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Lack of Influence of Brain Catecholamines on Acute Effects of Barbiturates

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BOURN, W. M. AND C. E. REIGEL. *Lack of influence of brain catecholamines on acute effects of barbiturates.* PHARMAC. BIOCHEM. BEHAV. 13(5) 733–735, 1980.—Rats with cerebral electrode implants were tested for sensitivity to EEG burst suppression by intravenously-infused sodium methohexital following manipulation of brain catecholamine function. Although depletion of both norepinephrine (NE) and dopamine (DA) with 6-hydroxydopamine resulted in a slight increase in methohexital sensitivity (MHS), similar depletion with α-methyltyrosine did not alter MHS. In addition, desipramine, an agent which selectively blocks uptake of NE did not affect MHS. The results indicate that brain NE and DA exert little, if any, effect on brain responsiveness to the acute effect of barbiturates.

Barbiturate sensitivity Catecholamines Norepinephrine Dopamine

THE possibility that the CNS depressant effect of barbiturates is somehow related to endogenous catecholamine function is suggested by a number of findings. Tabakoff, *et al.* [13,14] demonstrated that noradrenergic function is a requirement for the development of tolerance to phenobarbital in addicted mice. This was accomplished by showing that brain barbiturate concentration was significantly lower upon recovery of righting reflex in addicted animals that had also received 6-hydroxydopamine intracerebroventricularly than in addicted animals that had not received 6-hydroxydopamine. Morgan *et al.* [9] related catecholamines to the barbiturate withdrawal syndrome in rats. In this work it was shown that in rats withdrawn from sodium barbital, norepinephrine (NE) concentration decreased in the cerebral cortex, thalamus, and hypothalamus, and dopamine (DA) concentration increased in the thalamus.

Although these studies tend to relate brain catecholamines to tolerance and dependence phenomena, but not necessarily to the acute effects of barbiturates, it is reasonable to speculate that an association exists between the brain mechanisms responsible for acute effects and the mechanisms involved with tolerance and dependence. Some experiments dealing with this have been performed, but the results appear to be somewhat in conflict. Among the results reported by Tabakoff *et al.* [13] was the finding that non-addicted mice which had received intracerebral injections of 6-hydroxydopamine recovered the righting reflex at the same brain concentration of phenobarbital as mice which had not been treated with 6-hydroxydopamine. This implies that catecholaminergic function is not directly related to the acute effects of a single dose of barbiturate. On the other hand, Wood and Laverty [18] found that catecholamine depletion with 6-hydroxydopamine produced a decrease in the pentobarbital sleeping time of rats. Although a change in sleeping time may be a result of a change in barbiturate sensitivity, it may also represent a change in the rate at which the drug is removed from the body. Thus, it could be interpreted to mean that the 6-hydroxydopamine treatment decreased sensitivity to pentobarbital provided that other factors such as altered drug metabolism or distribution were not responsible for the effect. The authors' interpretation of the latter results, however, is that the impairment of catecholaminergic systems produced a non-specific increase in excitability which reduced the sleeping time. They concluded that a direct relationship between catecholamine function and acute barbiturate effects does not exist.

The present experiments were performed to examine the possibility that manipulation of brain catecholamine function would alter responsiveness to acute high doses of the rapid-onset barbiturate methohexital.

METHOD

Male rats of Sprague-Dawley descent weighing 260 to 340 g received permanent bilateral stainless steel screw electrode implants to allow recording of an epidural EEG. At the time of surgery, one group of rats received an intracerebroventricular dose of 250 μg of 6-hydroxydopamine hydrobromide, (an agent which produces destruction of catecholaminergic nerve terminals) in 20 μl of normal saline containing 0.01% ascorbic acid. Another group received only the normal saline-ascorbic acid vehicle. One week later, the above two groups were challenged with methohexital according to the method described below. All other rats received no drugs until one week after electrode implantation surgery. At that time, rats in a third treatment group were injected intraperitoneally (IP) with a 3-dose regimen of α-methyltyrosine, 100 mg/kg, 4, 8, and 12 hours prior to challenge with methohexi-

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tal (this catecholamine synthesis inhibitor produces a temporary depletion of catecholamines); a fourth group was administered desipramine 10 mg/kg IP, 30 min prior to challenge with methohexital, a fifth group was administered normal saline IP at 4, 8 and 12 hours prior to methohexital challenge; and a sixth group was administered normal saline, IP 30 min prior to methohexital challenge.

