ELIMINATION OF MICROWAVE EFFECTS ON THE VITALITY OF NERVES AFTER ACTIVE TRANSPORT HAS BEEN BLOCKED

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We have previously reported that exposure to fairly low level microwave fields (an SAR of 10 W/kg at 2.45 GHz CW) would consistently lower the survival time of isolated frog sciatic nerves stimulated at high repetition rates (50 pulse pairs per sec.). The time course of the loss of excitability of the exposed nerve (as compared to its unexposed contralateral mate) is reminiscent of that seen when the active transport of sodium (Na) and potassium (K) is blocked by certain agents - such as the cardiac glycoside ouabain. In order to assess the role that microwaves may be having in interfering with, or counteracting, active transport we have performed a series of experiments in which the active Na-K pump has been substantially blocked by ouabain prior to microwave exposure. Our waveguide exposure and thermal control system is the same as that described previously (Abstracts of Scientific Paper, 1977 International Symposium on the Biological Effects of Electromagnetic Waves, Airlie, Virginia, 1977) and allows us simultaneously to observe the "run down" of a pair of nerves from opposite legs of the same frog, only one of which is exposed to the 10 W/kg microwave SAR. In order to achieve the fastest and most complete blockade of Na-K pump the paired nerves were soaked for 5 minutes in a high concentration (10^{-3} g/l) of ouabain, prior to stimulation at 50 ppps. The "rundown time course" was, as expected, accelerated in all ouabain treated nerves, but the microwave exposed nerve showed no significant further shortening of its survival time (out of 8 experiments, 3 showed slightly longer survival times for the exposed nerve, 2 for the unexposed nerve and 3 others were too close to call). An additional 3 control experiments on ouabain treated nerves, but without microwaves, showed essentially the same results - i.e., no significant difference in vitality between the paired nerves.

We have also repeated these experiments at a lower stimulation rate 5 ppps) wherein the survival time more closely approximates that of the untreated nerves stimulated at 50 ppps (1 or 2 hours versus 30 minutes or less for ouabain treated nerves stimulated at 50 ppps). Preliminary results at these lower stimulation rates indicate that there is still no significant difference in the survival time of paired nerves. The results of these studies, therefore, lend support to the view that the relative loss of excitability in microwave exposed nerves is related to an interference with, or counteraction of the Na-K pump.

Background

This study is an extension of our earlier investigation in which we found that frog sciatic nerves exposed to fairly low level microwave fields (10 W/kg CW at 2.45 GHz) would not survive as long as their unexposed mates (taken from the opposite leg of the same animal) when subjected to high rates of stimulation (50 pulse pairs per sec).[1,2] This difference in the "vitality" of the exposed and control nerves usually manifested itself only after an hour, or more, of repetitive stimulation. It seemed to us therefore, that the microwaves might be interfering with a relatively long term regulatory process - such as the maintenance of ionic concentration gradients - rather than directly affecting the excitation processes of the nerve (which should have manifested itself much sooner).

Nerves are only able to operate (i.e., fire action potentials) as long as a high concentration of potassium $(K)_i$ and a low concentration of sodium $(Na)_i$ ions are maintained inside the neuron relative to the outside concentrations. In the course of maintained activity, $(Na)_i$ tends to build up inside the cell while $(K)_i$ runs down. However, these trends are normally counteracted by the activity of a Na-K "pump" which is driven by ATP. If the function of this pump is compromised, the neuron can still fire thousands of times before the ionic "batteries" (i.e., the Na and K concentration ratios) are depleted enough to preclude excitation - thus giving a time course of "rundown" similar to that seen in our earlier microwave experiments.

The activity of the Na-K pump can readily be blocked by agents such as the cardiac glycoside, ouabain. We reasoned that if the Na-K pump was blocked by ouabain and if the microwave effects were based on interference with, or a counteracting of active transport, the effect ought to be largely eliminated. If on the other hand the "site of action" of the microwaves lay elsewhere (e.g., the ability of the active channel "gates" to open and close) then the reduced survival time of the exposed nerve, with respect to its unexposed mate, ought to still be manifest in ouabain treated nerves. To see which of these suppositions was the more correct one, we have extended our earlier experiments to include pairs of ouabain treated nerves.

