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Journal

Toxicology letters, 36(1)

ISSN

0378-4274

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Publication Date

1987-03-01

DOI

10.1016/0378-4274(87)90042-7

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Peer reviewed

TXL 01743

EFFECT OF TRIMETHYLTIN ON ORNITHINE DECARBOXYLASE IN VARIOUS REGIONS OF THE MOUSE BRAIN

(Ornithine decarboxylase; trimethyltin; tin; cerebral regions; brain area)

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(Received 20 July 1986)

(Revision received 27 August 1986)

(Accepted 1 November 1986)

SUMMARY

Male C57B1/6N mice, 8-10 weeks old were given a single oral dose of 0, 1.0 or 3.0 mg/kg body weight of trimethyltin hydroxide (TMT). Levels of ornithine decarboxylase (ODC) activity were measured in several brain areas, 1, 2 and 7 days later. The lower dose of TMT produced a decrease of ODC in the caudate nucleus and hippocampus at all time points studied. Hypothalamus, cerebellum and brain stem levels of this enzyme were unaltered. At the higher dose of TMT, ODC activity in hippocampus, cerebellum and brain stem were increased relative to controls at 1 and 2 days after treatment, while other regions were not significantly affected. These elevated ODC levels returned to control values within 7 days. Thus, trimethyltin treatment causes changes in ODC activity in a region and dose-specific manner.

INTRODUCTION

Alkyltins have a number of applications as polymer stabilizers, fungicides, surface disinfectants and antioxidants in rubber products [1,2]. An increasing worldwide production of organotin products is expected in the future [3]. One of

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Abbreviations: TMT, trimethyltin hydroxide; ODC, ornithine decarboxylase; DFMO, difluoromethyl ornithine.

the most toxic alkyltin compounds, trimethyltin (TMT), is an intermediate in the production of other tin compounds, and a number of cases of human poisoning after exposure to this compound have been reported [4,5].

TMT produces a relatively selective pattern of limbic system damage involving the hippocampus, amygdala, pyriform cortex, and brain stem in mice [6, 7] and rats [8]. This regional selectivity has been confirmed in studies of neurotransmitter-related parameters [9–11] and provides the opportunity to examine for metabolic changes in various brain areas after exposure of animals to TMT. Ornithine decarboxylase (ODC), the rate limiting enzyme of polyamine synthesis, is capable of rapidly responding in cerebral tissues that have been activated or damaged [12–14]. This property of ODC can be used to detect the targets of neurotoxic agents [15]. Inhibition of cerebral ODC can result in a blockade of certain trophic responses in the brain [16]. This implies that polyamine synthesis reflects regenerative response rather than damage per se.

In this report, the effect of 2 doses of TMT upon ODC levels in several brain regions of adult male mice has been studied at 3 time points. The purpose was to determine if neuropathological toxic doses of TMT would stimulate a regional regenerative response as evidenced by enhanced ODC activity.

MATERIALS AND METHODS

Animals and treatment

Male C57B1/6N mice (NCTR breeding colony) 8–10 weeks of age, weighing 22.0 ± 2.0 g were used for this study. Mice were housed 4 per plastic cage with wood chip bedding and maintained on a 12 h light dark cycle (light, 07.00 h; dark, 19.00 h) in a temperature-controlled ($20 \pm 1^\circ\text{C}$) room. Food (Purina Laboratory Chow, St. Louis, MO) and tap water were available ad libitum.

Mice were randomly subdivided into 3 groups of 18 animals each. TMT (ICN Pharmaceutical, K&K Labs, Plainview, NY) was dissolved in deionized water and administered in a single dose of 0, 1.0 and 3.0 mg/kg orally by gavage in a volume of 10 ml/kg. The LD_{50} of TMT has been reported to be 4.0 mg/kg using this strain of mouse. At the higher dose employed here (3.0 mg/kg) mortality was between 9 and 18% [17].

