NOREPINEPHRINE DETECTION IN THE ANTERIOR PITUITARY OF MICROWAVE IRRADIATED RATS

Kaoru IRIE and Teruko NOMOTO

Department of Pharmacology, Tokyo Women's Medical College,
Kawada-cho, Shinjuku-ku, Tokyo 162, Japan

Accepted March 1, 1981

Microwave irradiation has become an acceptable technique for assessing small quantities of neurochemical substances in the brain. These substances are acetylcholine, cyclic nucleotides, biogenic amines, enkephaline and γ -aminobutylic acid (1-4). Rapid inactivation of brain enzymes by microwave irradiation prevents postmortem changes of tissue substances. Although it is known that brain biogenic amines play an important role in neuroendocrine regulation (5, 6), the nature of amines in pituitary secretion of hormones is unknown. Other workers have found that catecholamine levels were either low or undetectable in the anterior pituitary (7, 8). We now report a useful microwave irradiation technique which enables determination of catecholamines in

the anterior pituitary as well as the hypothalamus of the rat.

Adult male rats of Wistar-Imamichi strain, weighing 360-430 g (experiment in Table 1) or 280-340 g (experiment in Fig. 1), were housed in an air-conditioned room with controlled light-dark cycle (light on 06:00-20:00). Food and water were available *ad libitum*. Rats were killed either by decapitation or by microwave irradiation (2450 MHz, 4kw, 1.2sec, by model TMW-6402(2), Tokyo Shibaura Electric Co., Ltd, Tokyo, Japan) during 10:00-12:00 a.m. The power of microwave used was one that would not destroy the median eminence but would produce heat-induced changes in the ventral part of the brain. The hypothalamus was dissected out in such a manner that the optic

Table 1. NE and DA in the anterior pituitary and the hypothalamus of rats killed by decapitation and by microwave irradiation

	Body weight (g)	Tissue weight (mg) %	Amine concentration (μ g/g tissue)			
			NE	%	DA	%
Anterior pituitary						
Decapitation	393.6 $\pm$ 4.2(22)	9.06 $\pm$ 0.19(22) 100	not detected		0.037 $\pm$ 0.000(6) 100	
Microwave	394.1 $\pm$ 5.1(16)	8.62 $\pm$ 0.27(16) 95	0.048 $\pm$ 0.009(4)		0.112 $\pm$ 0.009(4) 303	
p	N.S.	N.S.	0.05		0.001	
Hypothalamus						
Decapitation		32.1 $\pm$ 1.6(6) 100	1.250 $\pm$ 0.099(5) 100		0.351 $\pm$ 0.026(6) 100	
Microwave		27.2 $\pm$ 1.0(6) 85	1.666 $\pm$ 0.057(6) 133		0.554 $\pm$ 0.036(6) 158	
p		0.05	0.01		0.005	

Values given are the means $\pm$ SEM of determinations. The number of determinations is shown in parentheses. The values are expressed in percentage of the decapitation controls. Student's *t*-test was used for statistical analysis.

Fig. 1. NE and DA in the anterior pituitary and the hypothalamus of the rats with pretreated reserpine (3 mg/kg s.c., 20 hr) or pargyline (100 mg/kg s.c., 2.5 hr). Rats were killed by microwave irradiation. NE and DA were not detected in the anterior pituitary of the reserpinized rats. Results are shown as the mean $\pm$ SEM. *, $p < 0.05$, ***, $p < 0.005$ compared to saline group (Student's *t*-test).

chiasma would be the anterior limit, the mammillary bodies as the caudal limit, and 2 mm laterally from the midline and extended about 1.5 mm dorsally. Weights were 25–35 mg. Three to 4 (experiment in Table 1) or 2 (experiment in Fig. 1) anterior pituitaries were pooled and used for one determination of catecholamine contents. All tissues were kept at -20°C until used for measurement in the afternoon of the same day. Catecholamines were extracted by the method of Keller et al. (9), with slight modification. Catecholamines were adsorbed to alumina and eluted into 50 μl of 0.1 N HCl and 10 μl of the eluate was injected onto a high performance liquid chromatography with an electrochemical detector system (YANACO L-2000 and VM-101, Yanagimoto Mfg. Co. Ltd., Kyoto, Japan). The column was packed with Yanapack-ODS-N (Yanagimoto Mfg. Co. Ltd.), the applied voltage was +0.6v, and the mobile phase was 0.1 M phosphate buffer pH 3.1.

