ELSEVIER

Neuroscience Letters 240 (1998) 93–96

Neuroscience Letters

Suppressive effects of low-power laser irradiation on bradykinin evoked action potentials in cultured murine dorsal root ganglion cells

Keita Jimbo, Kosuke Noda, Kazuo Suzuki, Kentaro Yoda*

Department of Bio-Medical Engineering, Tokai University, 317 Nishino, Numazu 410–03, Japan

Received 30 May 1997; received in revised form 7 November 1997; accepted 8 December 1997

Abstract

The effects of Ga-Al-As diode laser (830 nm, 16.2 mW) irradiation on the distal portion of the processes of cultured murine dorsal root ganglion (DRG) neurons associated with C-fibers were studied by patch-clamp whole-cell recording of membrane potentials at the cell body. The chemical as well as laser light stimulations were limited to the processes of the neuron isolated from the cell body with a separator. The action potentials elicited by bradykinin (BK) in the cell body were reversibly suppressed by the irradiation of laser light. The laser irradiation may block the conduction of nociceptive signals in primary afferent neurons. The present experimental method offers a simple and easy to use procedure for studying the pain relief effects by laser irradiation. © 1998 Elsevier Science Ireland Ltd.

Keywords: Low-power laser; Cultured neurons; Dorsal root ganglion; C-Fibers; Bradykinin; Action potential; Pain relief

Low-power laser irradiation has been used for the treatment of various kinds of pain [12,13]. However, the mechanisms of the pain relief effects of laser irradiation have not yet been completely understood in spite of the intensive studies by many groups. The mechanisms proposed so far are diverse and include: (1) an increase in peripheral blood flow [5], (2) the inhibition of release of algesic substances at the nerve endings [7], (3) changes in conductivity of primary afferent neurons [10,14,16–18], and (4) an activation of descending inhibitory pathways from higher centers [8,15]. In the traditional studies most experiments were carried out with animals or human volunteers. Therefore, the experimental systems were too complex to estimate the dose of laser irradiation and the intensities of noxious stimuli. To avoid the complexities, the utilization of cultured cells as experimental systems is beneficial though it has some limitations.

The purpose of the present study is to establish a simple experimental system suited for the fundamental studies on pain relief effects of laser irradiation. In the present study, a combination of the cultured murine dorsal root ganglion (DRG; peripheral afferent neuron) neuron and bradykinin (BK; algesic compound) was selected as a model for pain relevant system. In the clinical laser therapy, the cell bodies of peripheral afferent neurons are not stimulated by laser light but nerve fibers are stimulated. In the present experiments the processes of neuron were stimulated separately from the cell body using a separator, and the effects of Ga-Al-As diode laser irradiation to the processes were analyzed by intracellular recording of membrane potentials at the cell body. Free nerve endings in the peripheral tissue are activated by algesic substances released from cells damaged by noxious stimuli. In particular, BK is produced at injury sites by enzymatic cleavage from large plasma proteins, exciting peripheral unmyelinated C-fibers and thin myelinated A-δ fibers [2]. BK is known to depolarize cultured nociceptive neurons [1,4].

Young adult (7–8 weeks) male mice (Jcl:ICR) were killed by deep anesthesia with diethyl ether. About 40–45 DRGs were aseptically removed from a mouse. DRGs were dissociated in 2 ml of Ham's F12 medium with 0.2% col-

* Corresponding author. Tel.: +81 559 681111 ext. 4529; fax: +81 559 681156; e-mail: kyoda@wing.ncc.u.-tokai.ac.jp

0304-3940/98/$19.00
PII S0304-3940(97)00935-X

lagenase at 37°C for 90 min in a shaking bath. After a further 15 min incubation in Ca^{2+}- and Mg^{2+} free Hanks' balanced salt solution (HBSS) containing 0.25% trypsin, the tissues were triturated in HBSS containing trypsin inhibitor (0.05 mg/ml) and added to a 30% Percall solution. They were then subjected to density gradient centrifugation $200 \times g$ for 4 min, resulting in a yield of more than 5×10^4 neurons per mouse with high purity.

