Pharmacology Biochemistry & Behavior, Vol. 36, pp. 207–210. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

Ibotenic Acid Lesions of the Basal Forebrain Cholinergic System Retard Amygdala Kindling

DONALD P. CAIN AND DWIGHT J. STEWART

Departments of Psychology and Clinical Neurological Sciences
University of Western Ontario, London, Ontario, Canada N6A 5C2

Received 30 November 1988

CAIN, D. P. AND D. J. STEWART. *Ibotenic acid lesions of the basal forebrain cholinergic system retard amygdala kindling.* PHARMACOL BIOCHEM BEHAV 36(1) 207–210, 1990.—The effect of ibotenate lesions of septum and substantia innominata on electrical kindling of the amygdala was investigated in rats. The lesions significantly retarded kindling in the absence of a change in initial seizure sensitivity. This suggests that cholinergic neurotransmission can contribute to, but is not crucial for, amygdala kindling, and that a major component of the cholinergic circuitry involved in amygdala kindling may originate in the septum or substantia innominata or both.

Kindling Seizures Ibotenic acid Acetylcholine

CONSIDERABLE evidence links cholinergic function and epileptic phenomena. Cholinergic agonists and anticholinesterases can evoke or intensify seizure activity (11,13), and repeated administration of initially subconvulsant doses of the cholinomimetic carbachol can kindle generalized convulsions (21). Carbachol kindling and dietary choline facilitate subsequent electrical kindling (4,15), and prophylactic administration of cholinergic antagonists retards kindling (1, 2, 5, 23). Collectively, these data suggest that cholinergic mechanisms play a role in amygdala kindling.

The location of the cholinergic circuitry that is of importance for kindling is unknown. Results obtained with histochemical procedures that stain for cholinergic markers indicate that there are no cholinergic cell bodies in the amygdaloid complex (14). However, the amygdaloid complex and a number of neuroanatomically related structures that are probably involved in kindling receive afferents from cholinergic neurons originating from the medial septum and in or near the substantia innominata (SI) (10, 14, 18). In an initial attempt to identify cholinergic circuitry that participates in amygdala kindling we have selectively destroyed the septum and SI by injection of ibotenic acid prior to amygdala kindling. This treatment results in the excitotoxic destruction of neural cell bodies with sparing of fibers of passage, and avoids the confounding effect of seizures that accompany lesions produced by other excitotoxic agents such as kainic acid (8).

Male hooded rats of the Royal Victoria strain weighing 250–350 g served as subjects. They were housed individually and maintained on ad lib food and water and a 12/12-hr light/dark cycle. They were anesthetized with 60 mg/kg sodium pentobarbital IP and placed in a stereotaxic apparatus. For the lesioned group ibotenic acid was stereotaxically injected into the medial septum (coordinates: +4.8 mm from bregma, midline, −7.0 mm below skull surface) and the left caudal SI (−0.92 mm from bregma, 2.5 mm from midline, −7.5 mm below skull surface) (16,17). Each injection contained 10 μg ibotenic acid in 1 μl Locke's solution (without glucose). For the control group equivalent injections of Locke's solution were made. Injections were made over 6 or 15 min using an infusion pump. These lesion procedures result in substantial functional damage to forebrain cholinergic systems with much less mortality than typically occurs as a result of bilateral electrolytic lesions of SI (13, 19, 20).

After a minimum 14-day recovery period the subjects received implantation of a bipolar electrode into the left basolateral amygdala using standard stereotaxic techniques. The electrodes were constructed of twisted Teflon-insulated nichrome wire 127 μm in diameter that was soldered to small contacts. The contacts were placed in a headcap assembly that was attached to the skull with small screws and dental acrylic. At least one week after recovery from surgery the subjects were connected to a polygraph and Grass S88 electrical stimulator for determination of afterdischarge threshold (ADT). An initial current of 40 μA base-to-peak was applied, which subsequently was raised in small steps until an AD was observed on the polygraph. The ADT was defined as the weakest current that would evoke an AD of 4 sec or longer. The current consisted of biphasic square wave pulses, each 1.0 msec in duration, at 60 Hz and a total duration of 1 sec. All subjects were stimulated once daily at their ADT until a stage 5 convulsion occurred.

