Effect of pentylenetetrazol on the carbaryl-induced changes of serotonin metabolism in rat-brain hypothalamus

S. K. RAY and M. K. PODDAR

Department of Biochemistry, University College of Science,
35 Ballygunge Circular Road, Calcutta University,
Calcutta 700-019, India

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Carbaryl (200 mg/kg or 400 mg/kg, p.o.) significantly elevated serotonin (5-HT) (57-109%) and 5-hydroxyindoleacetic acid (5-HIAA) (60-78%) levels at 1.0 h in the hypothalamic region of adult male rat brain. Further, administration of carbaryl (200 mg/kg, p.o.) for different time intervals (0.5 h, 1.0 h, and 2.0 h) revealed that both 5-HT and 5-HIAA levels elevated maximally at 0.5 h in hypothalamus. These regional 5-HT and 5-HIAA levels were not significantly affected with pentylenetetrazol (PTZ) at any time after its treatment. But simultaneous administration of carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) reduced the carbaryl-induced elevation of both 5-HT and 5-HIAA levels. Measurement of (i) probenecid-induced (200 mg/kg, i.p.) accumulation and (ii) pargyline-induced (75 mg/kg, i.p.) depletion of hypothalamic 5-HIAA level in the absence or presence of carbaryl (200 mg/kg, p.o.) and/or PTZ (60 mg/kg, s.c.) revealed that (a) carbaryl enhanced the synthesis as well as the breakdown of 5-HT, (b) PTZ had no effect on either of these processes of 5-HT, and (c) carbaryl-induced increased catabolism of 5-HT became normal in the presence of PTZ.

Carbaryl (1-napthyl N-methylcarbamate) is an extensively used carbamate insecticide. Acute intoxication of the rat by this insecticide was reported to inhibit brain cholinesterase activity (1,2) and increase brain serotonin (5-HT) synthesis (3) as well as the level of 5-hydroxyindoleacetic acid (5-HIAA) (3,4). In a recent study it has been shown that this insecticide enhances the synthesis as well as the breakdown of central catecholamines (5). Pentylenetetrazol (PTZ), a well-known convulsant, mediates its action through the involvement of central serotonergic (6-8) and catecholaminergic (9) systems. Recently we have also shown that this drug (PTZ) becomes less potent in the presence of carbaryl at the level of brain catecholamine metabolism (10). These observations led us to study the effect of carbaryl and/or PTZ in hypothalamus (a serotonergic neuron-rich region) of rat brain at the level of 5-HT metabolism.
Materials and Methods

Adult male albino Charles Foster rats (body wt. 120-150 g) maintained at a temperature of 28 °C with normal laboratory diet and water ad libitum, were divided into four groups. Group I - Rats were subjected to a single oral (p.o.) administration of carbaryl (10% w/v, suspended in ground-nut oil) either at different dosages (200 mg/kg and 400 mg/kg) for 1.0 h or at a single dosage (200 mg/kg) for different time intervals (0.5 h, 1.0 h, and 2.0 h). Group II - Rats were treated with a convulsive dose of PTZ (60 mg/kg, s.c.). Group III - Rats were simultaneously treated with carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) Group IV - Rats of this group were treated with the equivalent amount of corresponding vehicles of PTZ (s.c.) and/or carbaryl (p.o.). To study the synthesis of 5-HIAA, rats were either treated with carbaryl (200 mg/kg, p.o.) and/or PTZ (60 mg/kg, s.c.) or their corresponding vehicles for 1.0 h to rats pretreated with probenecid (200 mg/kg, i.p.) for 0.5 h. To study the efflux of 5-HIAA from the hypothalamic region, pargyline (75 mg/kg, i.p.) was administered to the rats immediately after treatment either with carbaryl (200 mg/kg, p.o.) and/or PTZ (60 mg/kg, s.c.) or with their corresponding vehicles and the rats were sacrificed after 1.0 h. All the rats were sacrificed by cervical dislocation between 1000 and 1200 h. The brain tissues were immediately taken out and dissected under ice-cold (0-4°C) conditions in order to get the hypothalamic region according to the method described by Poddar and Dewey (11). 5-HT and 5-HIAA were assayed spectrofluorometrically by the method of Snyder et al. (12) and Haubrich and Denzer (13) respectively. Carbaryl (99.7% pure) was obtained from Union Carbide Ltd., Bhopal, India. PTZ was obtained from Boehringer-Knoll, Bombay, India.

Results

It is evident from Table I that a single oral administration of carbaryl at both lower (200 mg/kg, p.o.) and higher (400 mg/kg, p.o.) dosages caused a significant elevation of both 5-HT (57-109%) and 5-HIAA (60-78%) levels in the hypothalamic region. Figs. 1 and 2

<table>
<thead>
<tr>
<th>Nature of Dose</th>
<th>5-HT Level (%)</th>
<th>5-HIAA Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.00 ± 15.00a</td>
<td>100.00 ± 7.5</td>
</tr>
<tr>
<td>Carbaryl 200</td>
<td>157.00 ± 15.00a</td>
<td>160.00 ± 11.34c</td>
</tr>
<tr>
<td>Carbaryl 400</td>
<td>209.00 ± 22.00b</td>
<td>178.00 ± 16.57b</td>
</tr>
</tbody>
</table>