The dispersed neurons were washed three times with F12 containing 10% fetal bovine serum (FBS), and seeded onto the 35 mm culture dishes coated with poly-L-lysine. Cell density was adjusted to about 3×10^3 cells/cm^2. DRG neurons were kept in medium and allowed to adhere to the substrate for 4 h in a humidified atmosphere containing 5% CO_2 at 37°C. The culture solution with cellular debris in it was removed and replaced with fresh medium containing 10% FBS and 100 ng/ml 7S nerve growth factor (NGF). After 24 h, the culture solution was removed and replaced with a serum-free Ham's F12 culture medium containing 5 μg/ml insulin, 5 μg/ml transferrin, 2×10^{-8} M progesterone, 0.1 mM putrescine, 3×10^{-3} M selenium, and 100 ng/ml NGF. Medium change was carried out every other day. In DRG culture, cells of smaller than 25 μm in diameter were used in all experiments. Because of cell size (smaller than 25 μm), resting potential characteristics (–45 mV), and longspike duration (10–15 ms), the processes of the cells used in the experiments might be unmyelinated C-fibers [3,11].

Membrane potentials (intracellular potential) were recorded using the patch-clamp technique. Voltage recordings were obtained by the whole-cell current-clamp method [6]. The experiment was done on the stage of the microscope using a warm-plate at 30°C. The dish solution for recording contained (in mM): NaCl 145, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.13, glucose 10, HEPES 10 (pH 7.4). The patch-pipette solution contained (in mM): KCl 140, $MgCl_2$ 2.0, HEPES 10, EGTA 0.5 (pH 7.4).

Separation of the process from the cell body (diameter smaller than 25 μm) was done by putting a separator in culture dish as described in our previous literature [11]. Cells used for separation were those of the third day after seeding, which had long processes. We carefully selected a neuron without network formation. Upon stimulation by BK limited to the processes, action potentials were recorded at the cell body. The separator was made of thin aluminum foil (15 μm thick) coated with epoxy-resin insulator and the frame of the separator-compartment was made of Teflon. To check that the solution outside the separator-compartment did not pass into inside throughout the experimental period, the dye (trypan blue) was used, and no diffusion inside was confirmed prior to experiments. The solution outside the separation was perfused slowly, in which BK was added using micropipette. The final concentration of BK in the dish was adjusted to 10 μM evoking a single spike of action potential, by adding 50 μl of 200 μM BK solution into the dish.

Infrared light (wave length 830 nm, output power 16.2 mW, focal distance 4.5 mm) was generated from the Ga-Al-As diode laser irradiation system (Tokyo Iken, Four-Luck DL-130, Japan). Fig. 1 shows a continuous irradiation applied to the distal portion of a process separated from the cell body by a separator. The irradiation area was round with a diameter of about 75 μm. The energy density (J/cm^2) was estimated as the total energy divided by the irradiation area. The total energy of irradiation (J) was calculated as the product of the power (W) and the duration of irradiation (s).

In the first place, the effects of laser irradiation on the cultured DRG neuron were examined without BK stimulation. After the electrical stimulation with depolarization pulse to the cell body was confirmed to evoke the action potential, irradiation was done to the cell body for 2 min. The frequency of the spike was enhanced after irradiation (Fig. 2A), except for the case in which the irradiation was limited to the process (Fig. 2B). This experiment was done three times with different neurons resulting in similar recordings.

