Pharmacology Biochemistry & Behavior, Vol. 19, pp. 309–312, 1983. © Ankho International Inc. Printed in the U.S.A.

Loss of Cholinergic Neurons in the Rat Neocortex Produces Deficits in Passive Avoidance Learning[1]

EITAN FRIEDMAN, BARBARA LERER AND JOAN KUSTER

Departments of Psychiatry and Pharmacology, New York University, School of Medicine 550 First Avenue, New York, NY 10016

Received 2 December 1982

FRIEDMAN, E., B. LERER AND J. KUSTER. *Loss of cholinergic neurons in the rat neocortex produces deficits in passive avoidance learning.* PHARMACOL BIOCHEM BEHAV **19**(2) 309–312, 1983.—Bilateral kainic acid lesions of the ventral globus pallidus produced a significant and selective cortical decrease in choline acetyltransferase activity in the rat brain. When lesioned and control subjects were compared on performance of a step-through passive avoidance task, lesioned rats showed a marked retention deficit 24 hr after the initial training trial. This experimentally-induced memory deficit associated with a cortical cholinergic neuronal loss resembles the deficits in senile dementia of the Alzheimer type and may provide a useful animal model for studying the disease.

N. basalis lesion Cortical choline acetyltransferase Memory deficit Alzheimer disease

IN SENILE dementia of the Alzheimer type (SDAT), the characteristic decrease in cognitive function is correlated with reduced activity of brain choline acetyltransferase (CAT), the synthetic enzyme for the neurotransmitter acetylcholine [27]. Diminished CAT activity in SDAT has been observed in various brain regions including the neocortex [8,32]. In cortex, the reduction in this presynaptic marker of cholinergic neurons is not accompanied by changes in postsynaptic cholinergic receptors [9, 26, 28, 32] and is probably due to loss of cholinergic afferents rather than to loss of intrinsic cholinergic neurons [25,29].

The nucleus basalis of Meynert in the substantia innominata gives rise of cholinergic neurons which project diffusely to neocortex [16, 20, 24, 31]. A substantial reduction in nucleus basalis neurons was recently documented in a patient with SDAT [33]. Johnston *et al.* [17, 18] have shown that destruction of cell bodies in the area of the nucleus basalis in the rat (i.e., the ventral globus pallidus) is associated with a specific loss of cholinergic neuronal markers, primarily in fronto-parietal cortex.

These findings along with the large body of evidence implicating the central cholinergic nervous system in memory and learning [10, 11, 14] have suggested to us that cortical cholinergic synapses may mediate certain cognitive functions. The present study examined the effects of disruption of presynaptic cortical cholinergic neurons on the performance and retention over 24 hr of an inhibitory learning (passive avoidance) task in rats.

METHOD

Surgery

Male Sprague-Dawley rats weighing 200–250 g were maintained on a 12-hr light-dark cycle and allowed free access to food and water. Prior to surgical procedures, subjects were anesthetized with Nembutal (50 mg/kg IP). Bilateral lesions were made by direct stereotaxic application of 1.0 μg kainic acid (pH 7.1; Sigma) in 1 μl 0.2 M sodium phosphate buffer. Coordinates for the lesions were 0.7 mm posterior to bregma, 2.7 mm lateral to the midline, and 7.0 mm ventral to the brain surface. Control animals were treated in the same manner up to the point of the neurotoxin injection. In all cases the success of the lesion was assessed by measurement of brain CAT activity. Only animals which exhibited selective cortical CAT deficits without altered striatal enzyme were included in the analysis of the behavioral data.

