USE-DEPENDENT INHIBITION OF POLYMODAL C-FIBER CUTANEOUS SENSORY UNITS IN CATS BY LIDOCAINE AND N-PROPYLAJMALINE

N. A. Mangusheva, L. V. Baidakova, and S. V. Revenko

UDC 616.5-018.1-02:615.211]-07

KEY WORDS: C-fibers; nociception; lidocaine; N-propylajmaline

The initial stage of the nociceptive system in animals and man is a special group of neurons, which form C- and A-delta axons [2, 11]. In warm-blooded animals these sensory cutaneous units are polymodal: they are excited by intensive mechanical, thermal, and chemical stimuli, virtually identical in intensity with those inducing defensive reflexes, and in man, of inducing pain also. An important feature of the C-fiber polymodal mechanosensory and thermosensory units (CMT-units) is their ability to undergo low-frequency excitation under the influence of nonadhesive chemical stimuli [1-3]. The discharge frequency of CMT-units, corresponding to the transition from a nonadhesive to an adhesive chemical stimulus, is close to 2 Hz [1, 3]. Responses of CMT-units characteristically have low frequencies (under 10 Hz), even those to adhesive chemical stimulation [1-3, 15].

The ability of CMT-units to generate a subnociceptive low-frequency discharge suggests that for local analgesia all that is necessary is to lower the discharge frequency of these units to a certain minimal level, without the necessity of completely locking conduction or impulse generation. This could be achieved with the aid of certain local anesthetics or antiarrhythmics, with use-dependent (UD) action. During voltage clamping of an excitable membrane these substances, ionized (tertiary) amines or their permanently charged quaternary analogs, evoke chronic blockade of Na-channels at rest and also in response to infrequent testing stimuli. An increase in the frequency of stimulation leads to almost complete suppression of sodium currents, but if the frequency is lowered, these currents are restored to a level determined by the tonic block [5-10, 14]. As long ago as in 1975, the UD-block of ionic channels was suggested as a promising method of controlling arrhythmia and pain [5]. The role of the UD-block during the action of antiarrhythmics is generally accepted, but the contribution of UD-inhibition of C-fibers to local anesthesia is evidently not significant [7]. Could UD-inhibition of cutaneous CMT-units arise during their excitation by pain-inducing stimuli?

We found inhibition of this kind during the action of the tertiary amine lidocaine and the quaternary derivative of the antiarrhythmic ajmaline – N-propylajmaline. UD-inhibition of CMT-units broadens our ideas on the mechanisms of local anesthesia.

EXPERIMENTAL METHOD

Responses of 11 CMT-units were tested in nine cats anesthetized with chloralose (40 mg/kg) and urethane (6 mg/kg). Signals of single C-afferents of a cutaneous nerve of a pelvic limb were recorded by the standard method [1, 3]. Sensory endings of CMT-units were found in skin in the medial region of the leg. Thresholds of mechanical and thermal sensitivity were 25-50 g/mm^2 and 45-50°C respectively. The conduction velocity along C-fibers was 0.7-2.0 m/sec. For application of the test solutions an area of skin with the receptor was separated from the subcutaneous fatty areolar tissue. The test solutions were introduced into the subcutaneous cavity thus formed as a single procedure or continuously through

Group for Biomechanics and Regulation of the Circulation, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 248-250, March, 1992. Original article submitted July 8, 1991.

 0007-4888/92/0003-0316$12.50

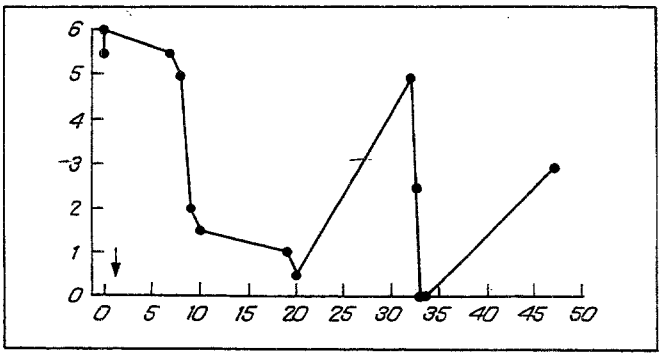

Fig. 1. Use-dependent action of lidocaine on C-fiber polymodal cutaneous sensory unit in cat. Abscissa, time (in min); ordinate, mean discharge frequency (in Hz), excited in sensory unit by mechanical stimulus of 50 g/mm^2. Arrow indicates time of single injection of solutions with lidocaine (0.1%).

a cannula. The hole in the skin required for the injection was based 1 cm distally to the receptor. The composition of the control Ringer's solution was (in mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, Tris 5.0, 37°C, pH 7.4. The test solutions were made up in Ringer's solution.

