Increase in Brain Tryptophan and 5-Hydroxytryptamine on Administration of Phenothiazines to Rats

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A major factor regulating the synthesis of the neurotransmitter 5-hydroxytryptamine in the brain is the availability of the precursor amino acid, tryptophan. The $K_m$ of tryptophan hydroxylase (L-tryptophan mono-oxygenase, EC 1.14.16.4) is of the same order of magnitude as the brain concentration of tryptophan (Gal, 1974). Uptake of tryptophan into the brain is believed to be regulated by that fraction of serum tryptophan that is freely diffusible, rather than by the total serum tryptophan concentration (Etienne et al., 1976) and by the concentrations in serum of those amino acids that compete with tryptophan for the uptake mechanism (Gessa & Tagliamonte, 1974). It therefore seems likely that measurement of diffusible serum tryptophan and the concentration of those amino acids that compete with tryptophan may allow the relative rate of brain 5-hydroxytryptamine synthesis to be predicted.

It has been shown previously (Bender, 1976a) that administration of chlorpromazine to rats results in a decrease in the total serum tryptophan concentration, and a decrease in the proportion of tryptophan bound to serum albumin, so that the concentration of diffusible tryptophan remains at or above the control value. At the same time there is a decrease in the total serum amino acid concentration. These serum changes are accompanied by an increase in the uptake of tryptophan into the brain and an increase in the synthesis of 5-hydroxytryptamine.

Previous work from this laboratory has also shown that a number of anti-schizophrenia drugs all had effects on serum tryptophan concentrations, albumin binding of tryptophan and total serum amino acid concentrations similar to those shown by chlorpromazine (Bender, 1976b). It would therefore be predicted that these drugs would lead to an increase in brain tryptophan uptake and 5-hydroxytryptamine synthesis. Confirmation of this prediction is presented here for three anti-schizophrenia drugs chemically related to chlorpromazine: prochlorperazine, chlorprothixene and thioridazine. At the same time, two further phenothiazines, which have no significant anti-schizophrenia action, trimeprazine and promethazine, have also been tested.

Female Courtauld Institute rats, weighing 90–100 g, were used for these experiments. They were deprived of food, but not water, for 24 h, and then the drugs, dissolved in 0.15 M-NaCl, were administered by intraperitoneal injection, at 09:00 h. Control animals received NaCl solution alone. The animals were killed by decapitation between 13:00 and 14:00 h. Blood was collected for preparation of serum, and livers and brains were rapidly dissected out and frozen in liquid N$_2$. They were stored at $-20^\circ$C until required for analysis. Total serum tryptophan was measured by the method of Denckla & Dewey (1967), tryptophan binding to albumin by the equilibrium-dialysis method of Bender et al. (1975), total serum amino acids by the method of Maeda & Tsuji (1973); brain tryptophan and 5-hydroxytryptamine were separated and measured by the method described previously (Bender, 1976a) and liver tryptophan oxygenase [L-tryptophan–oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] activity was measured by a modification of the method of Knox et al. (1966).

Table 1 shows the effects of the drugs on serum tryptophan concentration, albumin binding of tryptophan and total serum amino acid concentrations. As reported previously (Bender, 1976b) the anti-schizophrenia phenothiazines led to a decrease in total serum tryptophan and a decrease in the proportion of tryptophan bound to serum albumin, so that the concentration of freely diffusible serum tryptophan was the same as, or slightly higher than, in control animals. The two phenothiazines without anti-schizophrenia action both led to a slight increase in total serum tryptophan. Trimeprazine, but not promethazine, decreased the percentage of tryptophan freely diffusible.
Table 1. Effects of phenothiazines on serum and brain parameters

