SEROTONIN DEPLETION WITH P-CHLOROPHENYLALANINE IN THE CAT: EFFECTS ON CARBACHOL-INDUCED DEFENSIVE BEHAVIOR AND REGIONAL BRAIN AMINE CONTENT

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Abstract. Serotonin (5-HT) depletion with p-chlorophenylalanine (p-CPA) (3 × 150 mg/kg/12 h i.p.) in the cat resulted in an increase in the carbachol-induced growling response 36 h after the last injection. Besides important depletion of 5-HT in the anterior and posterior hypothalamus, midbrain and amygdala, and 5-hydroxyindoleacetic acid (5-HIAA) in the anterior and posterior hypothalamus and midbrain, dopamine (DA) level significantly increased in the amygdala and posterior hypothalamus with tendency to increase in the anterior hypothalamus and midbrain. No changes in noradrenaline (NA) level were observed. The results indicate that the carbachol-induced defensive behavior significantly increased as a consequence of reduction in the 5-HT system activity. The changes in DA content indicate that there are interactions between the 5-HT/DA systems.

INTRODUCTION

Numerous studies have demonstrated that various manipulations leading to the lowering of serotonin (5-HT) level in the brain or the blocking of serotonergic transmission bring about an increase in motor activity, as well as a rise in the general emotional arousal, enhanced irritability and aggression, and also hypersexuality. These effects have been obtained after blocking 5-HT synthesis by means of p-chlorophenylalanine (p-CPA) administration (8, 11, 16, 18, 20, 28-30, 33), after nuclei raphe lesions (3, 6, 15, 25, 34-36), after a diet lacking in tryptophan (9,
32) as well as after blocking 5-HT receptors (1, 10, 17). On the basis of these data it has been generally assumed that the 5-HT system exerts an inhibiting influence on motor and motivational behavior, and above all, on different types of emotional-defensive responses. However, the 5-HT system does not function independently but in connection with other neurotransmitter systems in the brain; there exist mutual and multidirectional functional interactions between them (13, 14, 22, 24). The functional interactions between the 5-HT and catecholaminergic systems have only been partly studied and being supported mainly by the results of pharmacological investigation they require to be confirmed by biochemical research in still many cases. In addition, few studies have been conducted on cats (2, 8, 10, 11, 25) which possess a number of different biological properties as compared to rats.

The present investigation was undertaken in order to give answer to the following questions: (i) what influence the decrease of 5-HT level in the brain exerts on the carbachol-induced emotional-defensive response, which is the best representative of cat’s behavior in natural conditions after being exposed to threat, (ii) whether and what correlations exist between defensive behavior evoked by intrahypothalamic carbachol injections and neurochemical processes in the “emotional areas” of the brain (hypothalamus, midbrain and amygdala).

MATERIAL AND METHODS

Subjects and surgery

The experiments were carried out on 11 cats of either sex. All cats had chronically bilaterally implanted cannulas to the hypothalamus according to the stereotaxic coordinates of Sőrder and Niemer’s atlas (27): A = 13.0, L = 1.5, H = -3.0. Other details of the surgery and micro-injection procedure were described earlier (26).

Drugs

Carbachol (carbachol puriss, Koch-Light) was dissolved in 0.9% NaCl solution and injected bilaterally 4 μg in 1 μl into each part of the hypothalamus. p-Chlorophenylalanine (DL-p-chlorophenylalanine, Sigma) was prepared in a concentration of 150 mg/3 cm³ in a 0.9% NaCl. The animals were given i.p. p-CPA for a total of 450 mg/kg, according to the schedule: 150 mg/kg 08:00 p.m.; 150 mg/kg 08:00 a.m. and 150 mg/kg 08:00 p.m. the next day. A control group was treated with the solvent in the same way in a volume of 3 cm³/kg.
**Experimental procedure**

The intensity of the emotional-defensive behavior evoked by bilateral carbachol injections into the hypothalamus was evaluated by recording the total number of growls, the total time of their duration, the total time of vocalization, and latency period of growling response (see 4 for details). The response was considered completed if a growl was not followed by another within 3 min. All the cats were tested one time for the growling response, and it was an initial (control) level of the carbachol-induced emotional-defensive behavior. Next, the cats were injected i.p. with p-CPA (experimental group, $n = 5$) and i.p. with $0.9\%$ NaCl (control group, $n = 6$), and the intensity of the carbachol-induced growling response was measured on the 12th and 36th h after p-CPA or NaCl injections. Six hours after the last test for the carbachol-induced growling response all cats were killed by decapitation; their brains were rapidly removed and four structures, i.e. the anterior hypothalamus (HA) (frontal planes A 11.0-15.0), the posterior hypothalamus (HP) (frontal planes A 7.0-10.0), the midbrain central grey matter (GC) (frontal planes A 1.0-5.0), and the amygdala (AM) including parts of nuclei cortico-medialis and basolateralis (frontal planes A 11.0-14.0) were separated with dissection and kept frozen. Concentrations of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in HA, HP, GC and AM were determined spectrofluorometrically according to the method of Earlier and Leonard (7).

**Statistics**

Biochemical results were elaborated by the Student $t$ test for unrelated data; the significance of changes in the growling response was evaluated by the Student $t$ test for paired data.

**RESULTS**

Thirty six hours following p-CPA injection a considerable rise of post-carbachol emotional-defensive response was observed as reflected by an increase in the number of growls by $81.4\%$ ($P < 0.02$) and the time duration of growling by $65.3\%$ ($P < 0.01$) (Fig. 1A). No significant changes in any of the parameters of the growling response in the control group after NaCl injection were observed (Fig. 1B).

