EFFECT OF IMIPRAMINE ON CENTRAL 5-HYDROXYTRYPTAMINE TURNOVER AND METABOLISM IN RATS

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Abstract—The levels of [3H]-5-HT and [3H]-5-HIAA in P2-fraction from rats treated with a single dose of imipramine were lower at 2, 5 and 10 min after intraventricular administration of [3H]-5-HT, compared with control animals, though these levels were statistically insignificant. Thereafter, however, the values became higher than in the controls. After long-term administration of imipramine, the levels of [3H]-5-HT and [3H]-5-HIAA in P2-fraction were, at any time after intraventricular administration of [3H]-5-HT, significantly lower than those in the fraction from controls. The endogenous contents of tryptophan and 5-HIAA were not altered either with a single dose or by long-term administration of imipramine, however, the content of endogenous 5-HT was decreased by long-term administration of imipramine. These results indicate that the rate of disappearance of 5-HT from presynaptic neurons was accelerated by long-term administration of imipramine.

The major neurochemical mechanism involved in the action of imipramine and other related tricyclic antidepressants has been ascribed to an inhibition of 5-hydroxytryptamine (5-HT) and/or norepinephrine (NE) at the neuronal membrane, after which there is an increase in the synaptic levels of these amine transmitters (1–5). However, these data were acquired with acute administration of a single dose, while the clinical efficacy of these drugs has been observed after about 3 weeks administration.

In a previous study from our laboratory, we found that a single i.p. administration of imipramine produced an inhibition in the uptake of intraventricularly administered [3H]-5-HT into synaptosomal fractions, however, the effect was transient and 10 min later the level of [3H]-5-HT in the fraction from imipramine-treated rats was higher than that in the fraction obtained from the non-treated rats (5). These results indicate that the rate of disappearance of 5-HT from nerve-endings is decreased after a single dose of imipramine. Thus, we attempted to determine if there are differences between the effects of single and long-term administrations of imipramine on 5-HT turnover and metabolism in brain.

MATERIALS AND METHODS

Effect of imipramine on the uptake of intraventricularly administered 5-HT: 5-Hydroxy-[G-3H]-tryptamine creatinine sulphate ([3H]-5-HT, 12.4 Ci/mmol) was obtained from The Radiochemical Centre, Amersham. Imipramine hydrochloride was generously donated from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). In acute experiments, male Wistar
rats (200-260 g) were given imipramine (30 mg/kg i.p.) and 30 min after injection, [3H]-5-HT dissolved in 1.5 μl of saline containing 0.02% ascorbate was given intraventricularly in a dose of 0.75 μCi through an implanted cannula. In chronic experiments, male Wistar rats (135-140 g) were given (at 10:00 a.m. and 5:00 p.m.) imipramine (10 mg/kg) or saline solution (10 ml/kg i.p., twice daily for 3 weeks) and were housed 5 per cage in a room with a controlled temperature (24±1 °C) and humidity (55±5%), with food and water ad libitum. Thirty min after the final injection, [3H]-5-HT was given intraventricularly, as described for the acute experiments.

The animals were decapitated 2 to 180 min after [3H]-5-HT injection and the whole brain (except cerebellum) was rapidly removed and dissected. The tissue was homogenized with 10 vols. of ice-cold 0.32 M sucrose in a homogenizer with a Teflon pestle driven mechanically at 1,200 rev/min (clearance 0.25 mm). The homogenates were centrifuged at 4°C for 10 min at 900 g. The pellet was washed twice with 0.32 M sucrose and the washings were added to the supernatant which was then centrifuged at 11,500 g for 20 min to produce a crude mitochondrial P2 fraction as a pellet. [3H]-5-HT and [3H]-5-hydroxyindoleacetic acid ([3H]-5-HIAA) in P2-fraction were extracted by the method of Curzon & Green (6) and the radioactivity in the fraction was determined in a model 3320 Packard Tri-Carb liquid scintillation spectrometer and corrected for efficiency by external standardization.

Effect of imipramine on the uptake of intraventricularly administered 5-HT: Male Wistar rats of either 120-150 g (in acute experiment) or of 105-110 g (in chronic experiment) were used. Imipramine and saline solution were given i.p. as described above. The animals were decapitated 15 min to 24 hr after imipramine or saline injection (after final injection in chronic experiments) and the whole brain (excluding cerebellum) was rapidly removed, dissected and homogenized with 9 vols. of acidified n-butanol made up of 0.85 ml conc. hydrochloric acid per liter of n-butanol. Tryptophan was measured by the method of Denckla & Dewey (7). The determination of 5-HT and 5-HIAA was made according to the method of Curzon & Green (6).

RESULTS

Effect of imipramine on the uptake of intraventricularly administered 5-HT: The results are shown in Figs. 1 and 2. Intraventricularly administered 5-HT was rapidly taken up into the P2-fraction. Thus, in control rats, within 2 min after injection of [3H]-5-HT, the amount of 5-HT was approx. 80% of the highest value at 10 min. A rapid decrease was observed between 10 to 30 min and thereafter, a slow decrease was evident up to 180 min (Fig. 1). Rate of increase and decrease of [3H]-5-HIAA in the P2-fraction after intraventricular administration of [3H]-5-HT was similar to that seen with [3H]-5-HT (Fig. 2). Compared with control animals, 5-HT and 5-HIAA levels in P2-fractions from rats treated with a single dose of imipramine (30 mg/kg) were lower at 2, 5 and 10 min after 5-HT administration, though these were statistically non-significant. Thereafter, however, the values became higher than those of the control. In contrast, after long-term administration of imipramine, the levels of both [3H]-5-HT and [3H]-5-HIAA in P2-fractions were, at any time
after intraventricular administration of [3H]-5-HT, significantly lower than those in the fraction from controls (Figs. 1, 2). Effect of imipramine on 5-HT metabolism.

