RADIATION RESEARCH 55, 153–159 (1973)

Effects of Microwave Irradiation on Enzymes and Metabolites in Mouse Brain*

STANLEY R. NELSON

Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103

NELSON, S. R. Effects of Microwave Irradiation on Enzymes and Metabolites in Mouse Brain. *Radiat. Res.* 55, 153–159 (1973).

After decapitation and exposure of the mouse head to microwave radiation, seven of the eight brain enzymes studied were inactivated. The remaining enzyme, myokinase, retained about 10% of control activity. Brain metabolite levels were also altered by microwave radiation and these changes were used indirectly to obtain information about sensitivity of enzymes to heat inactivation. ATP hydrolyzing enzymes were relatively resistant to heat since inactivation was delayed long enough for half the available ATP to be used. P-creatine kinase was inactivated early during microwave treatment since P-creatine, usually depleted in seconds, was only reduced 30% in the anoxic brain. Hexokinase appeared most sensitive to heat since glucose, rapidly used in the anoxic brain, remained at control levels after microwave irradiation. Glycogen decrease and G-6-P elevation show brain phosphorylase was not immediately inactivated in experimental mice. Low lactate levels in the anoxic, treated brains indicate the flow of glycolytic intermediates was quickly blocked at one or more steps between G-6-P and lactate. Since fructose diphosphate was depleted in brain from the irradiated, anoxic head, aldolase was resistant to early inactivation. The metabolite changes observed after decapitated heads were irradiated were also observed when the heads of intact mice were treated and the changes occurred in both cortex and subcortex of these mice.

INTRODUCTION

Microwave irradiation at high energy levels is lethal to small animals. Deichmann (*1*) reported that CNS stimulation is characteristic of microwave toxicity with clonic convulsions and respiratory failure being the terminal events. These toxic changes may be related to enzyme changes since enzyme inactivation is known to occur seconds after exposure to high levels of microwave energy. Schmidt *et al* (*2*) reported adenyl cyclase and phosphodiesterase were inactivated in rat brain after rats were exposed to microwaves for 20 sec. Brain cholinesterase was denatured after rats were treated for 10–15 sec but death occurred at 3–5 sec (*3*). Since death occurred before these enzymes were inactivated it was possible other critical enzymes were more sensitive to heat inactivation. Since the energy requirements of brain are high even a brief interruption of glycolysis would quickly

* This work was supported by National Institutes of Health Grants NB 07369 and NB 22359

TABLE I

EFFECT OF MICROWAVE HEAT ON ENZYME ACTIVITY IN MOUSE BRAIN

	Enzyme activity		
	Control	*Experimental*	
	mmoles per kg/hr		% of control
Hexokinase	853 ± 112[a]	2.3 ± 0.4	0.27
Aldolase	998 ± 39	2.0 ± 0.2	0.20
P-fructokinase	263 ± 20	1.1 ± 0.2	0.41
Pyruvate kinase	5635 ± 160	34.4 ± 4.2	0.61
Lactate dehydrogenase	3574[b]	<4.0[b]	<0.10
Adenylate kinase	164 ± 42	15.6 ± 2.1	9.50
P-creatine kinase	890 ± 158	<1.0	<0.11
Phosphorylase	37 ± 8	0.03 ± 0.01	0.08

The heads of decapitated mice were placed in a microwave oven 5 sec. The brains were removed and samples homogenized in phosphate buffer, 0.1 *M*, pH 7. The control mice were decapitated and the heads frozen immediately at −150°C. The frozen samples were stored at −70°C prior to use.

[a] Each value is the mean for samples from 3 or 4 mice ± S.E.

[b] The value is the mean of 2 determinations.

cause an energy deficit. This is a report on the early effects of microwave irradiation on enzymes related to glycolysis. The change in energy substrate levels, before the enzymes are denatured, were used indirectly to obtain information on enzyme activity during the first few seconds of irradiation.