Mean values significantly different from control, aP < 0.05, bP < 0.01, cP < 0.005. Control values (ng/mg, wet wt.) for hypothalamic 5-HT and 5-HIAA contents were 1.97 ± 0.29 and 1.57 ± 0.12 respectively.
show the effect of carbaryl (200 mg/kg, p.o.) and/or PTZ (60 mg/kg, s.c.) at different time intervals on the steady-state levels of 5-HT and 5-HIAA respectively. It appears from Fig. 1 that carbaryl (200 mg/kg, p.o.) significantly increased the levels of 5-HT at 0.5 h (140%) and 1.0 h (57%). A significant elevation of 5-HIAA level was also demonstrated at 0.5 h (65%) and 1.0 (60%) under similar conditions (Fig. 2). Treatment of rats either with PTZ or with carbaryl and PTZ did not cause any significant change in the levels of either 5-HT or 5-HIAA (Fig. 1 and 2). The accumulation of 5-HIAA induced by probenecid (a blocker of 5-HIAA transport from brain (14)) was taken as an index to study the effect of carbaryl (200 mg/kg, p.o.) and/or PTZ (60 mg/kg, s.c.) on the formation of 5-HIAA (Table 2). Table 2 shows that probenecid induced a significant further increase in accumulation of 5-HIAA after carbaryl treatment. No significant change in the probenecid-induced accumulation of 5-HIAA level was demonstrated in the brain region of rats treated either with PTZ or with carbaryl and PTZ. The depletion of 5-HIAA level induced by pargyline (a monoamine oxidase inhibitor) may be taken as an index to study the efflux of 5-HIAA from brain (Table 2). Table 2 shows that carbaryl and/or PTZ did not affect the efflux of 5-HIAA from the hypothalamic region of the brain.
Table 2. Effect of PTZ (60 mg/kg, s.c.) on the probenecid-induced (200 mg/kg, i.p.) accumulation and pargyline-induced (75 mg/kg, i.p.) reduction of 5-HIAA in the hypothalamic region of normal and carbaryl-treated (200 mg/kg, p.o.) rats

Carbaryl and/or PTZ or their corresponding vehicles were administered to the rats pretreated for 0.5 h with probenecid. All the rats were sacrificed at 1.5 h after probenecid. Probenecid-induced accumulation of 5-HIAA is about 45% of the corresponding vehicle-treated group. Pargyline was administered to the rats immediately after the treatment with carbaryl and/or PTZ or their corresponding vehicles. All the rats were sacrificed at 1.0 h after pargyline. Pargyline reduced the 5-HIAA level by about 39% with respect to the corresponding vehicle-treated group. Values are means ± S.E.M., n = 4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5-HIAA concentration(%)</th>
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<tbody>
<tr>
<td>Probenecid</td>
<td>100.00 ± 10.86</td>
</tr>
<tr>
<td>Probenecid + PTZ</td>
<td>116.21 ± 18.27</td>
</tr>
<tr>
<td>Probenecid + carbaryl</td>
<td>145.52 ± 11.85*</td>
</tr>
<tr>
<td>Probenecid + carbaryl + PTZ</td>
<td>86.89 ± 15.17</td>
</tr>
<tr>
<td>Pargyline</td>
<td>100.00 ± 8.16</td>
</tr>
<tr>
<td>Pargyline + PTZ</td>
<td>85.71 ± 12.24</td>
</tr>
<tr>
<td>Pargyline + carbaryl</td>
<td>114.28 ± 10.61</td>
</tr>
<tr>
<td>Pargyline + carbaryl + PTZ</td>
<td>97.96 ± 8.16</td>
</tr>
</tbody>
</table>

*P < 0.05, mean value significantly different from that of the group treated with probenecid alone.

Fig. 2. Effect of carbaryl (○), PTZ (△), and carbaryl + PTZ (●) on the steady-state level of hypothalamic 5-HIAA at different time intervals. 100% at 0.0 h represents the control value of 5-HIAA (1.57 ± 0.12 ng/mg wet wt.). Mean values significantly different from control, *P < 0.005, **P < 0.001.
Discussion

A significant elevation of hypothalamic 5-HT level after carbaryl administration either at lower or at higher dosage may be due to (i) increased synthesis or (ii) decreased breakdown of 5-HT. But the second assumption is unlikely as carbaryl increases the level of 5-HIAA (Table 1) without affecting its efflux process (Table 2). Thus it may be suggested that administration of carbaryl increases the synthesis as well as the breakdown of 5-HT. The increased catabolism of 5-HT may further be supported by an increase in the probenecid-induced accumulation of 5-HIAA (Table 2) and a significant stimulation of monoamine oxidase activity (5). The lack of significant change in (i) the steady-state level of 5-HT and 5-HIAA (Figs. 1 and 2), (ii) probenecid-induced accumulation of 5-HIAA level (Table 2), and (iii) pargyline-induced depletion of 5-HIAA (Table 2) in this brain region of rat treated with either PTZ or carbaryl and PTZ suggests that PTZ alone or in conjunction with carbaryl does not affect the metabolism of 5-HT in the hypothalamic region of the rat brain. Thus finally it may be concluded that a single oral administration of carbaryl increases the turnover of 5-HT in the hypothalamic region of rat brain and this carbaryl-induced increase in 5-HT turnover is reduced towards normal when rats are simultaneously treated with carbaryl and PTZ.

Acknowledgement

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References