IS THE BLOOD-BRAIN BARRIER ALTERED BY RF IRRADIATION?

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In previous studies from other laboratories, it was reported that RF radiation at low power densities produced an increased permeability in the blood-brain barrier (BBB) in rats. We have studied this phenomenon in mice, using as test compounds (T-cpds), fluorescein (FCN) and amino acids not normally found in mice. Following i.p. injection of the T-cpds, the animals were bled, killed, perfused, and the brains removed. Clear deproteinized extracts of plasma and of brain homogenates were analyzed for FCN with a Turner spectrofluorometer, and for amino acids with a Beckman amino acid analyzer. The concentration of T-cpds in the brain and plasma of RF-irradiated and other experimental animals was compared to that of non-treated controls.

Following exposure to CW or pulsed RF radiation at 918 MHz and at average power densities ranging from 2.5 to 132 mW/cm^2 (ave SAR = 0.22 W/kg per 1 mW/cm^2 incident power density), no significant increase in the BBB permeability could be detected. Subsequent experiments have been directed toward assessing the susceptibility or sensitivity of the mouse BBB as a system for testing RF radiation-induced biological changes. In substitution for RF radiation, the effects of whole-body heating and of i.p. injections of glycerol, urea, metaraminol (a pressor agent), and dimethyl sulfoxide (DMSO) have been examined with respect to any resulting changes in BBB penetration in mice. In the heating experiment, mice were placed in an incubator at 50^{o} for 22 to 25 minutes. In the experiments using the various pharmacological agents, a pretested, maximally-tolerated dose of the compound was injected i.p. In none of the experiments, except those using DMSO were we able to demonstrate an increase in BBB permeability. With DMSO, we observed a significant increase in BBB permeability for the first time in treated mice.

Since several investigators have reported RF radition-induced increases in BBB permeability in rats, we are now comparing the responses of rats with those of mice under closely controlled, comparable conditions, to determine whether there is a fundamental BBB difference in these two widely used experimental animals.

Three previous studies have been carried out on blood-brain barrier (BBB) effects in which the investigators found evidence for increased permeability of the BBB toward fluorescein and radioactive mannitol, following RF radiation exposure at low power densities. Further, it was reported that pulsed RF radiation was substantially more effective than continuous wave (CW) radiation. Since these initial findings, however, other investigators have been unable to confirm an increased permeability of the BBB under similar or identical experimental conditions.

Our rationale in approaching these studies included the goal of developing and utilizing highly objective and effective biochemical methods in the study of the BBB phenomenon and to combine such methodology with carefully regulated and controlled RF radiation exposure and dosimetry. We have used mice for most of our studies, but are now comparing the response in both rats and mice under comparable conditions.

Development of Methodology. The test compounds selected for potential BBB penetration studies were fluorescein (FCN), which had been used in the work of Frey and others, and amino acids, which represent low molecular weight molecules. Previous reports in the use of FCN stated that it was a high molecular weight FCN-protein complex which was passing the BBB into the brain tissue. We now believe that this is not necessarily the case.

FCN Measurements. In the development of needed methodology, we have shown that FCN (and amino acids) pass rapidly into the blood stream in the mouse following i.p. injection, and that most, or a substantial portion, of the FCN in the blood is present in the free form and is not bound to plasma proteins. We prepared plasma and brain tissue samples by deproteinization and homogenization to give extracts which were then clarified by high-speed centrifugation. Such extracts were analyzed for both FCN and amino acid content. A Turner spectrofluorometer was used for determinations of FCN in both plasma and brain tissue samples at pmole/ml levels.

Amino Acids. Several amino acids that are not normally found in mice, but that are closely related to those that are present, were screened. A combination was selected which included amino acids that do, and those that do not, normally cross the BBB. These were separated and accurately measured with an amino acid analyzer using specially developed analysis procedures. These amino acids were then included in the FCN-amino acid injection mixture to test for possible increased BBB permeability following RF radiation or other experimental treatment.

RF Radiation Procedures. Our procedure for RF radiation experiments has been to expose separate groups of animals to sham, CW, and pulsed (100 pps, 10μs/pulse) RF radiation at 918 MHz for 30 minutes. For each of these three groups, four mice in a specially designed, circular, sectioned styrofoam cage were placed in the circularly polarized

waveguide developed by Guy and Chou, for sham or RF exposure. The animals were then immediately injected with the FCN-amino acid mixture. At 5 minutes post injection, blood samples were rapidly taken by retro-orbital bleeding and the mouse was killed within 30 to 45 seconds with CO_2. The vascular system was then efficiently perfused with cold saline, and the brain removed. Analysis of both plasma and brain samples established the concentration of the FCN and amino acids in these samples and permitted the calculation of the brain content, if any, as a percent of the plasma level.

Radiation Exposure Results. We have carried out several RF radiation exposure experiments, using 2.5 and 33 mW/cm^2 average power density, both CW and pulsed, and also using 132 mW/cm^2 CW. The ave. SAR, determined by twin well calorimetry, was 0.22 W/kg per mW/cm^2 incident power density. The SAR (tail area), measured by themographic techniques, was 1.01 W/kg per mW/cm^2 incident power density. These exposure conditions included energy levels which are considered to be "non-thermal" in effect as well as those which obviously caused body temperature elevation. In all experiments the specific concentration of test compounds in the brain (brain concentration relative to plasma concentration) for the radiated animals has been in the same range as was obtained for the non-radiated controls. Thus, in our studies so far, there has been no demonstration of increase in BBB permeability toward the test compounds used, resulting from RF exposure.

Concentration of Test Compounds. In most studies, the same relative dose of FCN used by other investigators, has been employed in our studies. However, we have now determined that 25-fold higher doses of FCN can be tolerated by mice, and we are therefore looking for RF radiation effects using these higher dose. A comparable increase in the concentration of the amino acids utilized in the injection mixture has also been employed.

Whole-Body Heating. Our RF radiation results have consistently shown the absence of an increase in BBB peremeability. Such results might be more emphatic if more were known about the conditions that are required to produce a non-radiation induced increase in BBB permeability. The reports by Carl Sutton and the suggestions of James H. Merritt and Don R. Justeson, resulting from work with rats, prompted us to test whole-body heating with mice. Following injection of the test compounds, mice were placed in a preheated 50° incubator. After a controlled period of heat-exposure, the rectal temperatures were checked and the plasma and brain samples were analyzed. The heat-exposure altered the rate of passage of the test compounds from the peritoneal cavity into the blood, but there was no significant increase in the specific concentration of these substances in the brain as a result of the incubator treatment that produced hyperthermia in the mice.

Pharmacological Compounds. The effects of i.p. injections of urea, glycerol, metaraminol (a vasopressor), and dimethyl sulfoxide (DMSO) on the BBB permeability in mice have been investigated. None have been successful in altering the BBB permeability except DMSO. With the use of DMSO we observed, for the first time in experimentally treated mice, an alteration of the BBB. The rate of passage of one amino acid (thienylalanine) into the brain was markedly increased ($P<0.01$, Student's t-test), while that of another (glucosamine) was significantly decreased. Two other test compounds (FCN, galactosamine) were unchanged.

Rats vs Mice. Since several investigators have reported RF radiation-induced increases in the BBB permeability in rats, we are now comparing the responses of rats with those of mice under closely controlled comparable conditions, to determine whether there is a fundamental BBB difference in these two widely used experimental animals. If such a difference exists, it would be more difficult to anticipate or extrapolate the nature of a similar response in humans.