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Effects of Bilateral Cholinotoxin Infusions on the Behavior and Brain Biochemistry of the Rats

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MÄNNISTÖ, P. T., P. TUOMAINEN, O. KUTEPOVA, S. A. BORISENKO, N. ZOLOTOV AND T. VORONINA. *Effects of bilateral cholinotoxin infusions on the behavior and brain biochemistry of the rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 33-40, 1994. – We examined behavioral and biochemical specificity and the general usefulness of the proposed rat model of Alzheimer's disease. Bilateral infusions of ethylcholine aziridinium (AF64A) into the basal magnocellular nuclei caused a deterioration of learning in passive and active avoidance tests, increased emotional reactivity, and decreased motoric activity. Choline acetyltransferase activity was decreased by 22% in the frontal cortex but increased by 8-10% in the hippocampus and hypothalamus. Noradrenaline and dopamine levels in the frontal cortex were decreased by 20%. In striatum, dopamine and its metabolites were strongly suppressed (by 50-60%). Also striatal noradrenaline (−48%) and 5-hydroxytryptamine (−34%) were significantly decreased. Hypothalamic 5-hydroxytryptamine was increased (+25%). Bilateral AF64A lesions decreased significantly (by 14-20%) activities of prolyl endopeptidase, dipeptidyl peptidase II and IV in hippocampal and frontal cortical brain homogenates. These results show that AF64A can be used to induce long-term learning deficits in the rat. However, striatal amine levels are also strongly suppressed, and are reflected as hypomotility and increased emotional reactivity. These changes may limit the usefulness of the rat model. Universally decreased peptidase activities offer interesting views regarding the role of peptidase inhibitors in amnestic disorders.

AF64A (ethyl choline mustard aziridinium) Kainic acid Ibotenic acid Active avoidance Passive avoidance Basal magnocellular nucleus Choline acetyltransferase Prolyl endopeptidase Dipeptidyl endopeptidases Catecholamines

INJECTIONS of fiber-sparing excitatory neurotoxins, like kainic acid and ibotenic acid, into the basal magnocellular nuclei have been used as models of specific deafferentation of cerebral cortex (6,18,20,28). Such lesions cause severe alterations in the pre and postsynaptic cholinergic activity in the neocortex leaving hippocampus intact (12,13,52).

The newest nonexcitatory toxin used for the same purpose is ethylcholine aziridinium ion (AF64A), which may theoretically offer some advantages over the excitatory amino acids (15,20). Notably, AF64A has been claimed to selectively lesion cholinergic nerve terminals owing to its ability to utilize the choline transport system (15,20). No systematic comparisons have been made between the efficacy of AF64A and various other toxins. Further, only limited information is available on the behavioral and biochemical consequences of the bilateral cholinergic lesions induced by AF64A.

We have examined behavioral and biochemical specificity of a novel rat model of Alzheimer's disease using bilateral destruction of the basal magnocellular nuclei of Meynert. In behavioral studies, various doses of AF64A were tested on passive avoidance behavior. We also compared the effects of AF64A with those of two excitatory toxins, kainic acid and ibotenic acid, on impairment of active avoidance. We analyzed other aspects of the behavior, including reaction to handling, motoric activity, active avoidance, of the rats after lesions produced by AF64A. Cholinergic, aminergic, and peptidase activities were assessed from several brain regions to evaluate the selectivity of the cholinergic lesion. Brain pepti-

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dases were also studied, considering their role in the metabolism of neuropeptides (e.g., substance P, neurotensin, thyrotropin-releasing hormone, vasopressin) (32) and proteins, including processing of β-amyloid protein (46). In Alzheimer's disease, the activities of a variety of peptidases in cerebrospinal fluid are high (2). Some studies have proposed that inhibition of prolyl endopeptidase or dipeptidyl peptidase IV improved the cognitive functions in various amnestic models (4,30,55).

The preferred site of action of AF64A is directed to the cholinergic nerve endings rather than the perikarya (15,20). There are also cholinergic terminals in the basal magnocellular nuclei (36). However, local infusions, instead of intracerebroventricular administration, should limit the cholinergic lesion to the basal nuclei and to their projections.

METHOD

Animals

Male rats (Han/Kuo Wistar), weighing 180–250 g, were obtained from the colony in the Department of Pharmacology and Toxicology, University of Helsinki, Finland. They were housed five per cage and had free access to tap water and regular laboratory pellets. The lights were turned on at 0700 h and off at 1900 h.

