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Bilateral Injection of Fasciculin Into the Amygdala of Rats: Effects on Two Avoidance Tasks, Acetylcholinesterase Activity, and Cholinergic Muscarinic Receptors

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QUILLFELDT, J., S. RASKOVSKY, C. DALMAZ, M. DIAS, C. HUANG, C. A. NETTO, F. SCHNEIDER, I. IZQUIERDO, J. H. MEDINA, R. SILVEIRA AND F. DAJAS. *Bilateral injection of fasciculin into the amygdala of rats: Effects on two avoidance tasks, acetylcholinesterase activity, and cholinergic muscarinic receptors.* PHARMACOL BIOCHEM BEHAV **37**(3) 439–444, 1990.—These experiments examined the effects of the bilateral injection of fasciculin-2 (FAS), a natural acetylcholinesterase (AChE) inhibitory peptide, into the amygdala of rats on acquisition and retention of two avoidance behaviors. Intraamygdala injection of FAS (150 ng/amygdala) produced a pronounced and long-lasting inhibition of AChE activity: 85% and 74% on day 2 and day 5, respectively. After 48 hr, FAS-treated animals showed no changes in training or test session performance in a step-down inhibitory avoidance task (training-test interval was 24 hr). In a 2-way shuttle avoidance task, intraamygdala FAS slightly reduced retention test performance without modifying training session scores. Two and five days after FAS injections into the amygdala, the density of muscarinic receptor decreased about 50% as measured by the specific bindings of ^{3}H-quinuclidinyl benzilate and ^{3}H-oxotremorine. No alterations were observed in the apparent dissociation constants. On the other hand, the central-type benzodiazepine receptor population of the amygdala remained unchanged, suggesting that FAS microinjection did not produce damage to neuronal components of these nuclei. In conclusion, the results presented have indicated that a clear-cut and long-lasting inhibition of AChE activity in the amygdala is not accompanied by a facilitation of learning and memory of two different avoidance tasks. Compensation of the increased cholinergic activity by a down-regulation of muscarinic receptors could account for these findings.

Fasciculin Memory Amygdala Acetylcholinesterase Muscarinic receptor binding

RECENT evidence suggests that the amygdala is important for the modulation of memory processes (24,25). Bilateral section of the stria terminalis, or injection of a variety of substances into the amygdala alters memory consolidation (24); lesions of the amygdala severely disrupt acquisition and retention (35). The amygdala receives cholinergic afferents from the nucleus basalis of Meynert and sends cholinergic fibers to the cortex and other brain regions (14,28). There is evidence that brain cholinergic muscarinic systems are important for acquisition, consolidation, and retrieval. Systemic administration of scopolamine, at low doses, hinders acquisition (32), consolidation (29) and retrieval (6) of avoidance behavior in animals. In humans, scopolamine is known to induce memory deficits that have been taken as models for the amnesia of organic brain syndromes (5). On the other hand, cholinomimetic substances like arecoline, oxotremorine (16), or anticholinesterase agents (the best studied of which is physostigmine) (5) are well known to enhance acquisition, and/or consolidation and retrieval in a variety of tasks in laboratory animals and humans. In clinical trials, physostigmine, and recently, tetrahydroaminoacridine (THA), another AChE inhibitor, have shown their potential usefulness in the treatment of amnesia of Alzheimer disease and other organic syndromes (3, 5, 8).

Dajas and his collaborators (31) discovered two peptides, called fasciculins, in the venom of the snake *Dendroaspis angusticeps* (green mamba), which strongly inhibit acetylcholinesterase (AChE). One of these peptides, fasciculin-2 (FAS), has a potent

and long-lasting action on AChE in brain structures (9). In vitro it causes around 95% inhibition of hippocampal AChE activity (31) and its in vivo injection inhibits striatal AChE locally for periods of at least one week (90% inhibition on day 1, 50% inhibition on day 7) (9). FAS also shows a very fine discriminative power between unsuspected subtypes of AChE across different structures of the brain and different species, inhibiting some and having no effect on others (20). These characteristics define FAS as a very promising cholinergic tool.

The present study examines the effect of bilateral injection of FAS into the amygdaloid nucleus of the rat on acquisition and retention of two forms of avoidance behavior in the rat, on the AChE activity of the amygdaloid nucleus, and on the binding properties of ^{3}H-quinuclidine (^{3}H-QNB) and ^{3}H-oxotremorine (^{3}H-OXO) to cholinergic muscarinic receptors in the same structure.