TABLE 1

EFFECTS OF IBOTENIC ACID LESIONS ON AMYGDALA KINDLING

Group	N	AD Threshold (μA)	Initial AD Duration (sec)	Final AD Duration (sec)	ADs to Stage 5	Damage Rating
Experimental	7	53.6 ± 6.4	5.4 ± 0.9	47.6 ± 7.9	16.7 ± 2.1*	1.6 ± 0.3*
Control	7	67.9 ± 13.0	7.6 ± 1.2	61.0 ± 13.5	9.9 ± 0.5	0.1 ± 0.1

Measures are means ± S.E.M.
*Different from control group, $p<0.01$.

At the end of testing the subjects were perfused with cold saline followed by formol sucrose ammonia, and the brains were removed and prepared for sectioning on a freezing microtome. Alternate sections were stained with cresyl violet/luxol fast blue and prepared for acetylcholinesterase (ACHE) histochemistry using the technique described by Stewart *et al.* (19). Sections from all subjects were rated on a 3-point scale for damage to septum and SI and density of ACHE stain in the ipsilateral amygdala and neocortex by one of us (D.J.S.) who did not know the results of the kindling phase of the experiment (see below). All subjects discussed below had electrode placements in or on the border of the basolateral amygdala, but not in or on the border of the cortical amygdala (12).

The results are presented in Table 1. The experimental group kindled significantly slower than the control group ($p<0.01$, *t*-test), and exhibited somewhat attenuated mean initial and final AD durations. However, the differences in AD duration and threshold between the groups did not reach statistical significance ($p>0.05$, *t*-tests).

The histological ratings ranged from 0 (no detectable damage in septum or SI apart from the needle track, or loss of ACHE staining) to 3 (marked lateral septum and SI damage and marked loss of ACHE staining in amygdala and neocortex in lesioned hemisphere compared to unlesioned hemisphere). All but one of the experimental brains showed clear evidence of cell loss in the lateral septum and the lesioned SI, and moderate to strong loss of ACHE staining in the basolateral amygdala in the lesioned hemisphere compared to the unlesioned hemisphere. Lesioned and control brain sections stained for acetylcholinesterase are illustrated in Fig. 1. Comparison of ACHE staining in lesioned and control rats revealed normal levels of staining in the unlesioned hemisphere of the lesioned group. There was no evidence of cell loss in the basolateral amygdala of any rat. One control rat was found to have a small amount of septum and SI damage. The difference in the mean damage rating (Table 1) was statistically significant ($p<0.01$, *t*-test). The correlation between the damage rating and rate of kindling to stage 5 for the experimental group was not significant ($p>0.05$).

The main result of this study is the finding that ibotenic acid lesions of the septum and SI significantly retard kindling of the basolateral amygdala. The fact that initial measures of seizure sensitivity did not differ between the groups suggests that the retardation effect of the lesions was not secondary to a decrease in seizure sensitivity.

Previous attempts to study the effects of lesion damage on cholinergic afferents to the amygdala on amygdala kindling have yielded mixed results. Walker *et al.* (22) electrolytically lesioned the lateral preoptic area but failed to observe a retardation in the rate of amygdala kindling. Kimura *et al.* (13) found that large electrolytic lesions of the SI led to high rates of mortality, and were forced to abandon this approach. In an acute assessment of the effects of large electrolytic lesions of SI in rats previously kindled in the amygdala, Kimura *et al.* (13) found that the lesions almost completely suppressed the kindled response. Chronic and acute lesions of the diagonal band of Broca, medial septum, interpenduncular nucleus, or the habenula did not significantly affect the seizures, suggesting that the retardation effect was specific to the SI.