Bilateral Destruction of the Basal Magnocellular Nuclei of Meynert

The rats were anaesthetized with chloral hydrate (350 mg/kg IP) and placed in the stereotaxic instrument (David Kopf, Tujunga, CA). Guide cannulas made out of 23 gauge needles were inserted bilaterally 2.0 mm above the target. The injection needle was lowered into the final target (coordinates: A 6.7, V 5.0, L ±3.0, according to König and Klippel Atlas (31). All toxins were infused bilaterally, simultaneously at both sides, using a Sage syringe pump (model 351, Sage Instruments, Cambridge, MA), in total volume of 1 μl/side/5 min. After infusions, the needle was retained in the final position for an extra 2 min. The final dilutions were made in physiological saline. AF64A was from Research Biochemicals Inc., Natick, MA. It was activated by alkaline treatment (15) and used within 8 h; the dose was usually 57.5 ng/nucleus. In dose-response studies, 34.5, 57.5, 115, and 230 ng/nucleus were used. Kainic acid; 125 ng/nucleus, and ibotenic acid; 750 ng/nucleus, were from Sigma, St. Louis, MO. Kainic acid and ibotenic acid were dissolved in saline. The pH of the solutions was about 4.4, the same as that of the saline, and osmolality was between 260 and 300 mOsm/kg. In preliminary studies, 1500 ng of kainic acid and 250 ng of ibotenic acid were also tried. The former caused convulsions in most animals and did not differ from 750 ng. We used two types of control rats: one group was sham-operated and infused with saline and another group was left intact. We did not find any difference in their behavior.

Behavioral Studies

Emotional reactivity of the rats was studied according to Brady and Nauta (8) using a scoring from 0 to 4 for each of the following 6 parameters: 1) response to handling; 2) response to threat and 3) to the press by forceps; 4) degree of muscular strength; 5) degree of vocalization; and 6) defecation. These tests were performed 4 weeks after the lesions. This study was done twice.

Locomotor activity, including rearing, of single rats was measured for 10 min using dark photoelectric motility boxes (68 × 68 × 40 cm) connected to a computer (Kungsbacka Mät- & Reglerteknik AB, Kungsbacka, Sweden). These studies were done at 2, 3, and 6 weeks after lesions in two parts: first, six (control) and seven (lesion) rats were studied at 2, 3, and 6 weeks, then five (control) and seven (lesion) more rats were studied only at 3 weeks.

In the passive avoidance test, we used a two-compartment box. One end of the box was black and dark inside; the other transparent and well illuminated. There was a guillotine door between the two compartments. In the first day, the animals were allowed to freely explore the box for 5 min. In the second day, they were placed into the illuminated compartment. Usually the rats moved to the dark compartment within a few seconds, and were given there a series of electric shocks through the wired floor (80 V, 50 ms pulses for 3 s). Animals that did not enter the dark compartment within 5 min were excluded (about 10%). Exactly 24 h later (on the third day), the rats were again placed to the illuminated compartment, and the time lag (maximum 300 s) before entering to the dark compartment was recorded. If the rats remembered the unpleasant experience, they delayed their moving to the dark compartment. Testing was done between 1700 and 2000 h.

The one-way active avoidance test. A regular shuttle-box (Campden-Instruments, London, UK) was used. After a 5-min accommodation, the rats were trained to pass through the round opening to another compartment within a 4-s period when a light stimulus was turned on. If they did not pass during the light signal, electric shocks (0.8 mA) were continuously given (maximum time 5 s) through the grid floor to their feet. If the animals escaped from the compartment before the light stimulus, they were returned by hand and the trial was started again. If the animals escaped the compartment after the electric shock, they were allowed to stay in the other compartment for 40 s, and then returned by hand before the new trial. Each session consisted of 10 presentations of the light stimulus, and only one session was given in 1 day. Animals were trained not more than 6 days.

Biochemistry

After the experiments, the rats were decapitated, the brains removed, and frontal cortical and hippocampal choline acetyltransferase activities measured (16).

Dopamine, dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), noradrenaline, and 5-hydroxytryptamine (5-HT) in the hypothalamus, frontal cortex, and striatum were analyzed by high-pressure liquid chromatography using electrochemical detection as described earlier (34). Detection limits were 20 pg per injection (20 μl) for dopamine and DOPAC and 40 pg per injection for HVA and 0.1 ng/injection for 5-HT.

