Pharmacology Biochemistry & Behavior, Vol. 41, pp. 211–214. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

RAPID COMMUNICATION

Parenteral Domoic Acid Impairs Spatial Learning in Mice

B. F. PETRIE,*[1] C. PINSKY,† N. M. STANDISH,* R. BOSE† AND G. B. GLAVIN†‡

**Department of Psychology, Red Deer College, Red Deer, Alberta, Canada*
†Department of Pharmacology and Therapeutics, and ‡Department of Surgery, Faculty of Medicine
University of Manitoba, Winnipeg, Manitoba, Canada

Received 06 August 1991

PETRIE, B. F., C. PINSKY, N. M. STANDISH, R. BOSE AND G. B. GLAVIN. *Parenteral domoic acid impairs spatial learning in mice.* PHARMACOL BIOCHEM BEHAV **41**(1) 211–214, 1992.—The present study is the first to examine the effect of a single intraperitoneal injection of the neuroexcitotoxin domoic acid on learning in mice. Compared to saline controls, animals exposed to domoic acid (2.0 mg/kg) showed significant impairment on the acquisition of the place task in the Morris water maze. Observation of swim paths taken by mice searching for the underwater platform revealed a failure on the part of the domoic acid-exposed mice to select the appropriate problem solving strategies. The results, along with neuroanatomic work done here and elsewhere, suggest that impairment of acquisition and retention of this spatial navigation task by domoic acid, involves a neuropathology that includes not only the hippocampus, but other limbic, and possibly extralimbic brain regions.

Domoic acid Acquisition Spatial navigation Learning Hippocampus Morris water maze
Neuroexcitant amino acid Neurotoxin Neuroexcitotoxin Mouse Place task Mussel toxin

THE neuroexcitant amino acid domoic acid, identified as the agent responsible for an outbreak of mussel shellfish poisoning in Canada, in late 1987 (1, 4, 23), has been investigated extensively in both humans (6, 18, 23, 29, 30, 34) and animals (1–3, 7–11, 19–22, 25–28, 32, 33). Many human victims of the poisoning showed an unmistakable selective loss of ability to remember recent events, but few displayed other cognitive defects or dementia (29, 30, 34). A recent report (26) has shown that intrahippocampal microinfusion of domoic acid in rats impairs both learning and memory in the Morris water maze test (16) of spatial navigation. In the present study, we report that compared to saline-treated controls, animals exposed to a single intraperitoneal (IP) injection of domoic acid show significant impairment on the acquisition of the place task in the Morris water maze.

METHOD

Twelve experimentally naive DBA-derived mice (6 male; 6 female; 25–35 g) were selected at random, from the Red Deer College colony for this study, and divided into two groups (6 mice; 3 males, 3 females in each group). Subsequent to the division of the mice into groups, IP injections, in volumes of 10 ml/kg., were administered as follows: Group 1 animals received an initial single injection of saline followed 60 minutes later by a second injection of saline. Group 2 animals received an initial single injection of domoic acid (2.0 mg/kg; Sigma) followed 60 minutes later by an injection of saline.

The spatial learning ability of the 12 mice was assessed in the place version of the Morris water maze (16). Swimming trials in both groups began 60 minutes after their second injection. Both groups were swum daily, undergoing a regimen of 4 trials per day for 14 days. A trial consisted of placing a mouse, by hand, into the water, with its head toward the wall of the circular pool (diameter: 1.5 m), at one of four starting locations: north; south; east; or west, around the pool's perimeter. Within each block of 4 trials, every mouse started at each of the 4 locations. If, on a particular trial, a mouse found the platform, it was permitted to remain there for 10 seconds. A trial was terminated after 60 seconds if the mouse failed to find the platform. At the end of each trial, the animal was returned to a holding cage, and approximately 5 to 8 minutes elapsed before it began the next trial. Swim paths for all animals were recorded by an observer, who remained at a desk, located at the north end of the pool. For the first 7 days of the study, the platform was located in the centre of the SE quadrant of the pool. On day 8 of the study, the platform was moved to the centre of the NW quadrant of the pool where it stayed for the remainder of the experiment. Daily swimming ceased after 14 days.