Next, the effects of laser irradiation to the process of the cultured DRG neuron were examined under BK stimulation. Following the electrical stimulation (70 pA, 500ms) that evoked action potentials, 10 μM BK was applied to a process of DRG neuron, by which action potentials were elicited in the cell body. Then, laser light was irradiated to the same process for 1 min (1 J/cm^2) and 10 μM BK was subsequently applied to the same process, having no action potential observed in the cell body (Fig. 3). BK application was repeated after a 1-min interval. Two minutes after the irradiation, action potentials were again observed by BK stimulation. On the contrary, no action potential was

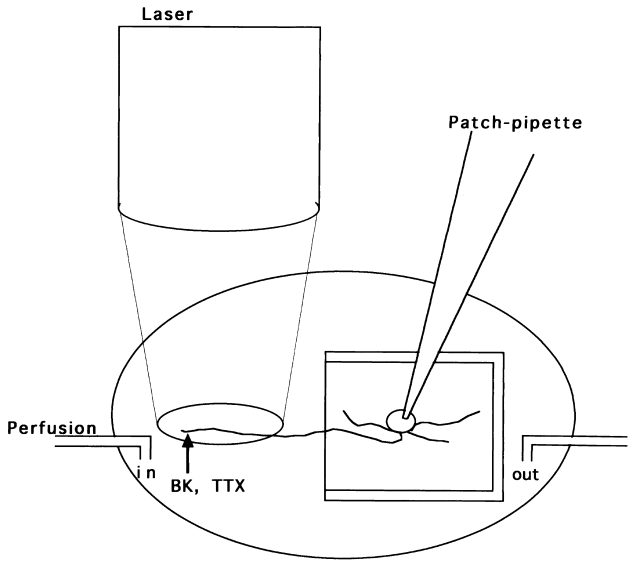

Fig. 1. Experimental diagram for the separation and laser irradiation. The separator was put in the dish to isolate a process from the cell body. The BK stimulation and laser irradiation were applied at the distal portion of a process separated from the cell body by a separator.

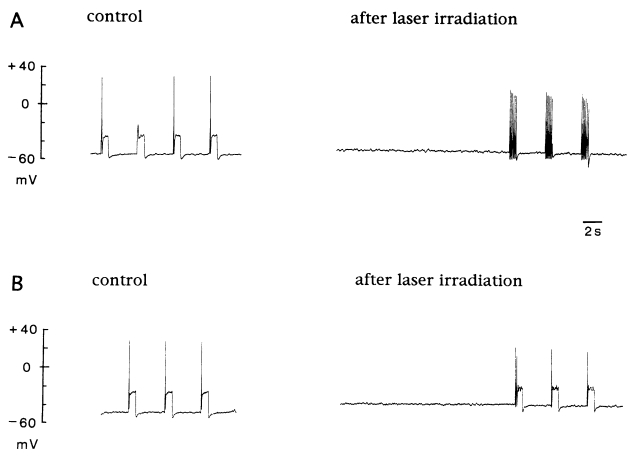

Fig. 2. Effects of laser irradiation on the membrane potential. (A) The cell body was irradiated by laser light for 2 min. (B) The process was irradiated by laser light for 2 min. Action potentials were evoked by electrical stimulation with depolarization pulse (70 pA, 750 ms duration) to the cell body.

observed when BK application and 1-min laser irradiation were done in turn for 5 min. These experiments were repeated with three different neurons, and results were similar as described above.

After confirmation of action potentials elicited in the cell body by the application of 10 μM BK limited to a process of the cultured DRG neuron, the effects of a Na^+-channel blocker Tetrodotxin (TTX; 6 μM) were examined, which resulted in the depression of the depolarization induced by subsequent BK applications.

Since the discharge frequency of action potentials was not changed by the laser irradiation to the process but increased remarkably by the irradiation to the cell body, the irradiation should be limited to the distal portion of the process to examine the effects under nearly same physiological conditions as practical clinical cases.

Low-power laser irradiation suppressed reversibly the action potentials induced by BK in a time-dependent and energy-dependent manner. Thus, it is suggested that the low-power laser irradiation may block the conduction of nociceptive signals in primary afferent neurons associated with unmyelinated C-fibers. Some different methods other than electrophysiology should be required to elucidate the molecular mechanism of the blockage.

The fact that TTX depressed the action potentials evoked in the process by BK suggests the inhibition of the activation of TTX sensitive Na^+-channel on the membrane of the process or the inhibition of the conductance along the process, but the activation of Na^+-channels has definitely to be confirmed by voltage-clamp experiments.

