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EFFECT OF PENTYLENETETRAZOL ON CARBARYL-INDUCED CHANGES IN STRIATAL CATECHOLAMINES

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Abstract—Administration of pentylenetetrazol (PTZ) (60 mg/kg, s.c.) to normal or carbaryl (200 mg/kg, p.o.) treated adult male albino rats produced characteristic changes in the steady-state levels of striatal dopamine (DA), noradrenaline (NA) and homovanillic acid (HVA) at different time intervals (0.5, 1.0 and 2.0 hr). The elevation of striatal NA level was found to be more pronounced with PTZ than that produced by carbaryl. Treatment of rats with PTZ alone caused a significant elevation of DA levels only at 2.0 hr without any significant change in the level of HVA at any time interval. Carbaryl which did not have any significant effect on striatal DA level produced an elevation of HVA at 0.5 hr and 1.0 hr in striatum. The simultaneous administration of PTZ and carbaryl, under similar conditions, caused a marked reduction in the level of NA at 0.5 hr and DA at 1.0 hr without any significant effect on (i) both the amine levels at 2.0 hr and (ii) HVA level at any of the time intervals. Measurement of (a) α-methyl-*p*-tyrosine (α-MpT) (250 mg/kg, i.p.) induced depletion of striatal DA and NA, (b) FLA-63 (25 mg/kg, i.p.) induced disappearance of NA, (c) pargyline (75 mg/kg, i.p.) induced reduction and probenecid (200 mg/kg, i.p.) induced accumulation of striatal HVA in the presence or absence of PTZ and/or carbaryl revealed that: (1) PTZ or carbaryl alone caused a significant increase in the turnover of striatal DA; (2) the turnover of striatal NA was significantly increased after PTZ treatment but not after carbaryl administration; (3) the simultaneous administration of carbaryl and PTZ, on the other hand, attenuated (a) PTZ- or carbaryl-induced increase in metabolic activity of the striatal dopaminergic system, and (b) the enhanced anabolic activity of striatal noradrenergic system caused by PTZ, but failed to affect the enhanced utilization of striatal NA induced by PTZ alone.

Pentylenetetrazol (PTZ) is a powerful convulsant which finds its use as a central stimulant. Recently several workers have implicated the involvement of catecholamines in PTZ-induced convulsions [1–3]. Acute intoxication by the anticholinesterase agent, carbaryl (a frequently used pesticide), has also been shown to interfere with neurotransmitter metabolism [4, 5]. Recently we have reported that carbaryl increased central catecholamine metabolism [6]. In the present investigation we were interested in studying the effect of PTZ, if any, on carbaryl-induced changes in brain striatal catecholamines.

MATERIALS AND METHODS

Adult male albino rats (Charles Foster strain) weighing 120–150 g, maintained at a temperature of 28 ± 1° with normal laboratory diet and water *ad libitum* were taken for this study. The rats were divided into four groups. Each group contained six or eight animals. Group I: rats were treated with a convulsive dose of PTZ (60 mg/kg, s.c.); group II: a single dose (200 mg/kg) of carbaryl (10% w/v, suspended in groundnut oil) was administered orally (p.o) to the rats; group III: rats were simultaneously treated with PTZ (60 mg/kg, s.c.) and carbaryl (200 mg/kg, p.o.); group IV: rats of this group were treated with the corresponding vehicles of PTZ (s.c.) and/or carbaryl (p.o.). All the rats were sacrificed by cervical dislocation (between 10 a.m. and 12 noon) at different time intervals (0.5, 1.0 and 2.0 hr) after treatment. The brain was quickly removed and kept on ice (0–4°). The corpus striatum region of brain was dissected out according to the method described by Poddar and Dewey [7]. All biochemical parameters were measured using the corpora striata from two animals for each determination. To study the effects of the above treatments on turnover of dopamine (DA) and noradrenaline (NA) in the corpus striatum, the following inhibitors of the metabolic pathway of catecholamines were used: (a) α-methyl-*p*-tyrosine (Sigma), an inhibitor of the rate limiting enzyme tyrosine hydroxylase; (b) Pargyline (Sigma), an inhibitor of monoamine oxidase; (c) FLA-63 (Astra), an inhibitor of dopamine-β-hydroxylase; and (d) Probenecid (Merck, Sharp & Dohme), a known blocker of the acid metabolite transport from brain. The dose used and experimental techniques followed were described in detail under the respective tables. The DA and NA concentrations were estimated by the spectrofluorometric method of Welch and Welch [8]. Striatal homovanillic acid (HVA) was extracted and estimated spectrofluorometrically by the method of Haubrich and Denzer [9] and Anden *et al.* [10] respectively. Monoamine oxidase (MAO) activity of the mitochondrial fraction was assayed using tyramine as substrate by the method of Green and Haughton [11] as modified by Guha [12]. The results were statistically analysed, using a two-tailed Student's *t*-test. The significance of the result was calculated with respect to control unless otherwise mentioned. Carbaryl (obtained

from Union Carbide Ltd., Bhopal, India) was of Technical Grade (99.7%). PTZ was obtained from Boehringer-Knoll.

