Comparison of Microwave Irradiation at 986 Versus 2450 MHz for *In Vivo* Inactivation of Brain Enzymes in Rats

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***Abstract*—The pattern of enzyme inactivation in the brains of rats sacrificed by exposure to high-intensity microwave irradiation at 2450 MHz in a closed-waveguide structure is markedly altered by rotation of the rat. At 986 MHz, the pattern is relatively insensitive to rotation. These data suggest that use of lower frequencies may reduce regional variability of enzyme inactivation and lessen the requirement for immobilization during sacrifice.**

INTRODUCTION

EXPOSURE to high-intensity microwave irradiation at 2450 MHz has been widely used as a rapid sacrifice technique which inactivates brain enzymes, reduces or eliminates post-mortem change in certain heat-stable substrates, and leaves the brain in a condition suitable for regional dissection [1]–[6]. Examples of heat-stable substrates which are subject to rapid post-mortem change are gamma aminobutyric acid (GABA), acetylcholine (Ach), cyclic 3′5′ monophosphate (cAMP), and cyclic 3′5′ guanosine monophosphate (cGMP). These compounds are distributed throughout the brain and are considered to be "neurotransmitters" (GABA and Ach) or "second messengers" (cAMP and cGMP) involved in mediation of neuronal activity [7]–[9]. To assess the levels in various brain regions accurately, it is essential to rapidly and uniformly inactivate the enzymes which normally synthesize or degrade these substrates.

The microwave systems used have varied in terms not only of power output but of applicator design [1]–[6] as well. Microwave applicators are the physical interface between the load (animal) and the microwave field. In most cases, they consist of a waveguide section into which the animal is placed within some kind of a plexiglass holder. The precise position and orientation of the animal within the particular waveguide at the time of exposure have been found to be critical determinants of the pattern of enzyme inactivation throughout the brain.

It is well known that the field distribution and the accompanying heating pattern within a load exposed in a microwave field are dependent upon not only the shape and dielectric properties of the load and its orientation with respect to the field, but also on the frequency of the microwave field. Our laboratory has previously observed that heat deposition [10] and the resultant pattern of enzyme inactivation [11] in the rat brain at 2450 MHz with two different applicator designs is nonuniform, and have noted energy distribution to be markedly affected by angular position of the rat head. Accordingly, we find it necessary to immobilize all animals prior to sacrifice. One way to reduce the sensitivity of the heating patterns to rotation would be to diminish the coupling to the microwave electric field by placement of the animal's head at a magnetic field maximum $\lambda g/2$ in front of a short-circuiting end plate in a terminal waveguide section. In this location, the electric field is at a minimum, and thus the magnetic field is minimally perturbed by the dielectric load (the rat head). Additionally, on the axis of the waveguide, the H field is fairly uniform in all directions. It is believed that this is the reason for the relative insensitivity to rotation. It is furthermore necessary that the dimensions of the volume to be heated occupy no more than a small fraction of a guide wavelength. This is necessary to ensure that the fields in the object to be heated cannot vary substantially due to standing waves. This implies the use of lower frequencies and hence longer wavelengths. A concomitant disadvantage of this approach is the difficulty of coupling energy into a load of biological constituency. This occurs because the small load (relative to a wavelength) results in a high Q cavity which is difficult to impedance match and because the magnetic field does not couple easily to a dielectric load. A way of alleviating the problem with high Q is to expose a longer mass, such as roughly half the length of the animal. Moreover, at 986 MHz, it is necessary to place the rat head on the longitudinal axis of the waveguide where the magnetic field is most uniform.

Because immobilization by physical restraint is a potent stressor which causes significant neurochemical changes

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[12], [13], a technique of irradiation independent of orientation of the rat would be extremely useful. The present study was undertaken to clearly demonstrate the effects of angular orientation on the spatial distribution of energy in the rat brain during exposure at 2450 MHz and to examine these same factors at a lower frequency, 986 MHz.[1] We decided that the pattern of energy deposition could be inferred from the pattern of enzyme inactivation, using the cytochemical technique of Nachlas [14] for succinic dehydrogenase. Cytochemical techniques have been employed previously to demonstrate enzyme inactivation in the brain with exposure to a microwave field at 2450 MHz [15], and we chose succinic dehydrogenase because of its relatively uniform distribution throughout the brain.

