DISSECTION OF UNDAMAGED SINGLE NERVE FIBERS WITH AN ULTRASONIC MICROSCALPEL

L. Ya. Selektor, L. M. Shapovalova,
S. V. Revenko, and L. D. Zaborovskaya

UDC 612.815.014.421.086.12

KEY WORDS: Ranvier node; ultrasound; microsurgery

Dissection of single myelinated nerve fibers for electrophysiological research is a laborious procedure. The nerve trunk is dissected in Ringer's solution on a dissecting slide under the microscope with the aid of thin needles and microscalpels with a tip 10-30 μ in diameter [6]. Efforts are made to avoid microtraumas, which adversely affect the functional characteristics of the Ranvier nodes [4]. Trauma arises most frequently when the interaxonal connective tissue is divided. The reasons for this are as follows. Because of bending of its thin blade the microscalpel does not exert sufficient pressure on the tissue, so that repeated attempts are needed to divide the tissue, by rocking and tilting the blade, and choosing the most advantageous position of the instrument. The divided tissue adheres to the microscalpel, so the preparation is pulled on when the instrument is removed from the tissue.

To overcome these difficulties the writers have used an ultrasonic microscalpel to dissect single nerve fibers. It was necessary to verify that this technique is atraumatic, because ultrasonic irradiation of nerve tissue can damage nerve fibers [3].

EXPERIMENTAL METHOD

The ultrasonic microscalpel consists of a microblade, fashioned from a sewing needle, connected to the vibrator of the mass-produced UZKh-201 apparatus for ultrasonic surgery [1]. During preparation of the microscalpel a steel strip was first welded to the vibrator, made of titanium alloy, after which the microblade was soldered to it with tin (Fig. 1A). The length of the cutting part of the microscalpel was made as small as possible (about 700 μ) to reduce whorl-formation around the blade during work.

In the intial stages of the work a single nerve fiber, dissected by the traditional method, was irradiated with ultrasound by moving the vibrating blade of the microscalpel along the fiber at a distance of 100-200 μ from it. During work with these fibers (three experiments) no visible differences were found in the sodium current and the leakage current compared with unirradiated preparations. It was therefore possible to proceed to the next task, which was direct dissection of nerve fibers with the aid of the ultrasonic microscalpel.

The sciatic nerve of a frog (*Rana ridibunda*) was dissected on a dissecting slide under the MBS-9 binocular microscope with maginfication of 56. The nerve membrane (epineurium) was divided by the traditional method by means of micro-scissors with blades of different lengths [5]. Single nerve fibers and micro-strands were separated by dissecting needles and the conective tissue along the fiber selected for isolation was divided by the ultrasonic microscalpel (Fig. 1B), by laying it on top of the tissue, pressing it against the glass, and switching on ultrasonic vibrations with a frequency of 44 kHz for a short time (not more than 1 sec). The amplitude of the vibrations of the blade was set to be very small (under 5 μ), so that no miniature walls of fluid appeared around the vibrating blade.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Research Institute of Biological Testing of Chemical Compounds, Kupavna, Moscow Region. A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 12, pp. 761-762, December, 1985. Original article submitted February 28, 1985.

0007-4888/85/0012-1763$09.50

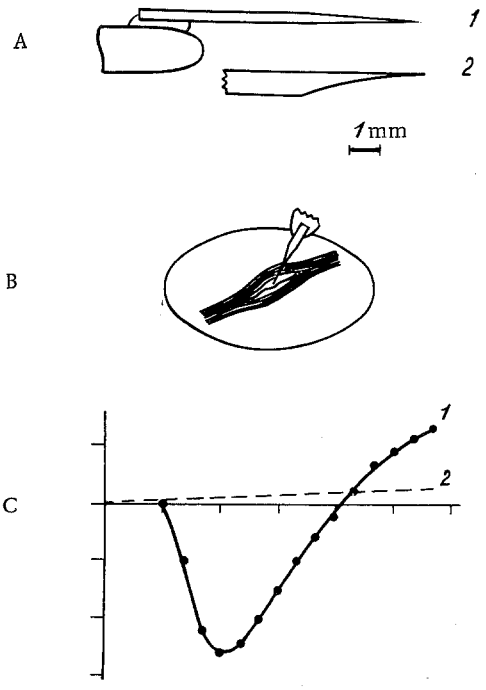

Fig. 1. Dissection of single fiber with ultrasonic microscalpel and current-voltage characteristic curve of sodium channels. A) Blade of ultrasonic microscalpel, soldered to tip of vibrator: 1) plan, 2) side view; B) cutting connective tissue in a bundle of nerve fibers; C) peak values of sodium current (1) and leakage current (2). Holding potential -80 mV.

EXPERIMENTAL RESULTS

The state of nerve fibers dissected by means of the ultrasonic microscalpel was assessed with respect to the current-voltage characteristic curve of the sodium channels, recorded by the voltage clamp method (Fig. 1C). Potassium channels were blocked by cesium ions, diffused into the exoplasm through the divided ends of the nerve fiber, dipped in isotonic CsCl solution. A sensitive parameter of the state of the sodium channels of the fiber is the ratio of its maximal sodium conductance (g_{Na}) to its leakage conductance (g_l) [4]. For the fiber under examination, with a reversal potential of the sodium current E_{Na} = +50 mV, this ratio is 28, which is not much different from the standard value of 30 [5]. About the same value for g_{Na}/g_l also was obtained for three other fibers dissected by means of the ultrasonic microscalpel.