RESULTS

Figures 1 and 2 showed the effect of PTZ (60 mg/kg, s.c.) and/or carbaryl (200 mg/kg, p.o.) on the striatal DA and NA levels at different time intervals (0–2 hr). It appears from Fig. 1 that a single dose of PTZ (60 mg/kg, s.c.) significantly elevated (36%) striatal DA level at only 2.0 hr. This PTZ induced elevation of DA level was significantly decreased (25%, $P < 0.005$ compared to PTZ treated group) and became normal when PTZ was administered along with carbaryl (200 mg/kg, p.o.). Carbaryl alone did not produce any significant change in the striatal DA level at only 2.0 hr. This PTZ-induced which failed to exhibit any significant change in the striatal DA concentration at 1.0 hr after the individual treatment with carbaryl or PTZ, showed a significant decline in this parameter ($P < 0.005$, 0.05 and 0.05 compared to vehicles, PTZ and carbaryl treated groups respectively) after the simultaneous treatment with carbaryl and PTZ. Figure 2 reveals that PTZ produced a time-dependent significant increase in the striatal NA level. This enhanced level of NA (0.5–2.0 hr) was not observed when the rats were treated with PTZ in presence of carbaryl; rather there was a significant decline in NA concentration at 0.5 hr (45%, $P < 0.001$ compared to control and 53%, $P < 0.001$ compared to PTZ treated group) and 2.0 hr (56%, $P < 0.005$ compared to PTZ treated group). Carbaryl alone also produced an elevation of striatal NA levels at 2.0 hr (54.77%). The simultaneous treatment of rats with carbaryl and PTZ, on the other hand, reduced (30%, $P < 0.05$ compared to carbaryl treated group) the NA level at 2.0 hr induced by carbaryl alone.

The effect of carbaryl and/or PTZ on the utilization of DA and NA in striatum have been studied by measuring α-M_pT- or FLA-63-induced disappearance of catecholamine(s). Table 1 indicates that PTZ alone accelerated the α-M_pT-induced disappearance of both DA (60%) and NA (55%). This acceleration of α-M_pT-induced depletion of DA and NA levels were significantly decreased by 26% ($P < 0.001$) and 31% ($P < 0.05$) respectively (compared to α-M_pT + PTZ treated group) when PTZ was treated along with carbaryl. Carbaryl alone under these conditions accelerated the α-M_pT-induced depletion of DA (40%) and NA (32%). Table 2 shows that the FLA-63-induced decrease of NA level was significantly augmented with PTZ (58%) or PTZ in presence of carbaryl (60%) but not with carbaryl alone.

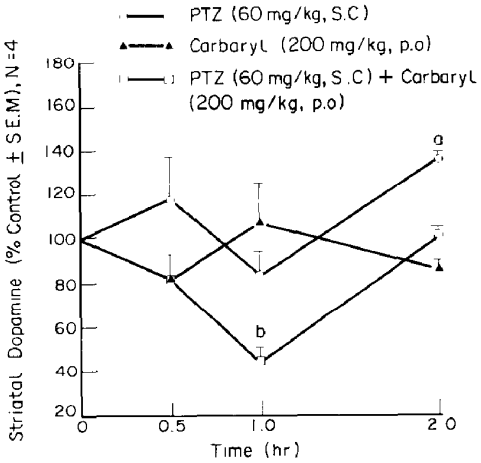

Fig. 1. Effect of PTZ, carbaryl and PTZ + carbaryl on striatal DA level at different time intervals. Each point represents the mean ± S.E.M. of 4 separate determinations. Each determination was carried out with two animals. The average control value of DA was 7.14 ± 0.44 μg/g wet wt. Significantly different from control, $^aP < 0.001$, $^bP < 0.005$.