Behavioral Testing

Animals were trained and tested 14–16 days post-operatively on a 24 hr passive-avoidance task. Each rat was individually placed in the lighted front compartment of a two-compartment shuttle box. Electromechanical switching circuitry was used to measure the subject's latency to enter the dark compartment of the shuttle box. When a subject had been in the dark rear compartment for 5 sec, a 1.5 mA scrambled foot-shock (Lafayette) was applied to the metal grid bars of the floor until the subject escaped through the doorway to the lighted front compartment. If the subject

[1]Preliminary results have been presented at the 12th Annual Society for Neuroscience meeting, Minneapolis, MN, October 31, 1982 and The 10th Aharon Katzir-Katchalsky Conference on the Aging of the Brain, Mantova, Italy, March 26, 1982. This research is supported in part by U.S.P.H.S., RSDA MH 00208, grants 5T 32MH15137 and MHCRC MH 35976.

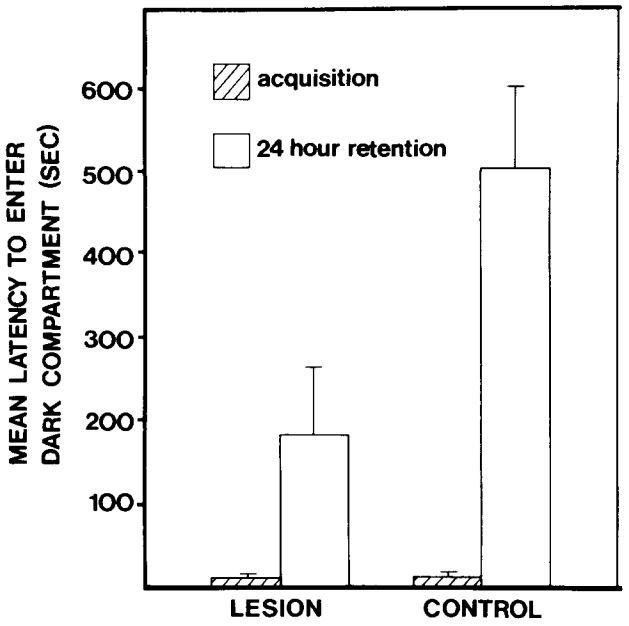

FIG. 1. Mean latency to enter dark compartment during acquisition (training) and retention testing for control and kainic acid lesioned rats. The bars represent the mean±SEM obtained from 6 animals each. Retention, measured 24 hr post training, was significantly ($p<0.05$) different in the lesioned group.

remained in the lighted compartment for 60 sec, it was removed from the shuttle box and returned to its home cage. If the subject re-entered the dark compartment during the 60 sec interval, the subject received another escapable footshock and the 60 sec timer was restarted. Retention of the passive avoidance task was measured 24 hr after training by replacing a rat in the lighted front compartment and measuring the latency to enter the dark rear compartment. The test session ended when a subject entered the rear compartment or after 600 sec had elapsed.

General motor activity was measured in a dark circular compartment with six pairs of infrared photosensor circuits (Lehigh Valley Electronics). Subjects were individually placed in the activity compartment for a single 30 min session on the day preceding passive-avoidance training. Digital counters kept track of the number of times the photosensor beams were broken and counts were recorded after each consecutive 5 min period. All behavioral testing was conducted during the middle portion of the diurnal light cycle.

Biochemistry

CAT activity was measured in various brain areas in order to assess the extent and the distribution of cholinergic neuronal loss produced by the lesion. Fifteen rats were sacrificed by decapitation 1 week after a unilateral injection of kainic acid and the brains were quickly dissected over ice. The olfactory tubercules, cingulate cortex, and the cortex below the rhinal sulcus were discarded and the remaining cortex was divided into frontal, parietal and posterior regions as described previously [19]. Hippocampus and striatum were also dissected for CAT analysis. CAT activity in these brain regions was measured under saturating conditions for both choline and ^{14}C-acetylcoenzyme A, as previously described [21]. Statistical significance was determined by the two-tailed Student's t test.

TABLE 1

DEFICITS IN BRAIN CHOLINE ACETYLTRANSFERASE (CAT) RESULTING FROM UNILATERAL KAINIC ACID INJECTION IN THE NUCLEUS BASALIS

	Percent CAT Reduction*
Frontal Cortex (n=15)	47†
Parietal Cortex (n=15)	35†
Posterior Cortex (n=11)	17†
Striatum (n=10)	6
Hippocampus (n=8)	5

*Mean control values (nmoles/mg tissue/hr); frontal cortex=5.36; parietal cortex=5.37; posterior cortex=5.03; striatum=22.12; hippocampus=6.68.

