inhibited *in vitro* by its product [18]. It is unclear how important this inhibition would be *in vivo*.

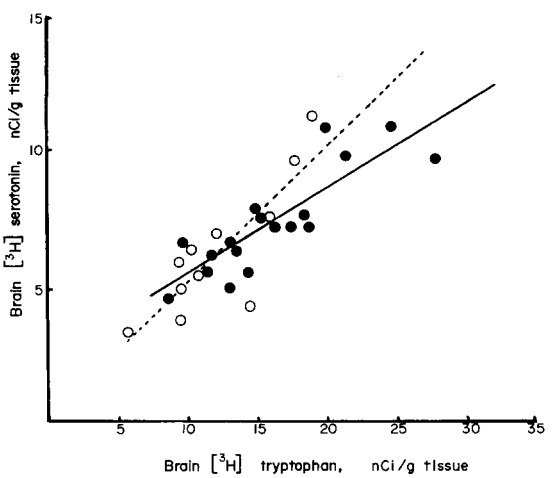

Fig. 3. The relationship between brain tryptophan uptake and serotonin accumulation in chlorpromazine-treated rats. Open circles and the broken line represent animals treated with a single dose of chlorpromazine hydrochloride (10 mg/kg body wt). Filled circles and the solid line represent animals treated with the same dose of the drug for 11 days.

Although a single dose of chlorpromazine led to a reduction in total serum tryptophan, this was apparently compensated for by a displacement of tryptophan from albumin binding, and a decrease in the concentrations of those amino acids which compete with tryptophan for uptake into the brain, so that there was an increase in brain serotonin synthesis following chlorpromazine administration. When the drug was given repeatedly over several days, serum tryptophan returned to normal, but total amino acid concentration remained depressed; under these conditions there was a greater increase in brain serotonin synthesis than following a single dose of chlorpromazine, providing evidence of the role of competing amino acids in serum as a regulatory factor for brain serotonin synthesis [2, 3]. However, as well as the availability of tryptophan in the brain, another factor appears to be involved in regulation of brain serotonin synthesis; this appears to be feed-back inhibition by serotonin.

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