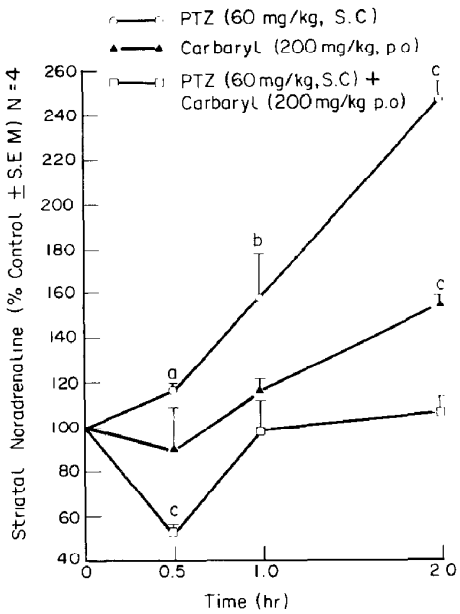

Fig. 2. Effect of PTZ, carbaryl and PTZ + carbaryl on the striatal NA level at different time intervals. Each point represents the mean ± S.E.M. of 4 separate determinations. Each determination was carried out with two animals. The average control value of NA was 1.89 ± 0.085 μg/g wet wt. Significantly different from control, $^aP < 0.02$, $^bP < 0.05$, $^cP < 0.001$.

Measurement of striatal HVA at different time intervals (0–2 hr) after PTZ (60 mg/kg, s.c.) and/or carbaryl (200 mg/kg, p.o.) administrations (Fig. 3) reveals that carbaryl alone significantly elevated the concentration of HVA at 0.5 hr (49.5%) and 1.0 hr (70%) whereas PTZ in the presence or absence of carbaryl failed to produce any significant change in HVA at any of these time intervals (compared to control). Further, it may be noted that the carbaryl-induced elevation of HVA at 1.0 hr was significantly reduced towards normal ($P < 0.005$, compared to carbaryl treated group) under carbaryl + PTZ treated condition. Administration of pargyline (75 mg/kg, i.p.), a MAO inhibitor, significantly reduced (50%) the striatal HVA concentration at 0.5 hr (Table 3). This pargyline-induced decline in striatal HVA was significantly potentiated with PTZ alone but not with carbaryl or carbaryl + PTZ. It appears from Table 4 that carbaryl and/or PTZ produced no appreciable effect on the striatal MAO

Table 1. Effect of PTZ (60 mg/kg, s.c.), carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) + carbaryl (200 mg/kg, p.o.) on α-M_pT-induced depletion of DA and NA levels in the rat brain striatum

Treatment	DA level (%)	NA level (%)
α-M_pT	100 ± 4.50	100 ± 1.30
α-M_pT + PTZ	40 ± 2.10*	45 ± 3.20*
αM_pT + carbaryl	60 ± 1.94†	68 ± 3.32†
α-M_pT + PTZ + carbaryl	56 ± 2.40†‡	62 ± 4.96†§

Results are expressed as mean ± S.E.M. of three separate determinations. A suspension (6%, w/v) of α-M_pT in normal saline was administered (i.p.) to all rats at a dose of 250 mg/kg 6 hr before sacrifice. PTZ and/or carbaryl were administered to these rats 2 hr prior to sacrifice. α-M_pT alone was found to reduce the striatal DA and NA levels by about 50% and 36% respectively, compared to corresponsing vehicle-treated groups.

Significantly different from only α-M_pT treated group, *$P < 0.02$, †$P < 0.05$.

Significantly different from α-M_pT + PTZ treated group, ‡$P < 0.01$, §$P < 0.05$.

activity at 1.0 hr. Treatment of rat with probenecid (200 mg/kg, i.p.), an inhibitor of acid metabolites' transport from brain [13] increased the accumulation of striatal HVA by 53% (Table 5). This probenecid-induced accumulation of striatal HVA was further increased with PTZ (29%) or carbaryl (45%). The simultaneous administration of rat with carbaryl and PTZ did not produce any significant change in the probenecid-induced accumulation of HVA. Moreover, it appears from Table 5 that the probenecid-induced accumulation of striatal HVA in presence of carbaryl + PTZ did not significantly differ from that observed in presence of either PTZ or carbaryl alone.

DISCUSSION

Recently from this laboratory, it was reported that carbaryl at doses of 200 and 400 mg/kg, p.o. stimulates the striatal dopaminergic system [5, 6]. Although the higher dose of this pesticide exhibited a more pronounced effect than the lower dose [6] the present investigation evaluated the effect of PTZ in the presence or absence of the lower dose (200 mg/kg, p.o.) of carbaryl in order to avoid the severity of tremor and to eliminate non-specific effects, if any, at the higher dose of carbaryl.