EXPERIMENTAL RESULTS

Values of responses of CMT-units to mechanical stimulation with a Frey's hair, developing a pressure of 50 g/mm^2, are shown in Figs. 1 and 2. The response of the CMT-unit was characterized by an average discharge frequency, determined for a 2-sec interval, counting from the beginning of mechanical stimulation (Fig. 1). The structure of the response of the CMT-unit is represented by histograms with a bin width of 0.33 sec (Fig. 2). The number of spikes in the bin, divided by the size of the bin (i.e., the local frequency), was plotted along the ordinate. At the beginning of the experiments the control Ringer's solution was injected and it was confirmed that repeated mechanical stimuli applied at an interval of 2-5 sec, evoke identical responses of the CMT-unit (Fig. 1, beginning of the curve, and Fig. 2a). For this verification two or three stimuli at 50 g/mm^2 and with a duration of 2-3 sec were used. With such stimuli no UD-inhibition of responses of the CMT-unit was present.

In the experiment illustrated in Fig. 1, at the 2nd minute a single injection of Ringer's solution containing 0.1% lidocaine was given. Development of the inhibitory action of lidocaine was monitored by subjecting the CMT-unit to mechanical stimulation. It can be seen that even infrequent stimulation, with intervals of not less than 1 min between stimuli, sharply increased the action of lidocaine (for comparison 2-7 and 7-10 min). Characteristically the duration of excitation of the CMT-unit inhibited by lidocaine was reduced (Fig. 2b, c). After a long pause (20-32 and 33-47 min) the response was largely restored (Fig. 2d, e). Application of two stimuli in the course of the 32nd minute (Fig. 1 and Fig. 2d, e) led to complete suppression of the response (Fig. 2f). This fragment of the experiment shows that conditions are possible under which UD-inhibition is well marked virtually without any tonic weakening of excitability of the CMT-unit. The action of lidocaine was completely abolished by rinsing out the subcutaneous cavity with Ringer's solution for 30 min.

A UD-inhibitory action on a CMT-unit also was exhibited by the antiarrhythmic N-propylajmaline (NPA), and, moreover, in significantly lower concentrations (0.01%) than for lidocaine (Fig. 3). In addition, NPA evoked stronger UD-inhibition than lidocaine: a single mechanical stimulation was sufficient for the CMT-unit to become virtually inexcitable toward a repetitive mechanical stimulus, applied at intervals of 5 sec. Moreover, the response to the first stimulation after a pause consisted of only 1-3 spikes. After the pause the response was partly restored. UD-inhibition of a CMT-unit by NPA was consistently reproduced on repetition of cycles of stimulation and pause (9-17 min). The action of NPA is completely reversible: rinsing with Ringer's solution led to restoration of responses of the CMT-unit, including to a series of repetitive stimuli (45th minute of the experiment).

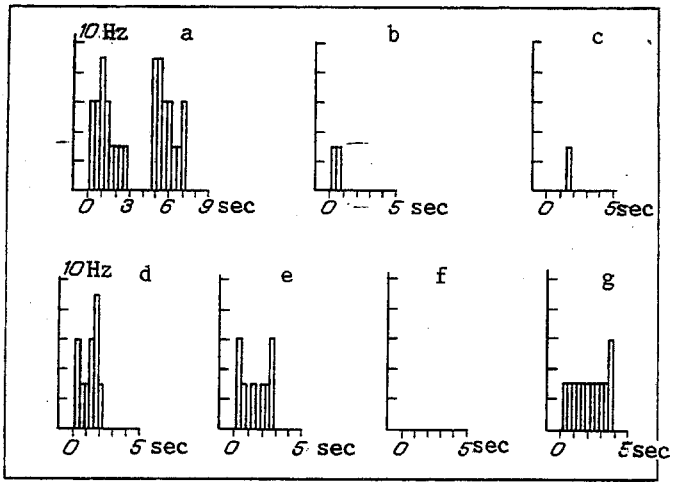

Fig. 2. Changes in histograms of discharges of a polymodal sensory unit during application of repeated mechanical stimuli at 50 g/mm^2 under normal conditions and during the action of 0.1% lidocaine. The same sensory unit as in Fig. 1: a) series of two stimulations in control Ringer's solution. Histograms (b-g) shown in frequency units and correspond to moments of time, in minutes: 19 (b), 20 (c), 32 (d), 32.5 (e), 33 (f), and 47 (9). Bin 0.33 sec.

