Effect of helium-neon laser on fast excitatory postsynaptic potential of neurons in the isolated rat superior cervical ganglia

Hua Mo (莫 华)[1], Ping He (何 萍)[2], and Ning Mo (莫 宁)[2]

[1]*Department of Physics;* [2]*Department of Pharmacology, Guangxi Medical University, Nanning 530021*

Received January 16, 2004

The aim of this study is to further measure the effect of 632.8-nm helium-neon laser on fast excitatory postsynaptic potential (f-EPSP) of postganglionic neurons in isolated rat superior cervical ganglia by means of intracellular recording techniques. The neurons with f-EPSP were irradiated by different power densities ($1-5$ mW/cm^2) laser. Irradiated by the 2-mW/cm^2 laser, the amplitude of the f-EPSP could augment ($P < 0.05$, paired t test) and even cause action potential at the end of the first $1-2$ minutes, the f-EPSP could descend and last for $3-8$ minutes. But the amplitude of the f-EPSP of neurons irradiated by the 5-mW/cm^2 laser could depress for the irradiating periods. The results show that: 1) the variation of the amplitude of f-EPSP caused by laser is power density-dependent and time-dependent; 2) there exist the second-order phases in the interaction of the helium-neon laser with neurons. These findings may provide certain evidence in explanation of the mechanisms of clinical helium-neon laser therapy.

OCIS codes: 140.0140, 330.5370, 170.0170.

Low intensity laser therapy has been used in a variety of clinical trials and remains a controversial issue due to its uncertain mechanism[1,2]. In order to research the mechanism of low intensity laser biostimulation on neurotransmission, we investigated helium-neon laser influential with sympathetic ganglia. In previous work[3], we studied the effects of helium-neon laser with wavelength of 632.8 nm on membrane conductance of neurons with stable fast excitatory postsynaptic potential (f-EPSP) in isolated rat superior cervical ganglia. The experiment showed the variation of the membrane conductance of neurons with stable f-EPSP irradiated by laser with power densities of 2 and 5 mW/cm^2. In the experiments, we attempted to identify the effects of 632.8-nm helium-neon laser on f-EPSP of postganglionic neuron of the isolated rat superior cervical ganglia by means of intracellular recording techniques.

Adult wister rats ($250-300$ g, aged $3-4$ months) were used in this study. The animals were killed and their right and left suprior cervical ganglia, together with their cervical sympathetic nerve trunks, were rapidly excised and transferred to the recording chamber. The ganglia were superfused with Krebs solution of the following composition (in millimolar): NaCl, 117; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 11.5[4]. The solution was equilibrated with 95% O_2 and 5% CO_2 and the temperature of the solution was maintained at 34.0 ± 0.5 °C. Intracellular recording was obtained from ganglion cells by means of fiber-containing glass micro-electrodes filled with 3-mmol/l KCl solution, which had resistance of $15-40$ MΩ. Electrodes were connected via a bridge-balance headstage to an amplifier (W-701,WPI) permitting current injection. Amplified signals were either displayed in an SBR-1 oscilloscope or recorded on an LBS-2B recorder. Cell input membrane conductance was measured from the slope of the current-voltage (I-V) relationship of the cell. The cervical sympathetic (preganglionic) nerve trunk was drawn into a suction electrode for orthodromic stimulation. Single electrical stimulation applied to the cervical sympathetic trunk elicits a f-EPSP in the ganglion cells[5,6]. The ganglia in the recording chamber were irradiated by laser beams with different intensities. Numerical results are expressed as a mean±SEM (standard error of mean) and statistical analysis was performed using the paired t test.

Determined from electrotonic potentials of the cells caused by constant hyperpolarizing current pulses, the membrane conductance and membrane time constant of the cells were 16.5 ± 7.4 nS (cell number $n = 45$) and 6.7 ± 1.8 ms.

Single electrical stimulation (frequency 2 Hz, duration 0.1 ms, voltage $5-10$ V) applied to the preganglionic nerve trunk elicits the f-EPSP in the ganglion cells. The f-EPSP had an amplitude of $4-20$ mV and duration of $7-12$ ms after a latency of $6-7$ ms ($n = 42$). With constant stimulation, the I-V relation curve was plotted from electric tension potentials of the cells during the period of the f-EPSP. The estimated slope membrane conductance of the cells measured from the I-V relationship was 26.8 ± 6.2 nS ($n = 42$). The membrane time constant of the cells was 6.3 ± 2.1 ms.

In contrast to the enhancing effect at different intensities of laser at 1, 2, 3, 4, and 5 mW/cm^2, 32 of the 42 cells examined with f-EPSP were used. Table 1 summarizes the percentage increase in the amplitude of f-EPSP induced by laser ($1-5$ mW/cm^2). It can be seen from Table 1 that the variation of the amplitude of f-EPSP caused by laser was power density-dependent. Laser at 2 mW/cm^2 or smaller power densities increased the amplitude of f-EPSP, and laser at 3 mW/cm^2 or greater power densities reversibly depressed the amplitude of f-EPSP to a certain degree.

