PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 30–32 (1975)

Increased Hypothalamic Norepinephrine in Genetically Hypertensive Rats following Administration of Diphenylhydantoin (38470)

G. M. LEW
(Introduced by W. B. Quay)

Department of Anatomy, Michigan State University, East Lansing, Michigan 48823

The mechanisms and loci of action of diphenylhydantoin (DPH) remain obscure although the drug has been widely used in the treatment of epilepsy and has assumed prominence in the treatment of rhythm disorders of the heart. DPH has also been used in the treatment of essential hypertension to lower blood pressure (1). Recent evidence (2) indicates that DPH can alter the uptake and binding of catecholamines in rat brain. Since the effect of this drug on neurotransmitters warrants additional investigation, this experiment was carried out to determine any changes in norepinephrine (NE) concentration in different regions of the brain and in sympathetically innervated organs of genetically hypertensive (GH) rats following administration of DPH.

Materials and Methods. GH rats (New Zealand strain) were bred in one of two windowless air-conditioned rooms at a temperature of approximately 23°. These rooms were maintained under artificial illumination (one from 6 AM to 8 PM EST, and the other from 2 PM to 4 AM EST, each day). The rats were fed on Wayne Blox (Allied Mills) and drinking water *ad libitum*. Male GH rats, 6 mo old, with a mean body weight of 315 ± 3 g and a mean blood pressure of 174 ± 4 mm Hg, were used in the experiment. The systolic blood pressure (BP) of each rat was measured by a tail-cuff method with the aid of a Narcobiosystems pneumatic pulse transducer and temperature control unit, without the use of anesthesia. Blood pressure recordings were made on at least three separate occasions and at the same time each day at 2- to 3-day intervals, during a 10-day period preceding the experiment. Body weights were recorded at the time of blood pressure measment. The animals were sacrificed by rapid decapitation in the late portion of the light and dark phases (at 9 hr of light and 9 hr of darkness), 4 hr after ip injection of DPH (sodium salt) (1 mg/10 g body wt in 1 ml distilled water to experimental GH rats) and after ip injection of 1 ml 0.85 % saline to control GH rats. In order to eliminate possible influences of circadian changes in catecholamine content no more than one drug-treated and one control GH rat was sacrificed at any one time. The arbitrary dissection boundaries of the brain regions were as described previously (3). The hearts and adrenal glands were quickly removed, freed of fat, blotted and frozen in vials on dry ice. The organs and brain regions were weighed while frozen prior to analysis of catecholamine contents. Separation and fluorometry of catecholamines followed the method of Anton and Sayre (4) with the modifications of Laverty and Taylor (5). The significance of the difference between mean values was determined by the Student–Fisher t test.

Results. These are summarized in Table I. The hypothalamic NE concentration in DPH-treated GH rats was found to be significantly higher ($P < .01$) than that in control GH rats, 4 hr following administration of the drug and only in the late portion of the light phase. The concentration of NE in the cerebellum was also found to be increased in the experimental GH rats, in the late portion of the light phase. This increase, although not statistically significant, resulted in a significant circadian difference ($P < .01$) between NE levels at the late light and late dark phases, which was not seen in the controls. Changes in NE concentration in the medial lower brainstem resulted in a circadian difference in NE levels which was found to be of marginal statistical significance ($P < .02$).

Discussion. The present data definitely show that DPH is capable of increasing NE concentration in the hypothalamus. They

TABLE I. EFFECTS OF DPH ON NE CONTENTS OF BRAIN REGIONS AND ORGANS OF GENETICALLY HYPERTENSIVE RATS ACCORDING TO TIME OF DAY.[a]