Ornithine decarboxylase assay

Six animals were randomly selected from each group at each time point and were killed by decapitation at 1, 2, or 7 days after TMT administration. Brains were quickly removed and dissected following the guidelines of Glowinski and Iversen [18] into caudate nucleus, hippocampus, hypothalamus, cerebellum, and brain stem, placed on dry ice and held at -70°C .

ODC was assayed by measurement of evolved $^{14}\text{CO}_2$ from [*carboxyl*- ^{14}C]-ornithine (55.9 mCi/mmol, New England Nuclear, Boston, MA) [19]. Frozen brain

regions were weighed and homogenized in 1.5 ml of 0.04 M Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at $26\,000 \times g$ for 10 min and the supernatant obtained. 0.9 ml of the supernatant was added to 50 μ l pyridoxal phosphate solution (1 mM) and 50 μ l [^{14} C]ornithine in 0.045 M dithiothreitol. The final ornithine concentration was 2.5 μ M. Incubation (37°C, 30 min) was carried out in a sealed tube and was terminated by injection of 1 ml 2 M acetic acid into the reaction mixture. Evolved $^{14}\text{CO}_2$ was trapped on a paper wick containing hyamine suspended above the reaction mixture [19]. The decarboxylation process was linear for up to 1.5 h under these conditions. Decarboxylation, not attributable to ODC, was determined by running a parallel incubation in the presence of 5 mM difluoromethyl ornithine (DFMO), a specific inhibitor of ODC [20]. Activity was expressed as the difference between total decarboxylative activity and that in the presence of DFMO. This correction for non-specific decarboxylation was less than 15% of total counts. The activity of frozen samples was around 70% of the corresponding value of tissues that had not been frozen [21].

Statistical evaluation

A non-parametric analysis, the Mann-Whitney *U*-test was used since overall heterogeneity of variance was apparent. The accepted level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

A single low dose of TMT (1.0 mg/kg body weight) caused a significant decrease in the ODC activity of caudate nucleus and hippocampus at all time points studied (Table I). In contrast, the higher TMT dose (3 mg/kg) caused an increase in the cerebellum, brain stem and hippocampus (Table I). This increase was seen 1 and 2 days after TMT administration, but ODC values were not significantly different from the corresponding control levels after 1 week. The loci and magnitude of the transient increases were largely in accord with morphological reports of damage restricted to the brain stem and hippocampus in mice exposed to 3.0 mg/kg, a dose of TMT identical to that used here [6,7]. However, no morphological changes in the cerebellum have been described by this treatment. The prolonged depression in ODC levels in the caudate nucleus and hippocampus of mice receiving the lower dose of TMT was unexpected. There have been no prior reports of a depression of basal ODC levels of the mature brain. This enzyme is at a very low level in the adult undamaged nervous system, where little mitotic or regenerative activity is taking place [22]. A dose of 3 mg/kg TMT in mice has been found to initially cause a decrease and then, later an increase in motor activity relative to untreated controls [23]. Gross observation of the mice used in these studies revealed that at the time of sacrifice, mice receiving the lower dose of TMT were lethargic while mice treated with 3 mg TMT/kg body weight exhibited tremor. This behavioral difference may

TABLE I

ODC ACTIVITY IN BRAIN REGIONS AT VARIOUS TIMES AFTER TREATMENT OF MICE WITH TMT

Enzyme activity is expressed as nmol CO₂ evolved/h/g tissue. Each value represents a mean derived from 5-6 animals $\pm$ SEM.