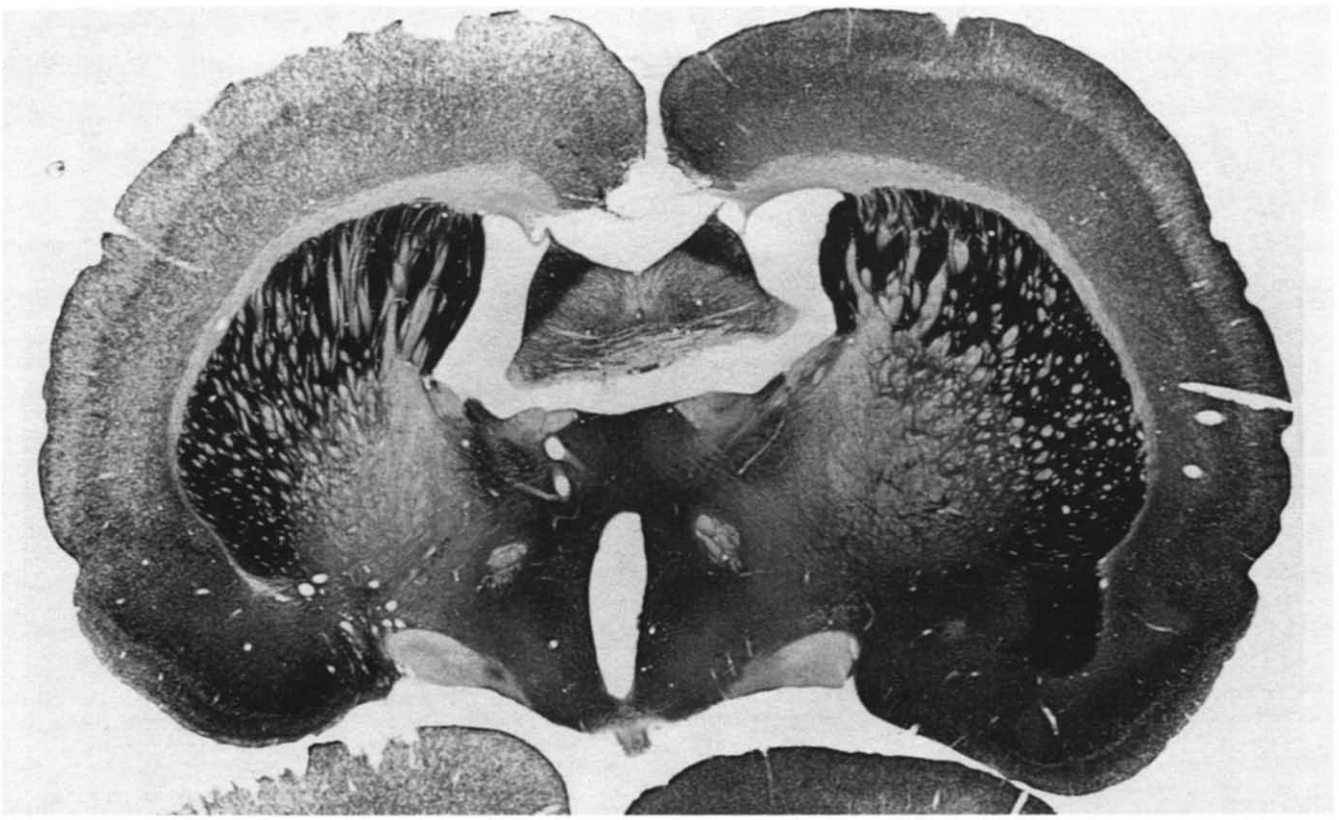
It is not clear why Walker *et al.* (22) failed to observe a chronic retardation effect, whereas Kimura *et al.* (13) observed a strong acute seizure suppression effect. It is possible that the electrolytic lesions made by Walker *et al.*, which did not lead to the expected high rate of mortality that typically results from electrolytic damage to the SI, failed to destroy circuits involved in amygdala kindling. Our results, which were obtained with a unilateral lesion technique that damages cholinergic input to the amygdala with low rates of mortality, are consistent with the acute data reported by Kimura *et al.* (13).