Rats were tested for methohexital sensitivity by an adaptation of the "silent second" method of Wahlstrom [16,17], a highly reproducible method for determining sensitivity of animals to depressant drugs. In this method, methohexital was infused into the tail vain of the restrained rats using a Harvard infusion pump while the ECoG was being monitored and recorded. The first one-second period of ECoG burst suppression is the end point, and is an indicator of the dose of methohexital required to produce near-death CNS depression. This end point is referred to herein as "methohexital sensitivity" or "MHS." Methohexital solution was infused at a rate of 7.88 mg/kg/min. The volume rate was held constant at 0.197 ml/min., and the concentration of methohexital adjusted according to the body weight of each animal. This was accomplished by diluting one ml of a solution of 20 mg/ml of methohexital (the concentration which would be required for an animal weighing 500 g) to a volume appropriate for the weight of each animal. For example, if an animal weighed 250 g, the final volume would be 2.00 ml; for an animal weighing 300 g the final volume would be 1.67 ml, etc. ECoG was monitored with a Grass Model 7d Polygraph using a 7 p5B EEG Preamplifier with low and high half-amplitude filters at 1 and 75 Hz, respectively. Additional rats received similar doses of 6-hydroxydopamine or α-methyltyrosine, and were killed for brain catecholamine assay in order to verify the depleting effect of the two drugs.

Catecholamine assays were performed using the high pressure liquid chromatographic method of Felice *et al.* [4] using a Bioanalytical Systems Model LC-54 high pressure liquid chromatograph with a reverse-phase C_{18} column and Model LC-12 electrochemical (amperometric) detector.

RESULTS

Brain NE and DA were severely depleted by 6-hydroxydopamine or α-methyltyrosine (Table 1). Following 6-hydroxydopamine administration, there was a slight increase in MHS such that the dose required to produce burst suppression was reduced by 18%. On the other hand, neither desipramine nor α-methyltyrosine produced any effect on MHS (Table 2).

TABLE 1

INFLUENCE OF 6-HYDROXYDOPAMINE OR α-METHYLTYROSINE ON BRAIN DOPAMINE AND NOREPINEPHRINE*

	Norepinephrine	Dopamine
6-Hydroxydopamine	39.1±4.3†	33.4±5.8†
α-Methyltyrosine	30.7±1.4†	27.8±1.2†

*Reported as percent of control ± SEM. Control concentrations were 424 ± 131 (DA) and 263 ± 52 (NE) ng/g ± SEM of wet tissue. See text for doses and treatment regimens.

†Significantly different from control at $p<0.0005$. For all groups, n=4.

TABLE 2

INFLUENCE OF 6-HYDROXYDOPAMINE, α-METHYLTYROSINE OR DESIPRAMINE ON METHOHEXITAL SENSITIVITY

	6-Hydroxydopamine	α-Methyltyrosine	Desipramine
Control†	14.3±0.5 (8)	17.4±0.6 (9)	15.1±0.6 (8)
Treated	11.3±0.3‡ (9)	17.7±1.4 (6)	17.6±1.4 (8)

*Reported as mg/kg to produce at least one second of EEG burst suppression ± SEM. The number of animals for each group is in parenthesis.

†Separate normal saline controls were run for each treatment since routes of administration and dose regimens were different for each treatment. See text for dosages and treatment regimens.

‡Significantly different from control at $p<0.001$, according to Student's t for unpaired comparisons.

DISCUSSION

Evidence linking barbiturate tolerance and dependence to brain catecholamine function, particularly norepinephrine, could be explained on the basis of an indirect relationship. Norepinephrine in the central nervous system serves as an inhibitory modulator of experimental convulsive seizures induced in the rat by several methods, including maximal electroshock, intense sound, and chemoshock [2, 3, 5, 7]. Generally, drugs which enhance noradrenergic function tend to suppress seizures, and drugs or lesions which interfere with noradrenergic function tend to increase severity of-and/or susceptibility to convulsive seizures. (For an extensive review of the topic, see Jobe, P. C., [6]).

It would be expected that the noradrenergic inhibitory system would be recruited during the hyperexcitable state of barbiturate withdrawal in an attempt to suppress seizure activity. This would be in keeping with the increased catecholamine turnover rate during withdrawal suggested by the work of Morgan *et al.* [10]. Conversely, the chronic depressed state of continued administration of barbiturates might decrease the need for activity of this endogenous inhibitory system. Indeed, Waddingham *et al.* [15] demonstrated an increase in β-adrenergic receptors in the brain of mice following chronic exposure to phenobarbital, which would be expected if transmitter release was decreased. Although the latter change would be expected to make noradrenergic inhibition more effective, the reports that animals become more susceptible to sound induced convulsive seizures and in some cases experience spontaneous convulsions during withdrawal indicate that this system is not adequate to entirely suppress withdrawal seizures. Furthermore, it is noteworthy that interference with noradrenergic function during withdrawal results in exacerbation of sound-induced seizures; likewise, enhancement of noradrenergic function with the uptake blocker desipramine provides protection [1].