Brain region	TMT dose (mg/kg)	Time after dosing (days)					
		1		2		7	
Caudate nucleus	0	21.3 $\pm$ 4.5		15.0 $\pm$ 4.4		18.9 $\pm$ 3.5	
	1	2.7 $\pm$ 1.4*		2.5 $\pm$ 1.1*		6.6 $\pm$ 2.9*	
	3	15.4 $\pm$ 5.2		11.5 $\pm$ 1.8		29.8 $\pm$ 16.1	
Hippocampus	0	66.2 $\pm$ 10.1		51.4 $\pm$ 4.4		65.8 $\pm$ 5.5	
	1	20.4 $\pm$ 6.8*		10.0 $\pm$ 3.0*		16.9 $\pm$ 3.7*	
	3	140 $\pm$ 10*		772 $\pm$ 85*		52.8 $\pm$ 19.2	
Hypothalamus	0	14.1 $\pm$ 3.2		19.1 $\pm$ 3.2		17.8 $\pm$ 4.2	
	1	11.2 $\pm$ 2.0		17.6 $\pm$ 0.6		17.0 $\pm$ 5.4	
	3	8.3 $\pm$ 2.7		25.8 $\pm$ 6.5		14.5 $\pm$ 1.8	
Cerebellum	0	81.7 $\pm$ 3.5		51.6 $\pm$ 11.5		74.2 $\pm$ 9.2	
	1	124 $\pm$ 28		52.5 $\pm$ 10.1		64.9 $\pm$ 12.4	
	3	183 $\pm$ 55*		367 $\pm$ 164*		80.2 $\pm$ 11.6	
Brain stem	0	87.5 $\pm$ 9.6		84.2 $\pm$ 9.5		106 $\pm$ 20	
	1	93.9 $\pm$ 22.0		88.2 $\pm$ 11.5		74.2 $\pm$ 9.5	
	3	586 $\pm$ 212*		565 $\pm$ 79*		144 $\pm$ 19	

* That value differs from corresponding zero-dose control ($P < 0.05$).

be related to our finding of a biphasic response of ODC in the hippocampus, using dose-magnitude as a variable.

The response of ODC levels to altered physiological conditions is generally in an upward direction. However, corticosterone administration has been shown to depress adrenal ODC levels below their basal value [15]. This may be due to the inhibition of ACTH secretion by corticosterone. The prolonged reduction of ODC in some brain areas, after administration of the lower dose of TMT, may reflect an inhibition of a chronic trophic influence that is normally present. Thus low levels of TMT may not be sufficient to stimulate repair mechanisms but may cause lesser changes in the central nervous system. On the other hand, higher levels of this neurotoxic agent may cause sufficient derangement of homeostasis so as to initiate adaptive and regenerative processes.

ACKNOWLEDGEMENTS

We would like to thank Ms. Sabreen Rahmaan for skilled technical assistance and Ms. Barbara Jacks and Ms. Virginia Gauldin for expert secretarial help.