The results are means±s.D. with the number of determination in parentheses.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/animal)</th>
<th>Serum tryptophan (µM)</th>
<th>Diffusible tryptophan (%)</th>
<th>Serum total amino acids (µM)</th>
<th>Liver tryptophan oxygenase (munits/g wet wt.)</th>
<th>Brain tryptophan (nmol/g wet wt.)</th>
<th>Brain 5-hydroxytryptamine (nmol/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 24)</td>
<td>—</td>
<td>96.8 ± 7.7</td>
<td>11.4 ± 1.7</td>
<td>2.24 ± 0.19</td>
<td>320 ± 35</td>
<td>96.7 ± 20.2</td>
<td>2.47 ± 0.36</td>
</tr>
<tr>
<td>Chlorpromazine (n = 8)</td>
<td>1</td>
<td>62.8 ± 10.4†</td>
<td>28.3 ± 6.5*</td>
<td>1.82 ± 0.11†</td>
<td>294 ± 28†</td>
<td>95.5 ± 7.5</td>
<td>2.87 ± 0.35†</td>
</tr>
<tr>
<td>Prochlorperazine (n = 8)</td>
<td>2</td>
<td>61.8 ± 17.3†</td>
<td>17.6 ± 6.6*</td>
<td>2.52 ± 0.23*</td>
<td>313 ± 32</td>
<td>141.7 ± 31.8*</td>
<td>2.99 ± 0.28*†</td>
</tr>
<tr>
<td>Chlorprothixene (n = 8)</td>
<td>4</td>
<td>59.4 ± 8.8†</td>
<td>26.1 ± 9.9*</td>
<td>1.53 ± 0.09†</td>
<td>245 ± 50†</td>
<td>200.6 ± 46.3*</td>
<td>4.44 ± 0.73*†</td>
</tr>
<tr>
<td>Thoridazine (n = 8)</td>
<td>6</td>
<td>49.7 ± 6.4†</td>
<td>36.2 ± 10.2*</td>
<td>1.82 ± 0.23†</td>
<td>322 ± 52</td>
<td>197.8 ± 26.4*</td>
<td>5.60 ± 0.53*†</td>
</tr>
<tr>
<td>Promethazine (n = 8)</td>
<td>10</td>
<td>104.0 ± 8.2</td>
<td>11.6 ± 1.6</td>
<td>1.50 ± 0.14†</td>
<td>257 ± 16†</td>
<td>148.5 ± 28.9*</td>
<td>3.23 ± 0.47*†</td>
</tr>
<tr>
<td>Trimeprazine (n = 8)</td>
<td>10</td>
<td>134.1 ± 12.2*</td>
<td>8.1 ± 1.1†</td>
<td>2.02 ± 0.22†</td>
<td>289 ± 24†</td>
<td>177.3 ± 26.5*</td>
<td>2.81 ± 0.24*†</td>
</tr>
</tbody>
</table>

* Significantly greater than control animals, P<0.02, by Student’s t test.
† Significantly less than control animals, P<0.02, by Student’s t test.
Apart from promethazine, which significantly increased total serum amino acid concentration, all the drugs led to a decrease in total serum amino acids.

It is possible that the decrease in total serum tryptophan could be due to increased catabolism catalysed by liver tryptophan oxygenase. However, as shown in Table 1, the activity of this enzyme was unchanged in animals treated with prochlorperazine or thioridazine, and significantly decreased in other groups. Thus although the fall in serum tryptophan after administration of the anti-schizophrenia drugs cannot be explained by a change in the activity of tryptophan oxygenase, the decreased activity of this enzyme might explain the increase in serum tryptophan concentration after promethazine or trimeprazine administration. None of the drugs tested had any effect in vitro on the activity of tryptophan oxygenase, even at concentrations very much higher than might be expected in vivo after administration.

Brain tryptophan and 5-hydroxytryptamine concentrations are also shown in Table 1. As reported previously, chlorpromazine had no effect on the concentration of tryptophan in the brain, although it has been shown to increase the uptake of trace amounts of labelled tryptophan (Bender, 1976a). All the other drugs significantly increased the brain tryptophan concentration, and all, including chlorpromazine, increased the concentration of 5-hydroxytryptamine.

These results can be considered to give further evidence for the hypothesis that both diffusible serum tryptophan (rather than the total serum tryptophan concentration) and the concentrations of competing amino acids are important factors in the regulation of brain 5-hydroxytryptamine synthesis. Thus the increase in tryptophan diffusibility, despite a decrease in total serum tryptophan, was sufficient to lead to an increase in brain tryptophan uptake and 5-hydroxytryptamine synthesis in the prochlorperazine-treated animals, in which the total serum amino acid concentration was significantly increased compared with control animals. Similarly, with promethazine, where there was a marginal increase in diffusible serum tryptophan, and trimeprazine, where the increase in total serum tryptophan was countered by a decrease in the proportion freely diffusible, so that the concentration of diffusible tryptophan was the same as in control animals, the decrease in total serum amino acids was apparently sufficient to allow increased brain tryptophan uptake and 5-hydroxytryptamine synthesis.

It appears that a decrease in total serum amino acid concentration may be a general effect of phenothiazines, although effects on serum tryptophan concentration and displacement of tryptophan from albumin binding seem to be limited to those phenothiazines with anti-schizophrenia action. It is possible that the effect on 5-hydroxytryptamine synthesis may be connected with the anti-schizophrenia action of these drugs, and that 5-hydroxytryptaminergic neurons may be involved in schizophrenia.

P. M. C. was supported in this work by a grant from The Wellcome Trust. The drugs used were generous gifts from the manufacturers: chlorpromazine and prochlorperazine, May and Baker, Dagenham, Essex, U.K.; chlorprothixene, Roche Products, Welwyn Garden City, Herts., U.K.; thioridazine, Sandoz Products, Leeds, W. Yorks., U.K. Promethazine and trimeprazine were obtained from the hospital dispensary.

Bender, D. A. (1976a) Biochem. Pharmacol. 25, 1743–1746
Bender, D. A. (1976b) Biochem. Soc. Trans. 4, 98–100