The significant increase in (a) the steady state level of HVA (Fig. 3) and (b) the probenecid-induced accumulation of HVA (Table 5) without any significant change in pargyline-induced depletion of HVA (Table 3) after oral administration of carbaryl (200 mg/kg) clearly suggests that carbaryl increases

Table 2. Effect of PTZ (60 mg/kg, s.c.), carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) + carbaryl (200 mg/kg, p.o.) on FLA-63-induced disappearance of striatal NA

Treatment	NA level (%)
FLA-63	100 ± 9.89
FLA-63 + PTZ	42 ± 7.52*
FLA-63 + carbaryl	117 ± 5.0
FLA-63 + PTZ + carbaryl	40 ± 8.54†‡

Results are expressed as mean ± S.E.M. of three separate determinations. A suspension (1%, w/v) of FLA-63 in normal saline was administered (i.p.) to rats at a dose of 25 mg/kg 2.5 hr before decapitation. PTZ and/or carbaryl was administered to these rats before 0.5 hr of their sacrifice. FLA-63 alone was found to reduce the striatal NA level by about 50% at 2.5 hr compared to the vehicle treated groups.

Significantly different from only FLA-63 treated group, *$P < 0.01$, †$P < 0.02$.

Significantly different from FLA-63 + Carbaryl treated group, ‡$P < 0.005$.

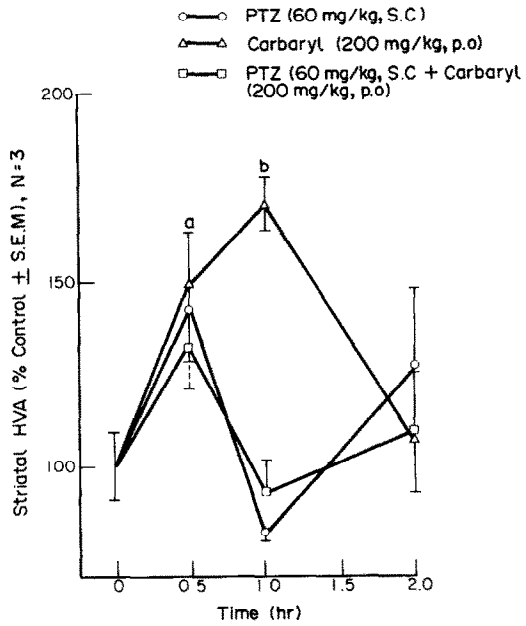

Fig. 3. Effect of PTZ, carbaryl and PTZ + carbaryl on the striatal HVA level at different time intervals. Each point represents the mean ± S.E.M. of 3 separate determinations. Each determination was carried out with two animals. The average control value of HVA was 0.43 ± 0.03 μg/g wet wt. Significantly different from control, *$P < 0.05$, $^b P < 0.005$.

Table 3. Effect of PTZ (60 mg/kg, s.c.), carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) + carbaryl (200 mg/kg, p.o.) on the pargyline induced decline of striatal HVA level

Treatment	HVA level (%)
Pargyline	100 ± 10.21
Pargyline + PTZ	62 ± 6.20*
Pargyline + carbaryl	81 ± 10.45
Pargyline + PTZ + carbaryl	82 ± 9.73

Results are expressed as mean ± S.E.M. of three separate determinations. An aqueous solution of pargyline (5%, w/v) was administered (i.p.) at a dose of 75 mg/kg to the rats treated immediately before with PTZ and/or carbaryl. All rats were decapitated 0.5 hr after pargyline treatment. Pargyline was found to reduce the striatal HVA level by 50% at 0.5 hr compared to the vehicle treated groups.

* Significantly different from pargyline treated group, $P < 0.05$.

Table 5. Effect of PTZ (60 mg/kg), s.c.), carbaryl (200 mg/kg, p.o.) and PTZ (60 mg/kg, s.c.) + carbaryl (200 mg/kg, p.o.) on probenecid-induced accumulation of HVA

Treatment	HVA level (%)
Probenecid	100 ± 3.03
Probenecid + PTZ	129 ± 1.63*
Probenecid + carbaryl	145 ± 8.18†
Probenecid + PTZ + carbaryl	112 ± 8.79

Results are expressed as mean ± S.E.M. of three separate determinations. An aqueous solution of probenecid (10%, w/v) of neutral pH was administered (i.p.) to all rats at a dose of 200 mg/kg 1.5 hr before decapitation. PTZ and/or carbaryl was administered to these rats 1.0 hr before their decapitation. Probenecid alone was found to elevate the striatal HVA level by about 53% at 1.5 hr compared to vehicle treated groups.