Three peptidase (prolyl endopeptidase, E.C. 3.4.21.26; dipeptidyl peptidase II, E.C. 3.4.14.2; and dipeptidyl peptidase IV, E.C. 3.4.14.5) activities from hypothalamic, hippocampal, and frontal cortical brain homogenates were measured fluorometrically using Aminco-Bowman spectrofluorometer (19,27,56). The fluorogenic substrates used were *N*-benzyloxycarbonyl-Ala-Pro-4-methylcoumarine-7-amide; Lys-Ala-4-methylcoumarine-7-amide tosylate and Gly Pro-4-methyl-coumarine-7-amide tosylate, respectively. The substrates were kindly synthesized by Dr. V. F. Pozdnev (Institute of Biological and Medical Chemistry, Russian Academy of Medical Sciences, Moscow).

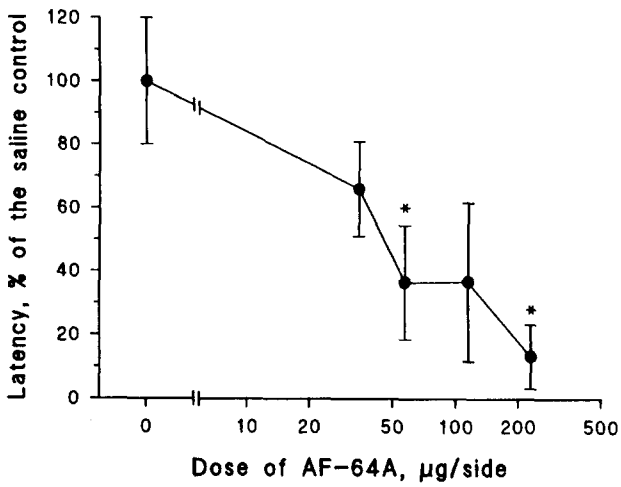

FIG. 1. The effect of various doses of AF64A (34.5, 57.5, 115, and 230 ng/nucleus) on the passive avoidance latency time. The studies were performed 2-3 weeks after the lesions (n = 5-8). Statistics: $*p < 0.05$, $**p < 0.01$ vs. the intact control animals.

Protein content of the brain homogenates were analyzed using the dye-binding method (7).

Histology

Because the brains were usually used for biochemistry studies, only two rats were subjected to histological examination. The anesthetized (sodium pentobarbital, 30 mg/kg and diazepam, 1 mg/kg) rats were perfused through the left ventricle with buffered 4% formaldehyde (0.1 M phosphate buffer, pH 7.3). After removing, the brains were immersed in 20% sucrose overnight, then frozen and cut in cryostat (−18°C). The 10 µm sections were examined with a light microscope.

Statistics

Arithmetic means, SEMs, and SDs were calculated. The learning results (percent of correct responses) were treated several ways. Both a two-way ANOVA (day, treatment) and a repeated ANOVA were run using a Systat statistical software (54). Because the two-way ANOVA did not add anything to the analysis given by the repeated ANOVA, only the latter results are given. At each day of training, also a one-way (treatment) analysis of variance (ANOVA) was performed using Pharmacological Calculation System software (48). Finally, the performance of the rats was also evaluated calculating areas under learning (percent of correct responses)-time (days) curves according to trapezoidal rule. Student's t-test was used to compare two means and Newman-Keuls test to compare the differences between three or more means (after ANOVA) (48).

RESULTS

Effect of Various Doses of AF64A on the Passive Avoidance

It appeared that 34.5 ng/side of AF64A did not affect the passive avoidance test while 57.5/side and 230 ng/side were quite effective when studied 2-3 weeks after operation (Fig. 1). Because 230 ng caused high mortality, the dose of 57.5 ng was selected for further studies.