METHOD

Animals

Ninety to 120-day-old male rats from our own Wistar-derived albino rats breeding stock (weights: 250 to 300 g) were used. Before and after the injection they have free access to food and water and are maintained on a light-dark cycle of 12/12 hr.

Experimental Drugs

Fasciculin was produced by one of us (Federico Dajas) at the Instituto de Investigaciones Biologicas Clemente Estable in Montevideo. Fasciculin was purified according to Karisson *et al.* (20) and, in this work, fasciculin 2 (FAS) was utilized. FAS is the most potent fasciculin as inhibitor of AChE (20).

Surgical Procedures

The animals were anesthetized with IP tionembuthal (40 mg/g) and placed in a stereotaxic frame. Through a skull hole, the needle (31 gauge) connected to a Hamilton syringe (5 μl) and attached to the stereotaxic injector was gently lowered into the amygdala according to the following coordinates (bregma system): AP = −0.32; LL = +0.45 and DV = 0.72.

A solution (0.3 μl) of 1.5 mg/ml of FAS were injected during 1 min on each structure, summing up a total dose of 150 ng of FAS per structure (300 ng/animal). The needle was withdrawn slowly after 1 min. The recuperation period, after the injection, was 48 hr.

Control animals were injected with saline for behavioral tasks; for biochemical assays both saline-injected and intact animals were used.

Exact placement of the site of injection (lesion) and local inhibition of AChE by FAS were verified by a histochemical technique (21).

Behavioral Procedures

The same animal was submitted consecutively to two different behavioral tasks, in a sequence currently used in our laboratory: training in step-down inhibitory avoidance task in the second day (after injection) and testing in the third; training in 2-way shuttle avoidance task in the fourth day and testing in the fifth; based in this design, we made the biochemical assays 2 and 5 days after injection, trying to evaluate parameters in the beginning and in the finish of the "useful" period of the animal.

Step-down inhibitory avoidance was performed in a 50 × 25 × 25 cm Plexiglas box whose floor was a grid of 1-mm caliber parallel bronze bars spaced 10 mm apart. The left side of the grid was covered by a 5-cm high, 7 × 25 cm wood platform. Latency to step down from the platform placing the four paws on the grid was measured. In the training session, a 2-sec, 60-Hz, 0.4-mA footshock was delivered as soon as the animal stepped down; in the test session, no footshock was given. Training-test interval was 24 hr. Test minus training step down latency was taken as a measure of retention; the test session was terminated when the animals stayed on the platform 180 sec more than they did on the training session.

Two-way shuttle avoidance was performed in an automatic 50 × 25 × 25 cm opaque Plexiglas shuttle box whose floor was the same grid described above (without the platform). Both the training and the test sessions consisted of 20 tones, each one followed by foot-shock trials. Training-test interval was 24 hr in each session, the intertrial interval was varied at random between 10 and 50 sec; the tone was a 5-second, 60-dB, 1-KHz tone, and the footshock (0.5 mA) was automatically delivered immediately after each tone unless the animals shuttled to the tone across a 2-mm high wood hurdle placed at the midline of the grid. In the training session we permitted a 3-min habituation period (without any stimulus) and in the test just 1 min. Test minus training difference in performance of shuttle (avoidance) responses was taken as a measure of retention.

Biochemical Assays

AChE assay. The animals were killed by decapitation and their brains quickly dissected in an ice-cold plaque. The amygdalae were dissected by current methods of our laboratory, weighed and homogenized in 0.1 M phosphate buffer with triton 0.5%, pH = 7.3, and centrifuged. The supernatant was used for assessing AChE activity according to the method of Ellman modified by Augustinsson *et al.* (4).

Protein determination, necesary here and in the binding assay, was performed by the method of Lowry *et al.* (23) using bovine serum albumin as standard.

Radioligand Binding Assay

For the binding assays, a crude synaptosomal membrane fraction of both amygdalae from each animal was obtained according to the methods currently used in the laboratory (12).

3H-QNB binding assay. Muscarinic cholinergic receptors were measured using ^{3}H-L-quinuclidinyl benzilate (QNB, 33.1 Ci/mmol, NEN). Typically, membranes (0.1 mg prot./ml) were resuspended in 50 mM Na/K phosphate buffer (pH 7.4). Aliquots of 1 ml were incubated with varying concentrations of the ligand (0.05–1.50 nM) at 37°C for 1 hr. At the end of the incubation, the membranes were collected on Whatman GF/B filters with a manifold filtration apparatus; filters were washed rapidly two times with the same phosphate buffer and dried. Radioactivity was measured in a Traco scintillation spectrophotometer after the addition of 3 ml PPO/xylene as the scintillator. Specific binding was determined by subtracting from total binding the amount bound in the presence of 10 μM atropine.