Table 1 shows norepinephrine (NE) and dopamine (DA) content in the hypothalamus

and anterior pituitary. NE in the anterior pituitary was not detectable in tissues from decapitated rats (less than 0.015 $\mu\text{g/g}$ tissue), though it was detected in microwave-irradiated rats (0.048 ± 0.009 $\mu\text{g/g}$). DA concentrations in the anterior pituitary were detected to be significantly higher in microwave-irradiated rats than that in decapitated ones (0.112 ± 0.009 $\mu\text{g/g}$ and 0.037 ± 0.000 $\mu\text{g/g}$ respectively). Both NE and DA levels in the hypothalamus were also significantly higher after microwave irradiation (Table 1). The high content of these amines in microwave-irradiated brains was much more than expected as assessed by the loss of wet weight of the microwave-irradiated tissues. The anterior pituitary, which is separated from the brain, lost only 5% of tissue weight after microwave irradiation (Table 1). The difference between microwave-irradiated and decapitated rats in the wet weight of the hypothalamus was 15%. The excess NE concentration in the hypothalamus of the microwave-irradiated rats was 33% and that of DA was 58%.

The following experiment was performed to confirm that NE detected in the anterior pituitary of the microwave-irradiated rats was not artifactual. Rats were pretreated by reserpine or a monoamine oxidase inhibitor, pargyline. Reserpine (3 mg/kg, s.c., Apoplone® injection, Daiichi Seiyaku Co. Ltd, Tokyo, Japan) was administered 20 hr prior to experiment. Another group of rats were given pargyline (100 mg/kg, s.c., Pargyline hydrochloride, Nakarai Chemicals Ltd, Kyoto, Japan) 2.5 hr prior to experiment. Control rats were given saline (s.c., 2.5 hr). All rats were killed by microwave irradiation and levels of catecholamines in the anterior pituitary or the hypothalamus were determined. Levels of NE and DA in the reserpinized rats were markedly decreased in the hypothalamus and were not detected in the anterior pituitary (Fig. 1). In the

pargyline-pretreated rats, the catecholamine levels were increased in both tissues (Fig. 1). These results indicate that the catecholamines detected in the microwave-irradiated tissues were of a physiological source.

According to the reports of Saavedra et al. (8) and Ben-Jonathan et al. (7), NE was undetectable and DA was detected in the anterior pituitary of the rat. Our study is consistent with these works in the case of decapitation. In microwave-irradiated rats, however, both DA and NE were detected consistently in the anterior pituitary. The content of NE was about one half of DA.

Both NE and DA levels in the hypothalamus were higher in the microwave-irradiated rats than those in the decapitated animals. The high-content of these amines in microwave-irradiated tissues is likely the result of a heat-induced inactivation of monoamine oxidase activity (2).

Meyerhoff et al. (10) stated that there was a structural disruption in the microwave-irradiated tissue and suggested that DA diffused from regions high in DA to adjacent regions low in DA. Sharpless and Brown (11) observed a diffuse catecholamine fluorescence in the microwave-irradiated tissue and they considered that DA had spread from one region to the adjacent area. However, this result was not confirmed by our study (12) as catecholamine fluorescence of the microwave-irradiated tissue was not demonstrated. Thus, any histochemical observation of catecholamine fluorescence in the microwave-irradiated tissue seems to be doubtful at present. Other workers (13) showed that there was no significant tissue structure breakdown after microwave irradiation. In our histochemical study (12) using the Falck-Hillarp method, catecholamine fluorescence was not observed in the microwave-irradiated tissue.

