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Limbic Forebrain Toxin Trimethyltin Reduces Behavioral Suppression by Clonidine

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MESSING, R. B., V. DEVAUGES AND S. J. SARA. *Limbic forebrain toxin trimethyltin reduces behavioral suppression by clonidine.* PHARMACOL BIOCHEM BEHAV **42**(2) 313-316, 1992.—Trimethyltin (TMT) at moderate doses selectively damages hippocampus and related olfactory cortex and produces learning and memory impairments. TMT also increases forebrain β-adrenergic ligand binding; this could be ancillary to reduced noradrenergic neurotransmission, which in turn could be involved in the cognitive deficit caused by TMT. If this hypothesis is correct, then the α_1-adrenergic agonist clonidine, which inhibits noradrenergic neurotransmission in normal subjects, should be less behaviorally effective after TMT poisoning. Thus, rats treated with water vehicle or TMT (6 mg/kg, PO) were given saline or clonidine IP (5, 10, or 20 μg/kg) 30 min before placement in a hole-board apparatus. Exploratory activity was reduced in controls by 10 or 20 μg/kg. Clonidine at 10 μg/kg was ineffective in rats given TMT. At 20 μg/kg, an apparent reduction in exploratory activity was not significant because variability of responding was higher after TMT treatment. The results suggest an impairment in noradrenergic neurotransmission following TMT poisoning.

Clonidine Exploratory activity Trimethyltin

THE organometal neurotoxin trimethyltin (TMT) produces a relatively specific lesion of the hippocampus and related olfactory cortical structures (5-7) when administered systemically. A moderate dose of TMT also produces an increase in forebrain binding of the β-adrenergic ligand dihydroalprenolol (21), as well as learning impairments reminiscent of those seen in aged animals (8,20). β-Receptor upregulation may be an indication of impaired noradrenergic neurotransmission (30), which could be involved in the learning deficit.

The central noradrenergic system, that is, the locus coeruleus (LC)-forebrain noradrenergic projection, appears to function primarily as a system for control of vigilance and/or selective attention, thereby modulating cognitive processes (24-26). Electrical activity of the LC is under the inhibitory control of α_2-adrenoreceptors (1). The α_2-agonists clonidine and guanfacine reduce catecholamine metabolism in LC, while the antagonists piperoxane and yohimbine increase it (22). Behaviorally, α_2-agonists such as clonidine exert locomotor suppressive effects (31), while small doses of the antagonists yohimbine or idazoxan can increase locomotor activity under appropriate circumstances (9,17,31). Finally, low doses of clonidine impair learning (12,14), while the α_2-antagonists yohimbine or idazoxan facilitate learning and memory retrieval (9,17,24,27).

However, in impaired animals and humans clonidine may exert a facilitatory effect on cognitive processes. Thus, clonidine exerts a positive effect on the amnesia associated with Korsakoff's psychosis (18,19) and clonidine and guanfacine facilitate performance of aged monkeys in a delayed nonmatching to sample task (2,3). These results suggest that when the LC-forebrain noradrenergic system is impaired by Korsakoff's psychosis (18,19) or normal aging (32) the suppressant action of the α_2-agonists via an inhibition of the LC are decreased; the primary effect of the drugs may therefore be as postsynaptic agonists at forebrain noradrenergic receptors (2,3).

If this general hypothesis is correct, then the α_2-adrenergic agonist clonidine, which produces behavioral suppression associated with reduced LC activity and forebrain norepinephrine release in normal rats, would be expected to be less effective in animals given TMT. Accordingly, rats were tested in a hole-board apparatus, which provides the opportunity to separate exploratory activity related to environmental novelty (examination of the holes) from generalized locomotor activity (11).

METHOD

Male Long-Evans rats (obtained from Centre d'Élevage R. Janvier, Le Genest St. Isle, France) weighing from 230-300 g were treated with water vehicle or TMT chloride (6 mg/kg of