^{3}H-Oxotremorine (^{3}H-OXO) binding assay. For the binding of ^{3}H-OXO (84.9 Ci/mmol, NEN) the membranes were resuspended in 25 mM Tris-HCl buffer plus 5 mM $MgCl_2$ (pH 7.4) to reach a concentration of 0.75 mg/ml (30). For each assay, aliquots of 0.25 of the membrane suspension were incubated using various concentrations of the ^{3}H-ligand (0.5–6.0 nM). The incubations were carried out at 4°C for 3 hr and the assays were done in triplicate. The assay was terminated by the addition of 5 ml of ice-cold buffer and the separation of the bound ligand was done by rapid filtration on Whatman GF/B glass fiber filters presoaked, for 60 min, with

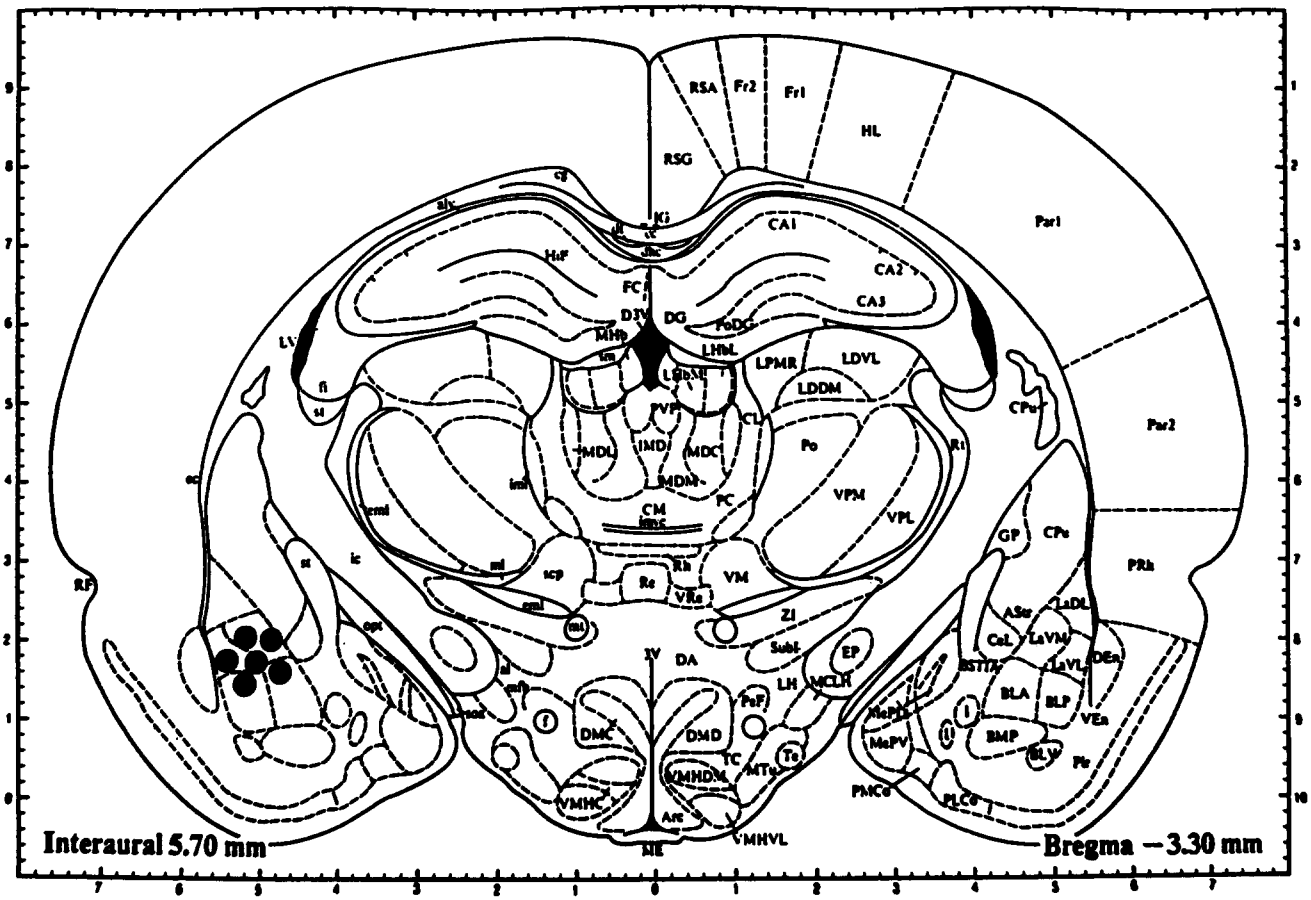

FIG. 1. Location of the FAS injections in the amygdaloid nucleus. [Taken from Paxinos and Watson (27) with permission.]

a solution of 0.01% polyethylenemine, followed by a washing twice with 5 ml of the same buffer. Nonspecific binding was determined in the presence of 10 μM atropine. The filters were dried and, after the addition of 3 ml of PPO-xylene as scintillation fluid, were counted in a Tracor spectrometer.

^{3}H-Flunitrazepam binding assay. Central type benzodiazepine receptors were measured using ^{3}H-flunitrazepam (^{3}H-FNZ, 81.8 Ci/mmol, NEN) as previously reported (26). Briefly, for each assay, triplicate samples containing 0.2 mg protein were incubated at 4°C for 60 min with 8 mM ^{3}H-FNZ. Specific binding was calculated as the difference between total binding and a blank done in the presence of 3 μM clonazepam or 3 μM flunitrazepam and represented over 80% of total binding. The assays were terminated by filtration through Whatman GF/B glass fiber filters, with 3 washes of 3 ml each of incubation medium. Filters were dried and counted with PPO-xylene.

Statistics