MATERIALS AND METHODS

A commercial microwave cooking oven (Litton Industries, Model 500) was used to generate the microwaves. The oven operated at 2450 MHz and had a 1000 watt input to the magnetron. Albino male mice (Ha/ICR, A. R. Schmidt Co., Madison, WI) were decapitated and the heads placed on a support attached to the inside of the oven door or they were anesthetized with phenobarbital (200 mg/kg/i.p.) and shielded so only the head was exposed to the microwaves. After irradiation, brain samples (50–70 mg of cortex) were removed from the unfrozen brain of experimental mice and the samples were weighed at room temperature. The samples were homogenized in cold (0°C) 0.3 *M* $HClO_4$, centrifuged and the supernatant neutralized with $NaHCO_3$. Control mice were decapitated and the heads frozen in Freon 12 (CCl_2F_2) cooled to −150°C in liquid N_2. The brain samples were dissected from the frozen brain and weighed at −20°C. Neutralized tissue extracts were prepared using acid-methanol (−20°C) as the initial solvent (*4*).

ATP, P-creatine, glucose, glucose-6-P, fructose diphosphate and lactate were measured using enzymatic-fluorometric methods described by Lowry *et al.* (*5*). Glycogen levels were measured according to Passonneau *et al.* (*6*); values are given in glucosyl units.

The enzyme activities of eight enzymes were based on the initial velocity of each enzyme reaction at 25–28°C. The auxiliary enzymes and cofactors used,

TABLE II

BRAIN METABOLITE LEVELS AFTER EXPOSURE TO MICROWAVE RADIATION

Group[a]	Brain					
	ATP	*P-Cr*	*Gly*	*Glu*	*Lact*	*G-6-P*
	mmoles per kg					*μmoles per kg*
Control—(Head frozen at 0 time)	2.55[d]	3.14	2.74	1.70	2.47	91
	0.08	*0.07*	*0.19*	*0.05*	*0.09*	*6*
Ischemic anoxia—(Head frozen 20 sec after decapitation)	2.10[c]	0.79[c]	2.32	0.26[c]	3.51[c]	29[c]
	0.10	*0.20*	*0.24*	*0.07*	*0.22*	*8*
Microwave 5 sec—(Room temp. 3 min)	1.61[c]	2.45	1.80[c]	1.87	1.84[b]	117[b]
	0.13	*0.46*	*0.08*	*0.12*	*0.17*	*6*
Microwave 3 sec—(Room temp. 5 min)	1.64[c]	2.20[c]	2.06[c]	1.87	1.60[c]	180[c]
	0.20	*0.19*	*0.05*	*0.11*	*0.17*	*10*

[a] After decapitation the mouse heads were treated in the manner indicated above. In the microwave irradiated groups, the head was placed on a platform on the oven door at the time of decapitation. The microwave energy was activated with door closure; the interval between decapitation and onset of heating was 1 to 2 sec. Abbreviations used are: *P-Cr*, phosphocreatine; *Gly*, glycogen; *Glu*, glucose; *Lact*, lactate; *G-6-P*, glucose-6-P.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] Each value is the mean for samples from 4 to 6 mice. S.E. is given in italics.

along with their concentrations, were those given by Lowry *et al.* (7). For the adenylate kinase measurement, the substrates and cofactors were those used for P-creatine kinase except P-creatine was omitted.

RESULTS

Microwave Irradiation and Brain Temperature

High temperatures are reached within the brain during exposure to microwave radiation. Five mice were decapitated and the heads were exposed to microwave radiation for 5 sec. As each head was removed a temperature probe was inserted into the brain through the foramen magnum. The mean temperature ± S.E. was 90.5 ± 0.1.

Microwave Irradiation and Enzyme Activities

A 5 sec exposure of the mouse head to microwave radiation caused nearly complete inactivation of all but one of the enzymes studied (Table I). After microwave irradiation, the activities of hexokinase, aldolase, P-fructokinase, pyruvate kinase, lactate dehydrogenase, P-creatine kinase and phosphorylase were less than 1% of control levels. Adenylate kinase was more resistant with nearly 10% of the enzyme activity left after microwave treatment.

Substrate breakdown was unlikely after treatment of the brain since enzymatic activity was largely absent. For this reason, in preparing tissue for metabolite measurement, brains from mice exposed to microwaves were removed and samples dissected and weighed at room temperature (see methods). If residual enzyme activity was significant, one would expect lower metabolite levels with longer incubation, but a 2 min extension of incubation did not change the metabolite levels (Table II).

Microwave Irradiation and Metabolites

The levels of the major energy reserves in heated brains were compared with the levels in control and ischemic–anoxic brains. ATP levels were 37% lower in the heated brains then in the control tissue (Table II). This change was greater than occurred in brain anoxic for 20 sec. The rapid conversion of high energy phosphate from P-creatine to ATP in the ischemic–anoxic tissue is greatly reduced with microwave irradiation (Table II). Glucose, which was rapidly used in the ischemic–anoxic brains was not changed when the brains were exposed to microwaves immediately after decapitation. Conversely, glycogen was not significantly reduced in the ischemic–anoxic tissue, but a significant reduction in glycogen occurred after decapitation and microwave treatment (Table II).