In the p-CPA-treated animals a very big decrease of 5-HT level occurred, in HA by $89\%$, in HP by $94\%$, in GC by $95\%$ and in AM by $86\%$. It was accompanied by a drop of 5-HIAA level, in HA by $46\%$, in HP by $57\%$ and in GC by $80\%$, whereas there were no statistically significant changes in AM though a tendency to rise appeared. The
Fig. 1. The effect of i.p. p-CPA (A) and 0.9% NaCl (B) administration on growling response evoked by intrahypothalamic carbachol injections. Mean latent period of growling response (L), mean number of growls (N), mean time duration of growling (T) and mean time duration of vocalization response (D) ± SEM. All values are expressed as a percentage of initial level before p-CPA or NaCl treatment. Differences from initial values: * $P < 0.02$, ** $P < 0.01$ (paired t statistics).

level of NA increased only in AM by 103% ($P < 0.01$), and the level of DA increased considerably in HP by 129% ($P < 0.01$) as well as in AM by 53% ($P < 0.02$) with a tendency to rise in HA and GC (Table I).

**Table I**

Regional brain concentrations of NA, DA, 5-HT and 5-HIAA in p-CPA-treated cats

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>Amine content in μg/g wet tissue (mean±SEM)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Control (n-6)</td>
<td>HA</td>
<td>0.754±0.104</td>
</tr>
<tr>
<td>p-CPA (n-5)</td>
<td>0.642±0.056</td>
<td>2.124±0.887</td>
</tr>
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<td></td>
<td></td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Control (n-6)</td>
<td>HP</td>
<td>0.509±0.090</td>
</tr>
<tr>
<td>p-CPA (n-5)</td>
<td>0.541±0.116</td>
<td>1.475±0.242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>Control (n-6)</td>
<td>GC</td>
<td>0.336±0.043</td>
</tr>
<tr>
<td>p-CPA (n-5)</td>
<td>0.232±0.042</td>
<td>0.718±0.105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Control (n-6)</td>
<td>AM</td>
<td>0.304±0.048</td>
</tr>
<tr>
<td>p-CPA (n-5)</td>
<td>0.618±0.078</td>
<td>2.711±0.294</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.01$</td>
</tr>
</tbody>
</table>
No visible changes in the cats' general behavior either in the experimental chamber or in the home cage were observed. There was an absence of spontaneous aggression and we did not observe any enhanced motor activity, which as a rule occurs in p-CPA-treated rats (16, 18, 29-31, 33).

**DISCUSSION**

The obtained results showed that 36 h following p-CPA administration to the cats there appeared a significant increase in post-carbachol growling response (the number of growls and time duration of growling), which speaks for the rise of central emotional-defensive arousal (4, 5). The decrease of 5-HT level in all examined structures and decrease of 5-HIAA level in HA, HP and GC speaks for the fact that the change was closely correlated with reduced turnover of 5-HT, thus with diminished activity of the serotonergic system. Simultaneously, there occurred a considerable increase in DA level in HP and AM with a tendency to rise in HA by 104\% (NS) and GC by 28\% (NS) explicitly. The level of NA increased only in AM by 103\% (P < 0.01).

Our findings are congruent with some other authors' experiments which demonstrated that various types of emotional-defensive responses are intensified as a consequence of 5-HT decrease in the brain. However, unlike in the experiments performed on rats, in our investigation we never dealt with either spontaneous aggression or enhanced motor activity. We obtained identical effects after a chemical lesion of 5-HT dorsal raphe nuclei neurons (25).

In spite of the fact that the test of DA metabolites was not carried out, which was caused by the methodological limitations, and that we cannot determine changes in the turnover of this neurotransmitter, nevertheless, an increase in DA level clearly speaks for the rise of the amine synthesis, and consequently it indirectly points to intensified activity of the DA system. The increase of DA level occurring together with the diminished activity of the 5-HT system that we observed provides direct biochemical evidence of interactions between the 5-HT and DA systems. In the references these are the only data obtained in the experiments conducted on cats. The increase of DA level can be interpreted as a consequence of tonic inhibition reduction of the DA system by means of the 5-HT system, which for a long time has been suggested on the grounds of pharmacological studies providing only indirect evidence in favor of the above mentioned assumption (13, 14, 24). These findings, however, are not in agreement with the investigations of Reader et al. (21-23) who claimed that in p-CPA-treated rats the depression
of 5-HT and 5-HIAA levels is accompanied by a decrease of NA and DA in the frontoparietal cortex, hippocampus, midbrain, pons-medulla, cerebellum and spinal cord. These authors interpret the result as a consequence of releasing the NA and DA systems from the inhibitory control of 5-HT system, which leads to an enhanced release of these amines in the synaptic endings and in consequence to the decrease of their levels in the brain tissue. Perhaps there exists only an apparent discrepancy between our investigation and this of Reader et al. caused by the fact that these authors used high doses of p-CPA (2 × 400 mg/kg and 2 × 500 mg/kg); moreover they determined the content of amines 48 h after the last p-CPA injection. In our investigation the employed dose was 450 mg/kg, and the content of amines was determined after 42 h. Hence, the activity of a high p-CPA dose after a longer period of time might well result in depletion of catecholamines after the loss of tonic inhibition of the 5-HT system. It is not unlikely either that high p-CPA doses besides the blocking of tryptophan hydroxylase, inhibit some other enzymes, indirectly depressing the catecholamine synthesis as well. Such an activity of higher doses was indicated by Koe and Weissman (12) as well as by Miller et al. (19).

The absence of changes in NA level (except AM) as well as the lack of metabolites measurement of this amine did not allow us, unfortunately, to obtain the information if and what functional interactions occur between the 5-HT and NA systems.

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REFERENCES


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