Brain biogenic amines regulate the secretion of pituitary hormone by liberating

hypothalamic hormone or by acting directly on the pituitary (6). Recent findings (7, 14, 15) showed that DA concentration in the hypophysial portal blood was high and changed according to the endocrine profile. It was suggested that DA directly regulates the secretion of pituitary hormones. Determination of catecholamines in the anterior pituitary may provide further evidence to indicate the role of these amines in regulating pituitary hormone secretion. We are now assessing catecholamine levels in the pituitary as well as the hypothalamus, under various pharmacological and endocrinological conditions.

Acknowledgements: We thank Dr. T. Moroji of the Psychiatric Research Institute of Tokyo and Dr. Y. Maruyama of Japan Upjohn Research Laboratories for pertinent discussions, and Dr. T. Tonoue of Tokyo Women's Medical College for comments on the manuscript.

REFERENCES

- 1) Balcom, G.J., Lenox, R.H. and Meyerhoff, J.L.: Regional γ -aminobutylic acid levels in rat brain determined after microwave fixation. *J. Neurochem.* **24**, 609–613 (1975)
- 2) Moroji, T., Takahashi, K., Ogura, K., Toishi, T. and Arai, S.: Rapid microwave fixation of rat brain. *J. Microwave Power* **12**, 273–286 (1977)
- 3) Stavinoha, W.B., Weinstraub, S.T. and Modak, A.T.: The use of microwave heating to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. *J. Neurochem.* **20**, 361–371 (1973)
- 4) Weinstraub, S.T., Stavinoha, W.B., Pike, R.L., Morgan, W.W., Modak, A.T., Koslow, S.H. and Blank, L.: Evaluation of the necessity for rapid inactivation of brain enzymes prior to analysis of norepinephrine, dopamine and serotonin in the mouse. *Life Sci.* **17**, 1423–1428 (1975)
- 5) Nomoto, T., Irie, K. and Suzuki, M.: Roles of biogenic amines in the hypothalamo-pituitary-thyroid system in the rats. *Gunma Symp. on Endocrinology* **15**, 107–118 (1978)
- 6) Weiner, R.I. and Ganong, W.F.: Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.* **58**, 905–976 (1978)

- 7) Ben-Jonathan, N., Neil, M.A., Arogast, L.A., Peters, L.L. and Hoefer, M.T.: Dopamine in hypophysial portal blood: relationship to circulating prolactin in pregnant and lactating rats. *Endocrinology* **106**, 690–695 (1978)
- 8) Saavedra, J.M., Palkovits, M., Kizer, J.S., Brownstein, M.J. and Zivin, J.A.: Distribution of biogenic amines and related enzymes in the rat pituitary gland. *J. Neurochem.* **25**, 257–260 (1975)
- 9) Keller, R., Oke, A., Mefford, I. and Adams, R.N.: Liquid chromatographic analysis of catecholamines routine assay for regional brain mapping. *Life Sci.* **19**, 995–1004 (1976)
- 10) Meyerhoff, J.L., Kant, G.J. and Lenox, R.H.: Increase in dopamine in cerebral cortex and other regions of rat brain after microwave fixation: possible diffusion artifact. *Brain Research* **152**, 161–169 (1978)
- 11) Sharpless, N.S. and Brown, L.L.: Use of microwave irradiation to prevent postmortem catecholamine metabolism: evidence for tissue disruption artifact in a discrete region of rat brain. *Brain Research* **140**, 171–176 (1978)
- 12) Irie, K. and Nomoto, T.: Effects of microwave irradiation on catecholamine levels in hypothalamus or anterior pituitary: liquid chromatographic assay and histochemical study. *Japan. J. Pharmacol.* **29**, Supp. 194P (1979)
- 13) Maruyama, Y., Nakamura, R. and Kobayashi, K.: Effect of microwave irradiation on brain tissue structure and catecholamine distribution. *Psychopharmacology* **67**, 119–123 (1980)
- 14) Gudelsky, G.A. and Porter, J.C.: Release of newly synthesized dopamine into the hypophysial portal vasculature of the rat. *Endocrinology* **104**, 583–587 (1979)
- 15) Plotsky, P.M., Gibbs, D.M. and Neil, J.D.: Liquid chromatographic-electrochemical measurement of dopamine in hypophysial stalk blood of rats. *Endocrinology* **102**, 1887–1894 (1978)