Brain Research, 379 (1986) 147–150
Elsevier

BRE 21670

Inhibition of ornithine decarboxylase alters neurological responsiveness to a tremorigen

HUGH A. TILSON, DWAINE EMERICH and STEPHEN C. BONDY

Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 (U.S.A.)

(Accepted April 1st, 1986)

Key words: difluoromethylornithine — ornithine decarboxylase — chlordecone — *p,p'*-DDT — tremor — neuronal adaptation

Difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC; 200–800 mg/kg, s.c.), to rats has no detectable behavioral effects using a battery of tests to assess sensorimotor function. In contrast, the induction of tremor by chlordecone, a neurotoxic agent that affects neuronal ionic processes, is significantly attenuated by pretreatment with DFMO. The effects of DFMO on chlordecone-induced tremor were reversed by pretreatment with putrescine. DFMO had no effects on *p,p'*-DDT, a tremorigen having a mechanism of action different from chlordecone. These findings imply that polyamines may play a role in select neuronal processes.

Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the biosynthesis of the polyamines, putrescine, spermidine and spermine[13,20,23]. Polyamines appear to be essential for cell division and ODC is present in high concentrations in proliferating tissues[14,17]. The irreversible inhibitor of ODC, difluoromethylornithine (DFMO)[14], is under trial as an experimental drug in cancer chemotherapy[4,15,21].

Although basal levels of ODC are relatively low in the mature nervous system, they can be rapidly elevated in response to a variety of stimuli, including chemical or physical insults to the brain and electroconvulsive shock[2,16]. A role for ODC in the adult nervous system is further suggested by the finding that intraventricular injection of DFMO produces hyperexcitability, tremors, muscular rigidity, seizures and at lethal doses, histological damage to the hippocampus[6]. Systemic administration of polyamines such as spermidine and spermine to mice has been reported to depress spontaneous motor activity, enhance pentobarbital sleeping times, inhibit the writhing response induced by acetic acid and attenuate the effects of strychnine[18]. Similar results have been obtained following central administration of polyamines[5,19]. In spite of these observations, there has been little published concerning the possible neurobiological consequences of ODC inhibition in mature animals. The present series of experiments were undertaken to determine the effects of systemically administered DFMO on sensorimotor function of intact, mature animals.

Male, adult rats of the Fischer-344 strain were used in these studies. Initial experiments were performed to determine the amount of inhibition produced by 200 mg/kg DFMO s.c., a dose suggested by pilot studies to cause marked inhibition of ODC. ODC was assayed by the measurement of evolved $^{14}CO_2$ from DL-[1-^{14}C]ornithine (55.9 mCi/mmol; New England Nuclear, Boston, MA)[17]. Tissues were homogenized in 19 vols. of 0.04 M Tris-HCl pH 7.4 and the homogenate centrifuged (26,000 *g*) for 20 min. An aliquot (0.9 ml) of the supernatant was added to 50 μl of pyridoxal phosphate solution (0.001 M) and 50 μl DL-[1-^{14}C]ornithine in 0.0445 M diethiothreitol. Incubation was for 30 min at 37 °C in a sealed tube and labeled CO_2 was trapped on a paper wick containing hyamine, suspended above the reaction mixture. The decarboxylation reaction was

Correspondence: H.A. Tilson, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709, U.S.A.

linear with time under these conditions. Background decarboxylation not attributable to ODC was determined by running a parallel incubation in the presence of 5 mM DFMO, a specific inhibitor of ODC. For the determination of DFMO content in the brains of treated animals, 30 μl of radioactive DL-α-[3,4-^{3}H]DFMO (26.5 Ci/mmol; NEN, Boston, MA) was mixed with unlabeled DFMO to a specific activity of 132 μCi/mmol. This mixture was injected s.c. into rats in a final concentration of 200 mg/kg. Five hours after injection, the rats were decapitated and the brains removed, weighed, and dissolved in tissue solubilizers (NCS, Nuclear, Chicago) and radioactivity measured. At 5 h after 200 mg/kg DFMO, brain ODC was inhibited by 87 ± 0.5% and the cerebral concentration of DFMO was 0.39 ± 0.12 μM.

