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ABSTRACT (Block 19)

We have developed a biological model system for examining the effects of electromagnetic radiation at the cellular level. This system features 1) a simple bacterial model with plasmid DNA which can be manipulated at the molecular level, 2) an assay which includes biological amplification for detecting subtle alterations, and 3) low variability usually inherent in biological assays. This system will now be used to study the effects of nondestructive free electron laser (FEL) radiation on biological processes in whole cells.

Beta

We have tested the model system using microwave radiation and have been able to detect subtle changes in the plasmid-encoded β-galactosidase activity. The effect observed is not duplicated by bulk heating of the bacterial culture and there is no effect on purified β-galactosidase.

INTRODUCTION

There are many uses of lasers in medicine and free electron laser (FEL) radiation may add greatly to the arsenal of medical tools. The particular advantage of FEL radiation is that tunability of the laser allows targeting of very specific biological processes or molecules. In order to take full advantage of FEL radiation, however, more basic research needs to be done to define the biologically useful targets and the frequency of radiation required to affect those targets. In order to define possible targets for FEL radiation, we have designed a model system to study the effects of electromagnntic radiation on whole cells.

The difficulty in demonstrating unambiguous bioeffects of electromagnetic fields, along with the irreproducibility of prior work, suggests that, if nonthermal effects do exist, they are subtle. Acknowledgement of this elusive nature should be of prime importance to investigators in their choice and design of biological model systems. Instead, the previous research on the biological effects of low level electromagnetic radiation has been primarily phenomenological utilizing measurements of often complex traits. The failure of such experiments to unambiguously demonstrate the existence of nonthermal biological effects of microwaves is due to 1) the complexity of the measured traits which complicates sensitive quantitation, 2) the inability to detect small alterations in some specific processes, and 3) the ability of the studied organism to respond and adapt to induced changes. In short, the noise is too great to accurately measure the signal. Because small populations are used, random biological variability confounds data analysis. Many of the studied parameters can neither be amplified nor studied in real time (i.e. there is a delay between irradiation and assessment of any effect), and gross phenotypic traits do not readily yield to sensitive quantitation. Furthermore, proof of a nonthermal effect will require relating the observed effect to the mechanism(s) of interaction. This is not possible in most systems studied.

We do not mean to imply that prior research has not contributed to the understanding of the interaction of electromagnetic fields with biological systems. In fact, many intriguing results do suggest the existence of nonthermal effects and are a motivation to attempt to clearly define the mechanisms involved. However, the limitations of the biological systems previously employed must be recognized. Demonstration of the existence of nonthermal electromagnetic radiation-induced perturbations and unequivocal determination of the molecular mechanism(s) by which these perturbations occur will require a simple, well-characterized, quantifiable, and easily manipulated system whose processes can be examined individually at the molecular level. By demonstating that the radiation alters a quantifiable and specific molecular parameter, such as enzyme activity, the mechanism(s) of radiation-induced perturbations can be systematically investigated.

RESULTS OF THE PAST YEAR

The model system

For the study of the interaction of microwave radiation with biological systems, we have selected a model system that has several advantages over earlier approaches. The choice of a simple bacterial model, E. coli, was based on its ease of handling and the minimal facilities required, hopefully facilitating and making more attractive repetition of the work by interested groups. E. coli is easily manipulated physiologically and genetically, and, if electromagnetic fields are found to affect the system, a full range of biochemical and molecular biological techniques can be applied leading to deeper levels of understanding.

The choice of β-galactosidase activity as the measured endpoint is based on the prior understanding of that system, the number of processes involved, and the benefits accorded by the assay. In E. coli JM101 containing pUC8, β-galactosidase activity results from complementation of polypeptide products from the pUC8 lacZ gene and the F' episomal lacZΔM15 gene. The regulation of the E. coli lacZ gene is one of the best characterized genetic regulatory systems. Many molecular interactions are required to produce enzyme activity including those involved in replication, gene induction and transcription, and translation. Because so many processes in addition to enzyme kinetics are involved in determining the activity in the culture, the chances for demonstrating an effect are improved.