With the increase in anaerobic glycolysis seconds after decapitation, brain lactate levels increase (Table II). However, low lactate levels were present when the anoxic brain was treated with microwaves which indicates one or more of the enzymes between glucose and lactate are inactivated in the first few seconds of microwave exposure.

Glucose-6-P levels were nearly twice control levels in brains treated with microwaves 3 sec and a significant increase was present in the mouse head treated 5 sec (Table II). Glucose-6-P level in the anoxic brain was one-third the control level (Table II).

Brain Metabolite Levels in Cortex and Subcortex of Intact Mice.

The microwave effects of metabolite levels in brains of the intact mice were similar to those described for the decapitated animals. Brain glycogen was reduced in both cortex and subcortex of brain from microwave treated mice (Table III) but glucose levels were similar in the two groups. ATP levels in the treated mice were about 60% of control levels in both cortex and subcortex. P-creatine was significantly reduced in both areas of cerebrum in the heated tissue. Glucose-6-P was higher in subcortex from treated mice than controls but there was no significant difference in levels in cortex from these mice. Fructose diphosphate was nearly depleted and lactate was significantly elevated in the cortex of the experimental animals (Table III). The high brain glucose levels in this study may be due to anesthesia since Mayman *et al.* reported elevated brain and blood glucose in anesthetized mice (*8*).

DISCUSSION

After decapitation the energy reserves in the brain are normally used until they are exhausted (*5*) and the manner in which they are used was shown in the

TABLE III

BRAIN METABOLITE LEVELS AFTER A THREE SECOND EXPOSURE TO MICROWAVES

	Brain			
	Control		*Experimental*	
	Cortex	*Subcortex*	*Cortex*	*Subcortex*
	mmoles per kg		mmoles per kg	
Glycogen	2.92 ± 0.19[a]	2.64 ± 0.14	2.13 ± 0.16[b]	1.82 ± 0.12[c]
Glucose	4.24 ± 0.27	3.84 ± 0.17	4.48 ± 0.39	4.02 ± 0.10
ATP	2.80 ± 0.16	2.86 ± 0.08	1.65 ± 0.18[c]	1.59 ± 0.08[c]
P-creatine	4.22 ± 0.16	3.62 ± 0.17	3.05 ± 0.32[b]	2.89 ± 0.09[c]
G-6-P	0.11 ± 0.02	0.09 ± 0.01	0.13 ± 0.01	0.14 ± 0.01[c]
FDP[e]	0.16 ± 0.02	0.26 ± 0.04	0.013 ± 0.004[c]	0.011[d]
Lactate	1.06 ± 0.04	1.69 ± 0.21	1.36 ± 0.11[b]	1.21 ± 0.26

[a] The mean value for samples from 3 to 7 mice are given along with the S.E. Mice were anesthetized with phenobarbital 200 mg/kg. Control mice were frozen whole in liquid N_2 (−150°C). Experimental mice were shielded so only their heads were exposed to the microwave radiation.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] Mean value for samples from 2 mice.

[e] *FDP*, fructose diphosphate.

ischemic-anoxic brain (Table II). This progressive utilization of energy stores in the ischemic–anoxic brain was altered in the brains of mice exposed to microwaves. This interruption in metabolic activity was best observed by the change in glucose utilization. In the ischemic–anoxic brain, glucose was nearly depleted after 20 sec, but when the decapitated head was treated with microwaves 3 or 5 sec the brain glucose levels remained at control levels for 5 min (Table II).

The inactivation of the glycolytic enzymes was not surprising in view of the high temperatures reached in the brain. In addition, nonthermal effects may have occurred since enzyme changes (*9*) and behavioral alterations (*10*) have been reported which are believed to be unrelated to the generation of heat. Although the enzyme activities after microwave treatment could provide no information on the events occurring in the first few seconds of heating, the metabolite levels were more revealing. Treating the head with microwaves immediately after decapitation altered the metabolite levels in ways which indicated the sequence of enzyme inactivation.