Materials and Methods

Male 300-g Walter Reed strain rats were anesthetized with sodium pentobarbital and inserted into a plexiglass cylinder which was placed in one of four angular positions (0°, 90°, 180°, or 270°) within the waveguide. The rats were then exposed to either of two microwave frequencies: 2450 or 986 MHz. Four animals were studied under each of the eight combinations of frequency and angular positions. "Zero degrees" describes the rat in a normal prone position with the ear-to-ear axis horizontal (parallel to the longitudinal axis of the waveguide), 90° and 270° describe positions with the ear-to-ear axis vertical, and 180° implies the rat supine, or the inverse of 0°. Following exposure to microwave irradiation, the subjects were decapitated and the brains were removed and frozen on dry ice. The frozen brains were coronally sectioned at 10 μm, at -2°C in a cryostat. Sagittal sections were cut in matched animals to permit cytochemical estimation of enzyme activity in all major brain regions. The spatial distribution of heat loading and protein denaturation at the two frequencies studied can be inferred from the pattern of loss of succinic dehydrogenase activity. Brains of rats sacrificed by decapitation without exposure to microwaves were used to demonstrate the normal staining pattern.

For irradiation at 2450 MHz, the cylinder containing the rat was inserted into a WR 430 waveguide [10] applicator through a hole in the shorting end plate (Fig. 1). The longitudinal axis of the animal was coaxial to the long axis of the waveguide. The power source was modified [16] to control precisely the total energy delivered to each animal. Greater efficiency of inactivation was obtained in this applicator by exposing only the rat head to microwave irradiation. Animals were exposed for 2.3 s with 2.5-kW forward power (1–3 percent reflected). The power source was a Varian PPS-2.5 modified with electronic control for precise timing ($\pm$ 32 ms) and leveling of forward power ($\pm$ 2 percent). Frequency output was verified at 2440 $\pm$ 20 MHz with a Tektronix 1L20 spectrum analyzer. Prior to exposure, each animal was impedance-matched over the range 2420–2460 MHz to a low-power (10-mW) signal from a sweep generator, using a double-stub tuner. The power supply in the source was an unfiltered, full-wave rectified supply. During every exposure, the number of actual pulses (120/s) as well as both forward and reflected power were monitored (10, 16).

For microwave fixation at 986 MHz, anesthetized rats were placed in a plexiglass cylinder and the cylinder was inserted into a hole in the narrow wall of a WR 975 waveguide applicator (Fig. 2). The rat holder was eccentric, but the rat head was centered with respect to the vertical dimension of the narrow wall. In this applicator, the head and thorax of the rat were exposed to microwave irradiation at a placement 19.3 cm (one-half guide wavelength) from the shorting end plate. At this location of minimum electric fields the heating is caused by microwave currents induced by high magnetic fields. It is argued that the choice of longer wavelengths coupled

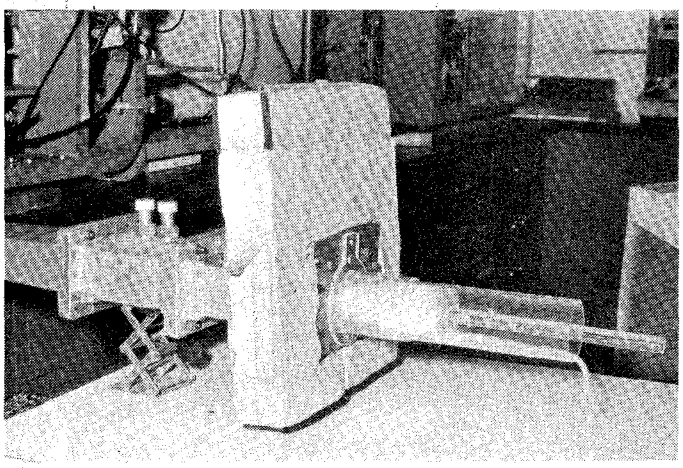

Fig. 1. Rat in applicator for exposure at 2450 MHz. The large square object surrounding the waveguide is part of the RF-absorbing material which is arrayed to completely surround the rat as a safety measure during actual exposure.

