Basolateral amygdala lesions block diazepam-induced anterograde amnesia in an inhibitory avoidance task

(benzodiazepine/memory/ibotenic acid)

CARLOS TOMAZ*, HEATHER DICKINSON-ANSON^{†‡}, AND JAMES L. MCGAUGH[†]

*Laboratory of Psychobiology, University of Sao Paulo, Ribeirao Preto, Brazil; and [†]Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717

Contributed by James L. McGaugh, January 2, 1992

ABSTRACT This experiment examined the effects of diazepam (DZP) on acquisition and retention of an inhibitory avoidance response by rats with excitotoxic-induced lesions of central (CE), lateral (LAT), or basolateral (BL) amygdala nuclei. Sham-operated and lesioned rats received i.p. injections of DZP (2.0 mg per kg of body weight) 30 min before training in a continuous multiple-trial inhibitory avoidance task. Retention was tested 48 h later. Acquisition was not impaired by the lesions or the DZP. Retention was impaired in animals with CE and LAT lesions in comparison with sham-operated controls. DZP impaired retention in the sham-operated controls as well as CE- and LAT-lesioned animals but did not affect retention in animals with BL lesions. These findings indicate that the DZP-induced anterograde amnesia for inhibitory avoidance training is mediated through influences involving the BL amygdala nucleus.

It is well known that benzodiazepines (BZDs) induce anterograde amnesia in humans (1, 2) as well as in animals trained in a variety of learning tasks (3–7). The memory-impairing effects of systemically administered BZDs appear to be due to influences on memory processes initiated during acquisition (8, 9) and not to sedation, state dependency, or motoric effects (4, 10–12).

Extensive evidence suggests that the anxiolytic effects of BZDs involve influences mediated by the amygdaloid complex. Intra-amygdala injections of BZDs produce anxiolytic effects comparable to those induced by systemic injections (13, 14). Furthermore, intra-amygdala injections of the BZD antagonist flumazenil attenuate the anxiolytic effects of systemically administered BZDs (15). The findings of several studies suggest that the anxiolytic effects of BZDs are mediated by the lateral (LAT)/basolateral (BL) amygdala nuclei. Injections of the BZDs chlordiazepoxide and midazolam into the LAT/BL amygdala induce anxiolytic effects, whereas injections of midazolam into the central (CE) amygdala are without effect (15–17). Such findings are consistent with evidence that BZD receptors are densely located in the LAT/BL amygdala (16, 18, 19).

Recent findings suggest that the memory impairing effects of BZDs also involve the amygdaloid complex. Bilateral intra-amygdala injections of flumazenil and midazolam enhance and impair, respectively, retention performance of rats trained in aversively motivated tasks (20; H.A.D.-A. and J.L.M., unpublished data). Furthermore, we recently reported that excitotoxic lesions of the amygdaloid complex block diazepam (DZP)-induced impairment of retention in an inhibitory avoidance task (21).

The evidence summarized above suggests that both the anxiolytic and the amnesic effects of BZDs are mediated by the amygdala. Furthermore, findings implicating the

LAT/BL amygdala nuclei in the anxiolytic effects of BZDs suggest that these nuclei might also mediate the memory-modulating effects of BZDs. To examine this implication, we investigated the effects of DZP on the acquisition and retention of an inhibitory avoidance response in rats with ibotenic acid (IBO)-induced lesions of the CE, LAT, and BL amygdala.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (200–225 g on arrival) from Charles River Laboratories were housed in a temperature-controlled (22°C) colony room and maintained on a 12-h light/12-h dark cycle (0700–1900 lights on) with food and water available ad lib. The rats were randomly assigned to one of four surgery groups: sham operated, CE lesion, LAT lesion, and BL lesion.