REFERENCES

- 1 W.T. Piver, Organotin compounds: industrial applications and biological investigation, *Environ. Health Perspect.*, 4 (1973) 61-79.
- 2 R. Kimbrough, Toxicity and health effects of selected organotin compounds: a review, *Environ. Health Perspect.*, 14 (1976) 51-56.
- 3 R.R. Wilkinson, Technoeconomic and environmental assessment of industrial organotin compounds, *Neurotoxicology*, 5 (1984) 141-158.
- 4 E. Fortemps, G. Amand, A. Bomboir, R. Lauwerys and E.C. Laterre, Trimethyltin poisoning: report of two cases, *Int. Arch. Occup. Environ. Health*, 41 (1978) 1-6.
- 5 W.D. Ross, E.A. Emmett, J. Steiner and R. Tureen, Neurotoxic effects of occupational exposure to organotins, *Am. J. Psychiatry*, 138 (1981) 1092-1095.
- 6 L.W. Chang, T.M. Tiemeyer, G.R. Wenger and D.E. McMillan, Neuropathology of mouse hippocampus in acute trimethyltin intoxication, *Neurobehav. Toxicol. Teratol.*, 4 (1982) 149-156.
- 7 L.W. Chang, T.M. Tiemeyer, G.R. Wenger and D.E. McMillan, Neuropathology of trimethyltin intoxication. III. Changes in the brain stem neuron, *Environ. Res.*, 30 (1983) 399-411.
- 8 A.W. Brown, W.N. Aldridge, B.W. Street and R.D. Verschoyle, The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat, *Am. J. Pathol.*, 97 (1979) 59-77.
- 9 D.L. DeHaven, T.J. Walsh and R.B. Mailman, Effects of trimethyltin on dopaminergic and serotonergic function in the central nervous system, *Toxicol. Appl. Pharmacol.*, 75 (1984) 182-189.
- 10 R.B. Mailman, M.R. Krigman, G.D. Frye and I. Hanin, Effects of postnatal trimethyltin or triethyltin treatment on CNS catecholamine, GABA, and acetylcholine system in the rat, *J. Neurochem.*, 40 (1983) 1423-1429.
- 11 S.F. Ali, W. Slikker, Jr., G.D. Newport and P.T. God, Cholinergic and dopaminergic alterations in the mouse central nervous system following acute trimethyltin exposure, *Acta Pharmacol. Toxicol.*, 59 (1986) 179-188.
- 12 G.A. Dienel and N.F. Cruz, Induction of brain ornithine decarboxylase during recovery from metabolic, mechanical, thermal or chemical injury, *J. Neurochem.*, 42 (1984) 1053-1061.
- 13 M.A. Cousin, D. Lando and M. Moguilewsky, Ornithine decarboxylase induction by glucocorticoids in brain and liver of adrenalectomized rats, *J. Neurochem.*, 38 (1982) 1296-1304.
- 14 S.C. Bondy, C.L. Mitchell, S. Rahman and G. Mason, Regional variation in the response of cerebral ornithine decarboxylase to electroconvulsive shock, *Neurochem. Int.*, (1986) (in press).
- 15 S.C. Bondy and J.S. Hong, Modulation of adrenal ornithine decarboxylase by chlordecone, *p,p'*-DDT and permethrin, *Neurotoxicology*, (1986) (in press).
- 16 L.F. Agnati, K. Fuxe, F. Benfati, M. Zou, C. Owman, N.H. Diemer, J. Kahrstrom, G. Toffano and M. Crimino, Effects of ganglionide GM1 treatment of striatal glucose metabolism, blood flow and protein phosphorylation in the rat, *Acta Physiol. Scand.*, 125 (1985) 43-53.
- 17 W. Slikker, Jr., S.F. Ali, J. Lipscomb and R. Denton, Time course alteration in tremor and muscarinic receptor binding produced by trimethyl tin, *Proc. West. Pharmacol. Soc.*, 28 (1985) 139-142.
- 18 J. Glowinski and L.L. Iversen, Regional studies of catecholamines in the rat brain. I. The disposition of ³H-norepinephrine, ³H-dopamine, and ³H-DOPA in various regions of the brain, *J. Neurochem.*, 13 (1966) 655-669.
- 19 D.H. Russell and S.H. Snyder, Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors, *Proc. Natl. Acad. Sci. U.S.A.*, 60 (1968) 1420-1427.
- 20 B.W. Metcalf, P. Bey, C. Danzin, M.J. Jung, P. Casara and J.P. Vever, Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C.4.1.1.17), *J. Am. Chem. Soc.*, 100 (1978) 2551-2553.

- 21 S.C. Bondy, Ornithine decarboxylase associated with a particulate fraction of rat brain, *Neurochem. Res.*, 11 (1986) 1653-1662.
- 22 T.R. Anderson and S.M. Schanberg, Ornithine decarboxylase in developing rat brain, *J. Neurochem.*, 19 (1972) 1471-1481.
- 23 G.R. Wenger, D.E. McMillan and L.W. Chang, Behavioral effects of trimethyltin in two strains of mice. I. Spontaneous motor activity, *Toxicol. Appl. Pharmacol.*, 73 (1984) 75-88.