Pharmacology Biochemistry & Behavior, Vol. 28, pp. 433–436. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Effects of Intraseptal Drug Administration on Pentobarbital-Induced Narcosis and Hippocampal Choline Uptake

JONATHAN ZUCKER,*[1] DIANNE CALKINS,* JADWIGA ZABAWSKA,† HENRY LAI† AND AKIRA HORITA†‡

*Departments of *Anesthesiology, †Pharmacology, and ‡Psychiatry and Behavioral Sciences University of Washington School of Medicine, Seattle, WA 98195*

Received 3 June 1987

ZUCKER, J., D. CALKINS, J. ZABAWSKA, H. LAI AND A. HORITA. *Effects of intraseptal drug administration on pentobarbital-induced narcosis and hippocampal choline uptake.* PHARMACOL BIOCHEM BEHAV **28**(4) 433–436, 1987.—Effects of injection of drugs into the septum on pentobarbital anesthesia were investigated in the rat. Intraseptal microinjection of bicuculline (5 μg), arecoline (2 μg), and phenylephrine (5 μg) shortened, MK-212 (5 μg) prolonged, and atropine (2 μg) had no significant effect on the duration of pentobarbital-induced loss of righting reflex. Bicuculline and arecoline increased and MK-212 reduced hippocampal cholinergic activity as measured by change in hippocampal sodium-dependent high-affinity choline uptake after intraseptal drug injection. It is concluded that activation of the septal-hippocampal cholinergic pathway might be an important neuromechanism for recovery from pentobarbital-narcosis.

Choline uptake Pentobarbital narcosis Septal-hippocampal cholinergic pathway

PENTOBARBITAL depresses the activity of the septal-hippocampal cholinergic pathway. Sodium-dependent high-affinity choline uptake [10] and the turnover rate of acetylcholine [11] in the hippocampus are reduced in pentobarbitalized animals. In a previous publication [13] we have hypothesized that the duration of pentobarbital narcosis is inversely related to cholinergic activity in the septal-hippocampal pathway. Activation of the cholinergic pathway shortens pentobarbital-narcosis and depression of the pathway prolongs pentobarbital-narcosis. Septal-hippocampal cholinergic neurons are regulated by a variety of septal afferents, including GABA, substance P, norepinephrine [2], serotonin [9], and TRH [4]. Intraseptal microinjection of β-endorphin or morphine stimulates a subset of GABA-ergic inhibitory interneurons in the septum and causes a reduction in septal-hippocampal cholinergic activity [12] and a prolongation of the duration of pentobarbital narcosis in rats [3]. Intraseptal microinjection of substance P reduces septal-hippocampal cholinergic activity by a mechanism that does not involve GABA-ergic interneurons [12] and also causes a prolongation of the duration of pentobarbital narcosis in rats [13]. On the other hand intraseptal TRH antagonizes the reduction in septal-hippocampal cholinergic activity and shortens the duration of narcosis induced by pentobarbital [1]. In this study, we measured the duration of pentobarbital-induced narcosis in rats that had been microinjected intraseptally with either the cholinergic compounds atropine, arecoline, or a noradrenergic agonist phenylephrine, or a GABA antagonist bicuculline, or a serotonin agonist MK-212. We also measured hippocampal synaptosomal choline uptake in rats treated in the same way with either intraseptal atropine, arecoline, bicuculline, phenylephrine, or MK-212. The purpose of this study was to further test our hypothesis that the duration of pentobarbital-induced narcosis is inversely related to septal-hippocampal cholinergic activity.

METHOD

Animals and Surgery

Male Sprague-Dawley rats (250–300 g, from Tyler Laboratories, Bellevue, WA) were housed in a vivarium maintained on a 12-hr light-dark cycle (lights on between 8:00–16:00 hr) and provided with food and water ad lib. At least five days before the experiment, the rats were anesthetized with pentobarbital (50 mg/kg, IP), and a 23-gauge guide cannula was implanted stereotaxically in the brain and secured to the skull with stainless-steel screws and dental acrylic. The tip of the guide cannula was positioned 3.0 mm above the septal area. Coordinates of the injection site were: AP, +9.5 mm; V, +4.0 mm; and L, ±0.3 mm, with reference to the ear-bar in accordance with the rat brain stereotaxic atlas of Paxinos and Watson [8]. The animals were allowed to recover from the surgery in the vivarium and then were

[1]Requests for reprints should be addressed to Jonathan R. Zucker, M.D., Department of Anesthesiology, RN-10, University of Washington, Seattle, WA 98195.

moved to the research laboratory 15 hr before the experiment, for habituation to the experimental environment.