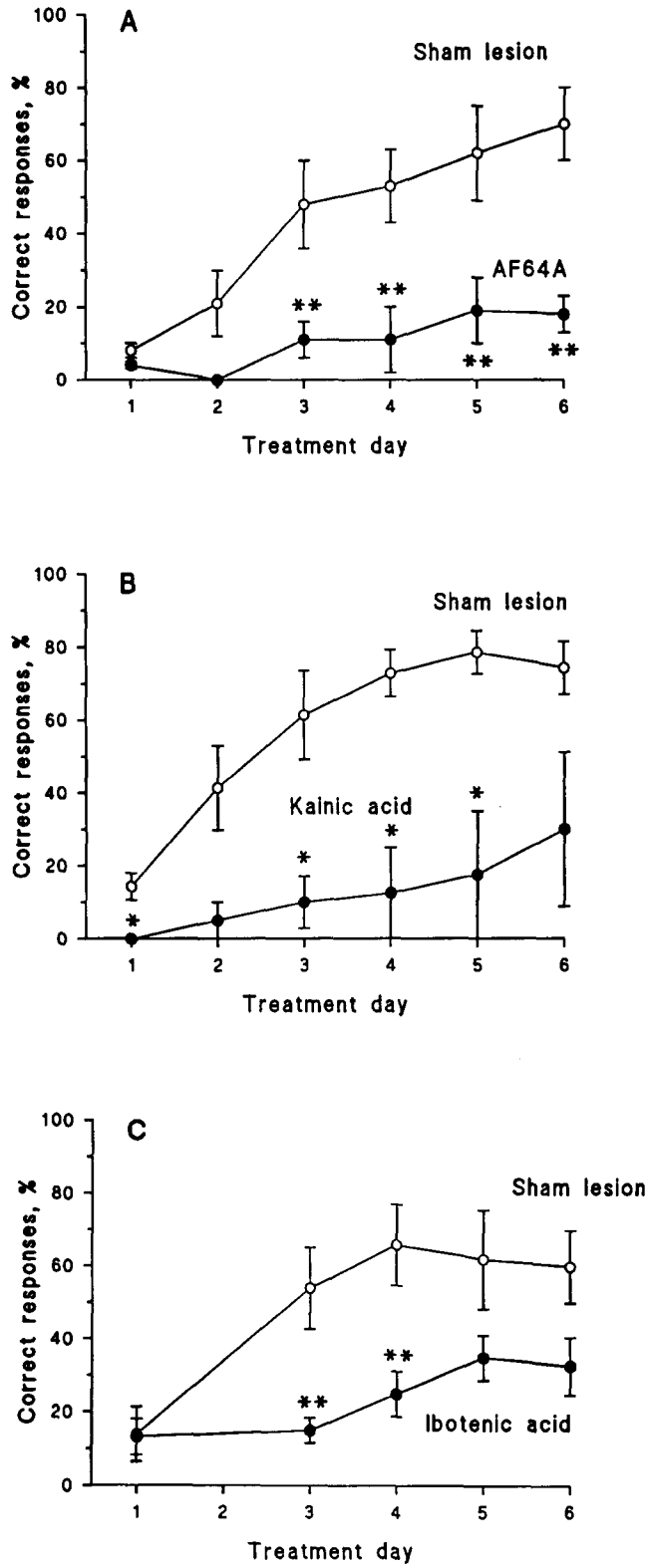

FIG. 2. The effect of AF64A (57.5 ng/site; A), kainic acid (125 ng/site; B), and ibotenic acid (750 ng/site; C) on the one-way active avoidance behavior performed starting 3-4 weeks after bilateral lesioning of the rats. Mean ± SEM (n = 5-7). The corresponding sham-operated control animals were infused saline. Statistics: $*p < 0.05$, $**p < 0.01$ vs. corresponding control at the same day.

Comparison of the Effects of the AF64A, Kainic Acid, and Ibotenic Acid Infusions on the Active Avoidance

The bilateral infusions of all three toxins worsened the learning results in the active avoidance test compared to the sham-operated, saline-infused control rats (Fig. 2). The statistics from repeated ANOVA are given in Table 1, showing significant effects of toxins, time, and their interaction. The areas under learning (percent of correct responses)-time (6 days) curves (percent of correct responses × days) values were decreased about 80% by both AF64A (57.5 ng/side) and kainic acid (125 ng/side) and 56% by ibotenic acid (750 ng/side) (Table 1). The effect of AF64A was quite consistent and we used only this toxin in further studies.