Subsequent behavioral studies were performed in rats using water vehicle, 400 or 800 mg/kg of DFMO. Injections were given s.c. in a volume of 1 ml/kg b.wt. The rats were tested for alterations in sensorimotor function 5 h later. Vertical and horizontal components of motor activity were independently assessed during a 30-min test session in black Plexiglas test chambers (22 × 44 × 18 cm) located within a light- and sound-attenuated test chamber. Arrays of 10 equally spaced infrared photodetector/emitter pairs were located 5.5 cm from the floor to measure horizontal activity and 13 cm from the floor to measure vertical activity. After testing motor activity, rats were assessed for their responsiveness to a novel acoustic stimulus using a commercially available reflex monitor. During testing each animal was placed into a small plastic cage enclosed within a light- and sound-attenuated chamber equipped with a ventilation fan. Test stimuli consisting of 10 consecutive 110 dB, 8 kHz tones superimposed on a continuous 75 dB white noise background were presented following a 2-min acclimation period in the test chamber. The stimulus duration was 200 ms and there was a fixed 15-s interstimulus interval. The startle response corresponded to the largest magnitude (from 0–1800 units) occurring within a 200-ms sampling period following onset of the tone. After the test for startle responsiveness, the rats were assessed for their responsiveness to a thermal stimulus using a commercially available hot-plate apparatus. During the test, each rat was picked up by the tail and gently placed onto the surface of the apparatus, which was maintained at 57.5 °C. The latency to lick one of the hindpaws was recorded. The animals were removed as soon as a response was made or after 45 s, whichever came first. Table I shows that DFMO had no effect on any of the behavioral measures taken.

The behavioral experiments indicated that DFMO had little or no effect on sensorimotor function of rats in the normal state. However, it was possible that these measures of neurological functioning may have been insensitive to more subtle effects of DFMO on nerve function. To assess this possibility, we studied the effects of DFMO on the neurological effects of a chemical known to produce its effects by interfering with ionic processes at the level of the nerve membrane. In these experiments, rats were dosed with 200 mg/kg of DFMO 30 min prior to receiving a tremorigenic dose of chlordecone (75 mg/kg, i.p.)[22]. This neurotoxicant has been reported to interfere with mitochondrial uptake of calcium and has been postulated to increase unbound Ca^{2+} intracellularly, which may lead to a destabilization of the membrane and increased transmitter release[3]. Five hours after exposure to chlordecone, rats were tested for tremorigenic activity by placing rats onto a platform attached to a load cell-transducer assembly[7,8]. The analog signal generated by the movement of the animal was processed by a Model 3582A Hewlett Packard spectral analyzer. A fast Fourier transformation of the signal was performed over a 1.3-min sampling period. Movement was quantified as power (–dBV) occurring from 0–25 Hz. In the present experiments, the average power of the curves was obtained for each rat and differences between groups were determined using analysis of variance (ANOVA) followed by Fisher's least significant difference test[24]. Fig. 1A

TABLE I

Effects of DFMO given s.c. on sensorimotor function of rats 5 h after injection

Average response ± S.E.M.[a].

Treatment (mg/kg, s.c.)	*Activity counts per 30 min*	*Average startle response per 10 trials*	*Latency to respond on hot-plate (s)*
Vehicle	1060 ± 114	766 ± 87	6.9 ± 0.6
400 mg/kg	1011 ± 71	785 ± 82	7.4 ± 0.7
800 mg/kg	818 ± 106	744 ± 124	7.5 ± 0.7

[a] Data are averages of 6 rats per group.