Intracerebral Injection Procedure and Study of Pentobarbital Narcosis

The rats were weighed and allowed to recover for 30 min from the handling. Then a challenge dose of sodium pentobarbital (50 mg/kg IP) was injected. At 30 min after the pentobarbital injection, a 30-gauge injection cannula was inserted to a point 3.0 mm beyond the tip of the guide cannula. Injection was begun 30 sec later, of either 1 μl of the test drug dissolved in sterile, pyrogen-free physiological saline, at neutral pH or 1 μl of the saline, at a rate of 2 μl/min. The injection cannula was withdrawn 30 sec after completion of the injection. The following drugs were studied: arecoline (Research Biochemical, Inc., Wayland, MA), bicuculline methiodide (Sigma Chemical Co., St. Louis, MO), phenylephrine (Sigma Chemical Co., St. Louis, MO), MK-212 (Merck, Sharp & Dohme, West Point, PA) and atropine (Merck & Co., Rahway, NY). The animals were placed on their backs after the intraseptal injection and the time of return of righting reflex was recorded. Timing was terminated when the animals could right themselves three times within 30 sec. We performed both saline and drug microinjections on each group of four rats caged together to minimize variations between cages of animals. All experiments were performed in the morning between 9:00–12:00 hr. After the experiment, the animals were decapitated and the injection site in the brain was verified by a 'blinded' observer by histologic inspection.

Measurement of Synaptosomal Choline Uptake

In a separate group of animals, cholinergic activity in the septal-hippocampal pathway was assessed by measurement of sodium-dependent, high-affinity choline uptake into hippocampal synaptosomes as previously described [13]. Rats were decapitated at 60 min after IP injection of sodium pentobarbital (i.e., 30 min after intraseptal injection). The hippocampus was then dissected on ice from the brain and placed in 20 volumes of ice-cold 0.32 M sucrose solution. The rest of the brain was placed in formaldehyde for later histological verification of the injection site. The hippocampus was homogenized with a glass pestle homogenizer with 10 up and down strokes and centrifuged at 1000×g for 10 min. The supernatant was centrifuged at 17,000×g for 15 min, and the resulting pellet was carefully resuspended in 20 volumes of 0.27 M sucrose solution. The incubating medium was either a buffer (containing dextrose, 4%; NaCl, 126 mM; Na_2HPO_4, 12.8 mM; KCl, 4.75 mM; $CaCl_2$, 1.27 mM; and $MgCl_2$, 1.42 mM; at pH 7.2) or an identical buffer containing 2 μM of hemicholinium-3 (Sigma Chemical Co., St. Louis, MO). Uptake of choline in the latter medium is the non-sodium-dependent uptake and was subtracted from the value of total uptake in the former medium to obtain the value of sodium-dependent uptake. Synaptosomal suspension in the amount of 0.1 ml was added to 0.9 ml of the incubation medium containing 0.3 μM choline chloride (Sigma Chemical Co.) and 0.4 μCi of [^{3}H]-choline (80 Ci/mmol, New England Nuclear, Boston, MA). The incubation was started by transfer of the samples from an ice bath to a water incubator at 38°C. After 4 min of incubation, the uptake reaction was terminated by transfer of the samples from the incubator back to the ice bath. The particulate matter in each sample was collected by centrifugation at 8000×g for 20 min. The remaining medium was decanted and the pellet surface was washed with 1 ml of ice-cold 0.9% saline. After removal of the saline, the pellet was dissolved overnight by addition of 0.7 ml of Protosol (New England Nuclear). All uptake experiments were performed in triplicate and radioactivity was determined by liquid-scintillation technique at 36 to 40% counting efficiency, with Econofluor (New England Nuclear) as the scintillant. Protein concentration in the synaptosomal suspension was determined by the method of Lowry *et al.* [5] with bovine serum albumin (Sigma Chemical Co.) as the standard.

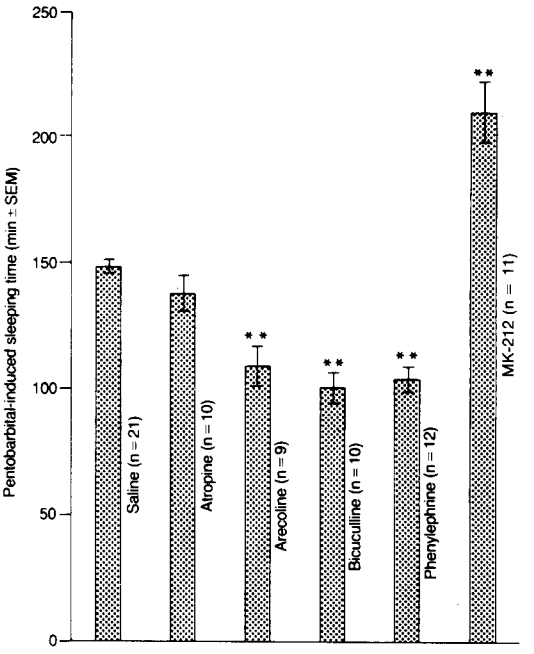

FIG. 1. Effects of intraseptal drug administration on duration of pentobarbital-induced sleeping time. One way analysis of variance showed significant treatment effect, $F(5,67)=35.0$, $p<0.001$. $^{**}p<0.01$, compared to data of intraseptal saline-injected controls.