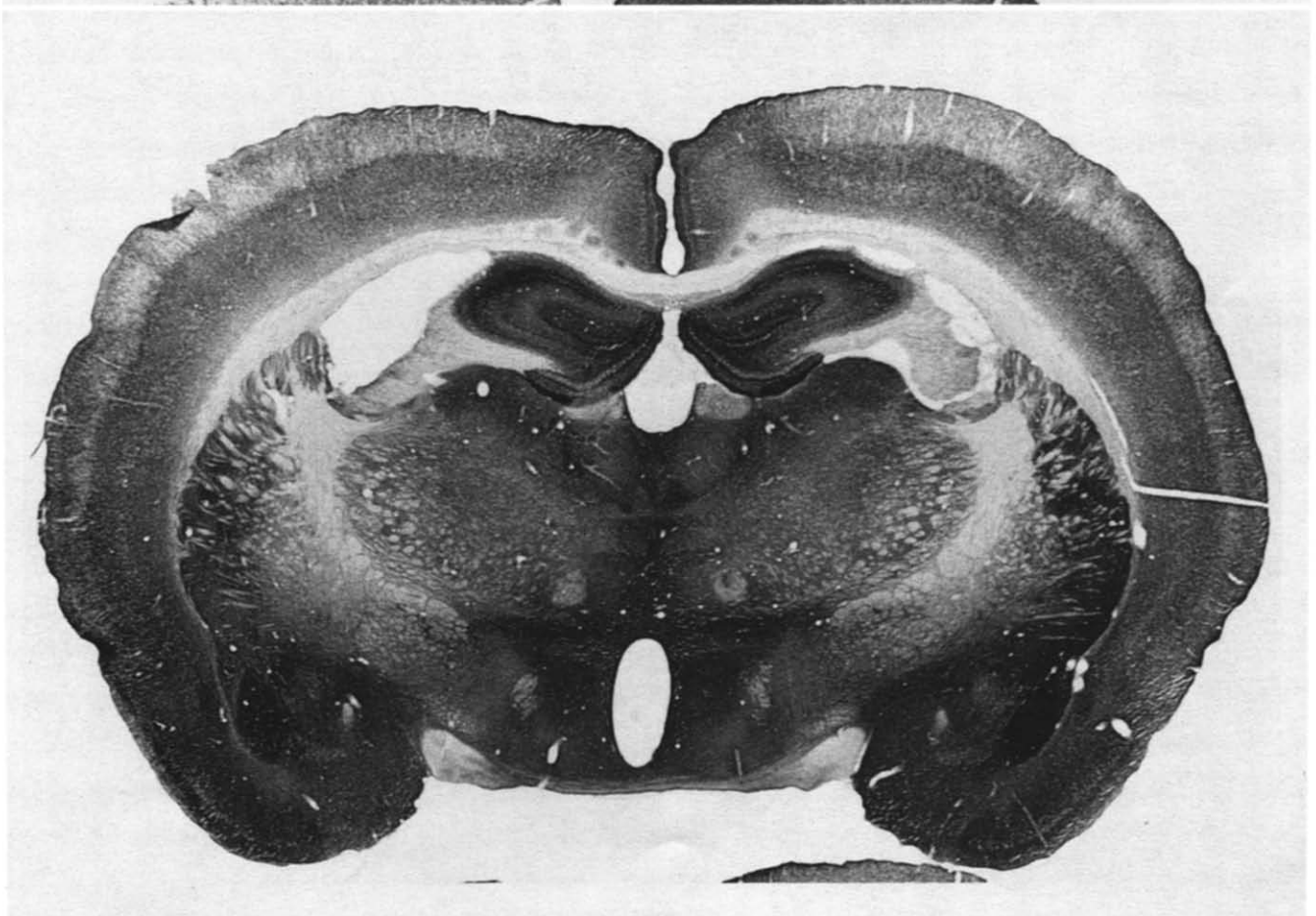
The retardation of kindling observed in this study is similar to that reported to result from the prophylactic administration of muscarinic antagonists (1, 2, 5, 23). Our results are consistent with these and other results linking muscarinic cholinergic function with kindled seizure development, and suggest that a major component of the cholinergic circuitry involved in amygdala kindling may originate in the septum or SI or both. However, in view of the fact that damage to the medial septum was minimal or absent, while damage to the lateral septum was generally pronounced among the experimental rats [similar to our previous observations, (20)], it is possible that the main contribution to the observed retardation effect was the reduction in cholinergic innervation of the amygdala and neocortex originating in the SI.

The fact that interference with cholinergic mechanisms in this and earlier studies did not completely block kindling indicates that cholinergic mechanisms contribute to, but are not crucial for kindling. Evidence obtained in our laboratory and elsewhere indicates that a number of excitatory neurotransmitters can contribute to amygdaloid kindling, and that kindling may normally proceed in part through a mechanism involving the summation of excitatory neurotransmission (5, 6, 9, 16). Thus, interference with a single excitatory neurotransmitter would be expected to retard but not block kindling. The contribution of other excitatory neurotransmitters as well as other possible mechanisms to the kindling process might account, in part, for our failure to find a significant relation between kindling rate and rated damage to cholinergic structures, and for the occasional failure of muscarinic antagonists to significantly retard kindling (3, 7, 16).

FACING PAGE

FIG. 1. Histological sections (acetylcholinesterase stain) through the amygdala in rats injected with ibotenic acid (top) and control solution (bottom). The injections were made in the hemisphere on the left. Note the marked paling of the neocortex and basolateral amygdala in the injected hemisphere (top), but normal staining in the control brain (bottom).





ACKNOWLEDGEMENT

Supported by a grant to D.P.C. from N.S.E.R.C. of Canada.