It has been reported that low-power laser irradiation selectively inhibits noxious signals in primary afferent neurons associated with Aδ- and C-fibers in vivo [17,18]. On the other hand, longterm effects have been shown by the in vivo experiments in which Ga-Al-As diode laser irradiation reduced the elevated mitochondrial density of the trigeminal

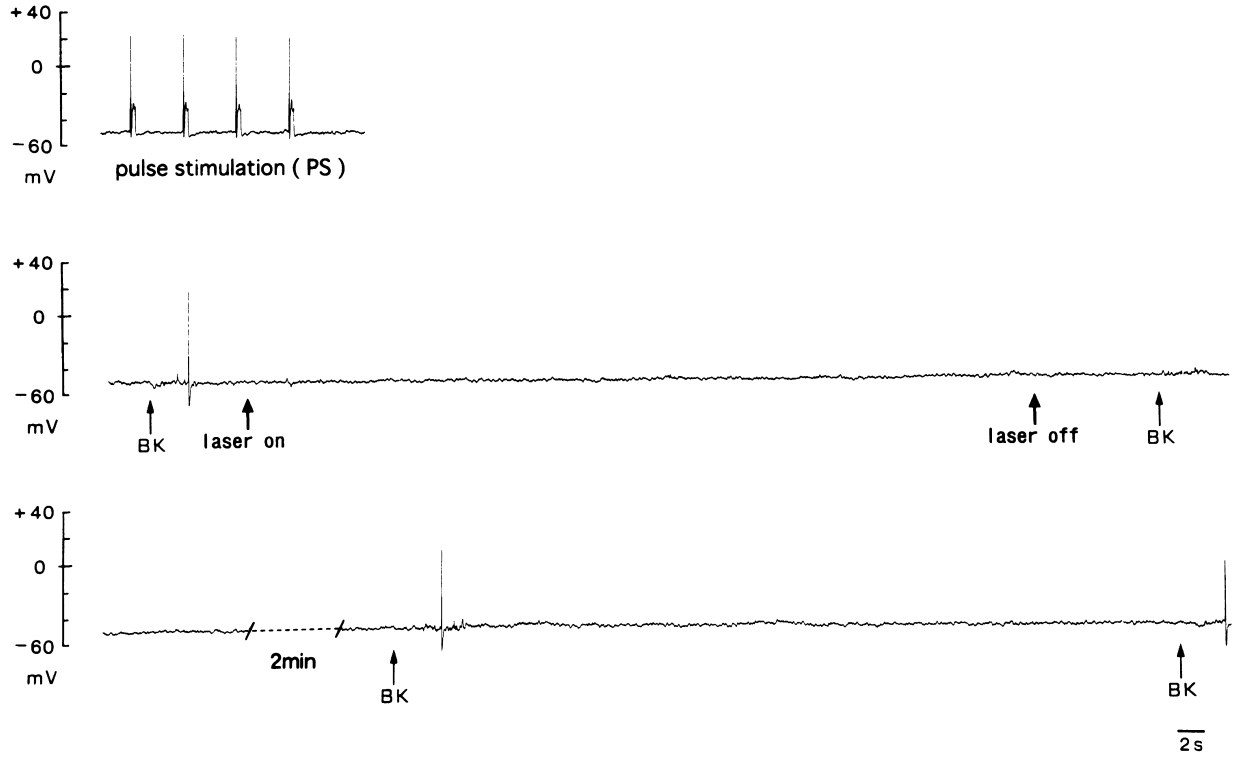

Fig. 3. Suppressing effects of laser irradiation on action potential induced by BK. The top trace shows action potential evoked by electrical stimulation (70 pA, 500 ms duration). The middle and bottom traces were obtained from continuous recording in the same cell. BK (10 μM) was applied to a process as shown by the arrow. The laser light was irradiated to the same process for 1 min.

ganglion cells and synaptic glomeruli of the spinal trigeminal nucleus of rat treated with BK by the subcutaneous injection into the maxillary trigeminal dermatome [9].

Although the effects of low-power laser irradiation are diverse, the present experimental method offers a simple and easy to use procedure for studying the underlying mechanisms involved in pain relief effects.