Surgery. After arrival, the rats were maintained in the animal colony room for 1 week before surgery. They were anesthetized with sodium pentobarbital (50 mg per kg of body weight, i.p.) and given atropine sulfate (0.4 mg/kg, i.p.). The skull was fixed to a stereotaxic frame (Kopf Instruments, Tujunga, CA) and bilateral lesions of either the CE, LAT, or BL were produced by IBO (Sigma; 1 mg per 100 μ l of phosphate buffer, pH 7.4). The IBO solution was back-filled into a 30-gauge needle, which was attached by a polyethylene tube to a 10- μ l syringe (Hamilton, Reno, NV) driven by a minipump (Sage Instruments, Boston). The needle was placed into either the CE [coordinates: anteroposterior (AP), –2.2 mm from bregma; mediolateral (ML), $\pm$ 4.1 mm from midline; dorsoventral (DV), –8.0 mm from dura; incisor bar, –3.3 mm from interaural line], LAT (AP, –3.0 mm; ML, $\pm$ 5.1 mm; DV, –7.5 mm; incisor, –3.3 mm), or BL (AP, –3.0 mm; ML, $\pm$ 5.1 mm; DV, –8.5 mm; incisor, –3.3 mm) amygdala (22) and IBO (0.25 μ g per 0.25 μ l of phosphate buffer) was injected over a period of 3.5 min. The injection needle was retained in place for an additional 5 min to maximize diffusion of the solution. Sham operations used the same general procedure except that the needle was lowered only to the level of the caudate/putamen (AP, –2.2 mm; ML, $\pm$ 4.5 mm; DV, –6.0 mm; incisor, –3.3 mm) and then removed immediately without injection of IBO. The animals were allowed to recover for 6–7 days before the onset of the training.

Apparatus and Procedures. The rats were trained on an inhibitory avoidance apparatus (23) located in a sound-attenuated room. The apparatus consisted of a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top, 6.4 cm wide at the floor) divided into two compartments separated by a sliding door that opened by retracting into the floor. The

Abbreviations: BZD, benzodiazepine; LAT, lateral; BL, basolateral; CE, central; DZP, diazepam; IBO, ibotenic acid; AP, anteroposterior; ML, mediolateral; DV, dorsoventral; CMIA, continuous multiple-trial inhibitory avoidance.

[‡]To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

FIG. 1. (Figure continues on the opposite page.)

FIG. 1. Representative photomicrograph of IBO lesions of the CE (A), LAT (B), and BL (C) amygdala nuclei. Arrows indicate area enlarged in *Insets*. (*Insets*, $\times 200$.)

starting compartment (31 cm long) was illuminated and the shock compartment (60 cm long) was dark.

A continuous multiple-trial inhibitory avoidance (CMIA) training procedure was used. On the training day, each animal received an i.p. injection of either DZP or vehicle 30 min before training. The rat was then placed in the starting compartment of the apparatus facing the guillotine door. When the rat turned away from the door, the door was opened and when the rat turned toward the open door, the timer was started. As the rat stepped with all four paws into the dark compartment, a footshock (0.55 mA) was delivered (maximum, 5 sec) until the rat escaped back into the starting compartment. The door remained open throughout the subsequent training period. During this period, whenever the rat reentered the dark compartment, shock was again delivered and was terminated when the rat escaped to the starting (safe) compartment. The rat was retained in the apparatus until it remained in the starting compartment continuously for 100 sec. The animal was then returned to its home cage. The latency of the initial entrance into the shock compartment and the total number of trials (i.e., entries) required to reach the acquisition criterion of 100 consecutive sec in the safe compartment were recorded. Retention was tested 48 hr after training. The rat was placed in the starting compartment, as in the training session, and the latency to step into the dark compartment (maximum, 600 sec) was recorded. Shock was not administered on the retention test trial.

Drug Administration. DZP (a generous gift from Hoffman-LaRoche) was suspended in a vehicle consisting of 0.9% saline plus two drops of Tween 80 and injected i.p. at 2.0 mg/kg in a vol of 1.0 ml/kg. To ensure a homogeneous solution, the suspension was agitated with a Vortex mixer immediately before each injection. The drug and control

vehicle solutions were coded so that experimenters were blind to the drug conditions.

Histology. The rats were anesthetized with an overdose of chloral hydrate and perfused intracardially with a 0.9% saline solution followed by 10% phosphate-buffered formalin. The brains were removed and placed in a 20% sucrose/10% formalin solution for at least 5 days, sectioned into 40- μ m slices (using a freezing microtome), and stained with thionin. The sections were examined under a light microscope. Determination of the location of the lesions was made according to the standardized atlas plates of Paxinos and Watson (22).