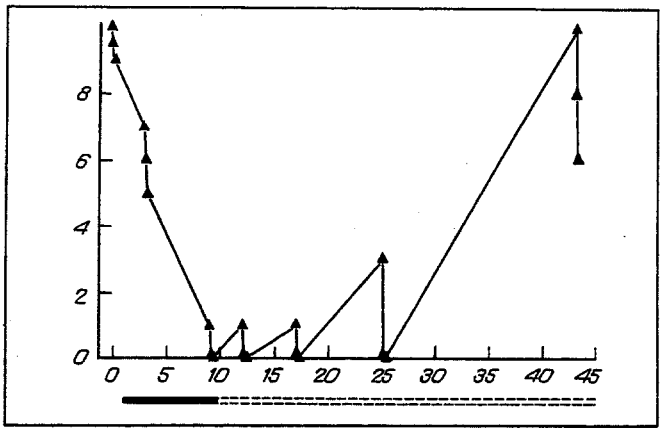

Fig. 3. Use-dependent action of N-propylajmaline (0.01%) on C-fiber polymodal sensory cutaneous unit in a cat. Abscissa, time (in min); ordinate, number of spikes excited in sensory unit by mechanical stimulus at 50 g/mm^2. Black band below indicates time of application of solution with NPA; unshaded band corresponds to rinsing with Ringer's solution. Mechanical stimulation applied in the form of series, each containing three stimuli with intervals of 5 sec.

The tertiary local anesthetic lidocaine and the permanently charged quaternary antiarrhythmic N-propylajmaline are known to induce a UD-block of sodium permeability of the nerve fiber membrane [5, 8, 10]. According to the modulated receptor theory [5, 8] the Na-channel receptor for amine blockers changes its affinity for the blocker during a change in the state of the channel from resting to open and to inactivated. The unblocking reaction depends on hypothetical analogous voltage-dependent states of the blocked channel. Movement of the blocker toward the receptor and away from it is limited: charged blockers reach the receptor from the inner side of the membrane via a hydrophilic pathway – an aqueous cavity of

the open channel, whereas uncharged blockers approach the receptor via the lipophilic route, independent of the state of the channel, i.e., through the membrane lipids [8]. In the course of electrical stimulation of the membrane, the velocities of the reactions of blocking and unblocking of the Na-channels change abruptly, and equilibrium of these reactions is achieved only after the action of a group of stimuli. The relationship between these reactions can be explained by dependence of the Na-channel block on use of the stimuli, and also by the effect of the frequency of stimulation on the rate of development of the block and the level achieved. In the case of application of depolarizing stimuli from the resting potential to a membrane (which corresponds to excitation of CMT-units in the present experiments), an increase in the frequency of stimulation leads to acceleration and deepening of the Na-channel block by local anesthetics and by antiarrhythmics. Further development of the UD-block theory [13] is linked with the choice of stricter limitations on movement of the blocker toward and from the receptor, and also with a decrease in the number of hypothetical (but not observed) states of the Na-channel (the guarded receptor theory).

For most local anesthetics and antiarrhythmics, the UD-block of the Na-channels of the myelinated frog fiber, evoked by the voltage clamping method, is observed starting from the frequency of 10 Hz [5, 8, 10, 14]. Under physiological conditions (without voltage clamp) the frequency-dependent block of discharges of myelinated frog fibers becomes appreciable only at a frequency of 40 Hz [6]. In experiments on the cervical sympathetic and vagus nerves of the rabbit in vitro, with recording of discharges of single C- and thin myelinated fibers UD-inhibition during the action of lidocaine also was observed only at frequencies higher than 40 Hz [7]. Considering that nociceptive stimulation of the human skin corresponds to frequencies of excitation of CMT-units lower than 10 Hz [15], the authors cited concluded that the role of UD-inhibition in local anesthesia is not significant. Marked UD-inhibition in the present experiments is connected with the fact that, by contrast with [7], the blockers were applied to the receptor zone of CMT-units. Incidentally, the quaternary NPA proved to be more effective than the tertiary lidocaine. This difference may be connected with differences in the ability of local anesthetics to diffuse through nerve cell membranes [12]. Since quaternary compounds are more effective (than tertiary) when acting on a nerve from which the perineurium has been removed [12], it can be tentatively suggested that the zone of UD-inhibition of CMT-units in our experiments consisted of its tissue terminals, deprived of their protective Schwann membrane.