†$p<0.001$ lesioned US control side by two-tailed t test.

RESULTS

Lesioned animals exhibited motor hyperactivity, profuse salivation, jumping, flailing the air with the front paws and "wheelbarrow" turning immediately and up to 6–10 hr following recovery from anesthesia. Following the hyperactivity stage, subjects displayed impaired motor reflexes (poor grooming, grasping, negative geotaxis); locomotor deficits persisted for 2–5 days following surgery. Aphagia and adipsia were observed for 6 to 10 days postoperatively; consequently, lesioned animals were sustained by intragastric feeding of Similac baby formula. Normal ingestion and locomotion resumed during the second week of recovery.

On the passive avoidance task, the initial latencies to enter the rear chamber on the training day were not significantly different in the two groups of rats; control and lesioned animals had latencies of 9.03±4.8 (S.E.M.) and 8.44±3.0 seconds respectively. The mean number of shocks required during training was not different in the lesioned and control groups (1.8 vs. 1.5). During retention testing, the mean re-entry latency for control subjects was 503.9±96 seconds and the mean re-entry latency for lesioned subjects was 176.1±88 ($p<0.05$) (see Fig. 1).

Rats from both the lesion and control groups were given an additional test to measure their sensitivity to scrambled footshock. Testing was administered on the day after the retention testing for the passive avoidance task in a Plexiglas box different from that used in the passive avoidance task. There was no apparent difference in the pain reactivity threshold for the two groups. Both lesioned and control subjects showed evidence of a pain threshold at about 0.3 mA using "flinch" and removal of at least two paws from the floor grid as criterion. Shocks were delivered in random order (with shocks ranging from 0.1 mA to 1.0 mA in 0.1 mA steps) for 1 sec every 60 sec.

Motor activity rates were not significantly different in the two groups. Mean motor activity during the first 5 min of the session was 82.4±8.6 and 79.9±8.0 counts/min for the lesion and control groups respectively ($p>0.2$).

The effect of kainic acid injection on regional CAT activity is shown in Table 1. Enzyme activity was reduced significantly ($p<0.001$) in the frontal (47%), parietal (35%) and

posteriors (17%) cortical areas. No significant alterations in CAT activity were obtained in the striatum and hippocampus of lesioned rats. The extent of the cholinergic deficit in the trained animals was routinely assessed in the cortex (frontal-parietal area) and was 30–50% reduced compared to control animals.

DISCUSSION

The present data confirm previous findings [17] which indicate that in the rat, cell bodies lying ventral to the globus pallidus provide extensive cholinergic innervation of neocortex. Moreover, our findings suggest that destruction of this innervation results in a performance deficit on a 24 hr passive avoidance task. The impairment seems to be that of retention and not of acquisition because the initial latenices on training day and the number of shocks required during training were not different in the lesioned and control groups. It remains possible that short retention test latencies may be secondary to changes in motor activity or to altered pain sensitvity; however, spontaneous locomotor activity and shock sensitivity measurements were not different in the experimental and control groups. The finding in lesioned animals of a decreased latency to enter the dark compartment 24 hr after training was therefore attributable to a retention deficit which may be related to a failure in memory processes; alternatively, the retention deficit observed in the lesioned animals may result from a failure of response inhibition.

