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SUPPRESSION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY IN VITRO AFTER LASER EXPOSURE

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Extracellular field potentials were recorded in the stratum pyramidale of CA3 in hippocampal slices prepared from male Sprague-Dawley rats. Electrical stimuli were delivered to s. radiatum of CA3. After stable responses were established, stimulus trains were delivered every 5 mm until stable triggered and spontaneous population bursts were elicited. The slices were then irradiated with a low power (25 mW) argon laser. No changes in the morphology or number of epileptiform bursts were found while the laser was on. However, when the laser was turned off, there was a highly significant reduction in frequency and morphology of bursts. These results indicate that exposure to light alters epileptiform activity within the hippocampus in vitro, and provides evidence indicating that the central nervous system is photosensitive.

Key words: Central nervous system, Low power laser, hippocampal epileptiform activity

Introduction

At present, most biomedical applications of laser rely on the observation that exposure to monochromatic light results in a thermal effect. However, photo-chemical phenomena remain largely unexplored although several observations indicate that neural tissue may be photosensitive. For example, brief exposure to 488-nm argon laser alters the firing pattern of isolated abdominal cells of Aplysia. This change is not due to heating because it occurs before there is a measurable increase in temperature. Very brief irradiation with a ruby laser (694 nm) results in a large increase in acetylcholine concentration in an isolate vertebrate nerve preparation and introduction of a helium-neon laser (625 nm) into rodent brain via fibre optics procedures behavioural and neurochemical changes. Irradiation of peripheral nerves in humans with a low power helium neon laser produces an evoked potential and depresses spinal reflexes. These events, too, are thought to be photochemical in nature.

We have studied the effect of the argon laser on an in vitro model of epileptiform hyperexcitability in the hippocampal slice. We now report a decrease in spontaneous and electrically evoked activity in this preparation after laser irradiation. This change occurs without a significant increase in temperature, and this report constitutes the first report indicating that laser irradiation can alter neuronal hyperexcitability.

Methods

Male Sprague-Dawley rats, 150 to 200 g, were decapitated and the hippocampi removed. Transverse slices, 625 μm thick, were cut on a McIlwain tissue chopper and incubated for at least 1 h in a holding chamber containing oxygenated (95% O₂—5% CO₂) artificial cerebrospinal fluid (ACSF) at room temperature. The ACSF composition was (in millimolar): NaCl, 120; KCl, 3.3; NaH₂PO₄, 1.23; NaHCO₃, 25; MgSO₄, 1.2; CaCl₂, 1.8; and dextrose, 10. Slices were studied individually in a submersion chamber perfused at a rate of 4 to 5 ml/mm with ACSF at 31°C. Each slice was stimulated electrically using a Grass S88 stimulator, SIU5 stimulus isolation unit and a sharpened, monopolar, platinum-irridium electrode, the tip of which was positioned in the stratum radiatum of CA3. Extracellular fields were recorded in the stratum pyramidale of CA3b-c using a 2 M NaCl-filled, glass microelectrode (1—15 MΩ), a high impedance D.C. amplifier and a digital oscilloscope. Permanent records were made from the oscilloscope with an XY plotter.

Stimulus-train induced bursting (STIB) was produced as described previously. Briefly, single stimuli (0.05 ms, monophasic, rectangular pulses) were applied at varying voltages to determine the intensity which evoked the maximum orthodromic population spike.

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Baseline stimuli of that intensity were then applied at 0.02 Hz for 20 min to ensure that the evoked response remained stable. Stimulus trains (60 Hz, 2 s duration) were then delivered every 5 min at twice the baseline intensity. Baseline stimulation was resumed between trains. Stimulus trains were continued until we noted stable, persistent epileptiform activity in the form of spontaneous and even 5-min triggered bursts between trains.

Separate experiments were conducted in order to ascertain whether laser irradiation at the intensity used on hippocampal slices caused significant membrane damage. The effect of a 5-mm exposure upon the intrasynaptosomal ionic calcium content within isolated synaptosomes was determined using a fluorescent probe fura-2 in a procedure developed specifically for synaptosomes.

The in vitro system was irradiated with a 25 mW single line argon ion laser with a wavelength of 488 nm. (Spectra Physics, Mountain View, California, model 162A-07) and a 262D power supply. The light was coherent, and beam diameter was 0.65—0.67 mm. Duration of irradiation was 4-5 mm. This exposure produced an increase of 0.2°C on a thermometer.

Results

The frequency of spontaneous bursts was quantified by expressing the number of bursts per mm in the presence and after laser exposure as a percentage of the control value before laser application. Group means and standard errors were calculated with each slice serving as its own control. Table 1 demonstrates that application of the blue laser produced no significant effect upon the frequency of spontaneous bursting. However, after laser irradiation was discontinued, there was a highly significant decrease in spontaneous burst frequency. This decrease was observed in 8/9 slices.

Exposure to laser energy was also associated with a decrease in the number and amplitude of population spikes within each spontaneous burst. Figure 1 shows a continuous record of one representative experiment. Before, during and after laser application individual spontaneous bursts were recorded at high speed. It was clear that the bursts recorded after the termination of laser irradiation was minimal (0.2°C). In summary, the results provide evidence indicating that exposure to monochromatic light results in a temporary reduction of the frequency and morphology of epileptiform discharges in area CA3 of the hippocampal slice.

Discussion

We have demonstrated a significant decrease in the frequency and an alteration in the morphology of epileptiform discharges in hippocampal slices as a result of irradiation with a 25 mW single blue line argon ion laser. Interestingly, all of the effects occurred after laser exposure (laser ‘off’) and not during exposure (laser ‘on’). The electrophysiological changes were not due to disruption of membrane function as there were no changes in synaptosomal calcium during or after laser exposure. The maximum temperature change during the experiment was minor (0.2°C). In summary, the results provide clear in vitro evidence indicating that exposure to monochromatic light results in a temporary reduction of the frequency and morphology of epileptiform discharges in area CA3 of the hippocampal slice.

Does exposure to such laser produce deleterious effects on the slices? In general, toxic agents such as kainic acid and bicuculline increase the excitability of hippocampal cells, are irreversible, and frequently increase calcium content. Exposure to laser has none of these characteristics. In fact, laser irradiation mimics the effects of anticonvulsant drugs such as benzodiazepines, barbiturates, and baclofen. These agents are thought to block GABA B receptors and laser irradiation may involve a similar neurochemical substrate.

The observation that neural tissue is photosensitive is surprising. Chromophores (optically active molecules) have been described in the retina and the skin, which share common embryological origins with neural tissue. More recently, rhodopsin kinase, an enzyme involved in photochemical transduction in the retina, has been shown to occur in mammalian pineal and, to a smaller extent, mammalian CNS. In classically pho-
tosensitive structures, rhodopsin kinase phosphorylates rhodopsin-like integral membrane receptors and is involved in signal transduction. Whether such a system operates in the CNS is unknown, but is certainly testable. This in vitro system affords an excellent model in which to study the action spectrum of the photochemical effect and the mechanism of transduction.

References