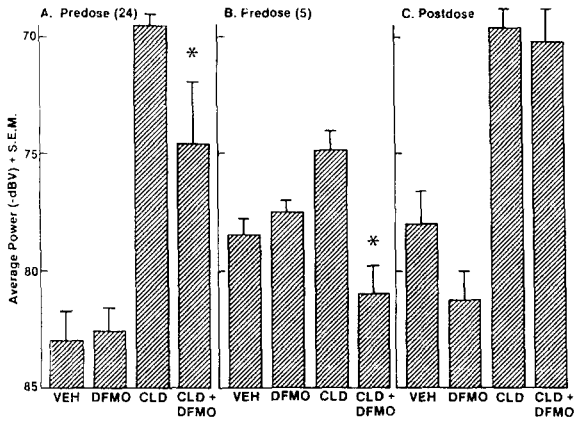

Fig. 1. Effects of DFMO pretreatment on power of movement generated by a tremorigenic dose of chlordecone (CLD). Data are average power units (-dBV) ± S.E.M. of 6–8 rats per group. If DFMO (200 mg/kg, s.c.) was given 30 min prior to chlordecone, significant attenuation of tremor was seen 24 (A) and 5 (B) h after chlordecone. If DFMO was given 19 h after exposure to chlordecone, no effect was seen (C). Data were analyzed for overall significance using ANOVA; asterisks indicate significant difference relative to chlordecone.

shows that chlordecone increased the average power of movement as determined by spectral analysis, while DFMO had no effect relative to controls. Rats treated 30 min previously with DFMO and given chlordecone had significantly less tremorigenic activity than rats given only chlordecone. In a subsequent experiment, rats were dosed with DFMO 30 min prior to receiving either corn oil or chlordecone and tremorigenic activity was measured 24 h after exposure to chlordecone. Fig. 1B shows that under these conditions, DFMO still significantly attenuated chlordecone-induced alteration of motor movement. However, if rats were first exposed to chlordecone followed by DFMO 19 h later, then no attenuation of tremor was seen 24 h after exposure to chlordecone (Fig. 1C).

We reasoned that if the interaction between DFMO and chlordecone were related to the inhibition of ODC, then the effects of DFMO on chlordecone-induced tremor should be attenuated with putrescine, the primary decarboxylation product of ornithine. Other investigators have reported that putrescine pretreatment can attenuate other effects produced by DFMO[10]. Putrescine has also been reported to reverse DFMO-blockage of traumatically induced brain edema[11]. In the following experiment, some rats were given 120 mg/kg putrescine s.c., in a volume of 1 ml/kg immediately before and at a different injection site than the DFMO injection (200 mg/kg). Tremorigenic activity of chlordecone was determined 5 h after i.p. injection. Fig. 2A shows that putrescine alone had no effect on the spectral analysis of movement, while chlordecone significantly increased the average power of the spectrum. DFMO significantly attenuated the tremor produced by chlordecone and this effect was reversed by coadministration of putrescine. Earlier experiments found that a dose of 60 mg/kg of putrescine was insufficient to affect the DFMO–chlordecone interaction (unpublished observation).

Subsequent experiments were performed to determine the generality of the observation that DFMO affects tremorigenic activity produced by neuronally active chemicals. In these studies, the effects of DFMO pretreatment on the tremorigenic activity of another tremorigen, *p,p'*-DDT were studied. This agent is believed to act by holding the sodium channel open once it is open, causing repetitive firing of the nerve[25]. Rats were pretreated with 200 mg/kg of DFMO s.c., followed 30 min later by 75 mg/kg of *p,p'*-DDT dissolved in corn oil and given by gavage in a volume of 1 ml/kg. Fig. 2B shows that DFMO had no significant effect on spectral analysis of movement, while *p,p'*-DDT produced a significant increase in the average power. Pretreatment with