Comparison between groups were made by two-way ANOVA (and Newman-Keuls test, when indicated) or nonpaired Student's *t*-test for the biochemical data and for the data of Shuttle Avoidance and Inhibitory Avoidance training; for the Retention values (training minus test step-down latency) and test session values of the Inhibitory Avoidance we used the nonparametric individual two-tailed Mann-Whitney U-test.

RESULTS

Figure 1 shows positions of the injection sites and surrounding tissue damage, as ascertained by postmortem histological examination using Nissl staining. Data on cholinesterase and binding (see below) complement histological examination indicating a good local AChE inhibition and the restriction of this pharmacological effect to the cholinergic neurons (data on benzodiazepine receptors, below).

Inhibition of Acetylcholinesterase Activity by Fasciculin

The injection of FAS (150 ng/structure) into the amygdaloid nucleus caused a pronounced and long-lasting inhibition of AChE activity measured in the same nucleus: 85% on Day 2, 74% on Day 5 (Fig. 2). The data agree with previous findings in the literature in which the effect of FAS on AChE activity was studied in other brain structure of the rat, the striatum (9) and the locus coeruleus (1).

Behavioral Effects of FAS

The behavioral effects of FAS are shown in Fig. 3 (Inhibitory avoidance) and Fig. 4 (Shuttle avoidance). FAS-treated animals showed no difference relative to controls in training or test session performance in the inhibitory avoidance task (Fig. 3). In the shuttle avoidance task, there was a slight but significant impair-

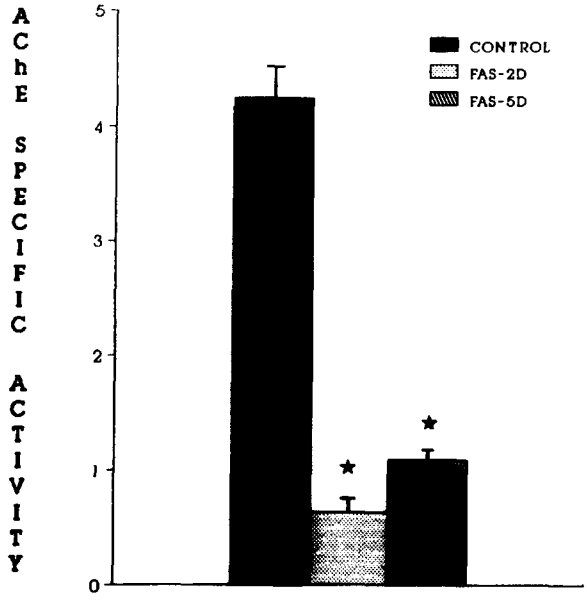

FIG. 2. Specific activities of AChE in amygdalae of rats. FAS-2d and FAS-5d are, respectively, 85 and 74% inhibited in relation to Control (intact) animals. Specific activity expressed, in nkat/mg of protein. Control: intact animals (N = 8); FAS-2d: FAS-injected animals 2 days after injection (N = 5); FAS-5d: FAS-injected animals 5 days after injection (N = 6). Data expressed as mean ± S.E.M. of AChE specific activity. *F(2,16) = 64.511 (ANOVA). The differences are significant in a Newman-Keuls test with $p<0.01$.