[1]Requests for reprints should be addressed to Dr. B. F. Petrie, Department of Psychology, Red Deer College, Box 5005, Red Deer, Alberta, Canada T4N 5H5.

TABLE 1

EFFECT OF TREATMENTS ON MEAN SWIMMING LATENCIES IN MICE

Days	Saline Mean Seconds (S.E.) (N=6)	Domoic Acid Mean Seconds (S.E.) (N=6)
1	51.9 (3.2)	58.8 (1.1)*
2	41.7 (3.6)	53.8 (3.1)†
3	16.6 (2.8)	35.5 (5.3)†
4	12.6 (2.2)	31.9 (4.3)‡
5	9.4 (1.2)	20.9 (3.4)†
6	10.2 (1.6)	25.7 (4.4)‡
7	10.5 (1.5)	19.6 (3.1)†
8	32.8 (4.5)	28.7 (4.1)NS
9	10.4 (2.4)	25.8 (4.7)†
10	12.1 (2.5)	21.7 (3.5)*
11	8.2 (1.1)	15.2 (3.4)*
12	9.2 (1.3)	15.1 (2.4)*
13	6.7 (0.9)	12.8 (2.6)*
14	8.6 (1.5)	15.6 (3.1)*

*$p<0.05$; †$p<0.01$; ‡$p<0.001$.
NS = not significant.

Comparisons between the 2 groups on latency to reach the platform were analyzed for each day by Student's *t*-test. Table 1 summarizes the results from the present study.

RESULTS

In this, the first study to examine the effects of domoic acid, administered IP, on acquisition of a spatial discrimination task, the data clearly indicate that on 13 of the 14 days, domoic acid-exposed mice were significantly slower in their performance in the pool, as compared to the saline control animals. The moving of the platform on day 8 proved to be the only day in which the saline animals were not significantly faster than the domoate-exposed mice in reaching the platform (Fig. 1).

Swim paths were also examined for routes taken while searching for the platform. It was apparent from these observations that the domoic acid-exposed mice never acquired the sophisticated search strategies observed in the control animals. Indeed, the domoic acid-exposed animals spent much more of their time in the pool engaged in circling behaviour, with some rudimentary attempts at crossing the pool. It appeared that the domoic acid-exposed mice encountered the platform only after swimming in a circuitous manner, while the saline-treated animals employed a search strategy that initially included circling, followed by a systematic criss-crossing of the pool. Once the platform was encountered and mounted, the saline-treated animals appeared to incorporate distal cues into an accessible spatial map that allowed them, on subsequent trials, to swim directly to the platform.

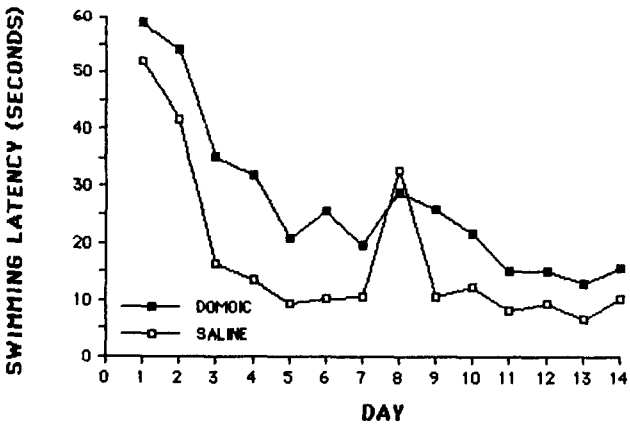

FIG. 1. Mean latencies for acquisition of place task discrimination after saline and domoic acid-saline treatment.