Statistics. Behavioral results were subjected to a 4×2 analysis of variance (ANOVA) with lesion (four levels) and drug (two levels) as the between-subjects variables. Acquisition trials are expressed as means $\pm$ SEM. Retention latencies were analyzed by Mann-Whitney U tests and are expressed as medians (interquartile range).

RESULTS

Histological examination of the slides indicated that the lesioned areas were characterized by loss of neurons and gliosis in the tissue. Representative IBO lesions of the CE, LAT, and BL nuclei are shown in Fig. 1.

In all of the animals included in the analyses for each lesion group, 50% or more of the specific nucleus was damaged bilaterally. The data of four animals were excluded because their lesions failed to meet this criterion and those of one additional animal were excluded because extensive damage was seen in adjacent amygdala nuclei. In several other animals, whose data are included in the analyses, minor damage was seen in adjacent amygdala nuclei. The behav-

ioral data of these animals did not differ from those of animals whose lesions were restricted to the targeted nuclei.

The groups did not differ in the step through latencies on trial 1 of the CMIA task [$F(3,67) = 1.33$; $P > 0.05$]. The median latencies of the groups were all between 3 and 5 sec. In addition, there were no significant differences among the groups in the number of trials required to reach the acquisition criterion [$F(7,67) = 0.901$; $P > 0.5$]. The median number of trials to criterion ranged from 1 to 2.

The retention test latencies are shown in Fig. 2. Analyses of the differences between the initial step-through training latencies and retention test latencies indicated that all groups showed significant retention of the task ($P < 0.001$ for all comparisons, repeated measures). A two-way ANOVA revealed significant drug treatment effect [$F(1,67) = 85$; $P < 0.0001$], significant lesion effect [$F(3,67) = 38$; $P < 0.0001$] and significant drug times lesion interaction [$F(3,67) = 26$; $P < 0.0001$]. A posteriori analyses with Mann-Whitney U tests indicated that retention performance was significantly impaired by CE and LAT lesions but was not affected by BL lesions. The retention latencies of the CE lesion-vehicle and LAT lesion-vehicle groups were significantly shorter than those of the sham-vehicle group ($P < 0.001$ and $P < 0.05$, respectively, Mann-Whitney U test). However, the latencies of the BL lesion-vehicle group did not differ significantly from those of the sham-vehicle controls ($P > 0.4$, Mann-Whitney U test).

Fig. 2 also shows that DZP impaired retention in the sham group, as well as the CE and LAT lesion groups, but did not affect retention of the BL lesion group. The retention latencies of sham, CE lesion, and LAT lesion rats given DZP were significantly shorter than those of their vehicle controls ($P < 0.001$, $P < 0.001$, and $P < 0.02$, respectively, Mann-Whitney U test). However, the retention latencies of the BL lesion-DZP group did not differ from those of either the BL lesion-vehicle controls ($P > 0.3$, Mann-Whitney U test) or the sham-vehicle group ($P > 0.7$, Mann-Whitney U test). Furthermore, the retention latencies of the BL lesion-DZP group were significantly higher than those of the sham-DZP, CE lesion-DZP, and LAT lesion-DZP groups ($P < 0.001$ for all comparisons).

DISCUSSION

The findings of this study indicate that (i) IBO-induced lesions of the CE, LAT, or BL amygdala nuclei did not impair acquisition of the CMIA task; (ii) injections of DZP administered systemically before training did not affect acquisition in either the controls or the amygdala-lesioned animals; (iii) retention was impaired in animals with lesions of the CE and LAT nuclei but the retention of animals with BL lesions was unimpaired; (iv) DZP impaired retention in controls as well as in animals with lesions of the CE and LAT amygdala nuclei; (v) BL lesions blocked the retention impairment induced by systemic injections of BZD.

Animals with lesions of the CE, LAT, or BL amygdala nuclei did not differ from the controls in acquisition performance and showed significant retention of the inhibitory avoidance response (although the retention performance of CE lesion and LAT lesion groups was poorer than that of the sham groups). These results agree with previous evidence from our laboratory indicating that an intact amygdala is not essential for either acquisition or retention of an inhibitory avoidance response (21, 24, 25). The present findings are also consistent with evidence indicating that acquisition and retention of an inhibitory avoidance task is not blocked by lesions of the stria terminalis, a major afferent/efferent amygdala pathway (26, 27).