Quantitation of β-galactosidase activity

The assay for β-galactosidase activity itself offers many useful features. The assay we have used for these initial studies is the simple inclusion of a chromogenic substrate in the growth medium. Hydrolysis of the substrate, such as nitrophenyl-β-D-galactoside (NPG) or 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) by β-galactosidase produces an easily quantitated chromophore. The substrate can be added at the beginning of irradiation, and quantitation can be achieved by non-invasive spectrophotometric methods; this approach gives three major benefits. First, since the chromophore is stable, the amount of chromophore produced represents the integral over time of β-galactosidase activity in the culture, giving biological amplification of any signal. This provides greater sensitivity than is possible by looking at the enzyme activity in the culture at only one time point. The second benefit of the assay is that chromophore production occurs over the course of irradiation allowing quantitation of β-galactosidase activity in real time, i.e. there are no delays between the time of irradiation and quantitation. A third and very important benefit of this assay is that complicated manipulations of the culture which can introduce experimental variations likely to mask small effects are avoided.

Assay variability

As previously mentioned, random biological variability has likely confounded many other similar investigations. Manipulations of biological samples do not allow for the same precision characteristic of the engineering aspects of these experiments, and the random errors may be as large as the induced effect. When complex cells or organisms, it is difficult to employ a large enough population to overcome the random biological variability. In our assay, large bacterial populations are used and a single culture can be split for control and irradiation eliminating the variability associated with multiple set-ups. This leads to minimal experimental errors within a single experiment. We find that within a group of identical samples set-up from one initial culture the largest difference observed between any two samples is 3%, although typically variations are less than 2%.

Effects of heat on the model system

In studying biological effects of nonionizing radiation, it is of primary importance to distinguish between thermal and nonthermal mechanisms. Even though heroic efforts can be made to maintain sample temperature, arguments of local heating are difficult to dismiss since energy is obviously deposited into the system, particularly with microwave fields. Therefore, in order to define the mechanism of any effect, the model system needs to be characterized as to the effect of heating. To determine the effect of increased temperature on our model system, we have set up a control experiment in which, according to our standard protocol, a single culture containing X-gal was aliquoted into several tubes which were randomly placed into water baths maintained at varying temperatures. Over the range of 35 to 38 , there are no effects of increasing temperature discernable above the variability inherent in the assay. Above 38 however, both cell growth and chromophore production are decreased relative to the lower temperatures. This information is critical in determining whether observed effects are nonthermal.

Microwave radiation increases β-galactosidase activity

Cultures of JM101 containing the plasmid pUC8 were maintained at 38 and irradiated with 2 mW/g of 2.55 GHz radiation for 6 hr. This caused a 7.3% increase in the β-galactosidase activity in the sample relative to the control. This effect is not duplicated by irradiating purified β-galactosidase. Since increasing the temperature above the 38 control level results in decreased β-galactosidase activity, these results suggest the effect of microwave radiation is nonthermal in nature.

DISCUSSION AND FUTURE DIRECTIONS

Given the past difficulty in demonstrating nonthermal effects of nonionizing electromagnetic radiation, it is apparent that, if such effects exist, they are likely to be subtle. Therefore, if attempts are to be made to search for small effects, the model system examined must be capable of allowing detection. The system described has the features required for sensitive quantitation of small effects. The primary advantages of our system are 1) there is biological amplification of the signal, 2) the assay does not perturb the system, and the assay requires no manipulations which can affect the measured parameters, and 3) a deeper level of understanding can be achieved through molecular biological techniques and physiological and genetic manipulation of the system.

This model system will be useful for studies on FEL radiation. Radiation of the cells with frequencies known to affect particular processes and molecules may prove very useful for defining new medical uses of FEL radiation. For example, Prohofsky has predicted vibrational modes in DNA that are important in DNA melting such as occurs during replication. These modes lie within the frequency range covered by the UCSB FEL facility and will be an exciting target to test.