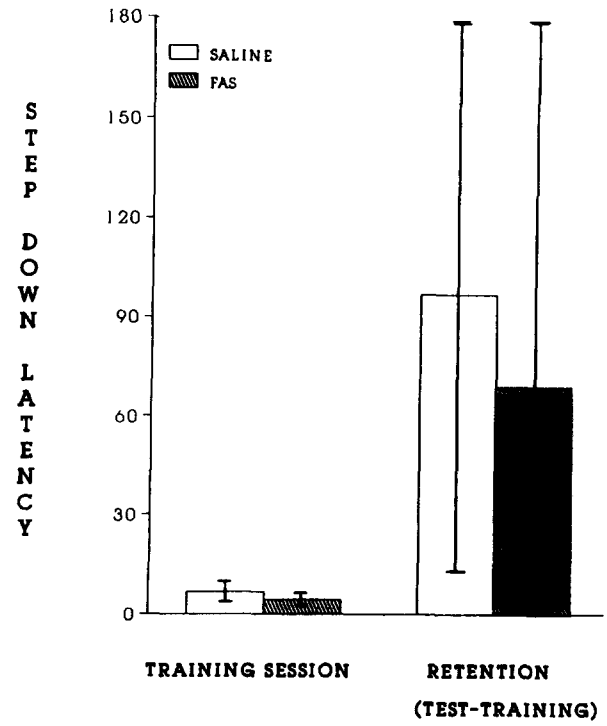

FIG. 3. Performance in a step-down inhibitory avoidance (shock 0.4 mA) of rats submitted to intraamygdalar FAS-injection (150 ng in each hemiamygdala). Training session was 48 hr after FAS-injection and test session was 24 hr after training (with a superior time limit of 180 sec more than training time). Data expressed in median (interquartile range) of step-down latency (sec). Saline groups, N = 15; fasciculin groups, N = 17. Differences among saline and FAS groups in training session are not significant in a Student's *t*-test ($p>0.05$); differences among saline and FAS groups in retention values are not significant in a Mann-Whitney test ($p>0.10$; U = 104.0).

ment of retention test performance in the FAS group, but no differences in training session performance (Fig. 4).

Receptor Binding

The effects of intraamygdala injection of FAS on muscarinic cholinergic and benzodiazepine receptors are summarized in Fig. 5. FAS-treated animals showed a significant decrease (about 50% in the maximal number of amygdala muscarinic receptors, saturation curves of ^{3}H-QNB and ^{3}H-OXO bindings revealed that these modifications are due to a reduced B_{max} with no alterations in the apparent dissociation constants. On the other hand, no changes were observed in the population of the central type benzodiazepine receptors of the amygdala nuclei.

DISCUSSION

FAS is a potent, specific and long-lasting acetylcholinesterase inhibitor (9,20). In the present experiment, it caused a pronounced inhibition of AChE activity in the amygdala, which lasted at least 5 days. Dajas *et al.* (10) found that FAS-induced AChE inhibition in the striatum lasts for at least 21 days.

Perhaps unexpectedly, this effect was not accompanied by a facilitation of learning and memory of two different avoidance behaviors in the present study. It is, in fact, accompanied by a lack of significant effect in step-down inhibitory avoidance performance (Fig. 3) and a significant but not very intense impairment in shuttle avoidance retention values (Fig. 4). It is important to note that the shock intensity used was such as to permit detection both of improvement and of impairment of retention: means and medians values were intermediate and did not reach floor or ceiling levels in control groups in both tasks. Abundant data from the literature show a pronounced enhancement of acquisition and/or retention of avoidance tasks in rats or mice, as well as of various forms of memory in humans or animals with reversible AChE inhibitors (13, 15–17). On the other hand, the present findings are similar to what can be observed after, say, a low dose of scopolamine given systemically (18). Prolonged increases in ACh activity could have exactly the opposite effect of transient increases in ACh activity induced by agonists (2, 11, 22).