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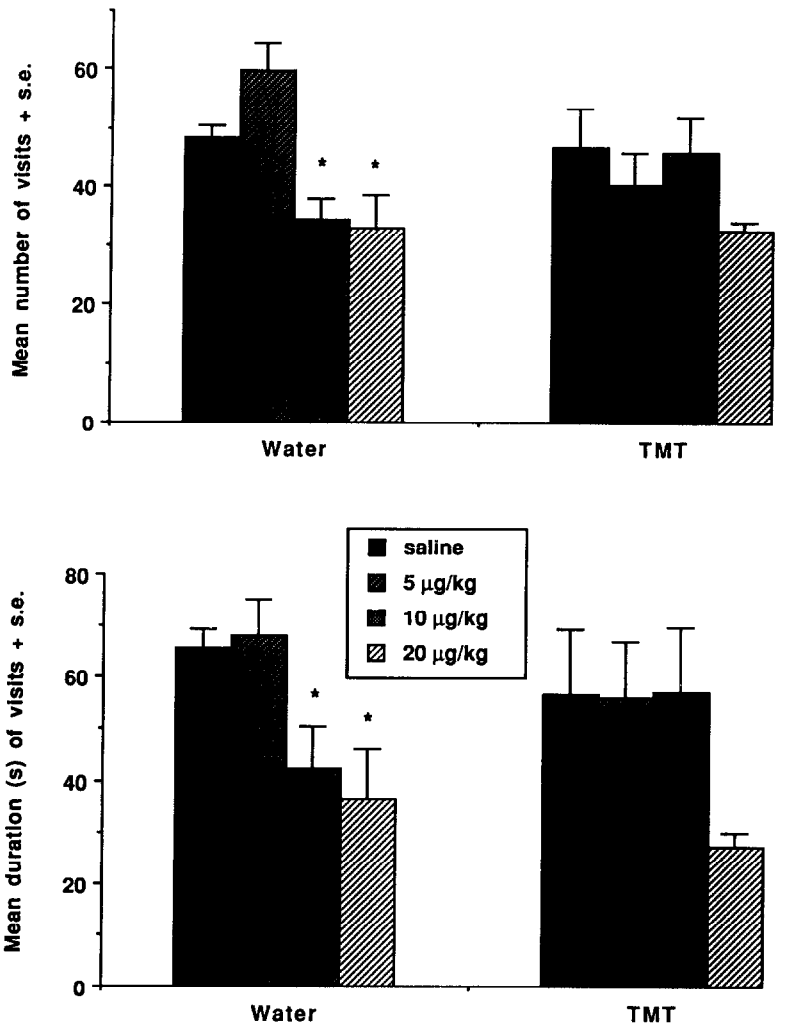

FIG. 1. Effects of clonidine on number of holes visited and duration of visits. Rats treated with water (n = 5/group) or 6.0 mg/kg TMT (n = 6/group) were given saline or clonidine IP 30 min before placement into the hole-board apparatus for 9 min. *$p < 0.05$ different from saline-treated animals by Dunnett's t-test (one tailed).

the TMT base, PO). Animals were housed in pairs, with an animal receiving the same treatment. Nineteen to 26 days after treatment, animals were given 0.9% saline vehicle or clonidine HCl IP (5, 10, or 20 µg/kg of the salt) (n = 5/group for water-treated rats; n = 6/group for TMT-treated rats) 30 min before being placed in a hole-board apparatus for 9 min. The hole board consisted of a box constructed of heavy beige plastic (60 m × 60 m × 35 cm high) divided into nine squares with nine holes (4 cm diameter) symmetrically cut in the floor at the center of each square. Photoelectric cells detected total horizontal exploration (squares entered), rearings, and numbers and durations of visits to the different holes. Data were collected through a custom-designed interface and stored in an Apple II computer.

Effects of clonidine were assessed by separate analyses of variance (ANOVAs) for animals treated with vehicle or TMT, followed by Dunnett's t-tests (one tailed) comparing rats treated with clonidine with saline-injected animals.

RESULTS

None of the rats treated with TMT exhibited signs of overt toxicity (convulsions, irritability, self-mutilation) associated with high doses of TMT (10,13), thus confirming that this dose produced moderate toxicity. As shown in Fig. 1, exploratory activity was significantly reduced in water-treated rats by 10 or 20 µg/kg clonidine for both number of visits, $F(3, 16) = 8.62$, $p = 0.001$, and duration of visits, $F(3, 160) = 4.58$, $p = 0.02$, to holes. In rats treated with TMT, there were no significant effects of clonidine on either the number of hole visits, $F(3, 20) = 1.49$, $p = 0.25$, or duration of visits, $F(3, 20) = 1.93$, $p = 0.16$. In these rats, in contrast to controls, there was clearly no reduction in exploratory activity at 10 µg/kg, while there was a clear reduction at this dose in rats treated with water. At the higher dose, the results are more ambiguous. There was greater within-groups error variance in the data for rats treated with TMT compared to animals