The ganglion cells with stable f-EPSP in the recording chamber were irradiated for $1-2$ minutes by laser with power density of 2 mW/cm^2. The amplitude of f-EPSP in 22 irradiated cells noticeably increased by $7-23$ mV,

Table 1. Effects of Laser Power Density on the Amplitude of f-EPSP Evoked in Cells of the Rat Superior Cervical Ganglion

Laser Power Density (mW/cm^2)	Cell Number (n)	Amplitude Percentage Change of f-EPSP*
1	6	14.67±3.04
2	10	33.15±8.67
3	6	−17.32±5.29
4	5	−21.71±2.41
5	6	−45.45±9.56

Negative values denote decrease over the original value. The values are expressed as mean±SEM.
*Statistically significant, $P < 0.05$, paired t test.

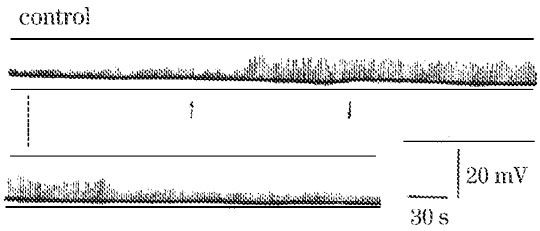

Fig. 1. An enhancement of the amplitude of f-EPSPs of cells irradiated by laser at 2 mW/cm^2 during 1 − 2 minutes (indicated by two arrows).

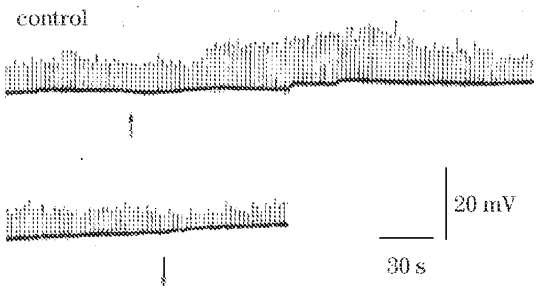

Fig. 2. A reduction of the amplitude of f-EPSPs of cells irradiated by laser at 2 mW/cm^2 during 5 minutes (indicated by two arrows).

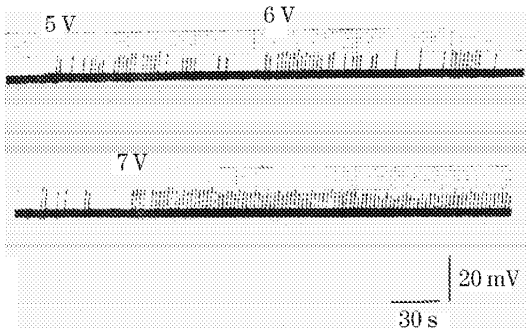

Fig. 3. The irradiation of laser at 2 mW/cm^2 decreased the intensity threshold and enhanced frequencies of electrical stimulation elicited f-EPSP.

this increase ranged from 13% to 75%, with a mean of 41% (Fig. 1). Some f-EPSPs reached the threshold to generate action potentials in 7 of the 22 cells. The duration of the f-EPSPs in 11 cells had a slight increase and the latency of the f-EPSP showed no change during irradiating period. Accordingly, the I-V relation curve of the cells irradiated for 1 − 2 minutes was plotted, and slope membrane conductance and membrane time constant of the cells were 34.6±5.4 nS ($n = 22$) and 6.5±1.8 ms, the membrane conductance had significant increase ($P < 0.05$, paired t test).

Under the above conditions, the ganglion cells with stable f-EPSP in the recording chamber continued to be irradiated for 3 − 8 minutes, and the amplitude of f-EPSP observed was noticeably decreased and membrane time constant was 7.0±2.6 ms ($n = 15$). Accordingly, the amplitude of f-EPSP was 2 − 8 mV, this decrease ranged from 50% to 60%, with a mean of 52% (Fig. 2). The duration and the latency of these f-EPSPs showed no change during the irradiating period.

When changing the power density of laser to 5 mW/cm^2 in the 5 cells irradiated for 1 − 2 minutes, the amplitude of f-EPSP and membrane conductance of the cells decreased.

In genenal, in our experiment the intensity threshold of electrical stimulation elicited f-EPSP is more than 8 V (freguency 2 Hz, duration 0.1 ms). But by laser irradiation at 2 mW/cm^2, a clear decrease of the intensity threshold was observed in the four examined cells, leading to enhanced frequencies of elicited f-EPSP (see Fig. 3).