This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan. We thank Professors K. Mizumura, M. Horikawa and M. Sakakibara for their helpful discussions.

[1] Baccaglini, P.I. and Hogan, P.G., Some rat sensory neurons in culture express characteristics of differentiated pain sensory cells, Proc. Natl. Acad. Sci. USA, 80 (1983) 594–598.
[2] Beck, P.W. and Handwerker, O.H., Bradykinin and serotonin effects on various types of cutaneous nerve fibers, Pflugers Arch., 347 (1974) 209–222.
[3] Cameron, A.A., Leah, J.D. and Snow, P.J., The electrophysiological and morphological characteristics of feline dorsal ganglion cells, Brain Res., 362 (1986) 1–6.
[4] Dunn, P.M., Coote, P.R., Wood, J.N., Burgess, G.M. and Rang, H.P., Bradykinin evoked depolarization of a novel neuroblastoma × DRG neurone hybrid cell line (ND7/23), Brain Res., 545 (1991) 80–86.
[5] Garrgoulitos, Z.F., Welch, A.J., Piller, K.R. and Aggarmal, S.J., Laser-irradiation-induced relaxation of blood vessels in vivo, Laser Surg. Med., 10 (1990) 524–532.
[6] Hamill, O.P., Marty, Y., Neher, E., Sakmann, B. and Sigworth, F.J., Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches, Pflugers Arch., 391 (1981) 85–100.
[7] Honmura, A., Yanase, M., Obata, J. and Haruki, E., Therapeutic effect of Ga-Al-As diode laser irradiation on experimentally induced inflammation in rats, Laser Surg., 12 (1992) 441–449.
[8] Kreczi, T. and Klingler, D., A comparison of laser acupuncture versus placebo in radicular and pseudoradicular pain syndromes as recorded by subjective responses of patients, Acupunct. Electro-Ther. Res., 11 (1986) 207–216.
[9] Maeda, T., Morphological demonstration of low reactive laser therapeutic pain attenuation effects of the gallium aluminum arsenide diode laser, Laser Ther., 1 (1989) 23–26.
[10] Miura, A. and Kawatani, M., Effects of diode laser irradiation on sensory ganglion cells from the rat, Pain Res., 11 (1996) 175–183.
[11] Noda, K., Ueda, U., Suzuki, K. and Yoda, K., Excitatory effects of algesic compounds on neuronal processes in murine dorsal root ganglion cell culture, Brain Res., 751 (1997) 348–351.
[12] Plog, F.M.W., Biophysical application of the laser beam. In H.K. Koebner (Ed.), Lasers in Medicine, Wiley, Chichester, 1980, pp. 21–37.
[13] Pontinen, P.J., Low Level Laser Therapy as a Medical Treatment Modality, 1st edn., Art Urpo., Tampere, 1992, pp. 11–211.
[14] Rochkind, S., Nissan, M., Lubart, R., Avram, J. and Bartal, A., The in-vivo-nerve response to direct low-energy-laser irradiation, Acta Neurochirurg., 94 (1988) 74–77.
[15] Shimoyama, M., Iijima, K., Mizuguchi, T., Shimoyama, M. and Hori, Y., Effects of low power laser irradiation on electrophysiological characteristics of dorsal root ganglion neurons of rats, Pain Res., 6 (1991) 111–115.
[16] Snyder-Mackler, L., The effect of helium-neon laser on latency of sensory nerve, Phys. Ther., 65 (1985) 737.
[17] Tsuchiya, K., Kawatani, M., Takegishi, C., Sato, T. and Matsumoto, I., Diode laser irradiation selectively diminishes slow component of axonal volleys to dorsal roots from the saphenous nerve in the rat, Neurosci. Lett., 161 (1993) 65–68.
[18] Tsuchiya, K., Kawatani, M., Takegishi, C. and Matsumoto, I., Laser irradiation abates neuronal responses to nociceptive stimulation of rat-paw skin, Brain Res. Bull., 34 (1994) 369–374.