Data Analysis

Data on sleeping time (duration of loss of righting reflex) and data on synaptosomal choline uptake were analyzed by the one-way analysis of variance and in both cases, the difference between the drug-treatment groups and the saline-treated group was compared with the Newman-Keuls test. Difference at $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Data on the effect of intraseptal drug injections on pentobarbital-induced duration of loss of righting reflex (sleeping time) are presented in Fig. 1. Arecoline 2 μg, bicuculline 5 μg, and phenylephrine 5 μg all shortened the duration of pentobarbital-induced sleeping time, whereas MK-212 5 μg prolonged sleeping time and atropine 2 μg had no significant effect. The mean hippocampal choline uptake in saline-treated rats was 28.9±0.9 pmols/4 min/mg protein (±SEM). The data on choline uptake with intraseptal injection of arecoline, bicuculline, phenylephrine, and MK-212 and atro-

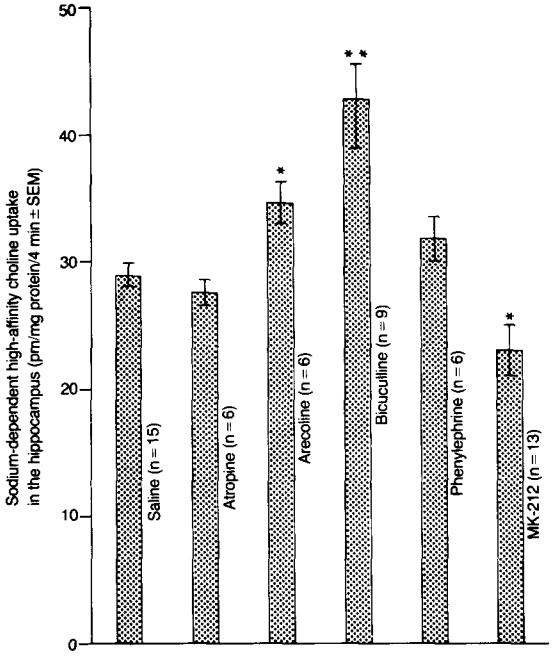

FIG. 2. Effects of intraseptal drug administration on sodium-dependent high-affinity choline uptake in the hippocampus. One way analysis of variance showed a significant treatment effect, $F(5,49)=11.3$, $p<0.001$. **, *$p<0.01$ and 0.05, respectively, compared to data of intraseptal saline-injected controls.

pine are shown in Fig. 2. Arecoline and bicuculline injections increased choline uptake and MK-212 injection decreased choline uptake. Intraseptal injection of phenyleprine and atropine had no significant effect on hippocampal choline uptake.

Inhibition of septal GABA-ergic [13] and activation of septal serotonergic [9] innervations increases and decreases the turnover rate of acetylcholine in the hippocampus, respectively. Our findings measuring hippocampal synaptosomal choline uptake after intraseptal bicuculline and MK-212 confirm the role played by these septal afferents on the septal hippocampal cholinergic pathway. Additionally, the finding that injection of arecoline intraseptally significantly increased hippocampal choline uptake in pentobarbitalized rats suggests that cholinergic innervations in the septum activate septo-hippocampal cholinergic neurons. The inability of intraseptal atropine to decrease hippocampal choline uptake suggests either a nicotinic receptor mechanism or a phasic, non-tonic role for this septal afferent system. Activation of septal noradrenergic [2] innervation has also been shown to increase the turnover rate of acetylcholine in the hippocampus. Since *in vitro* hippocampal synaptosomal choline uptake most probably reflects the state of *in vivo* cholinergic activity in the hippocampus just prior to decapitation [10], the inability to demonstrate significant change in hippocampal synaptosomal choline uptake after intraseptal injections of phenylephrine might be due to a variety of factors. The time of the peak change in hippocampal cholinergic activity and the duration of the effect after intraseptal phenylephrine injection are two possible factors. It is also possible that phenylephrine effects sleeping time via non-cholinergic mechanisms in the septum.