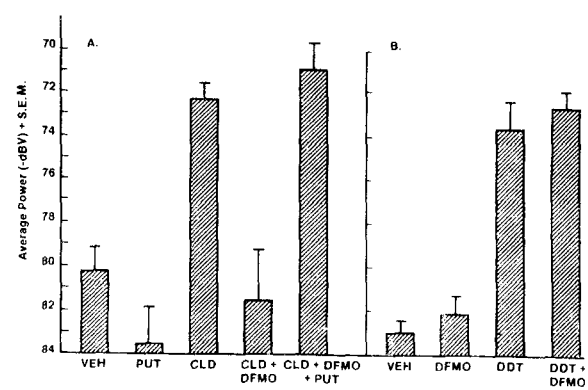

Fig. 2. Effects of putrescine on DFMO attenuation of chlordecone-induced tremor (A). Data are average power units (-dBV) ± S.E.M. of 6–8 rats per group. Putrescine (PUT; 120 mg/kg) was given s.c. at a different injection site than 200 mg/kg of DFMO. Tremorigenic activity was determined 5 h later. B shows that DFMO (200 mg/kg, s.c.) had no effect on the tremorigenic effect produced by 75 mg/kg of *p,p'*-DDT. Testing was performed 8 h after dosing with DDT. Data were analyzed for overall treatment effects using ANOVA. DFMO had no significant effect on tremorigenic activity of DDT.

DFMO had no significant effect on the tremorigenic activity produced by *p,p'*-DDT.

The data reported here are compatible with recent reports suggesting polyamines play a role in calcium mobilization in several tissues including the brain[12] and may be involved in modulating intracellular membrane fusion[9]. DFMO has been shown to inhibit calcium-stimulated release of D-aspartate from nerve endings while the concurrent presence of putrescine prevents this inhibition[1]. Thus, the induction of tremor by chlordecone and its apparent dependence on polyamines such as putrescine may be related to modulation of calcium fluxes within nerve tissue. This conclusion is in accord with the potential effects of chlordecone on intracellular calcium disposition[3]. The finding that DFMO had no effect on tremorigenic activity produced by *p,p'*-DDT while attenuating that of chlordecone lends further support to the conclusion that these two chlorinated insecticides have different mechanisms of action[22]. Finally, our results suggest that, although ODC is very low in the mature nervous system, polyamines appear to play a role in neuronal processes. Furthermore, these results suggest that care be taken in the treatment of individuals with DFMO[15,21], who may also be receiving pharmacological agents having effects on nerve function.

We thank Dr. P. McCann of the Merrell Research Center, Cincinnati, OH, for the DFMO used in this research. The technical assistance of Mr. Ronnie McLamb in performing some of these experiments and Mrs. Loretta Moore in typing this manuscript is gratefully acknowledged.

