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Effect of chlorimipramine on the metabolism of 5-hydroxytryptamine in the rat brain

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THE MECHANISM of action of tricyclic antidepressant drugs on central monoamine metabolism involves blockade of the membrane pump, probably at the level of the nerve cell membrane. Chlorimipramine has been found to be very effective in blocking the re-uptake of 5-hydroxytryptamine (5-HT).[1-5] It has been suggested that blockade of the re-uptake increases the amount of 5-HT available at receptor sites. Increased receptor activity might then induce a lower rate of 5-HT turnover via a negative feedback mechanism affecting 5-HT synthesis.[6]

Meek and Werdinius[7] inferred that chlorimipramine reduced 5-HT turnover by about 30 per cent. Utilizing probenecid to block the efflux of 5-hydroxyindoleacetic acid (5-HIAA), they found a decrease in 5-HIAA in chlorimipramine-pretreated animals. They found no alteration in endogenous levels of 5-HT after chlorimipramine, but no time course data were reported. Although the blocking of efflux of 5-HIAA after probenecid seems to be complete,[8] there is recent evidence that probenecid also affects brain tryptophan and 5-HT.[9,10] Nor is it known whether interactions of probenecid with other drugs might mask effects of chlorimipramine on 5-HT metabolism.

Because a sequence of different mechanisms over time may be involved in the effects of chlorimipramine, experiments to investigate the basic time course data of changes in endogenous levels of 5-HT and 5-HIAA after a single dose of chlorimipramine were undertaken.

Male Sprague–Dawley rats (150–250 g) were injected intraperitoneally with chlorimipramine (25 mg/kg) in a volume of 1·3 ml/kg. The animals were sacrificed by decapitation at various intervals and the brains were assayed for 5-HT essentially according to the method described by Andén and Magnusson,[11] and for 5-HIAA by the method of Jonsson and Lewander.[12] Standards were run with each assay and the values were corrected for recoveries.

TABLE 1. EFFECT OF CHLORIMIPRAMINE ON ENDOGENOUS LEVELS OF 5-HT AND 5-HIAA

Time (min)	5-HT (ng/g brain)		5-HIAA (ng/g brain)	
	Control	Chlorimipramine	Control	Chlorimipramine
15	511 ± 12 (7)	480 ± 15 (7)	293 ± 5 (7)	259 ± 5 (7)†
30	499 ± 7 (7)	547 ± 14 (8)‡	271 ± 7 (7)	249 ± 7 (8)‡
45	500 ± 15 (4)	538 ± 9 (5)‡	296 ± 13 (4)	233 ± 8 (6)§
60	473 ± 16 (5)	486 ± 14 (6)		
90	450 ± 10 (5)	440 ± 11 (6)		
120	448 ± 19 (5)	441 ± 25 (5)	320 ± 6 (6)	271 ± 10 (6)§
150	444 ± 9 (5)	430 ± 3 (5)	307 ± 5 (7)	240 ± 10 (8)†
180	463 ± 11 (7)	443 ± 9 (8)	313 ± 6 (7)	219 ± 9 (8)†
210	479 ± 12 (7)	444 ± 5 (8)‡	299 ± 6 (7)	247 ± 5 (8)†

* Each value represents the mean 5-HT or 5-HIAA level ± S.E.M. for the number of animals in parentheses.
† $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.005$.

The changes in endogenous levels of brain 5-HT and 5-HIAA with time after a single dose of chlorimipramine (25 mg/kg, i.p.) are summarized in Table 1. These data are more easily visualized when plotted as per cent of control values, as in Fig. 1. The drug initially causes small but significant increases in brain 5-HT at 30 and 45 min. Thereafter, 5-HT levels decline slightly but consistently over time and at 210 min this decrease becomes statistically significant. 5-HIAA levels show a significant decrease at all times studied, the most pronounced effect being observed at 180 min.

The cause of the effect of chlorimipramine on 5-HT is uncertain. Reflection on the time course (Fig. 1) suggests that the effect of chlorimipramine cannot be only a matter of re-uptake blockade

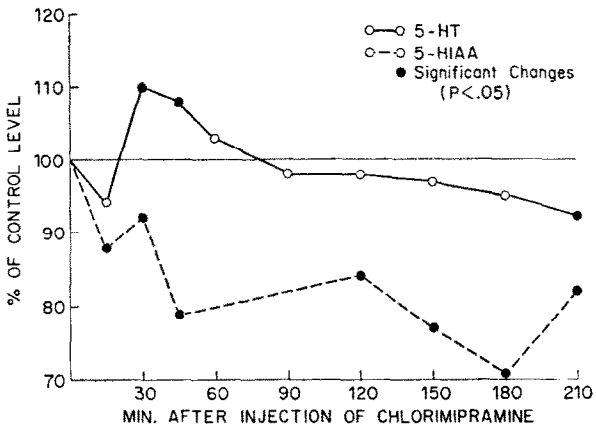

FIG. 1. Time course of the effect of 25 mg/kg of chlorimipramine on the metabolism of 5-HT. The changes in 5-HT and 5-HIAA are plotted as per cent of the control levels at each time. The absolute values are presented in Table 1.