DISCUSSION

The neuroanatomical damage produced by domoic acid has been investigated in mice (5, 27, 28) and rats (25, 32, 33). It appears that while rats administered 2.0 mg/kg IP show only transient behavioural signs suggestive of central nervous system disturbance (e.g., sluggishness and scratching stereotypy), limbic system damage (specifically hippocampal areas CA_1 and CA_3) is reported upon postmortem examination (32). Similarly, mice injected with domoic acid (4.9 mg/kg IP) exhibit damage in the caudate, lateral thalamic and hippocampal (especially CA_3) regions (27). Damage to the CA_1 and CA_3 areas of the hippocampus is consistent with impaired place task performance in the Morris water maze, and it appears that glutaminergic antagonists significantly reduce this cell loss (24). The animals in the present study that were injected with domoic acid showed behavioural signs of central nervous system disturbance, and also showed an extended cognitive impairment on the acquisition of a spatial discrimination task. It is reasonable to speculate that the damage done to the caudate, lateral thalamic and hippocampal regions by domoic acid was at least partly responsible for the impaired strategy selection seen in the domoic acid-exposed mice. It is conceivable that strategy selection is based upon motor control, visual cuing, and memory, and that damage to areas that appear to mediate, at least in part, these functions, may prove detrimental to the overall efficiency of the organism when attempting to arrive at an appropriate selection of strategy in the solution of a problem.

While it is clear that the hippocampus is a structure of paramount importance in memory, and that damage to it has been implicated in a number of neurodegenerative disorders in humans including Huntington's disease (12), and senile dementia of the Alzheimer's type (13,24), the relative contributions of the caudate, lateral thalamic, hippocampal and possibly other brain regions in the solving of a spatial problem have yet to be determined. Circumventricular brain regions are highly sensitive to kainate and domoate toxicity and can, in this regard, be more vulnerable than even the hippocampus to systemic administration of neurotoxins (5, 17, 33). Nevertheless, the role of circumventricular structures in response to parenteral domoic acid administration remains to be elucidated, especially with regard to the involvement of the mediobasal hypothalamus. It has been suggested (5) that domoic acid excitation of hypothalamic neurons may activate hypothalamic projections (15), and thereby cause "remote" damage to the limbic system. This suggestion is attractive because domoic acid, which appears to cross the blood-brain barrier with great difficulty (22), would have access to all structures within the circumventricular region (5, 17, 33). This latter circumstance would also account for the autonomic responses that ubiquitously accompany the central nervous system effect of parenterally administered domoic acid (2, 5, 8–10).

Thus the true nature of the ability of parenteral domoic acid to impair spatial task learning may reside in the neuroexcitotoxin's proclivity to damage a multipartite system of which the hippocampus is only one important component. Such an integrated

system for spatial learning and cognition would support Krechevsky's (14) theory. Krechevsky proposed that task acquisition could be divided into two phases, a presolution phase and a solution phase. In the presolution condition, animals experiment with various "attempted solutions" or "hypotheses" from which an appropriate behavioural pattern is eventually selected and used in the solution phase. This capacity to perform well on learning tasks is probably dependent upon the existence of innate mechanisms that can be usefully applied to the task (31). It is therefore conceivable that the neuroanatomical damage caused by domoic acid may be located in areas of the central nervous system that are responsible for the selecting, and sequencing, from a variety of potential strategies, the appropriate method or methods necessary for the most efficient solution of the problem. Damage might be severe in some regions (e.g., hypothalamus; hippocampus), and moderate in others, a possibility that could explain the varying degrees of dementia and memory loss seen in the human victims of the amnestic mussel poisoning epidemic.

