MICROWAVE IRRADIATION SACRIFICE: APPLICATION IN NEUROCHEMICAL RESEARCH

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Abstract

Temperatures, inactivation rates of adenyl cyclase, phosphodiesterase and cholinesterase, and levels of acetylcholine and cyclic AMP were measured in rat brain following microwave sacrifice. Results indicate that such sacrifice results in rapid and simultaneous inactivation of enzyme systems throughout the brain. Levels of acetylcholine and cyclic AMP in brain compare favorably to those obtained following other means of sacrifice.

Introduction

Analysis of compounds such as acetylcholine (ACh), choline, cyclic AMP and other substances in brain require rapid inactivation of enzyme systems due to the rapid post-mortem changes in levels which have been shown to occur. Ideally such inactivation should occur in situ and should be rapid and simultaneous throughout the brain. In addition, pre-sacrifice and sacrifice stress should be minimal and post-sacrifice dissection of brain into discrete anatomical areas is very desirable. Microwave irradiation sacrifice (MIS), first suggested by Stavinoha[1], appears to be a promising approach in obtaining these goals.

Results

Measurement of the rate of temperature rise[2] in brain following MIS indicate a rapid increase, with temperatures of 70-80°C being reached in 15-20 seconds in a 200-250 gm rat. Measurement of cholinesterase[2] (table 1) following MIS indicates that complete and simultaneous inactivation occurs throughout brain. Similar results were obtained for inactivation of phosphodiesterase and adenyl cyclase activity[3]. Complete enzyme inactivation in brain corresponds to a brain temperature of 60-75°C.

Measurement of the levels of ACh in brain following MIS yielded values which are higher than those generally appearing in the literature[2] (table 2). The corresponding value for free choline (28.7 nmoles/mg whole brain) was significantly lower than previous literature values for free choline. Levels of cyclic AMP in brain and brain areas[3,4] (table 3) are comparable to those obtained by rapid freezing.

Discussion

Temperature and enzyme activity measurement in brain indicate that rapid and simultaneous inactivation of enzymatic activity occurs during MIS. Levels of ACh and choline following MIS yield values which are interpreted as more closely approaching true in vivo levels. ACh values, which decline rapidly post-mortem, are higher than those generally found in the literature, indicating that post-mortem loss is reduced. Choline values, which rise rapidly post-mortem, are significantly lower than previous values. The MIS value for choline (28.7 nmoles/gm) closely approximates the estimated in vivo value for free choline as determined by Dross and Kewitz[5]. Similarly, cyclic AMP values, which rise rapidly post-mortem, are comparable to those obtained by rapid freezing. However, unlike freezing, MIS permits rapid, accurate dissection into discrete brain areas prior to analysis. MIS can be applied to animals with chronically implanted brain cannulae without loss of brain tissue. Reasonable localization of brain constituents is preserved as indicated by injection of dyes through such cannulae prior to MIS. Dye distribution does not vary significantly in MIS animals versus control animals.

MIS does, however, destroy fine cell structure thereby rendering measurement of subcellular distribution of ACh and cyclic AMP unlikely. Thermally unstable compounds and enzyme activities also cannot be assessed in MIS animals. In addition, in the present MIS system, some transient heat mediated enzyme activation is doubtless occurring due to the low power density and the resultant relatively slow rate of inactivation.

Table I

TIME COURSE OF INACTIVATION OF CHOLINESTERASES IN VARIOUS AREAS OF RAT BRAIN[2]

Brain area	Duration of exposure to microwave irradiation				
	Control	5 sec	10 sec	15 sec	20 sec
Cortex	682±39*	722±14	557± 72	18±31	0
Midbrain-hypothal.	506±27	581±40	484±121	34±59	0
Hippocampus	352±10	373±62	342± 62	36±62	0

* µMoles ACh hydrolyzed/g tissue/h. Radiation was applied to 230 g male rats. Each value represents the mean ± standard deviation of 3 animals.

Table II

DISTRIBUTION OF ACh IN DISCRETE AREAS OF THE RAT BRAIN[2]

Area	Tissue wght (mg)	nmoles ACh/g	% Total brain wght	% Total ACh
Striatum	126± 5*	46.96±2.22	8.5±0.3	18.8±1.1
Hypothalamus	45± 2	34.12±1.67	3.1±0.2	4.5±0.3
Midbrain	169± 7	30.69±1.22	11.5±0.5	15.0±0.6
Brain stem	172± 6	27.37±1.22	11.8±0.5	13.9±0.4
Hippocampus	109± 3	24.24±0.51	7.4±0.2	8.0±0.4
Cortex	650± 7	18.95±0.46	44.1±0.3	35.9±1.1
Cerebellum	204± 3	6.55±0.93	13.8±0.2	4.4±0.9
Whole brain**	1474±12	23.54±0.79		

* ± S.E.M.
** Value calculated as sum of the areas.

Table III

AMOUNTS OF CYCLIC AMP IN BRAIN AREAS AFTER MICROWAVE IRRADIATION[4]

Area	Cyclic AMP (nmole/g)
Cerebellum	1.86±0.06
Brainstem	1.87±0.06
Hypothalamus	1.60±0.04
Midbrain	1.43±0.11
Hippocampus	0.84±0.09
Cortex	0.74±0.06

Values represent the mean and standard error of the mean from six separate determinations.

Conclusion

MIS has been shown to be a promising method for sacrifice of animals prior to analysis of heat stable compounds in brain. This method of sacrifice yields values for ACh, choline and cyclic AMP which more accurately reflect in vivo brain levels. In addition, such analysis can easily be performed on discrete brain areas, and advantage over "whole brain" studies. MIS causes an apparent reduction in pre-sacrifice and sacrifice stress in animals when compared to decapitation and/or freezing. Design and construction of a more powerful focused microwave system should greatly reduce inactivation time to under one second and will be designed as to further reduce or eliminate stress. Reduction or elimination of stress will prevent physiologically induced changes in level or turnover prior to sacrifice. Inactivation in less than one second will allow very minimal time for any changes to occur in in vivo levels of brain compounds during enzyme inactivation. Such a system will allow the most accurate determination of true in vivo levels and turnover rates of various heat stable neurotransmitters to date and will represent a significant advance in the area of neuroscience.

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