1 Bondy, S.C. and Walker, C.H., Polyamines contribute to calcium-stimulated release of aspartate from brain particulate fractions, *Brain Research,* in press.
2 Dienel, G.A. and Cruz, N.F., Induction of ornithine decarboxylase during brain recovery from metabolic, mechanical, thermal or chemical injury, *J. Neurochem.*, 42 (1984) 1053–1061.
3 End, D.W., Carchman, R.A. and Dewey, W.L., Neurochemical correlates of chlordecone neurotoxicity, *J. Toxicol. Environ. Health*, 8 (1981) 707–718.
4 Janne, J., Holtta, E., Kallio, A. and Kapyaho, K., Role of polyamines and their antimetabolites in clinical medicine, *Spec. Top. Endocrin. Metabol.*, 5 (1983) 227–293.
5 Genedani, S., Piccinini, G. and Bertolini, A., Putrescine has analgesic activity in rats, *Life Sci.*, 34 (1984) 2407–2412.
6 Genedani, S., Bernardi, M., Botticelli, A. and Bertolini, A., Convulsive syndrome induced by the intraventricular injection of alpha-difluoromethylornithine in rats, *Acta Pharmacol. Toxicol.*, 56 (1985) 250–253.
7 Gerhart, J.M., Hong, J.S., Uphouse, L.L. and Tilson, H.A., Chlordecone-induced tremor: quantification and pharmacological analysis, *Toxicol. Appl. Pharmacol.*, 66 (1982) 234–243.
8 Herr, D.W., Hong, J.S. and Tilson, H.A., DDT-induced tremor in rats: effects of pharmacological agents, *Psychopharmacology*, 86 (1985) 426–431.
9 Hong, K., Schuber, F. and Papahadjopoulos, D., Polyamines: biological modulators of membrane fusion, *Biochim. Biophys. Acta*, 732 (1983) 469–472.
10 Koenig, H., Goldstone, A. and Lu, C.Y., Polyamines regulate calcium fluxes in a rapid plasma membrane response, *Nature (London)*, 305 (1983) 530–534.
11 Koenig, H., Goldstone, A.D. and Lu, C.Y., Blood brain barrier breakdown in brain edema following cold injury is mediated by microvascular polyamines, *Biochem. Biophys. Res. Commun.*, 116 (1983) 1039–1048.
12 Koenig, H., Goldstone, A. and Lu, C.Y., β-adrenergic stimulation of Ca^{2+} fluxes, endocytosis, hexose transport, and amino acid transport in mouse kidney cortex is mediated by polyamine synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 7210–7214.
13 Mauldsley, D.V., Regulation of polyamine biosynthesis, *Biochem. Pharmacol.*, 28 (1979) 153–161.
14 Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Vevert, J.P., Catalytic irreversible inhibition of mammalian ornithine decarboxylase by substrate and product analogues, *J. Am. Chem. Soc.*, 100 (1978) 2551–2552.
15 Pegg, A.E. and McCann, P.P., Polyamine metabolism and function, *Am. J. Physiol.*, 243 (1982) c212–c221.
16 Pajunen, A.E., Hietala, O.A., Virransalo, E.L. and Piha, R.S., Ornithine decarboxylase and adenosylmethionine decarboxylase in mouse brain — effect of electrical stimulation, *J. Neurochem.*, 30 (1978) 281–283.
17 Russell, D.H. and Snyder, S.H., Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tissues, *Proc. Natl. Acad. Sci. U.S.A.*, 60 (1968) 1420–1427.
18 Sakurada, T., Imai, M., Tadano, T. and Kisara, K., Effects of bilateral olfactory bulb ablations on the polyamine levels in rat brain, *Jpn. J. Pharmacol.*, 26 (1976) 509–511.
19 Sakurada, T., Kohno, H., Tadano, T. and Kisara, K., Pharmacological actions of intracerebrally administered polyamines in mice, *Jpn. J. Pharmacol.*, 27 (1977) 453–460.
20 Shaw, G.G., The polyamines in the central nervous system, *Biochem. Pharmacol.*, 28 (1979) 1–6.
21 Sjoerdsma, A. and Schechter, P.J., Chemotherapeutic implications of polyamine biosynthesis inhibition, *Clin. Pharmacol. Ther.*, 35 (1984) 287–300.
22 Tilson, H.A., Hong, J.S. and Mactutus, C.F., Effects of 5,5-diphenylhydantoin (phenytoin) on neurobehavioral toxicity of organochlorine insecticides and permethrin, *J. Pharmacol. Exp. Ther.*, 233 (1985) 285–289.
23 Williams-Ashman, H.G. and Canellakis, Z.N., Polyamines in mammalian biology and medicine, *Perspect. Biol. Med.*, 22 (1979) 421–453.
24 Winer, B.J., *Statistical Principles in Experimental Design*, McGraw-Hill, New York, 1962.
25 Woolley, D.E., Neurotoxicity of DDT and possible mechanisms of action. In K.N. Prasad and A. Vernadakis (Eds), *Mechanisms of Action of Neurotoxic Substances*, Raven Press, New York, 1982, pp. 95–138.