This is the first experimental study to show that parenteral administration of domoic acid, a neuroexcitotoxin that found its way into foodstuff consumed by humans, is detrimental to learning. Parenteral administration, unlike intracerebral microinjection, provides for greater ecological validity in studying the damage that might accrue to many brain regions shortly after ingestion. This multifaceted degeneration would more likely resemble the neuropathology seen in clinical neurodegenerative disease states than would a regionally restricted model. Our present results might prove useful not only in determining the mechanisms of domoate toxicity, but also establishing the neuroexcitotoxin-treated mouse as an animal model with which to study the learning deficits found in senile dementia of the Alzheimer's type, as well as in other neurodegenerative disorders.

ACKNOWLEDGEMENTS

This work was supported by Health and Welfare Canada/NHRDP (C.P., G.B.G.), the Medical Research Council of Canada (C.P., G.B.G.), S.E.E.D. (N.M.S.), and RDC Special Projects Fund (B.F.P.).

REFERENCES

1. ARL Shellfish Toxin Team. Solving the toxic mussel problem. Can. Chem. News 40:15–17; 1988.
2. Bose, R.; Glavin, G.; Pinsky, C. Neurotoxicity and lethality of toxic extracts from Atlantic coast shellfish. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:559–562; 1989.
3. Bose, R.; Pinsky, C.; Glavin, G. Sensitive murine model and putative antidotes for behavioral toxicosis from contaminated mussel extracts. Can. Dis. Week. Rep. 16S1E(Suppl.; September):91–100; 1990.
4. Boyd, R.; Hogge, L.; Jamieson, W.; McLaren, J.; Quilliam, M.; Sim, P.; Thibault, P.; Wright, J. The role of mass spectrometry combined with reversed phase HPLC in the identification of an unusual shellfish toxin. Proc. Am. Soc. Mass. Spec. 36:165; 1988.
5. Bruni, J. E.; Bose, R.; Pinsky, C.; Glavin, G. Circumventricular organ origin of domoic acid-induced neuropathology and toxicology. Brain Res. Bull. 26:419–424; 1991.
6. Carpenter, S. The human neuropathology of encephalopathic mussel toxin poisoning. Can. Dis. Week. Rep. 16S1E(Suppl.; September): 73–74; 1990.
7. Debonnel, G.; Beauchesne, L.; de Montigny, C. Domoic acid, the alleged mussel toxin, might produce its neurotoxic effect through kainate receptor activation; an electrophysiological study of the rat dorsal hippocampus. Can. J. Physiol. Pharmacol. 67:29–33; 1989.
8. Glavin, G.; Bose, R.; Pinsky, C. Kynurenic acid protects against gastroduodenal ulceration in mice injected with extracts from poisonous Atlantic shellfish. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:569–572; 1989.
9. Glavin, G.; Pinsky, C.; Bose, R. Domoic acid-induced neurovisceral toxic syndrome: Characterization of an animal model and putative antidotes. Brain Res. Bull. 24:701–703; 1990.
10. Glavin, G.; Pinsky, C.; Bose, R. Gastrointestinal effects of contaminated mussels and putative antidotes thereof. Can. Dis. Week. Rep. 16S1E(Suppl.; September):111–115; 1990.
11. Glavin, G.; Pinsky, C. Kynurenic acid attenuates experimental ulcer formation and basal gastric secretion in rats. Res. Commun. Chem. Pathol. Pharmacol. 64:111–119; 1989.
12. Greenamyre, J. T. The role of glutamate in neurotransmission and in neuroleptic disease. Arch. Neurol. 43:1058–1063; 1986.
13. Greenamyre, J. T.; Young, A. B. Excitatory amino acids and Alzheimer's disease. Neurobiol. Aging 10:593–602; 1989.
14. Krechevsky, I. A study of the continuity of the problem-solving process. Psychol. Rev. 45:107–133; 1938.