ATP reduction was greater in the microwave treated than in the untreated ischemic-anoxic brains. Possible causes for this include a resistance of ATPases to heat inactivation and enhancement of ATP hydrolysis with temperature rise early in the course of treatment. Another possibility is that when neuronal activity is increased during microwave treatment (*1*) ATP was utilized more rapidly than it could be synthesized. In any case, the inability for the energy reserves to generate adequate ATP is apparent. P-creatine, which is generally one of the first reserves to be depleted when ATP is needed, was reduced only

30% (Table II). This indicates P-creatine kinase was inactivated early in the course of microwave treatment. Glucose, another rapidly depleted reserve in the anoxic brain (Table II), was not used in the microwave treated mice which suggests hexokinase is very heat-sensitive with inactivation occurring early at relatively low temperatures.

Glycogen levels were significantly reduced in the treated mouse brain and this may account for the elevation in glucose-6-P. With a decrease in glycogen of about 1 mmole/kg (glucosyl units), a comparable increase in glucose-6-P would be expected. Since a much smaller increase was observed, appreciable glucose-1-P may have accumulated due to inactivation of phosphoglucomutase or considerable glucose-6-P hydrolysis occurred before P-fructokinase was denatured. The low level of brain lactate in microwave treated mice suggests the flux of intermediate metabolites was largely blocked between glucose-6-P and lactate. Aldolase was probably not inactivated early because fructose diphosphate was nearly gone in brain from irradiated mice.

As indicated above, the brain metabolite changes due to microwave irradiation may be explained by the variable sensitivity of enzymes to microwaves. Although little is known of the direct effects of microwaves on enzymes it is known that enzymes vary with regard to resistance to heat denaturization. In general, enzyme denaturization can be viewed as an opening up of the protein molecule by separation of the peptide chains. The stability of each molecular species is governed by a variety of factors in the tissue which include pH, ionic strength, and the protective effect of substrates and other substances (*11*). Since the state of these factors in the brain tissue at the time of microwave irradiation could favor stability of some enzymes more than others, it is not surprising that differences in enzyme resistance to denaturization were observed in the studies reported.

ACKNOWLEDGMENT

The author thanks Mrs. Mary-Lucille Mantz for her skilled technical assistance.

RECEIVED: December 12, 1972

REFERENCES

1. W. B. DEICHMANN, Biological effects of microwave radiation of 24,000 megacycles. *Arch. Toxik.* **22**, 24–35 (1966).
2. M. J. SCHMIDT, D. E. SCHMIDT, and G. A. ROBISON, Cyclicadenosine monophosphate in brain areas: Microwave irradiation as a means of tissue fixation. *Science* **173**, 1142–1143 (1971).
3. D. E. SCHMIDT, R. C. SPETH, F. WELSCH, and M. J. SCHMIDT, The use of microwave radiation in the determination of acetylcholine in the rat brain. *Brain Res.* **38**, 377–389 (1972).
4. S. R. NELSON, O. H. LOWRY, and J. V. PASSONNEAU, Changes in energy reserves in mouse brain associated with compressive head injury. In *Head Injury. Conference Proceedings* (W. F. Caveness and A. E. Walker, Eds.), Lippincott, Philadelphia, 1966.
5. O. H. LOWRY, J. V. PASSONNEAU, F. X. HASSELBERGER, and D. W. SCHULTZ, Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. Biol. Chem.* **239**, 18–30 (1964).
6. J. V. PASSONNEAU, P. D. GATFIELD, D. W. SCHULTZ, and O. H. LOWRY, An enzymic method for measurement of glycogen. *Anal. Biochem.* **19**, 315–326 (1967).

7. O. H. Lowry, and J. V. Passonneau, The relationships between substrates and enzymes of glycolysis in brain. *J. Biol. Chem.* **239**, 31–42 (1964).
8. C. I. Mayman, P. D. Gatfield, and B. M. Breckenridge, The glucose content of brain in anesthesia. *J. Neurochem.* **11**, 483–487 (1964).
9. W. Sawicki, and K. Ostrowski, Nonthermal effect of microwave radiation *in vitro* on peritoneal mast cells of the rat. *Amer. J. Phys. Med.* **47**, 225–234 (1968).
10. N. W. King, D. R. Justesen, and R. L. Clarke, Behavioral sensitivity to microwave irradiation. *Science* **172**, 398–401 (1971).
11. M. Dixon, and E. C. Webb, *Enzymes*, 2nd ed., pp. 145–150. Academic Press Inc., New York. (1964).