**Fig. 1.** Effect of imipramine on the uptake of intraventricularly administered 5-HT. Rats were given a single dose of imipramine (30 mg/kg i.p., ○) or were given imipramine (10 mg/kg i.p. twice daily for 3 weeks, ▲) or saline solution (10 ml/kg, ◯). Thirty min after this injection, [3H]-5-HT was given intraventricularly and the radioactivity was determined in the P2-fraction. Values are the mean of 5 determinations, vertical line indicates S.E.M. *Significantly different from control. Ordinate—[3H]-5-HT in P2-fraction.

**Fig. 2.** Effect of imipramine on the uptake of intraventricularly administered 5-HT. Rats were given a single dose of imipramine (30 mg/kg i.p., ○) or imipramine (10 mg/kg i.p. twice daily for 3 weeks, ▲) or saline solution (10 ml/kg, ◯). Thirty min after injection, [3H]-5-HT was given intraventricularly and the radioactivity was determined in P2-fraction. Values are the mean of 5 determinations, vertical line indicates S.E.M. *Significantly different from control. Ordinate—[3H]-5-HIAA in P2-fraction.
TABLE 1. Effect of imipramine on 5-HT metabolism

<table>
<thead>
<tr>
<th>Time before sacrifice</th>
<th>Drugs</th>
<th>Tryptophan (g/g)</th>
<th>5-HT (g/g)</th>
<th>5-HIAA (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>Single</td>
<td>Control</td>
<td>1.58±0.08</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.59±0.08</td>
<td>0.58±0.07</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>Control</td>
<td>1.64±0.06</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.63±0.03</td>
<td>0.53±0.02*</td>
</tr>
<tr>
<td>180 min</td>
<td>Single</td>
<td>Control</td>
<td>1.52±0.07</td>
<td>0.56±0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.45±0.04</td>
<td>0.57±0.04</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>Control</td>
<td>1.59±0.08</td>
<td>0.59±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.55±0.09</td>
<td>0.50±0.02*</td>
</tr>
<tr>
<td>24 hr</td>
<td>Single</td>
<td>Control</td>
<td>1.56±0.08</td>
<td>0.55±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.48±0.09</td>
<td>0.59±0.06</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>Control</td>
<td>1.49±0.08</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>1.45±0.08</td>
<td>0.51±0.02*</td>
</tr>
</tbody>
</table>

*R: P<0.05

Rats were given a single dose of imipramine (30 mg/kg i.p.) or were given imipramine (10 mg/kg i.p.) twice daily for 3 weeks. Controls were given saline solution (10 ml/kg i.p.). Values are the means of 4 determinations, vertical line indicates S.E.M.

The results are presented in Table 1. The contents of endogenous tryptophan and 5-HIAA in the rat brain were not altered significantly either with a single dose or with long-term administration of imipramine. The content of endogenous 5-HT in the brain was not altered with a single dose of imipramine but was decreased significantly with long-term administration of this drug.

DISCUSSION

A single dose of imipramine tended to decrease the levels of [3H]-5-HT and [3H]-5-HIAA in P2-fractions at 2, 5 and 10 min after 5-HT administration, though these values were statistically non-significant. This decrease is probably due to uptake inhibition of [3H]-5-HT by imipramine. Subsequent increase in [3H]-5-HT and [3H]-5-HIAA in P2-fraction did contribute to the decreased rate in disappearance from the nerve-endings. The inhibition of 5-HT uptake by imipramine may increase the concentration of 5-HT at the receptor sites and with negative feedback mechanisms, decrease the rate of disappearance from the presynaptic neurons.

In contrast, after long-term administration of imipramine, the decreased levels of [3H]-5-HT and [3H]-5-HIAA in P2-fraction were observed up to 180 min after [3H]-5-HT injection. Furthermore, the content of endogenous 5-HT in the brain was significantly lower after a long-term administration of imipramine than after administration of saline. These results indicate that the rate of disappearance of 5-HT from presynaptic neurons was accelerated by long-term administration of imipramine. Although final conclusions cannot be drawn from our present results, we have obtained additional evidence that long-term administration of tricyclic antidepressants to rats produces a development of central
5-HT receptor subsensitivity (8). Therefore, it seems reasonable to conclude that long-term administration of imipramine, increases the levels of 5-HT at the synapse, induces a compensatory decrease in 5-HT receptor sensitivity, which in turn, by a positive feedback mechanism, accelerates the rate of disappearance of 5-HT out of presynaptic neurons. Whether such an acceleration in the rate of transmitter disappearance induced by long-term administration of imipramine is related to its clinical effect or only is a subsequent phenomenon to the development of central 5-HT receptor subsensitivity is now being investigated.

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REFERENCES