The present experiment in which 6-hydroxydopamine pretreatment lowered the silent second threshold would support the hypothesis that there is a relationship between catecholamines and acute effects of barbiturates. However, the slight decrease in methohexital threshold could easily be explained by a change in the relative volume of distribution for the barbiturate. All rats treated with 6-hydroxydopamine consumed very little food or water during the week prior to methohexital challenge, and had an average body weight 18% below control sham-operated animals. Since this weight difference would be mainly fat and body water, the change in

volume of distribution could easily account for the difference in methohexital sensitivity. This suggestion is supported by the fact that neither desipramine nor α-methyltyrosine changed the methohexital threshold.

Although norepinephrine and/or dopamine systems appear to be affected by some of the changes occurring in the brain during chronic treatment with the barbiturates and during subsequent withdrawal, it is our conclusion from the present results that neither norepinephrine nor dopamine has any direct role in the acute effects of barbiturates.

REFERENCES

1. Bourn, W. M. Influence of brain catecholamines on barbiturate withdrawal convulsions. *Soc. Neurosci. Abstr.* **4:** 133, 1978.
2. Bourn, W. M., L. Chin and A. L. Picchioni. The role of dopamine in sound-induced convulsions. *J. Pharm. Pharmac.* **30:** 800–801, 1978.
3. DeSchaepdryver, A. F., Y. Piette, A. L. Delaunois. Brain amines and electroshock threshold. *Archs int. Pharmacodyn. Thér.* **140:** 358–367, 1962.
4. Felice, L. J., J. D. Felice and P. T. Kissinger. Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. *J. Neurochem.* **31:** 1461–1465, 1978.
5. Jobe, P. C., A. L. Picchioni and L. Chin. Role of brain norepinephrine in audiogenic seizure in the rat. *J. Pharmac. exp. Ther.* **184:** 1–10, 1973.
6. Jobe, P. C. Pharmacology of audiogenic seizures. In: *The Pharmacology of Hearing: Experimental and Clinical Bases*, edited by R. W. Brown and E. A. Daigneault. New York: Wiley Interscience, 1980, in press.
7. Maynert, E. W., T. J. Marczynski and R. A. Browning. The role of neurotransmitters in the epilepsies. *Adv. Neurol.* **13:** 79–147, 1975.
8. Morgan, W. W. Effect of 6-hydroxydopamine pretreatment on spontaneous convulsions induced by barbital withdrawal. *Experientia* **32:** 489–491, 1976.
9. Morgan, W. W., K. A. Pfeil and E. G. Gonzales. Catecholamine concentration in discrete brain areas following the withdrawal of barbital dependent rats. *Life Sci.* **20:** 493–500, 1977.
10. Morgan, W. W., R. D. Haffman, K. A. Pfeil and E. G. Gonzales. Effects of synthesis inhibition on the levels of brain catecholamines in barbital-dependent rats. *Psychopharmacology* **46:** 41–44, 1978.
11. Morgan, W. W., K. A. Pfeil and E. G. Gonzales. Effect of α-methyl-p-tyrosine on the incidence of spontaneous convulsions observed following barbital withdrawal. *Neuropharmacology* **17:** 115–119, 1978.
12. Norton, P. R. E. The effect of drugs on barbiturate withdrawal convulsions in the rat. *J. Pharm. Pharmac.* **22:** 763–766, 1970.
13. Tabakoff, B., J. Yanai and R. F. Ritzmann. Brain noradrenergic systems as a prerequisite for developing tolerance to barbiturates. *Science* **200:** 449–451, 1978.
14. Tabakoff, B., R. F. Ritzmann, G. Altmans and P. L. Hoffman. Brain noradrenergic systems as a prerequisite for development of barbiturate tolerance. *Soc. Neurosci. Abstr.* **4:** 435, 1978.
15. Waddingham, S. W., R. Riffee, J. K. Belknap and J. R. Sheppard. Barbiturate dependence in mice: evidence for β-adrenergic receptor proliferation in brain. *Res. Commun. chem. pathol. Pharmac.* **20:** 207–220, 1978.
16. Wahlstrom, G. The interaction between spontaneous convulsions and tolerance to hexobarbital in the abstinence after chronic barbital treatments in the rat. *Life Sci.* **19:** 1817–1826, 1976.
17. Wahlstrom, G. The interaction between electrically-induced convulsions and tolerance in the abstinence period after chronic barbital treatments in the rat. *Psychopharmacology* **48:** 239–245, 1976.
18. Wood, J. M. and R. Laverty. Effect of depletion of brain catecholamines on ethanol tolerance and dependence. *Eur. J. Pharmac.* **58:** 285–293, 1979.