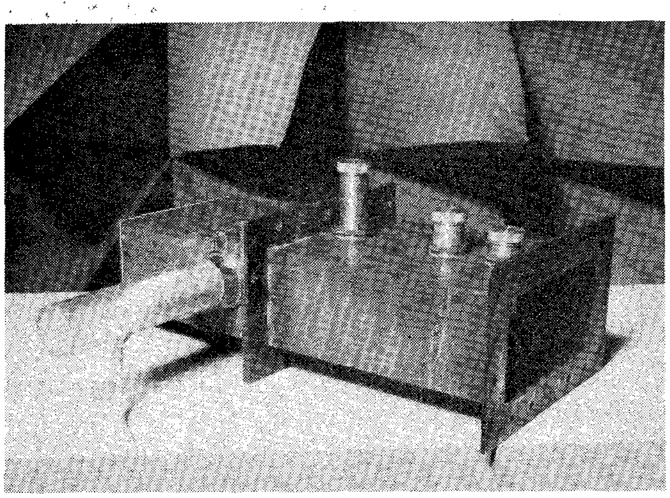

Fig. 2. Rat in applicator for exposure at 986 MHz.

[1]This frequency was used because of its ready availability on our high-power microwave source [Varian Klystron 4KM3000LR]. The general nature of the results at any of the L-band frequencies would be expected to be qualitatively similar to the results given here for 986 MHz.

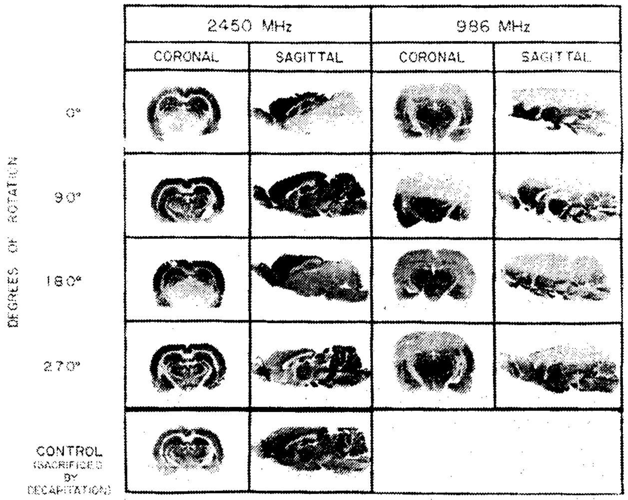

Fig. 3. Coronal and sagittal sections of rat brain following exposure at 2450 or 986 MHz at one of four angular positions (0°, 90°, 180°, or 270°).

with an insertion of a much larger mass at a location of reduced coupling to electric fields should result in heating of the exposed parts independent of rotation. Unlike the 2450-MHz applicator (Fig. 1) in which only the head is exposed to irradiation, the 986-MHz applicator exposes nearly half the animal's body. Exposure of a larger mass requires exposures of longer duration, but use of a higher powered source could reduce required exposure duration to 1 to 2 s. In this experiment, the power source was a Varian model 4KM3000LR, 986-MHz CW Klystron. Each animal was impedance matched with a triple stub tuner (Fig. 2) using a Hewlett–Packard model 8410A network analyzer, and then exposed for 6 s, with 1.75-kW forward power (1–3 percent reflected).