It is interesting to note that the passive avoidance task has previously been found to be sensitive to other manipulations of cholinergic transmission [3, 4, 5, 14, 22]. A large body of pharmacological literature has implicated the central cholinergic system in cognitive function in humans and animals. Central cholinergic blockade with the muscarinic antagonists atropine or scopolamine, or with acetylcholine synthesis inhibitors pyrrolcholine or hemicholinium-3, result in memory deficits [4, 6, 7, 14, 23]. On the other hand, cholinomimetic drugs administered to normal subjects improve performance on cognitive tasks and selectively reverse scopolamine-induced memory deficits [1,11]. The present data focuses on the role played by the specific cholinergic n. basalis-cortical pathway in the passive avoidance task. The kainic acid-induced cholinergic deficit was confined to cortical areas and did not include the hippocampus and striatum, two areas previously implicated in avoidance learning [15].

Pharmacological, biochemical and behavioral studies conducted in animals and humans suggest that impaired cholinergic neurotranmission is at least partly responsible for the cognitive decline observed during senescence [2, 12, 13, 30]. The evidence for a cholinergic deficit is particularly strong in SDAT patients who present marked losses in cognitive functions [8]. Cognitive dysfunction in SDAT has been correlated with marked reductions (50–90%) in cortical CAT activity [28]. These congitive and CAT deficits are also correlated with the extent of neuritic plaques and neurofibrillary tangles that histologically characterize SDAT [27].

The neurochemical and behavioral impairments induced by the kainic acid lesion suggest that a parallel may be drawn between the lesion effects in the rat and two important aspects of SDAT in humans—presynaptic cholinergic deficit and cognitive impairment. This lesion may be useful, therefore, in constructing an animal model to study the pathophysiology of SDAT and to develop new strategies for the treatment of the disease.

REFERENCES

1. Bartus, R. T. Evidence for a direct cholinergic involvement in the scopolamine induced amnesia in monkeys: Effects of concurrent administration of physostigmine and methylphenidate with scopolamine. *Pharmacol Biochem Behav* **9:** 833–836, 1978.
2. Bartus, R. T., R. L. Dean, B. Beer and A. S. Lippa. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217:** 408–417, 1982.
3. Bohdanecky, Z. and M. E. Jarvik. Impairment of one trial passive avoidance learning in mice by scopolamine methylbromide and physostigmine. *Int J Neuropharmacol* **6:** 217–222, 1967.
4. Buresova, O., J. Bures, Z. Bohdanecky and T. Weiss. Effect of atropine on learning extinction, retention and retrieval in rats. *Psychopharmacologia* **5:** 255–263, 1964.
5. Carlton, P. L. and B. Markiewicz. Behavioral effects of atropine and scopolamine. Pharmacological and biophysical agents and behavior. In: *Pharmacological and Biophysical Agents and Behavior*, edited by E. Furchtgott. New York: Academic Press, 1973, pp. 345–373.
6. Caulfield, M. P., D. H. Fortune, P. M. Roberts and J. K. Stubley. Intracerebroventricular hemicholinium-3 (HC-3) impairs learning of a passive avoidance task in mice. *Br J Pharmacol* **74:** 865, 1981.
7. Crow, T. and I. Grove-White. An analysis of the learning deficit following hyoscine administration to man. *Br J Pharmacol* **49:** 322–327, 1973.
8. Davies, P. and A. J. F. Maloney. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2:** 1403, 1976.
9. Davies, P. and A. H. Verth. Regional distributions of muscarinic acetylcholine receptor in normal and Alzheimer's-type dementia brains. *Brain Res* **158:** 385–392, 1978.
10. Davis, K. L. and J. A. Yesavage. Brain acetylcholine and disorders of memory. In: *Brain Acetylcholine and Neuropsychiatric Disease*, edited by K. L. Davis and P. A. Berger. New York: Plenum Press, 1979, pp. 205–213.
11. Drachman, D. A. Memory and cognitive function in man: Does the cholinergic system have a specific role. *Neurology (NY)* **27:** 783–790, 1977.
12. Drachman, D. A. and J. Leavitt. Human memory and the cholinergic system: A relationship to aging? *Arch Neurol* **30:** 113–121, 1974.
13. Gibson, G. E., C. Piterson and D. Jensen. Brain acetylcholine synthesis declines with senescence. *Science* **213:** 674–676, 1981.
14. Glick, S. D., A. M. Crane, L. A. Barker and T. W. Mittag. Effects of N-hydroxyethylpyrrolidinum methiodide, a choline analogue on passive avoidance behavior in mice. *Neuropharmacology* **14:** 561–564, 1975.
15. Glick, S. D. and S. Greenstein. Comparative learning and memory deficits following hippocampal and caudate lesions in mice. *J Comp Physiol Psychol* **82:** 188–194, 1973.
16. Gorry, J. D. Studies of the comparative anatomy of the ganglion basale of meynert. *Acta Anat* **55:** 51–104, 1963.
17. Johnston, M. V., M. McKinney and J. T. Coyle. Evidence for a cholinergic projection to neocortex from neurons in basal forebrain. *Proc Natl Acad Sci USA* **76:** 5392–5396, 1979.
18. Johnston, M. V., M. McKinney and J. T. Coyle. Neocortical cholinergic innervation: A Description of extrinsic and intrinsic components in the rat. *Exp Brain Res* **43:** 159–172, 1981.
19. Kelly, P. H. and K. E. Moore. Decrease of neocortical choline acetyltransferase after lesion of the globus pallidus in the rat. *Exp Neurol* **61:** 479–484, 1978.