TABLE 1
LOCOMOTOR ACTIVITY OF RATS TREATED WITH CLONIDINE

	Water	TMT
Squares entered		
Saline	192.4 ± 10.3	168.7 ± 14.8
5 μg/kg clonidine	176.6 ± 3.2	148.2 ± 11.3
10 μg/kg clonidine	170.6 ± 10.4	197.8 ± 16.6
20 μg/kg clonidine	165.2 ± 11.7	180.2 ± 10.8
Correlation with hole visits	$r = 0.46$	$r = 0.56$
	$p = 0.043$	$p = 0.005$
Rearing activity		
Saline	4.4 ± 0.4	6.7 ± 2.1
5 μg/kg clonidine	7.4 ± 1.3	7.3 ± 1.4
10 μg/kg clonidine	5.4 ± 1.2	6.3 ± 1.8
20 μg/kg clonidine	4.6 ± 0.5	5.6 ± 1.0
Correlation with hole visits	$r = 0.31$	$r = 0.095$
	$p = 0.19$	$p = 0.66$

See Fig. 1 legend for details. Values are mean ± SE.

treated with water, $F(20, 16) = 1.89$, $p = 0.10$ for number of visits, $F(20, 16) = 2.39$, $p < 0.05$ for duration of visits. This increase in variance was sufficient to result in no significant reduction in activity (as determined by Dunnett's t-tests) despite the fact that mean levels are about equal to those for water-treated animals.

No effect of these doses of clonidine was seen on horizontal activity (number of squares entered) or rearing activity for rats treated with water or with TMT (see Table 1). Furthermore, while (of necessity) there was a weak correlation of exploration of the holes with general horizontal locomotor activity, there was no correlation with rearing activity. Thus, the effect of clonidine at the low doses used is seen as a suppression of activity related to novel aspects of the environment rather than a general sedation or suppression of all locomotor activity.

DISCUSSION

The results demonstrate that TMT administration results in reduced sensitivity to clonidine-induced suppression of unconditioned exploratory activity related to environmental novelty. Taken together with the upregulation of forebrain β-receptors in rats given TMT (21), the present data suggest that TMT administration at moderate doses results in impaired functioning of central noradrenergic neurons, which may very well be involved in the cognitive impairment seen in these animals (see the introductory section).

Animals treated with moderate doses of TMT, that is, doses that do not lead to weight loss, convulsions, or other obvious sequelae, are similar, in important respects, to aged organisms. This is reflected biochemically in CNS noradrenergic impairments in aged and TMT-treated animals (21,32), as well as reduced hippocampal responsivity to glucocorticoids after TMT treatment (13,20) or in aged animals (23). Behaviorally, these animals exhibit learning impairments in delayed reinforcement autoshaping (8,20) that are similar to impairments in classical trace conditioning (15).

This suggest that the positive cognitive effects of α_2-agonists in impaired or aged subjects, in contrast to the negative effects in normal young subjects (see the introductory section), may be due, at least in part, to a reduced behavioral suppressant effect of these agents in impaired subjects and a consequently relatively greater functional agonistic action of these agents.

While higher doses of TMT produce degenerative changes throughout the neocortex and brainstem of rats (4), moderate doses do not (7). There are transient chromatolytic changes in brainstem after a moderate dose (6.0 mg/kg TMT base to Long-Evans rats) but with apparent recovery and normal morphology by 15 days posttreatment. It is therefore possible that TMT produces brainstem lesions and thus a direct effect on LC and noradrenergic neurotransmission.

However, the absence of positive evidence of neocortical and/or brainstem toxicity suggests that the increase in forebrain β-adrenoreceptor binding (21) and the decrease in sensitivity to the α_2-adrenergic agonist clonidine are most likely the result of an indirect reduction of noradrenergic neurotransmission by TMT. This may represent an adaptive process that serves to militate against the increased irritability and hyperreactivity to environmental stimuli and seizure susceptibility seen at higher doses (10,13). This is especially likely since a higher dose of TMT (7.5 mg/kg) was found not to induce β-receptor upregulation (21). Decreases in noradrenergic neurotransmission are associated with impaired generation of long-term potentiation (LTP) in the dentate gyrus (29), while norepinephrine enhances LTP (16). Thus, decreased noradrenergic neurotransmission may be an adaptive response to combat increased seizure activity that occurs after high-dose TMT treatment and apparently exacerbates neuronal damage (28).

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