A possible explanation for the lack of memory facilitation by FAS is provided by the receptor binding assay studies. A receptor that is chronically being activated may not be able to transmit information useful for memory processing. Behavioral alterations have been described as a consequence of intense AChE inhibition (33). The FAS treatment caused a marked decrease of the maximal number of muscarinic receptor binding sites, as measured by ^{3}H-QNB and ^{3}H-OXO binding. This decrease was seen both at 2 and 5 days after the FAS injection, and was not accompanied by changes in K_d. These results are in agreement with reports that show that treatment of animals with AChE inhibitors cause a decrease in ^{3}H-QNB binding sites in several brain regions (34). Thus, in effect, FAS reduced the number of available cholinergic receptor sites in the amygdala. These findings strongly suggest that intraamygdala neuronal systems would compensate the increased cholingeric activity producing a down-regulation of the muscarinic receptors and therefore blocking its behavioral expression. Excessive doses of physostigmine (2,11) or chronic treatments with cholinomimetics (22) have, in fact, also been reported

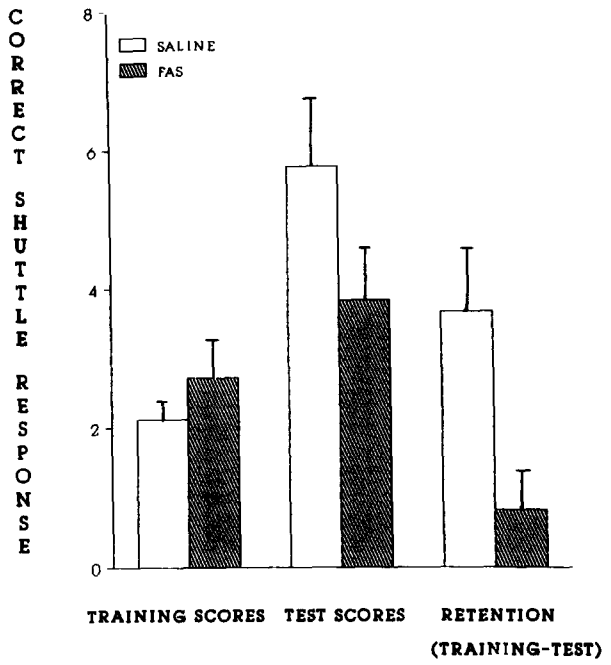

FIG. 4. Performance in a shuttle avoidance task of rats submitted to intraamygdalar FAS-injection (150 ng in each amygdala). Training session was 96 hr after FAS-injection (1 day after inhibitory Avoidance test) and test session was 24 hr after training. Data expressed as mean ± S.E.M. of correct shuttle responses. N = 17 animals per group. *Significant difference from correspondent saline group with $p<0.05$ in a Student's t-test.