20. Kievit, J. and H. G. J. Kuypers. Basal forebrain and hypothalamic connections to the frontal and parietal cortex in the rhesus monkey. *Science* **187:** 660–662, 1978.
21. Kuhar, M. J., M. J. Sethy, R. H. Roth and G. K. Agajanian. Choline: Selective accumulation by central cholinergic neurons. *J Neurochem* **20:** 581–592, 1973.
22. Meyers, B. Some effects of scopolamine on passive avoidance response in rats. *Psychopharmacologia* **8:** 111–119, 1965.
23. Ostfeld, A. M. and A. Aruguete. Central nervous system effects of hyoscine in man. *Exp Ther* **137:** 133–139, 1962.
24. Parent, A., A. Gravel and A. Olivier. The extrapyramidal and limbic systems relationship at the Globus Pallidus level: A comparative histochemical study in the rat, cat and monkey. *Adv Neurol* **24:** 1–11, 1979.
25. Perry, E. K. The cholinergic system in old age and Alzheimer's disease. *Age Aging* **9:** 1–8, 1980.
26. Perry, E. K., R. H. Perry, G. Blessed and B. E. Tomlinson. Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* **1:** 189, 1977.
27. Perry, E. K., B. E. Tomlinson, G. Blessed, K. Bergman, P. H. Gibson and R. H. Perry. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J* **52:** 1457–1459, 1978.
28. Reisine, T., H. I. Yamamura, E. D. Bird, E. Spokes and S. J. Enna. Pre and post-synaptic neurochemical alteration in Alzheimer's disease. *Brain Res* **159:** 477–481, 1978.
29. Rossor, M., H. Fahrenkrug, P. Emson, C. Mountjoy, L. Iverson and M. Roth. Reduced cortical choline acetyltransferase activity in senile dementia of Alzheimer type is not accompanied by changes in vasoactive intestinal polypeptide. *Brain Res* **201:** 209–253, 1980.
30. Sherman, K. A., J. E. Kuster, R. L. Dean, R. T. Bartus and E. Friedman. Presynaptic cholinergic mechanisms in brain of aged rats with memory impairments. *Neurobiol Aging* **2:** 99–104, 1981.
31. Shute, C. C. D. and P. R. Lewis. The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. *Brain* **90:** 497–520, 1967.
32. White, P., M. T. Goodhardt, J. P. Keet, C. R. Hiley, L. H. Carrasco, F. E. I. Williams and D. M. Bowen. Neocortical neurons in elderly people. *Lancet* **1:** 668–674, 1977.
33. Whitehouse, P. J., D. L. Price, A. W. Clark, J. T. Coyle and M. R. DeLong. Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol* **10:** 122–126, 1981.