The finding that retention was impaired by lesions of the CE and LAT nuclei, but not by lesions of the BL nucleus, suggests that these nuclei are differentially involved in influencing retention of inhibitory avoidance training. The BL does not appear to be involved in mediating either the learning or the retention of this task. Our finding of impaired retention in the CE- and LAT-lesioned groups agrees with those of previous studies indicating that damage to these nuclei attenuates fear conditioning (28–32). However, it should be noted that, in the present experiment, animals with lesions of the CE and LAT amygdala nuclei demonstrated significant retention—that is, retention was impaired, particularly in the CE lesion group, but it was not blocked.

Our findings indicating that DZP impaired retention in the nonlesioned control animals as well as in the animals with CE and LAT nucleus lesions are consistent with extensive evi-

FIG. 2. Median step-through latencies and interquartile ranges for the retention test conducted 48 h after inhibitory avoidance training using the continuous-trial procedure. Asterisks indicate difference from corresponding vehicle control group (*, $P < 0.05$; **, $P < 0.001$), circles indicate difference from BL lesion-DZP group ($P < 0.001$), and diamonds indicate difference from sham-vehicle group ($P < 0.05$; $P < 0.001$) ($n = 8$ –10 per group).

dence indicating that BZDs induce anterograde amnesia (3–7). We recently reported that *N*-methyl-D-aspartate-induced lesions of the amygdaloid complex blocked the impairing effects of DZP on CMIA retention (21). That study did not address the question of the role of specific amygdala nuclei mediating BZD-induced memory impairment. Our finding that lesions of the BL block DZP-induced anterograde amnesia, and that lesions of the CE and LAT do not, strongly suggests that the BL nucleus is a critical site for BZD-induced modulation of memory storage. Since an intact BL nucleus appears not to be required for the acquisition of this task, it seems likely that BZD-induced memory impairment is due to alteration of activity in brain regions influenced by projections from the BL nucleus. It is possible, although it seems unlikely, that the BZD effects on memory result from activation of BZD receptors in other brain regions and that such effects require concurrent activation of the BL nucleus. Although lesions of the CE and LAT nuclei impaired retention, the lesions of these nuclei did not prevent the retention-impairing effects of DZP. However, it should be noted that the degree of DZP-induced retention impairment observed in animals with lesions of the LAT nucleus was less than that of animals or those with CE lesions. Thus, although an intact CE nucleus is not required for DZP-induced anterograde amnesia, the LAT nucleus appears to have some influence on the DZP effects.

Previous work from our laboratory indicates that the amygdala is involved in mediating the effects on memory of treatments affecting several neurotransmitter and hormone systems, including noradrenergic, peptidergic opioid, and GABAergic systems (25, 26, 33–37). The findings of the present experiment provide additional support for the hypothesis that the amygdaloid complex plays a critical role in integrating neuromodulatory influences on learning and memory.

We would like to thank Dean Cestari and Erin Schuck for their invaluable technical assistance and Nancy Collett for assistance in preparation of the manuscript. This research was supported by a Fullbright scholarship (C.T.) and Grant MH12526 from the National Institute of Mental Health and National Institute on Drug Abuse and by Office of Naval Research Contract N00014-90-J-1626 (J.L.M.).