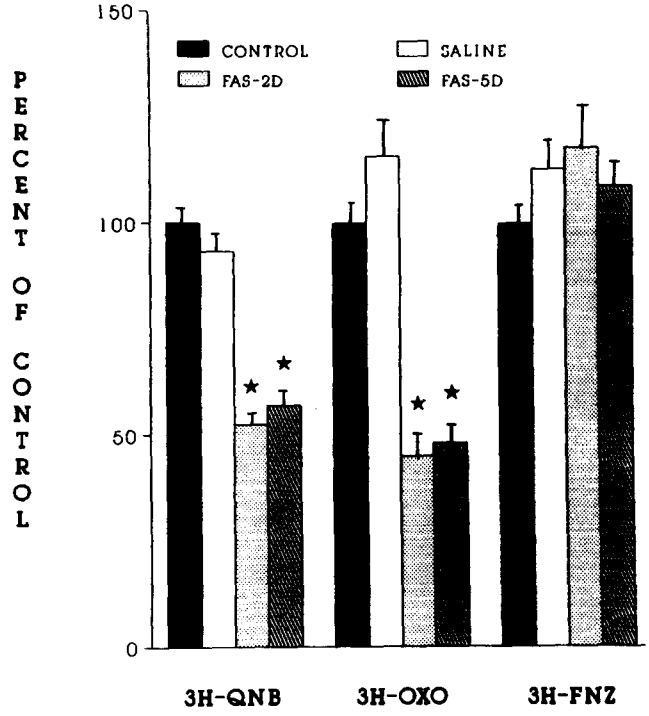

FIG. 5. Diagram representing the concentration of muscarinic cholinergic receptors as measured by ^{3}H-QNB and ^{3}H-OXO bindings at saturation (1 and 6 nM, respectively), and benzodiazepine receptors (^{3}H-FNZ binding at 8 nM) in the amygdala. The values are expressed as percentage and represented the mean ± S.E.M. from 3–9 independent experiments done by triplicate. Each experiment was performed in both amygdala from an individual animal. C = control; S = sham-operated; F2 = 2 days after FAS; F5 = 5 days after FAS. Control values of binding assays: ^{3}H-QNB = 2112.0 ± 154.8 fmol·mg prot.; ^{3}H-OXO = 165.8 ± 31.1 fmol·mg prot.; ^{3}H-FNZ = 917.7 ± 65.1 fmol·mg prot. *$F(2,16) = 38.4$ (QNB) and $F(2, 12) = 18.5$ (OXO) (ANOVA). The differences are all significant in a Newman-Keuls test with $p<0.01$ in comparison with control and sham-operated (saline) rats.

to cause memory impairments.

The effects of FAS on the cholinergic system of the amygdala seems to be selective, since no changes in the neuronal-type benzodiazepine binding sites was observed in FAS-treated amygdala neuron membranes. These data suggest that bilateral FAS microinjection did not produce a relevant damage to the neuronal components of the amygdala.

Alternatively, the AChE inhibition caused by FAS might have affected other systems in the amygdala aside from the cholinergic system. AChE, in fact, has been shown to hydrolyze a variety of neuropeptides (36), including opioid peptides (7). Enkephalins and β-endorphin have well-known participation in memory processes (19,24), and, too, have amnestic effects (18). In this sense. a putative change in the levels of neurpeptides produced by FAS could, eventually, block the facilitatory effect of cholinergic hyperactivity. This and other possibilities are under investigation in our laboratories.

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REFERENCES

1. Abo, V.; Vieira, L.; Silveira, R.; Dajas, F. Effects of local inhibition of locus coeruleus acetylcholinesterase by fasciculin in rats. Neurosci. Lett. 98:253–257; 1989.
2. Aigner, T. G.; Mishkin, M. The effects of physostigmine and scopolamine on recognition memory in monkeys. Behav. Neural Biol. 45(1):81–87; 1986.
3. Ashford, J. W.; Sherman, K. A.; Kumar, V. Advances in Alzheimer therapy: cholinesterase inhibitors. Neurobiol. Aging 10:99–105; 1989.
4. Augustinsson, K. B.; Eriksson, H.; Faijersson, Y. A new approach to determining cholinesterase activities in samples of whole blood. Clin. Chim. Acta 89:239–252: 1978.
5. Bartus, R. T.; Dean, R. L.; Pontecorvo, M. J.; Flicker, C. The cholinergic hypothesis: a historical overview, current perspective, and future directions. Ann. NY Acad. Sci. 444:332–358; 1985.
6. Brioni, J. D.; Izquierdo, I. Retention enhancement by pre-test β-endorphin and oxotremorine and its reversal by scopolamine. Behav. Neural Biol. 50(3):251–254; 1988.
7. Chubb, I. W.; Ranieri, E.; White, G. H.; Hodgson, A. J. The enkephalins are amongst the peptides hydrolyzed by purified acetylcholinesterase. Neuroscience 10:1369–1377; 1983.
8. Crook, T. Clinical drug trials in Alzheimer's disease. Ann. NY Acad. Sci. 444:428–436; 1985.
9. Dajas, F.; Bolioli, B.; Castello, M. E.; Silveira, R. Rat striatal acetylcholinesterase inhibition by fasciculin (a polypeptide from green mamba snake venom). Neurosci. Lett. 77:87–91; 1987.
10. Dajas, F.; Cervenansky, C.; Silveira, R.; Barbeito, L. Fasciculins: some aspects of their central nervous system anticholinesterase activity. In: Dolly, O., ed. Neurotoxins as tools in neurochemistry.