Previous reports suggested that intraseptal microinjection of drugs (substance P, β-endorphin) that depress the septal-hippocampal cholinergic pathway prolonged the duration of pentobarbital narcosis in rats [3,13] whereas intraseptal microinjection of drugs (TRH, bicuculline) that reversed pentobarbital-induced depression in this pathway, shortened the duration of pentobarbital narcosis [1]. The data presented in this report support the hypothesis that there is an inverse relationship between the duration of pentobarbital narcosis and septal-hippocampal cholinergic activity in rats. Drugs that activate the septal-hippocampal cholinergic pathway (TRH, arecoline, bicuculline) shorten the duration of pentobarbital narcosis, whereas drugs that depress this pathway (β-endorphin, substance P and serotonin) prolong the duration of pentobarbital narcosis. In all cases, increased septal-hippocampal cholinergic activity decreased pentobarbital-induced sleeping time and decreased septal-hippocampal cholinergic activity increased pentobarbital-induced sleeping time.

The most likely mechanism of action of pentobarbital that underlies its ability to produce anesthesia is its potentiation of the GABA receptor/chloride ion channel mechanism [7]. It is tempting to speculate that the intraseptal drugs act in the septum via GABA-ergic mechanisms to counteract or potentiate the effect of pentobarbital in the septum. This could be true for bicuculline and for β-endorphin [12], and it might be likely for serotonin, arecoline, and TRH; but substance P does not act via GABA-ergic mechanisms in the septum to produce its effect on hippocampal cholinergic activity [12]. Whether the septal-hippocampal cholinergic pathway activity is responsible for modulating the recovery from pentobarbital narcosis, or merely intrinsic hippocampal cholinergic mechanisms are responsible for this phenomenon is unclear. However, since greater than 80% of hippocampal cholinergic innervation is from the septum [6], this might be a moot point.

ACKNOWLEDGEMENTS

We are grateful to the Alcoholism and Drug Abuse Institute of the University of Washington for support of the work performed and to Gwen Bell for expert typing assistance.

REFERENCES

1. Brunello, N. and D. L. Cheney. The septal-hippocampal cholinergic pathway: role in antagonism of pentobarbital anesthesia and regulation by various afferents. *J Pharmacol Exp Ther* **219**: 489–495, 1981.
2. Costa, E., P. Panula, A. K. Thompson and D. L. Cheney. The trans-synaptic regulation of the septal-hippocampal cholinergic neurons. *Life Sci* **32**: 165–179, 1983.
3. Fatherazi, S., H. Lai, S. Kazi and A. Horita. Intraseptal morphine potentiates pentobarbital narcosis and hypothermia in the rat. *Pharmacol Biochem Behav* **23**: 505–507, 1985.
4. Kalivas, P. W. and A. Horita. Involvement of the septo-hippocampal system in TRH antagonism of pentobarbital narcosis. In: *Thyrotropin-Releasing Hormone*, edited by E. C. Griffiths and G. W. Bennett. New York: Raven Press, 1983, pp. 283–290.

5. Lowry, O. H., N. J. Roseborough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
6. Moroni, F., D. Malthe-Sorenssen, D. L. Cheney and E. Costa. Modulation of ACh turnover of the septal-hippocampal pathway by electrical stimulation and lesioning. *Brain Res* **150:** 333–341, 1978.
7. Olsen, R. W. GABA-benzodiazepine-barbiturate receptor interactions. *J Neurochem* **37:** 1–13, 1981.
8. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
9. Robinson, S. E. Effect of specific serotonergic lesions on cholinergic neurons in the hippocampus, cortex and striatum. *Life Sci* **32:** 345–353, 1983.
10. Simon, J. R., S. Atweh and M. J. Kuhar. Sodium-dependent high affinity choline uptake: A regulatory step in the synthesis of acetylcholine. *J Neurochem* **26:** 909–922, 1976.
11. Trabucchi, M., D. L. Cheney, G. Racagni and E. Costa. Pentobarbital and *in vivo* turnover rate of acetylcholine in mouse brain and in regions of rat brain. *Pharmacol Res Commun* **7:** 81–94, 1975.
12. Wood, P. L., D. L. Cheney and E. Costa. An investigation of whether septal γ-aminobutyrate containing interneurons are involved in the reduction in the turnover rate of acetylcholine elicited by substance P and β-endorphin in the hippocampus. *Neuroscience* **41:** 1479–1484, 1979.
13. Zucker, J. R., H. Lai and A. Horita. Intraseptal microinjections of substance P and analogs potentiate pentobarbital-induced narcosis and depression of hippocampal cholinergic activity. *J Pharmacol Exp Ther* **235:** 398–401, 1985.