Helium-neon laser with 1 − 5 mW/cm^2 power densities in this experiment belonged to low intensity laser in clinical trial. From the semiconductor thermometer in the recording chamber, the temperature variation of the ganglion cells irradiated by laser was less than ±0.1 °C. So, the changes of membrane conductance and of the amplitude of f-EPSP may be biostimulatory effects of helium-neon laser on ganglionic transmission, not thermal effects.

The fast EPSP is evoked by Nicotinic receptor on postsynaptic membrane activated by acetylcholine chloride (Ach) from presynaptic membrane. Single electrical stimulation of the cervical sympathetic trunk elicits in the ganglion cells an EPSP or multiple EPSPs of varying latencies. Among them a f-EPSP is the main type of ganglionic transmission in the sympathetic neurons. As we know, a photon of the 632.8-nm helium-neon laser is with the energy of 1.9 eV, which just equals to an energy unit of the process of metabolism. When the electric conductance zone of a cell is irradiated by helium-neon laser, according to the principle of resonance, quantum transition will occur, resulting in cell's metabolism to refresh arrangement. In order to further investigate the effect of laser on the ion conductivity of postsynaptic membrane, different ion concentrations of Krebs solution were used in our experiment. The test showed that in the case of high-K^+/low-Na^+ Krebs solution, the decreased f-EPSP amplitude noticeably increases after 2-min, 2-mW/cm^2 laser irradiation. Because f-EPSP is produced and controlled by Na^+/K^+-mediated ion channels in the cell membrane, and in the case of high-K^+/low-Na^+ Krebs solution, the ion conductivity of cell membrane generally decreases. The result indicated that the ion conductivity of postsynaptic membrane can be increased by 2-mW/cm^2 laser irradiation in short time. For this reason, by laser irradiation, increased membrane conduc-

tance would evoke an amplitude of f-EPSP to reach the threshold to discharge an action potential. It was therefore suggested that the helium-neon laser biological effect on the fast excitatory synaptic transmission would be influenced by membrane conductance changed by laser irradiation.

The fact of neurons with f-EPSP irradiated by laser with different power densities ($1 - 5$ mW/cm^2) showed that when the cells were irradiated by the 2-mW/cm^2 laser, the amplitude of f-EPSP could augment ($P < 0.05$) and even cause action potential at the end of the first $1 - 2$ minutes, and could descend and last for $3 - 8$ minutes later; when the cells were irradiated by the 5-mW/cm^2 laser, the amplitude of f-EPSP could depress for the irradiating period. It was indicated that the variation of the amplitude of f-EPSP caused by laser was laser power density-dependent and time-dependent. The results show that, in the case of lower laser intensity, there exist the second-order phases in the interaction of the helium-neon laser with neurons: firstly, the initial, short-time promotion or positive effect, and secondly, the late, long-time effect of inhibition or negative effect. These findings will help to find suitable dose for laser clinical treatment.

We can approximately approach the second-order phases with the mechanism of photobiology. Electron transport respiratory chain is an original accepter of light; the absorption of light alters the energy of molecules in the membrane chromophore of electron transport respiratory chain to some excited states as follows. 1) The irradiation of lower intensity or short-time laser activates chondriosome to compound adensine triphosphate (ATP) through adensine diphosphate (ADP): ATP↑[2,7,8], and ATP, as an excitatory neurotransmitter in the nervous system, mediates fast synaptic transmissiom[8] and increases the membrane conductor and the amplitude of f-EPSP in our experiment. 2) The irradiation of high intensity or long-time laser results in the photoactivation of chondriosome through photosensitizer (cytochrome) to produce photodynamic action and decreases the membrane conductor and the amplitude of f-EPSP in our experiment. This is the second-order phase and its behavior might be an essential feature that enables neurons to respond to low intensity laser irradiation. Our findings may provide certain evidence in explanation of the mechanisms of clinical helium-neon laser therapy.

This work was supported by the Natural Science Foundation of Department of Education, Guangxi, P. R. China under Grant No. 200028. H. Mo's e-mail address is mohuawuli@vip.sina.com.

References

1. Q. M. Luo, X. D. Liu, and Z. G. Li, Chin. J. Laser Med. Sury. **3,** 39 (1994).
2. T. C. Liu and S. H. Liu, Chin. J. Laser Med. Sury. **6,** 125 (1997).
3. H. Mo, P. He, A. X. Liang, and N. Mo, Acta Laser Biology Sin. **11,** 369 (2002).
4. N. Mo, N. J. Dun, and A. G. Karczmar, Neuropharmacology **24,** 1093 (1985).
5. N. Mo and N. J. Dun, Neuroscience Lett. **64,** 263 (1986).
6. N. J. Dun, Z. G. Jiang, and N. Mo, J. Physiol **357,** 37 (1984).
7. M. W. Salter, Y. de Koninck, and J. L. Henry, Prog. Neurobiol. **41,** 125 (1993).
8. R. J. Evens, V. Derkach, and A. Surprenant, Nature **357,** 503 (1992).