It is thus possible to dissect uninjured isolated amphibian nerve fibers with the aid of an ultrasonic microscalpel. The method is free from the disadvantages mentioned above. The connective tissue is divided at the first application of the instrument, and the excised fragments of tissue do not adhere to the vibrating blade. The advantages of the ultrasonic microscalpel when dividing the membranes covering nerve trunks of warm-blooded animals in vivo were demonstrated previously [2]. The present study confirms the efficacy of the ultrasonic microscalpel for performing fine microsurgical operations on nerves.

The authors are grateful to Professor V. M. Khayutin for his interest and support during preparation of this paper and to A. P. Leont'ev, Head of the Department of Ultrasonic Instruments, All-Union Research Institute of Medical Instrumentation, for making the instrument available.

LITERATURE CITED

1. M. D. Gurevich, V. I. Bogdanov, and M. I. Ferdman, Novosti Med. Tekh., No. 5, 4 (1981).
2. V. V. Ermishkin and S. V. Revenko, Byull. Éksp. Biol. Med., No. 11, 631 (1984).

3. A. V. Lonskii, P. O. Makarov, and B. S. Tuchkov, Tsitologiya, No. 11, 1401 (1969).
4. S. V. Revenko, O. S. Sotnikov, and B. I. Khodorov, Neirofiziologiya, 10, 400 (1978).
5. R. Stämpfly and B. Hille, in: Frog Neurobiology, Berlin (1976), pp. 1-32.

DELAYED-TYPE HYPERSENSITIVITY REACTION OF MICE TO XENOGENEIC RAT ANTIGEN

I. V. Lyadova and I. Yu. Chernyakhovskaya

UDC 612.6.02.017.4+612.112.94-17.1-063

KEY WORDS: delayed-type hypersensitivity; xenogeneic antigens; T lymphocytes

Xenogeneic transplantation into an adult animal induces a whole range of reactions leading to gradual destruction of the grafted tissue. In the modern view, cytotoxic T lymphocytes, T effector cells, and also delayed-type hypersensitivity (DTH) antibodies may participate in graft rejection [3, 8, 11, 12]. Depending on the nature of the graft, the method of transplantation (with vascular anastomoses or by free grafting), and also the level of sensitization of the recipient, each of the above components of the immune response may play a more or less important role in rejection. Evidence of the important role of DTH reactions in allograft rejection has recently been published [6, 7, 10]. The mechanisms of rejection of xenogeneic grafts have received much less study. Their interpretation is important in connection with the prospects for the use of xenografts in medical practice.

A method of induction of tolerance in adult mice to allogeneic and xenogeneic grafts of neonatal heart has recently been suggested in the writers' laboratory [2, 4, 5]. During a study of the immunologic status of animals tolerant to an allograft, depression of DTH to the corresponding alloantigens was observed [1]. A similar investigation conducted on animal tolerance to a xenograft was difficult, because no method of determining DTH against transplantation antigens of xenogeneic origin has yet been developed.

The aim of this investigation was to devise a scheme for induction of DTH to xenogeneic lymphocytes in mice.

EXPERIMENTAL METHOD

Male CBA mice of inbred lines, August rats, and guinea pigs aged 2-3 months, obtained from the nurseries of the Academy of Medical Sciences of the USSR-were used.

Suspensions of lymphocytes from the lymph nodes and spleen of mice, rats, and guinea pigs were prepared in medium 199 with the addition of anitbiotics (penicillin 100 U/ml, streptomycin 100 μg/ml and HEPES (0.005 M), filtered through Kapron filters, and washed by centrifugation at 200g for 10 min. The residue was resuspended and diluted to the required concentration.

To induce DTH, a scheme including sensitization of CBA mice followed by subcutaneous injection of the reacting dose of antigen, was adopted. The antigen was a suspension of splenic lymphocytes from August rats or guinea pigs. In preliminary experiments the mice were immunized intraperitineally with various doses of antigen (5×10^6, 10^7, 5×10^7 cells) and the test injection was given after 5, 7, 11, 14, or 21 days. Later, to sensitize the mice, 5×10^6 xenogeneic cells in Freund's complete adjuvant (FCA) were injected subcutaneously and the reaction was tested 5 days later. In all cases, to test the reaction, 5×10^6 xenogeneic spleen cells in a volume of 0.05 ml were injected into the plantar surface of the right hind limb, and the same number of syngeneic spleen cells was injected into the left hind limb. The reaction was recorded 24 h later as the difference between the volume of edema of the right and left limbs, measured with and MK-0-25 mm micrometer with an accuracy of 0.01 mm. A difference of 0.1 mm was taken as 1 unit of reaction.

In experiments to study adoptive local transfer of DTH, a suspension of lymphocytes was

Laboratory of Immunologic Tolerance, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. No. 12, pp. 762-765, December, 1985. Original article submitted March 4, 1985.

0007-4888/85/0012-1765$09.50