Succinic dehydrogenase activity was assessed cytochemically by the method of Nachlas, modified by addition of phenazine methosulfate to shorten incubation time. One slide from each of 8 different subjects, each representing one square of the experimental design matrix (4 angles × 2 frequencies, see Fig. 3) was stained simultaneously in each coplin jar. This assured precise comparability of incubation time and other factors affecting staining such as remaining potency of tetrazolium (formation of monoformazans), or presence of cytochrome inhibitors. In addition, one slide of brain tissue from un unexposed animal was included in each batch as a control.

Results

Four animals were individually exposed at each angle and frequency, and the brains were sectioned and stained as above. An exposure duration was deliberately selected to produce a gradient of enzyme inactivation in the brains of rats exposed to either frequency. As seen in Fig. 3, this gradient was markedly angle-dependent at 2450 MHz; absorption of energy was much greater at the 0° and 180° positions. At 986 MHz, however, using the applicator shown in Fig. 2, degree of rotation produced almost no effect on the pattern of enzyme inactivation; the dorsal portion of the brain absorbed most of the energy at all four angles of exposure. The sagittal sections confirm and extend the data provided by the coronal sections.

To date, the applications of high-intensity microwave irradiation as a technique in neurochemistry have relied on one nominal frequency: 2450 MHz. This frequency has the disadvantage of yielding a nonuniform distribution of energy dissipation within the rat brain [10], [11]. Furthermore, the pattern of energy deposition is drastically affected by angular position of the head. This latter consideration imposes the requirement of rigorous immobilization of subjects prior to sacrifice. The possibility must be considered that the stress of immobilization during sacrifice may introduce changes which confound biochemical data from brain or plasma of animals sacrificed by microwave irradiation. If conditions could be identified under which rats could be irradiated without the requirement for immobilization, a potential source of artifact could be eliminated. A ventilated plastic cage or some other means of confining the rats to an area within the applicator would be required, but the differences from home cage conditions could be easily minimized or adequate controls could be instituted. The 986-MHz applicator pictured in Fig. 2 is relatively insensitive to rotation of rats in the field. These data suggest that further exploration of the frequency parameter[2] could lead to an extremely rapid method of sacrifice with simultaneous inactivation of brain enzymes in the unrestrained rat. Such a technique would have widespread utility in the study of neurochemical correlates of behavior and physiology.

Acknowledgment

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The Electric-Field Probe Near a Material Interface with Application to the Probing of Fields in Biological Bodies

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Abstract—**A theoretical model is formulated to determine the effect of an interface between different media on the response of an electric-field probe. The model used provides a "worst case" estimate of the interaction between the probe and the interface. The effect of the interface on the response of the probe is examined as a function of the size of the probe, the insulation on the probe, the load admittance at the terminals of the probe, the dissipation in the surrounding medium, and the spacing between the probe and the interface. The use of electrically small bare and insulated probes to measure the field in the interior of biological bodies is discussed as an example. Measured results are shown to be in general agreement with the theory.**

I. Introduction

IN MANY APPLICATIONS an electric dipole is used as a probe to measure the component of an incident electric field parallel to its axis. When the dipole is located in an inhomogeneous body, such as a biological specimen, the response of the probe can change with its position as a result of the variation of the electrical constitutive parameters within the body. The constitutive parameters may be slowly varying continuous functions of the position or may change abruptly at a material boundary, such as at a muscle–fat interface or the boundary between tissue and air. Specifically, the response from the probe, i.e., the voltage V, is proportional to the component of the incident electric field parallel to its axis ($\hat{z}$)

$$V = K_e \boldsymbol{E}(\boldsymbol{r}) \cdot \hat{z} = K_e E_z(\boldsymbol{r}) \qquad (1)$$

and the proportionality factor K_e is a function of the constitutive parameters of the medium surrounding the probe ($\sigma_e, \epsilon_e, \mu \approx \mu_0$) including any abrupt changes in these parameters due to nearby boundaries. Note that the *incident field* $E_z(\boldsymbol{r})$ in (1) is the field at the point where the probe is located when the probe is absent; it is the field to be measured. The *total field* is the incident field plus the field that is scattered from the probe and scattered between the probe and the boundaries of the body.

Ideally, a probe is needed that has a proportionality

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