Behavioral Studies

It was found that bilateral lesions in the basal nuclei of Meynert (by 57.5 ng/nucleus of AF64A), caused besides a deterioration of learning in the passive and active avoidance tests (see above), significantly increased emotional reactivity, particularly vocalization and reaction to handling (Table 2) and time-dependent decrease of motoric activity compared to the intact control rats (Fig. 3). Hypomotility was significant at 6 weeks after AF64A-induced lesions compared to the control animals at the same time ($p < 0.05$). When the comparison was made to the situation at 2 weeks in the rats having lesions, their motility was further significantly reduced at 3 and 6 weeks (Fig. 3).

Biochemistry

Cortical choline acetyltransferase activities were 22% lower in the operated (AF64A lesions) than the intact control animals ($p < 0.01$) but both in the hypothalamus (+9.7%; $p < 0.05$) and hippocampus (+8.2%; $p < 0.01$) there were significant increases (Table 3).

Cortical dopamine and noradrenaline levels were decreased by about 36 (not significant) and 20% ($p < 0.05$), respectively, after AF64A lesions but 5-HT levels were not changed (Table 3).

TABLE 2

EMOTIONAL REACTIVITY SCORES OF THE RATS 4 WEEKS AFTER BILATERAL CHOLINERGIC LESIONS

Response Analyzed	Intact Control Rats	AF64A-Treated Rats
Handling	1.1 ± 0.2	2.8 ± 0.2*
Muscular strength	2.3 ± 0.3	2.5 ± 0.2
Response to the threat by forceps	1.4 ± 0.2	1.3 ± 0.1
Response to the press by forceps	1.9 ± 0.3	1.7 ± 0.1
Vocalization	1.5 ± 0.3	2.4 ± 0.2*
Defecation	1.3 ± 0.2	1.5 ± 0.1
Total scores	9.2 ± 1.4	11.3 ± 0.9*

Mean ± SEM. Number of animals was 16 in the intact control group and 31 in the group having lesions (57.5 ng/nucleus of AF64A).
Statistics: *$p < 0.05$ vs. intact control group (t-test). Each behavior was scored using a scale from 0 to 4.

Hypothalamic dopamine and noradrenaline levels were not changed but 5-HT was significantly increased (+25%; $p < 0.01$; Table 3).

In striatum, dopamine (−61%) and noradrenaline (−48%) levels were strongly suppressed compared to the control levels ($p < 0.01$). Striatal DOPAC was decreased from 1.85 ± 0.15 μg/g ($n = 12$) to 0.94 ± 0.11 μg/g ($n = 16$; $p < 0.01$) and HVA levels from 1.09 ± 0.057 μg/g ($n = 13$) to 0.51 ± 0.038 μg/g ($n = 35$; $p < 0.01$). Even striatal 5-HT was significantly decreased (−34%; $p < 0.01$).

Activities of all three peptidases (prolyl endopeptidase, dipeptidyl peptidase II and IV) were significantly decreased in the cortex and hippocampus (from −15 to −19%; $p < 0.01$) of the animals having AF64A lesions (Table 4).

TABLE 1

EFFECT OF VARIOUS TOXINS ON THE ACTIVE AVOIDANCE BEHAVIOR 3-4 WEEKS AFTER INFUSIONS

	Area Under Learning × Time (% Correct Responses × 6 Days)		Repeated ANOVA Statistics		
Toxin and Dose	Sham-Operated Control Rats Infused With Saline	Toxin-Infused Rats (Decrease, % of the Control) (t-Test)	Effect of Lesion	Effect of Time	Lesion × Time Interaction
AF64A 57.5 ng/side	239 ± 52	49 ± 18 (−79.5) $p < 0.01$	$F = 16.75$ $p < 0.001$	$F = 18.03$ $p < 0.001$	$F = 7.71$ $p < 0.001$
Kainic acid 125 ng/side	330 ± 39	62 ± 56 (−81.2) $p < 0.01$	$F = 16.33$ $p < 0.01$	$F = 10.12$ $p < 0.001$	$F = 2.73$ $p < 0.05$
Ibotenic acid 750 ng/side	253 ± 48	112 ± 16 (−55.7) $p < 0.05$	$F = 9.73$ $p < 0.05$	$F = 10.90$ $p < 0.001$	$F = 5.06$ $p < 0.001$

Mean ± SEM There were 5–7 rats per group. The summary results are given as areas under learning × time (% of correct responses × 6 days), based on the detailed results given in Fig. 2. For repeated ANOVA, F-values and corresponding p-values are given. The results of the two-way ANOVA were quite similar or gave higher levels of significance, and are not given here.