- Clarke, P. R. F., Eccersley, P. S., Frisby, J. P. & Thornton, J. A. (1970) *Br. J. Anaesth.* **42**, 690–697.
- Lister, R. G. (1985) *Neurosci. Biobehav. Rev.* **9**, 87–94.
- Arolfo, R. G. & Brioni, J. D. (1991) *Behav. Neural Biol.* **55**, 131–136.
- Cahill, L., Brioni, J. D. & Izquierdo, I. (1986) *Psychopharmacology* **90**, 554–556.
- Izquierdo, I., Pereira, M. E. & Medina, J. H. (1990) *Behav. Neural Biol.* **54**, 27–41.
- Jensen, R. A., Martinez, J. L., Vasquez, B. J. & McGaugh, J. L. (1979) *Psychopharmacology* **64**, 125–126.
- Venault, P., Chapouthier, G., Simiand, J., Dodd, R. H. & Rossier, J. (1987) *Brain Res. Bull.* **19**, 365–370.
- Decker, M. W., Tran, T. & McGaugh, J. L. (1990) *Psychopharmacology* **100**, 515–521.
- Theibot, M. (1985) *Neurosci. Biobehav. Rev.* **9**, 95–100.
- Davis, M. (1979) *Psychopharmacology* **62**, 1–7.
- Gamzu, E. R. (1988) *Psychopharmacol. Ser.* **6**, 218–229.
- McNamara, R. K. & Skelton, R. W. (1991) *Pharmacol. Biochem. Behav.* **15**, 911–914.
- Nagy, J., Zambu, K. & Decsi, L. (1979) *Neuropharmacology* **18**, 573–576.
- Shibata, K., Kataoka, Y., Gomita, Y. & Ueki, S. (1982) *Brain Res.* **234**, 442–446.
- Hodges, H. & Green, S. (1987) *Physiol. Behav.* **41**, 257–264.
- Niehoff, D. L. & Kuhar, M. J. (1983) *J. Neurosci.* **10**, 2091–2097.
- Thomas, S. R., Lewis, M. E. & Iversen, S. D. (1985) *Brain Res.* **342**, 85–90.
- Young, W. S. & Kuhar, M. J. (1980) *J. Pharmacol. Exp. Ther.* **212**, 338–346.
- Petersen, E. N., Braestrup, C. & Scheel-Kruger, J. (1985) *Neurosci. Lett.* **53**, 285–288.
- Da Cunha, C. D., Wolfman, C., Huang, C. H., Walz, R., Koya, R., Bianchin, M., Medina, J. H. & Izquierdo, I. (1990) *Braz. J. Med. Biol. Res.* **23**, 301–306.
- Tomaz, C., Dickinson-Anson, H. A. & McGaugh, J. L. (1991) *Brain Res.* **568**, 85–91.
- Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates* (Academic, Sydney, Australia), 2nd Ed.
- McGaugh, J. L., Introini-Collison, I. B. & Nagahara, A. (1988) *Brain Res.* **446**, 37–49.
- Cahill, L. & McGaugh, J. L. (1990) *Behav. Neurosci.* **104**, 532–543.
- McGaugh, J. L., Introini-Collison, I. B., Nagahara, A. H., Cahill, L., Brioni, J. D. & Castellano, C. (1990) *Neurosci. Biobehav. Rev.* **14**, 425–431.
- Liang, K. C. & McGaugh, J. L. (1987) *Psychobiology* **15**, 154–160.
- Liang, K. C., McGaugh, J. L. & Yao, H. (1990) *Brain Res.* **508**, 225–233.
- Hitchcock, J. & Davis, M. (1986) *Behav. Neurosci.* **100**, 11–22.
- Hitchcock, J., Sananes, C. & Davis, M. (1989) *Behav. Neurosci.* **103**, 509–518.
- Kapp, B. S., Frysinger, R. C., Gallagher, M. & Haselton, J. R. (1979) *Physiol. Behav.* **23**, 1109–1117.
- LeDoux, J., Cicchetti, P., Xagoraris, A. & Romanski, L. M. (1990) *J. Neurosci.* **10**, 1062–1069.
- Roosendaal, B., Koolhaas, J. M. & Bohus, B. (1990) *Behav. Brain Res.* **41**, 39–48.
- Ammassari-Teule, M., Pavone, F., Castellano, C. & McGaugh, J. L. (1991) *Brain Res.* **551**, 104–109.
- Brioni, J. D., Nagahara, A. H. & McGaugh, J. L. (1989) *Brain Res.* **487**, 105–112.
- Liang, K. C., Juler, R. C. & McGaugh, J. L. (1986) *Brain Res.* **368**, 125–133.
- McGaugh, J. L., Introini-Collison, I. B., Cahill, L., Kim, M. & Liang, K. C., in *The Amygdala*, ed. Aggleton, J. (Wiley, New York), in press.
- Cahill, L. & McGaugh, J. L. (1991) *Psychobiology* **19**, 206–210.