Significantly different from only probenecid treated group, *$P < 0.005$, †$P < 0.02$.

the breakdown of striatal DA. Further, the lack of any change in the steady state level of striatal DA (Fig. 1) under this condition apparently suggests that carbaryl increases the synthesis of striatal DA. In addition, the significant increase in the α-M_pT-induced disappearance of DA (Table 1) supports the suggestion that carbaryl increases the utilization of striatal DA. The failure of carbaryl to alter the FLA-63-induced disappearance of NA (Table 2), despite an increase in the α-M_pT-induced depletion of NA (Table 1) is paradoxical; we therefore suggest that carbaryl (i) does not affect the utilization of striatal NA and (ii) increases the conversion of DA into HVA (Table 5), eventually reducing the availability of DA for the synthesis of NA.

A time dependent increase in striatal NA (Fig. 2) with a significant increase of (a) probenecid-induced accumulation of HVA (Table 5), (b) α-M_pT-induced disappearance of DA and NA (Table 1) and (c) FLA-63-induced disappearance of NA (Table 2) after a single administration of PTZ suggest that this drug increases the metabolism of striatal DA as well as NA. The lack of any significant rise in the steady state level of HVA after PTZ administration (Fig. 3) may be explained by an increased efflux of this metabolite from this region (Table 3). It is noteworthy that the transport of HVA was enhanced after electroconvulsive shock in mice [14]. The observed increase in conversion of DA into HVA after administration of carbaryl or PTZ alone (Table 5) without any significant change in MAO activity (Table 4), indicates the possibility of an increased release of DA under the same conditions.

Simultaneous administration of carbaryl and PTZ reduces the levels of NA at 0.5 hr and DA at 1.0 hr (Fig. 1), while producing a significant increase in the α-M_pT-induced depletion of DA and NA (Table 1) and FLA-63-induced depletion of NA (Table 2). These results suggest that the simultaneous administration of drug and pesticide may increase the utilization of NA or DA with or without a decrease in the synthesis of these two amines. The lack of any significant change in the steady state level of HVA (Fig. 3) and probenecid-induced accumulation (Table 5) or pargyline-induced depletion (Table 3) of HVA does however discount the possibility of enhanced utilization of DA during treatment with PTZ and carbaryl. It is apparent from Tables 1 and 2 that the simultaneous administration of carbaryl and PTZ has a relatively greater impetus on the FLA-63-induced depletion of NA (60%) than on the αM_pT-induced depletion of NA (38%). Hence an increase in the α-M_pT-induced depletion of DA (Table 1) after the simultaneous treatment of carbaryl and PTZ may likely be due to a relatively greater conversion of striatal DA into NA. Further, the simultaneous administration of carbaryl and PTZ was observed to reduce (i) the PTZ-induced elevation of DA (2.0 hr) (Fig. 1) and NA (0.5 hr and 2.0 hr) (Fig. 2) and (ii) α-M_pT + PTZ-induced depletion of

Table 4. Effect of PTZ, carbaryl and PTZ + carbaryl on striatal MAO activity

Treatment	Duration of treatment (hr)	Dosage (mg/kg) and routes of administration	MAO activity ($\Delta O.D._{450}$/mg protein/hr) X 10^2
Control (Vehicle)	1.0	—	65.03 ± 3.96
PTZ	1.0	60 (s.c.)	54.73 ± 3.96
Carbaryl	1.0	200 (p.o.)	76.17 ± 1.46
PTZ + carbaryl	1.0	60 (s.c.) +200 (p.o.)	75.94 ± 2.34

Results are mean ± S.E.M. of four separate determinations.

DA and NA (Table 1) without changing the FLA-63 + PTZ-induced depletion of NA (Table 2) which may indicate that carbaryl + PTZ diminishes the effect of PTZ alone at the level of striatal DA metabolism and NA anabolism.

Thus from all these observations it may be concluded that the administration of PTZ in the presence of carbaryl (a) attenuates the enhanced metabolic activity of dopaminergic neurons produced by carbaryl or PTZ alone and (b) either accelerates the catabolic activity of noradrenergic neurons or reduces their anabolic activity induced by PTZ alone.

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