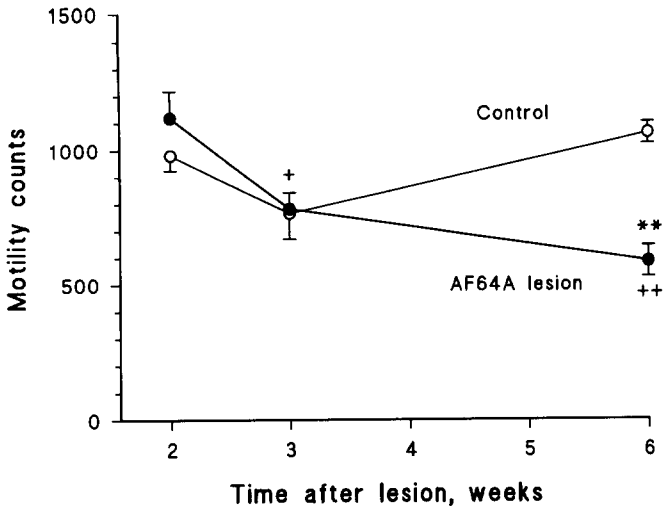

FIG. 3. Spontaneous motility of the rats made bilateral AF64A lesions in the basal magnocellular nuclei analyzed at 2, 3, or 6 weeks after lesions. Mean ± SEM (n = 7–14 in the lesion group and 5–11 in the intact control group). Statistics: $^{**}p < 0.01$ vs. the corresponding control group at the same time, $+p < 0.05$ and $++p < 0.01$ vs. the motility at 2 weeks of the animals having lesion.

Histology

In the two brains studied, the needle routes were partially visible. In the region of the basal nuclei there were easily identifiable lesions with a diameter of about 1 mm, containing disrupted cell debris and even some cavitation.

DISCUSSION

All toxins produced comparative lesions of the basal magnocellular nuclei, as judged from the equal results of the active avoidance learning. Kainic acid caused convulsions, although ibotenic acid was quite easy to use. We decided, however, to study AF64A in more detail, because it may theoretically have some advantage over the simple excitotoxic ibotenic acid. Notably, AF64A would utilize the high affinity choline uptake to enter selectively into the cholinergic nerve endings (15). We probably lost this advantage while infusing the toxin into the perikarya and not to the terminal area or the ventricular space. It is noteworthy though that there are cholinergic terminals also in the basal magnocellular nuclei (36) that are preferably injured by toxin. Local infusions would, however, limit the cholinergic lesion to the infusion site, in contrast to the intracerebroventricular administration, which may be assumed to affect all cholinergic nuclei. However, the infusion directly to the basal magnocellular nuclei inevitably raised a possibility to cause some nonspecific (noncholinergic) destruction effects to the structures originating from or passing through the infusion site (1,47) (see below).

The optimum dose of AF64A was 0.25 mM (57.5 ng/nucleus), about the same recommended before (15). The highest dose tested (230 ng/nuclei) was even more effective in shortening the passive avoidance retention time but it was also clearly toxic, killing 30% of the rats. AF64A caused only modest decrease of cerebral cortical choline acetyltransferase (about 22%), which is of the same magnitude as or slightly less than what has been described after the lesions of basal magnocellular nuclei (5,13,22,37,52). Interestingly, choline acetyl-transferase activities were, maybe compensatorily, increased in hippocampus and hypothalamus. The results were compatible with the toxin demonstrating nucleus selectivity, because hip-

TABLE 3

CHOLINE ACETYLTRANSFERASE ACTIVITY AND CONCENTRATIONS OF DOPAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE IN VARIOUS BRAIN AREAS IN INTACT CONTROL RATS AND IN RATS HAVING AF64A LESIONS

	Intact Control Rats	n	AF64A-Treated Rats	n
Choline acetyl transferase (nmol/min/mg protein)				
Frontal cortex	1.30 ± 0.09	24	1.01 ± 0.06*	59
Hypothalamus	0.93 ± 0.044	33	1.02 ± 0.05†	33
Hippocampus	1.71 ± 0.05	24	1.85 ± 0.03*	59
Dopamine, μg/g				
Frontal cortex	0.96 ± 0.50	7	0.61 ± 0.31	17
Hypothalamus	0.48 ± 0.023	22	0.44 ± 0.019	58
Striatum	11.65 ± 0.33	14	4.60 ± 0.40*	34
Noradrenaline, μg/g				
Frontal cortex	0.41 ± 0.024	22	0.33 ± 0.014†	57
Hypothalamus	1.86 ± 0.14	22	1.91 ± 0.08	58
Striatum	0.21 ± 0.019	14	0.11 ± 0.01*	18
5-Hydroxy-tryptamine, μg/g				
Frontal cortex	0.59 ± 0.05	19	0.59 ± 0.05	38
Hypothalamus	0.73 ± 0.033	14	0.91 ± 0.027*	31
Striatum	0.47 ± 0.018	14	0.31 ± 0.024*	17

Mean ± SEM.