REFERENCES

1. Albright, P.; Burnham, W. M.; Okazaki, M. Effect of atropine sulfate on amygdaloid kindling in the rat. Exp. Neurol. 66:409–412; 1979.
2. Arnold, P. S.; Racine, R. J.; Wise, R. Effects of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. Exp. Neurol. 40:457–470; 1973.
3. Blackwood, D.; Martin, M.; Howe, J. A study of the role of the cholinergic system in amygdaloid kindling. Psychopharmacology (Berlin) 76:66–69; 1982.
4. Cain, D. P. Bidirectional transfer of electrical and carbachol kindling. Brain Res. 260:135–138; 1983.
5. Cain, D. P.; McKitrick, D. J.; Desborough, K. A. Effects of treatment with scopolamine and naloxone, singly and in combination, on amygdala kindling. Exp. Neurol. 96:97–103; 1987.
6. Cain, D. P.; Desborough, K. A.; McKitrick, D. J. Retardation of amygdala kindling by antagonism of NMD-aspartate and muscarinic cholinergic receptors: Evidence for the summation of excitatory mechanisms in kindling. Exp. Neurol. 100:179–187; 1988.
7. Corcoran, M. E.; Wada, J.; Wake, A.; Urstad, H. Failure of atropine to retard amygdaloid kindling. Exp. Neurol. 51:271–275; 1976.
8. Coyle, J. T. Excitatory amino acid neurotoxins. In: Iverson, L.; Iverson, S.; Snyder, S. H., eds. Handbook of psychopharmacology, vol. 15. New York: Plenum Press; 1982:237–269.
9. Dennison, Z.; Cain, D. P. Retardation of amygdaloid kindling by and the behavioral effects of kynurenic acid, an antagonist of excitatory amino acids: A preliminary report. Soc. Neurosci. Abstr. 13:761; 1987.
10. Emson, P. C.; Paxinos, G.; Le Gal La Salle, G.; Ben-Ari, Y.; Silver, A. Choline acetyltransferase and acetylcholinesterase containing projections from the basal forebrain to the amygdaloid complex of the rat. Brain Res. 165:271–282; 1979.
11. Ferguson, J.; Cornblath, D. Acetylcholine epilepsy: Relationship of surface concentration, chronicity of denervation, and focus size. Exp. Neurol. 46:302–314; 1975.
12. Gilbert, M. E.; Gillis, B. G.; Cain, D. P. Kindling in the cortical nucleus of the amygdala. Brain Res. 295:360–363; 1984.
13. Kimura, H.; Kaneko, Y.; Wada, J. A. Catecholamine and cholinergic systems and amygdaloid kindling. In: Wada, J. A., ed. Kindling 2. New York: Raven Press; 1981:265–284.
14. Kimura, H.; McGeer, P.; Peng, J.; McGeer, E. Mapping of cholinergic systems in rostral forebrain of the rodent. In: Pupeu, G.; Ladinsky, H., eds. Cholinergic mechanisms. New York: Plenum Press; 1981:695–704.
15. McCann, K.; Cain, D. P.; Philbrick, D. J. Facilitation of kindled seizures in rats fed choline-supplemented diets. Can. J. Neurol. Sci. 10:47–49; 1983.
16. Meyerhoff, J.; Bates, V. Combined treatment with muscarinic and nicotinic cholinergic antagonists slows development of kindled seizures. Brain Res. 339:386–389; 1985.
17. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Orlando: Academic Press; 1986.
18. Saper, C. Organization of cerebral sortical afferent systems in the rat. II. Magnocellular basal nucleus. J. Comp. Neurol. 222:313–342; 1984.
19. Stewart, D. J.; MacFabe, D. F.; Vanderwolf, C. H. Cholinergic activation of the electroencephalogram: Role of the substantia innominata and effects of atropine and quinuclidinyl benzilate. Brain Res. 322:219–232; 1984.
20. Stewart, D. J.; Vanderwolf, C. H. Hippocampal rhythmical slow activity following ibotenic acid lesions of the septal region. I. Relations to behavior and effects of atropine and urethane. Brain Res. 423:88–100; 1987.
21. Vosu, H.; Wise, R. Cholinergic seizure kindling in the rat: Comparison of caudate, amygdala and hippocampus. Behav. Biol. 13: 491–495; 1975.
22. Walker, J.; Hirsch, S.; Crawford, I. Interruption of cholinergic pathways to the amygdala failed to alter electrical kindling. Exp. Neurol. 88:742–747; 1985.
23. Westerberg, V.; Corcoran, M. E. Antagonism of central but not peripheral cholinergic receptors retards amygdala kindling in rats. Exp. Neurol. 95:194–206; 1987.