15. Morgane, P.; Panskepp, J., eds. Handbook of the hypothalamus. vol. 1: Anatomy of the hypothalamus. New York: Marcel Dekker; 1979.
16. Morris, R. G. M. Spatial localization does not require the presence of local cues. Learn. Motiv. 12:239–260; 1981.
17. Olney, J. W. Excitotoxic amino acids: Research applications and safety implications. In: Glutamic acid: Advances in biochemistry and physiology. In: Filer, L. J.; Garattini, S.; Kare, M. R.; Reynolds, W. A.; Wurtman, R. J., eds. New York: Raven Press; 1979: 287–319.
18. Perl, T. M.; Bedard, L.; Kosatsky, T.; Hockin, J.; Todd, E. C. D.; McNutt, L. A.; Remis, R. S. Amnestic shellfish poisoning: A new clinical syndrome due to domoic acid. Can. Dis. Week Rep. 16S1E(Suppl.; September):7–8; 1990.
19. Pinsky, C.; Bose, R.; del Campo, M.; Sutherland, G.; Glavin, G.; Bruni, J. Kynurenate antagonism of domoic acid-provoked EEG seizure activity in the mouse. Eur. J. Pharmacol. 183:514–515; 1990.
20. Pinsky, C.; Bose, R.; Hall, A.; Glavin, G. Opioid involvement in epileptogenetic and neurovisceral activity. In: Quiron, R.; Jhamandas, K.; Gianoulakis, C., eds. Progress in clinical and biological research. vol. 328. New York: Liss; 1990:421–424.
21. Pinsky, C.; Glavin, G.; Bose, R. Kynurenic acid protects against neurotoxicity and lethality of toxic extracts from contaminated Atlantic coast mussels. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:595–598; 1989.
22. Preston, E.; Hynie, I. Transfer constants for blood-brain barrier permeation of the neuroexcitatory shellfish toxin, domoic acid. Can. J. Neurol. Sci. 18:39–44; 1991.
23. Quilliam, M. A.; Wright, J. The amnestic shellfish poisoning mystery. Anal. Chem. 61:1053–1060; 1989.
24. Rogers, B.; Barnes, M.; Mitchell, C.; Tilson, H. Functional deficits after sustained stimulation of the perforant path. Brain Res. 493:41–50; 1989.
25. Stewart, G. R.; Zorumski, C. F.; Price, M. T.; Olney, J. W. Domoic acid: A dementia-inducing excitotoxic food poison with kainic acid receptor specificity. Exp. Neurol. 110:127–138; 1990.
26. Sutherland, R. J.; Hoesing, J. M.; Whishaw, I. Q. Domoic acid, an environmental toxin, produces hippocampal damage and severe memory impairment. Neurosci. Lett. 120:221–223; 1990.
27. Tasker, R.; Connell, B.; Strain, S. Pharmacological, behavioural and morphological studies of domoic acid toxicity in vivo. Eur. J. Pharmacol. 183:959; 1990.
28. Tasker, R.; Connell, B.; Strain, S. M. Pharmacology of systemically administered domoic acid in mice. Can. J. Physiol. Pharmacol. 69:378–382; 1991.
29. Teitelbaum, J.; Zatorre, R.; Carpenter, S.; Gendron, D.; Cashman, N. Neurological sequelae of domoic acid intoxication. Can. Dis. Week Rep. 16S1E(Suppl.; September):9–13; 1990.
30. Teitelbaum, J.; Zatorre, R.; Carpenter, S.; Gendron, D.; Evans, A.; Gjedde, A.; Cashman, N. Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. N. Engl. J.

Med. 13:559–562; 1989.
31. Tinbergen, N. The study of instinct. New York: Oxford University Press; 1951.
32. Tryphonas, L.; Truelove, J.; Nera, E.; Iverson, F. Acute neurotoxicity of domoic acid in the rat. Toxicol. Pathol. 18:1–9; 1990.
33. Tryphonas, L.; Iverson, F. Neuropathology of excitatory neurotoxins: The domoic acid model. Toxicol. Pathol. 18:165–169; 1990.
34. Zattore, R. J. Memory loss following domoic acid intoxication from ingestion of toxic mussels. Can. Dis. Week Rep. 16S1E(Suppl.; September):101–104; 1990.