Brain regions and organs Groups	Time of day		Comparisons *P
	Late light phase	Late dark phase	
Medial lower brainstem control	0.37 ± 0.01 (8)[b] A	0.39 ± 0.04 (7) B	A-B n.s.[c]
			A-C n.s.
Experimental	0.31 ± 0.04 (7) C	0.42 ± 0.02 (7) D	C-D $P < .02$
			B-D n.s.
Hypothalamus control	1.15 ± 0.22 (7) E	1.50 ± 0.06 (8) F	E-F n.s.
			E-G $P < .01$
Experimental	1.62 ± 0.13 (7) G	1.37 ± 0.10 (8) H	F-H n.s.
			G-H n.s.
Cerebellum control	0.27 ± 0.02 (8) I	0.23 ± 0.01 (8) J	I-J n.s.
			I-K n.s.
Experimental	0.31 ± 0.02 (6) K	0.23 ± 0.01 (6) L	K-L $P < .01$
Heart control	0.32 ± 0.04 (8) M	0.29 ± 0.01 (8) N	M-N n.s.
			M-O n.s.
Experimental	0.29 ± 0.04 (6) O	0.32 ± 0.03 (6) P	O-P n.s.
			N-P n.s.
Adrenal gland control	165 ± 11 (7) Q	147 ± 17 (8) R	Q-R n.s.
			Q-S n.s.
Experimental	203 ± 30 (6) S	150 ± 11 (7) T	S-T n.s.

[a] Values are Mean ± S.E.M. μg NE/g tissue.
* P = probability based on Student-Fisher t test.
[b] Nos. in parentheses = nos. of animals.
[c] n.s. = difference not statistically significant

also indicate circadian differences in the effect of DPH on the neurotransmitter in the medial lower brainstem and cerebellum. Such data have not been reported previously. There are reports of an *in vivo* effect of DPH on the levels of 5-hydroxytryptamine in whole brain (6), although no definite correlation has yet been made between absolute levels of whole brain amines and degree of anticonvulsant effect. The results reported here are consistent with the findings of Hadfield (2) who reported that in slices of intact brain, DPH stimulated the uptake of dopamine in the hypothalamus. It was suggested that the binding of DPH to microsomes and synaptosomes may alter the synthesis or activity of ATPase, an enzyme controlling neurotransmitter uptake and binding. According to Azzaro (7) DPH possibly alters NE metabolism in cerebral cortex slices by inhibitory action on monoamine oxidase activity and the neuronal uptake system.

The present study opens up other possible explanations for the clinical effects of DPH. It is interesting to note that DPH administration resulted in circadian differences in the cerebellum in NE levels at the late light and late dark phases. A deficiency in hypothalamic and brainstem NE has been suggested as a possible factor in the production of hypertension and only treatments that increased brainstem NE were effective in reducing blood pressure (8). It is therefore possible that the action of this drug in increasing hypothalamic NE is involved in its effect on blood pressure. Additional investigation of the effects of DPH on neurotransmitters as a possible mechanism for the action of this drug is therefore fully warranted.

Summary. DPH administration to GH rats caused changes in NE concentration in the hypothalamus, cerebellum and medial lower brainstem which resulted in circadian differences in the effect of this drug on the catecholamine. These effects have not been reported previously. Further investigations on the effect of DPH on neurotransmitters are fully warranted.

1. Editor, J. Amer. Med. Ass. **143,** 939 (1950).
2. Hadfield, M. G., Archs. Neurol. **26,** 78 (1972).
3. Quay, W. B., Amer. J. Physiol. **215,** 1448 (1968).
4. Anton, A. H., and Sayre, D. F., J. Pharmacol. Exp. Therap. **138,** 360 (1962).
5. Laverty, R., and Taylor, K. M., Anal. Biochem. **22,** 269 (1968).
6. Anderson, E. G., Markowitz, S. D., and Bonnycastle, D. D., J. Pharmacol. Exp. Therap. **136,** 179 (1962).
7. Azzaro, A. J., Gutrecht, J. A., and Smith, D. J., Biochem. Pharmacol. **22,** 2719 (1973).
8. Yamori, Y., DeJong, W., Yamabe, H., Lovenberg, W., and Sjoerdsma, A., J. Pharm. Pharmacol. **24,** 690 (1972).

Received June 10, 1974. P.S.E.B.M. 1975, Vol. 148.