because the 5-HIAA levels are reduced no matter whether 5-HT is above, at or below control levels; the decline in 5-HT over time may indicate that the rate of its metabolism is altered or that its accessibility to monoamine oxidase (MAO) is affected or that it is being metabolized in a different pathway. Thus, one could offer the following hypothetical sequence of events. 5-HT initially (30–45 min) accumulates as a result of the blockade of re-uptake; this shift in the disposition of 5-HT is initially best measured by the diminished 5-HIAA at 15 min. The measurable accumulation of 5-HT at 30 and 45 min probably takes place at the extraneuronal receptor sites; these events over 45 min possibly induce an increased receptor stimulation. Negative feedback mechanisms might then initiate a reduction in the rate of 5-HT synthesis. This effect could be reflected in the trend toward a decrease of 5-HT, which becomes significant at 210 min.

The fact that 5-HIAA levels are reduced over the whole time course after one dose of chlorimipramine might be explained by the assumption that the drug prevents 5-HT from reaching MAO. However, the peak depletion of 5-HIAA is reached at 180 min after chlorimipramine. The same sequence of events as described for 5-HT could be postulated for 5-HIAA. Thus, the initial reduction between 30 and 45 min would merely be the result of the blockade of re-uptake: 5-HT accumulates extraneuronally and consequently 5-HIAA drops. If 5-HT synthesis is slowed down via a feedback mechanism at a later point in time, maximal depletion of 5-HIAA (30 per cent at 180 min) would reflect this. The assumption that a negative feedback mechanism exists can to an extent be tested by studies *in vivo*, already in progress, of the incorporation of label from radioactive precursors into the amine and its metabolite over time.

In a recent study, Sheard *et al.*[13] reported that chlorimipramine and other tertiary amine antidepressants depress the firing rate of midbrain raphe neurons. To explain this phenomenon, they postulated a negative feedback resulting from the accumulation of 5-HT at the synapse secondary to a blockade in the re-uptake of 5-HT. It is tempting to speculate whether the same sequence of events is responsible for both the biochemical changes reported above and for these electrophysiological observations. Thus, the accumulation of but small amounts of 5-HT at postsynaptic receptor sites would be considered as initiating the slowing of firing, which then results in a reduced rate of 5-HT synthesis. This reduced synthesis assumed to be contingent upon slowed neuronal firing, is reflected in the declining endogenous levels of the amine over time. In this connection, it would be important to know how long it takes the raphe neurons to recover from a single dose of chlorimipramine. Thus, a more accurate correlation between the biochemical and the electrophysiological events could be established.

While the immediate decrease of 5-HIAA levels after chlorimipramine may be solely ascribed to membrane blockade of 5-HT and hence inaccessibility to MAO, the question of a possible inhibitory effect of this compound on brain MAO remains. In two series of experiments, we investigated the activity of MAO, both *in vitro* and *in vivo*, in relation to chlorimipramine. MAO activity *in vitro* was assayed by the method of Robinson *et al.*;[14] mitochondria purified by differential and density gradient centrifugation[15,16] were used as enzyme source. The substrate was ^{14}C-serotonin. The linearity of the correlation between labeled 5-HIAA production and enzyme concentration was established in

preliminary experiments to determine appropriate conditions for the MAO assay. We found that chlorimipramine does inhibit MAO *in vitro* 30–40 per cent at a concentration of 10^{-4}M. This inhibition drops to 5–10 per cent at 10^{-5} and 10^{-6}M concentrations. Whether this degree of MAO inhibition *in vitro* has any physiological significance relative to the decreases in 5-HIAA reported here is still unknown. The experiments *in vivo*, in which we injected chlorimipramine intraperitoneally (25 mg/kg) and isolated the brain mitochondrial fraction at different intervals, showed no inhibition of MAO.

Although tricyclic compounds can accumulate at relatively high concentrations in the brain,[17] interpretation of data on their effects on brain MAO activity is difficult in the light of evidence for the existence of multiple forms[18–20] as well as an extraneuronal localization of the enzyme in brain.[21–24] The possible existence of an extraneuronal deaminating enzyme, wherever it be localized, renders the interpretation of the morphological site of biochemical events solely from endogenous 5-HIAA data hazardous. Here we simply report our data and raise the question about the possible role of MAO in the effects of chlorimipramine. Failure to detect inhibition of MAO *in vivo* does not necessarily rule out such a possibility, since (for example) a reversibly bound inhibitor could be washed out in the course of the manipulations associated with subcellular fractionation. A further explanation for the discrepancy between the data *in vitro* and *in vivo* could be that the drug is not accessible to the enzyme after systemic application. Experiments on the subcellular localization of radioactively labeled compounds, currently underway, may reveal whether they preferentially bind to any of the subcellular organelles.

These results show that the effects of chlorimipramine on endogenous brain 5-HT and 5-HIAA most probably involve different processes, re-uptake blockade and possible changes in the synthesis rate, depending on the time after the drug. The measured levels of endogenous 5-HT and 5-HIAA reflect the operation of different mechanisms over time.

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