England: Ellis Horwood, Ltd.; 1989.
11. Davis, K. L.; Mohs, R. C.; Rosen, W. G.; Greenwald, B. S.; Levy, M. I.; Horvath, T. B. Memory enhancement with oral physostigmine in Alzheimer's disease. N. Engl. J. Med. 308:721; 1983.
12. De Robertis, E.; Alberici, M.; Rodriguez de Lores Arnaiz, G.; Azcurra, J. M. Cell fractionation studies in brain. Life Sci. 5: 577–580; 1966.
13. Ellis, M. E.; Kesner, R. P. Physostigmine and norepinephrine: Effects of injection into the amygdala on taste association. Physiol. Behav. 27:203–209; 1981.
14. Fibiger, H. C.; Vincent, S. R. Anatomy of central cholinergic neurons. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987.
15. Flood, J. F.; Cherkin, A. Scopolamine effects on memory retention in mice: a model of dementia? Behav. Neural Biol. 45:169–184; 1986.
16. Flood, J. F.; Smith, G. E.; Cherkin, A. Memory retention: Potentiation of cholinergic drug combinations in mice. Neurobiol. Aging 4:37–43; 1983.
17. Gower, A. J. Enhancement by secoverine and physostigmine of retention of passive avoidance response in rats. Psychopharmacology (Berlin) 91:326–329; 1987.
18. Izquierdo, I. Mechanism of action of scopolamine as an amnesic. Trends Pharmacol. Sci. 10:175–177; 1989.
19. Izquierdo, I. Different forms of post-training memory processing. Behav. Neural Biol. 51:171–202; 1989.
20. Karlsson, E.; Mbugua, P. M.; Rodriguez-Ithurralde, D. Fasciculins, anticholinesterase toxins from the venom of green mamba *Dendroaspis angusticeps*. J. Physiol. (Paris) 79:232–240; 1984.
21. Koelle, G. B. The histochemical localization of cholinesterase in the central nervous system of the rat. J. Comp. Neurol. 100:211–236; 1954.
22. Louilis, C. C.; Dean, R. L.; Lippa, L. S.; Meyerson, L. R.; Beer, B.; Bartus, R. T. Chronic administration of cholinergic agents: Effects on behavior and calmodulin. Pharmacol. Biochem. Behav. 18:601–604; 1983.
23. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
24. McGaugh, J. L. Modulation of memory storage processes. In: Solomon, P. R.; Goethals, G. R.; Kelley, C. M.; Stephens, B. R.; eds. Perspectives on memory research. New York: Springer-Verlag; 1988:33–64.
25. McGaugh, J. L.; Liang, K. C.; Bennett, C.; Sternberg, D. B. Adrenergic influences on memory storage: interaction of peripheral and central systems. In: Lynch, G.; McGaugh, J. L.; Weinberger, N. M., eds. Neurobiology of learning and memory. New York: The Guilford Press; 1984:313–332.
26. Medina, J. H.; De Robertis, E. Benzodiazepine receptors and thyroid hormones: in vivo and in vitro modulation. J. Neurochem. 44: 1340–1344; 1985.
27. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. Sydney: Academic Press; 1986.
28. Price, J. L.; Russchen, F. T.; Amaral, D. G. The amygdaloid complex. In: Bjorklund, A.; Hokfelt, T.; Swanson, L. W., eds. Handbook of chemical neuroanatomy. vol. 5: Integrated systems of the CNS, Part I (hypothalamus, hippocampus, amygdala, retina). Amsterdam: Elsevier; 1987.
29. Quatermain, D.; Leo, P. Strength of scopolamine-induced amnesia as a function of time between training and testing. Behav. Neural Biol. 50(3):300–310; 1988.
30. Raskovsky, S.; Aguilar, J. S.; Jerusalinsky, D.; De Robertis, E. An ^{3}H-oxotremorine binding method reveals regulatory changes by guanine nucleotides in cholinergic muscarinic receptors of cerebral cortex. Neurochem. Res. 13:525–529; 1988.
31. Rodriguez-Ithurralde, D.; Silveira, R.; Barbeito, L.; Dajas, F. Fasciculin, a powerful anticholinesterase polypeptide from *Dendroaspis angusticeps* venom. Neurochem. Int. 5:267–274; 1983.
32. Rush, D. K. Scopolamine amnesia of passive avoidance: A deficit of information acquisition. Behav. Neural Biol. 50(3):255–274; 1988.
33. Russell, R. W. Cholinergic system in behavior: the search for mechanisms of action. Annu. Rev. Pharmacol. Toxicol. 22:435–463; 1982.
34. Russell, R. W.; Overstreet, D. H. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. Prog. Neurobiol. 28:97–129; 1987.
35. Schutz, R. A.; Izquierdo, I. Effect of brain lesions on rat shuttle behavior in four different tests. Physiol. Behav. 23:97–105; 1979.
36. Small, D. H. Acetylcholinesterase: Zymogens of neuropeptides processing enzymes? Neuroscience 29(2):241–249; 1989.