Methods

Our experiments were performed on paired sciatic nerves from small to medium specimens of Rana catesbeiana using a waveguide exposure system similar to that of Chou and Guy (1974).[3] The exposed nerve crossed the waveguide near (within 2 mm) the proximal (to the microwave source) surface of the surrounding Ringer's solution, whereas the other nerve (control) was positioned 5 cm distal to the surface and, was, therefore essentially unexposed.

The stimulating and recording electrodes were located outside the waveguide. This configuration eliminated any artifacts due to the electromagnetic fields interacting with the electrodes or recording equipment. A quarter guide wavelength of matching material with a dielectric constant of 6 was used to reduce the reflected power in the waveguide to less than 4% of the incident power. The incident and reflected powers were measured by using a dual directional coupler and power meters. The solution depth was 6 cm and can be considered as a medium of infinite depth. The fact enabled us to use the same relationship,

$$P_a = 4\alpha \frac{P_I - P_R}{A} e^{2\alpha x}$$

as Chou and Guy[3] to determine the specific absorption rates. Thus the nerves proximal to the source were exposed to 2450 MHz radiation at specific absorption rates of 100 W/kg, 50 W/kg, and 20 W/kg while the paired control nerve remained essentially unexposed. Saline was circulated through a water bath into a waveguide exposure chamber in order to maintain a constant temperature at both nerves. The temperature of both nerves was held at 24° $\pm$ 0.05°C throughout the experiment. These temperature measurements were made with small thermistors in the solution both during the exposure and immediately at the termination of exposure. The measurement using the two conditions agreed to within 0.1°C of each other.

In the present series of experiments, nerves were first soaked in a 10^{-3} g/l solution of ouabain octahydrate (Sigma) for 5 minutes. It was found that this unusually high concentration of ouabain was required in order to achieve a fairly rapid blockade of the Na-K pump. In preliminary trials with 10^{-5} or even 10^{-4} g/l of ouabain did not achieve such a rapidity of action consistently.

Results

As expected a sufficient dose of ouabain caused all of the nerves, whether exposed to microwaves or not, to "rundown" faster than untreated nerves. The time in which the compound action potentials (CAPs) decayed to half their original value (when stimulated at 50 ppps) averaged close to two hours in the untreated nerves but was reduced to less than 30 minutes after ouabain treatments. In three control experiments, in which neither nerve was exposed to microwaves, the half decay times were within 15% of each other.

In a series of eight experiments in which the bottom nerve of each ouabain treated pair was exposed to a microwave SAR of 10 W/kg, and stimulated at the usual 50 ppps, no significant difference in half decay times were seen (in 3 cases the exposed nerve lasted longer, in 2 cases, the unexposed nerve lasted longer and in the other 3 cases the difference between the two nerves was unmeasurable). On the average, the differences between the exposed and unexposed nerve was less than that seen in the control group.

An additional set of experiments has been carried out on ouabain treated nerve pairs stimulated at a lower rate (5 ppps) in order to achieve a time course of rundown that would be more comparable to that of the untreated nerves. These experiments, in which the half-decay times again approach two hours, also indicate that there is no significant difference between the survival times of the microwave exposed and the unexposed ouabain treated nerves.

Conclusions

These experiments shown no significant differences in the survival time of microwave exposed, versus unexposed, nerves after blockage of the Na-K pump by treatment with ouabain. These results thus suggest that the microwave effects on nerve vitality that we had previously seen, are associated with the decay of ionic gradients that are normally maintained by active transport. It is still possible, particularly from the results of the studies at 50 ppps, to conjecture that a very slow deterioration of the membrane excitation processes could also be at fault. The results of the experiments at the 5 ppps stimulus rate tend, however, to refute this notion since in these cases, there would be adequate time for such a phenomena to manifest itself, and yet it does not. Of course, if such a deterioration in the excitation processes were dependent both on the rate of stimulation as well as its duration, it could be argued that these experiments do not rule out the possibility altogether.

We can speculate that the supposed effects on the ionic gradients could be due to a direct interference with the operation of the Na-K pump (e.g., by blocking the action of the Na-K ATPase enzyme) than normal. On the other hand, it may also be possible that the microwaves are simply "overburdening" the pump by causing an additional influx of Na (and efflux of K, e.g., as consequence of currents due to rectification). Substantiating these speculations, and resolving between them, will require further experimental work.

REFERENCES

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