Statistics: $^{*}p < 0.01$, $^{\dagger}p < 0.05$ vs. corresponding intact control rats (t-test). Analyses were made 6–7 weeks after toxin infusions.

TABLE 4

ACTIVITIES OF THREE PEPTIDASES IN FRONTAL CORTEX AND HIPPOCAMPUS OF INTACT CONTROL RATS AND IN RATS HAVING AF64A LESIONS

Peptidase and the Brain Area Studied	Intact Rats	*n*	AF64A-Treated Rats	*n*
Prolyl endopeptidase (nmol/min/mg protein)				
Frontal cortex	0.47 ± 0.022	24	0.38 ± 0.013*	59
Hypothalamus	0.35 ± 0.016	33	0.33 ± 0.010	33
Hippocampus	0.51 ± 0.017	24	0.43 ± 0.015*	59
Dipeptidyl peptidase II (nmol/min/mg protein)				
Frontal cortex	0.015 ± 0.001	24	0.012 ± 0.003†	59
Hypothalamus	0.025 ± 0.003	33	0.024 ± 0.003	33
Hippocampus	0.020 ± 0.001	24	0.017 ± 0.001†	59
Dipeptidyl peptidase IV (nmol/min/mg protein)				
Frontal cortex	0.069 ± 0.003	24	0.056 ± 0.003*	59
Hypothalamus	0.058 ± 0.003	33	0.051 ± 0.002	33
Hippocampus	0.059 ± 0.003	24	0.051 ± 0.004*	59

Mean ± SEM.

Statistics: *$p < 0.01$, †$p < 0.05$ vs. corresponding intact control rats (t-test). Analyses were made 6–7 weeks after toxin infusions.

pocampus gets only minor cholinergic input from the basal magnocellular nuclei (9,45).

We want to point out that the specific functional consequences of the degeneration of the cholinergic cells in the basal forebrain still remain unsolved. Cholinergic cell losses (assessed as a decrease of frontal cortical choline acetyltransferase activity) after the local infusions of various excitotoxins do not necessarily correlate with impaired performance (e.g., choice accuracy, spatial delayed response, passive avoidance task), as thoroughly documented by Dunnett and coworkers (13) and Wenk and associates (53) and discussed by Fibiger (14), Nabeshima (41), and Dunnett and co-workers (12). Several facts may explain this discrepancy. The basal nuclei are heterogenous and their uniform destruction is difficult. Even if the lesions are successful, impairing the reference memory, no harm happens to hippocampal cholinergic innervation leaving working memory intact (41). It is also unfortunate that local toxin infusions may destroy some of the noncholinergic neurons originating from or passing through the basal nuclei (3). It seems that ibotenate causes more learning deficits (and also more noncholinergic lesions) than quisqualate (12–14, 41,53). Against early optimism (15), local infusions of AF64A apparently does not solve the selectivity problem [(1,26,38, 44), our results].

In contrast to the claims of some earlier papers, where only transient aminergic changes have been described, especially after intracerebroventricular infusions of AF64A (15,20,24, 50), the abundant aminergic alterations were quite permanent in our rats. Cortical noradrenaline was decreased by 20%, although hypothalamic 5-HT was increased by 20% still at 6–7 weeks. The most surprising finding was, however, that all striatal amines were strongly suppressed: dopamine and noradrenaline by 50–60%, 5-HT by 34%. Even striatal DOPAC and HVA were similarly decreased, suggesting that there was a serious disturbance of dopaminergic function, not a mere altered turnover.

Brain cholinergic and dopaminergic functions are intensively interacting (10,17,39). It should be pointed out, however, that the positive cholinergic input to s. nigra arises mainly from the pedunculopontine tegmental nuclei and not from the basal magnocellular nuclei of Meynert (9,23,45). Intrastriatal infusions of AF64A, which decreased choline acetyltransferase activity by 42%, also decreased dopamine levels by 59%, analyzed as soon as at 8 days after infusions (51). Intrastriatal infusions of AF64A were more toxic to dopamine cell bodies than ibotenate infusions. However, both toxins were even more deleterious to intrinsic cholinergic cells (40). In the latter study, the dialysis samples were collected already at 10 days postinfusion. The mechanism of the toxicity was supposed to be either a nonspecific damage to nigrostriatal dopamine terminals or loss of a stimulatory striatonigral feedback loop. Also, bilateral electrolytic lesions of the basal forebrain neurons have caused a clear decrease of cortical and striatal dopamine (35–43%) and noradrenaline levels (28–33%) analyzed at 2 weeks postlesion (3).

In our study, the general decrease of all striatal transmitters analyzed (dopamine, noradrenaline, 5-HT) supports the nonspecific destruction of the fibers passing through the infusion site, especially those heading to striatum (1,3,47). Indeed, there is a dense catecholaminergic network in the basal magnocellular nuclei (35). Our limited histological findings, showing general tissue disruption and even cavitation, favor the same view. The limited selectivity of AF64A to cholinergic neurons has been proposed by several groups (1,26,38,42). Apparently only few fibers leading to the frontal cortex or hypothalamus were passing through the infusion site, because the changes in the transmitter levels in these brain areas were minor or nothing.

The 50% loss of the striatal dopamine is probably not very serious, because in the animal model of Parkinson's disease, the aim is to reduce dopamine by more than 95% (43,49). However, it seems that even the loss of dopamine observed in the present study may have behavioral consequences such as decreased motility at 6 weeks or strengthened emotional be-

havioral at 4 weeks. In a previous study, ibotenic acid lesions did not alter the daytime locomotor activity at 2 weeks after surgery (21). However, electrolytic lesions of the anterior part of the basal forebrain have caused hypoactivity at 10 and 60 days postoperatively (29). It should also be pointed out, that using the 2-deoxyglucose technique, striatum has been shown to be involved in the early events of acquiring the passive avoidance reaction (11).

The analysis of the brain peptidase activities was interesting, considering the crucial role of endopeptidases in protein metabolism, including processing of β-amyloid protein (46). Also, activation and inactivation of various potent neuropeptides (e.g., substance P, neurotensin, thyrotropin-releasing hormone, vasopressin) are regulated by endopeptidases (32). There are also some direct connections of the endopeptidases to cognitive function. In cerebrospinal fluid of the patients having Alzheimer's disease, the activities of a variety of peptidases have been high (2). Several studies have proposed that inhibition of prolyl endopeptidase or dipeptidyl peptidase IV have improved the cognitive functions in various amnestic models (4,30,55). Finally, intrastriatal infusions of AF64A, which caused serious cholinergic and dopaminergic deficits, decreased the activity of neutral endopeptidase, also known as angiotensin converting enzyme (51).

We found that activities of all peptidase studied (prolyl endopeptidase, dipeptidyl peptidase II and IV) in frontal cortex and hippocampus were slightly (15–20%) but significantly decreased. This is apparently in contrast to some clinical findings. Hypothalamus was mostly saved from peptidase losses. The high peptidase levels in Alzheimer's disease may be the sign of the high proteolytic activity on the amyloid precursor protein and the subsequent enhanced production of neurotoxic β-amyloid protein (46). On the other hand, protease inhibition has caused some manifestations of aging and Alzheimer's disease in rodent and primate brain (25). It appears that the role of proteases and their inhibitors in cognitive processes is still far from clear. In the proposed model of Alzheimer's disease, however, where the basal magnocellular nuclei are bilaterally destroyed by AF64A, the peptidase activities are generally decreased.

In conclusion, these results show that AF64A induced a long-term learning deficits in the rat. However, also striatal amine levels were even more suppressed and reflected as hypomotility and increased emotional reactivity. Decreased peptidase activities in the frontal cortex and hippocampus add to the confusion about the proteolytic processing of β-amyloid precursors in the pathogenesis of Alzheimer's disease. The observed noncholinergic changes may limit the usefulness of the amnestic rat model. We want to point out, however, that even with the recognized limitations, this model has been useful in testing the efficacy of some novel drugs (33).

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