The search for UD-inhibition of CMT-units involves testing for the possibility of reducing the excitability of these units by means of a pharmacologic preparation to the subnociceptive level, corresponding to their excitation by nonadhesive stimuli. This kind of procedure could lead to local anesthesia without a total block of the nerve fibers, and with keeping open the possibility of low-frequency excitation of sensory units. The role of subnociceptive low-frequency discharges of CMT-units in the body is still unknown. If it proves to be that they correspond to the transmission of certain nonnociceptive signals (interoceptive, for example [3, 4]), in that case UD-inhibition of CMT-units could evoke differential local anesthesia, blocking only one (the nociceptive) function of the CMT-units. Partial, but not total, suppression of activity of CMT units during local anesthesia would seem to be important in the case of the long-term use of analgesics. Substances evoking use-dependent block of sodium permeability may prove to be effective instruments of such partial inhibition of CMT-units.

The authors are grateful to V. V. Ermishkin, L. L. Katalymov, V. M. Khayutin, and B. I. Khodorov for help with the work.

LITERATURE CITED

1. S. V. Revenko, V. V. Ermishkin, and L. Ya. Selektor, Neirofiziologiya, **20**, No. 2, 147 (1988).
2. S. V. Revenko, V. V. Ermishkin, and L. Ya. Selektor, Sensornye Sistemy, **2**, No. 2, 198 (1988).
3. L. Ya. Selektor, V. V. Ermishkin, S. V. Revenko, et al., Byull. Éksp. Biol. Med., No. 8, 183 (1988).
4. V. M. Khayutin, Problems in Interoception, Regulation of Physiological Functions and Behavior [in Russian], Leningrad (1976), pp. 230-247.
5. K. R. Courtney, J. Pharmacol. Exp. Ther., **195**, No. 2, 225 (1975).
6. K. R. Courtney, J. J. Kendig, and E. N. Cohen, Anesthesiology, **48**, No. 2, 111 (1978).
7. B. R. Fink and A. M. Cairns, Anesthesiology, **67**, No. 4, 477 (1987).
8. B. Hille, J. Gen. Physiol., **69**, No. 4, 497 (1977).
9. B. Khodorov, L. Shishkova, E. Peganov, et al., Biochim. Biophys. Acta, **433**, No. 2, 409 (1976).
10. B. I. Khodorov and L. D. Zaborovskaya, Gen. Physiol. Biophys., **2**, No. 4, 233 (1983).

11. B. Lynn, Textbook of Pain, ed. by P. D. Wall and R. Melzack, Edinburgh (1984), pp. 19-33.
12. J. M. Ritchie, B. Ritchie, and P. Greengard, J. Pharmacol. Exp. Ther., **150**, No. 1, 160 (1965).
13. C. F. Starmer, A. O. Grant, and H. S. Strauss, Biophys. J., **46**, No. 1, 15 (1984).
14. G. R. Strichartz, J. Gen. Physiol., **62**, No. 1, 37 (1973).
15. J. Van Hees and J. M. Gybels, J. Neurol. Neurosurg. Psychiat., **44**, No. 7, 600 (1981).

PARAMETERS OF THE SYSTEMIC HEMODYNAMICS IN CONSCIOUS RATS WITH ACUTE STREPTOZOTOCIN DIABETES

L. V. Kuznetsova, N. A. Medvedeva, and O. S. Medvedev

UDC 612.13:616.379-008.64

KEY WORDS: experimental diabetes mellitus; hemodynamics; vasodilatation

Disturbances of functions of the cardiovascular system are frequently observed in patients with diabetes mellitus. Among the important causes of these disturbances are multiple microangiopathies [7]. It is considered that microvascular lesions in diabetes may have a hemodynamic basis [9, 15], and they may arise as a result of vasodilatation in the initial stages of the disease, followed by elevation of the capillary pressure and increased blood flow [14, 15]. An increased blood flow has been found in many tissues during short-term insulin-dependent diabetes [6, 7, 10]. Functional microvascular changes may arise at the end of the prediabetic phase and may be preceded by degenerative changes in the vessels [3]. It has been suggested that in order to play a primary, key role, vasodilatation must be recorded on the 1st day of diabetes [12, 13].

This paper describes a study of changes in the basic parameters of the systemic hemodynamics – arterial blood pressure (BP), cardiac index (CI), calculated as the cardiac ejection per 100 g body weight, and the total peripheral vascular resistance (TPVR) – in conscious rats with acute streptozotocin-induced diabetes (24 h after injection of streptozotocin).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 350-450 g. The cardiac output was determined by the thermodilution method, using a "Cardiomax" instrument (USA). Through a catheter implanted beforehand in the jugular vein, 0.2 ml of cold physiological saline was injected into the animal. By means of a thermocouple implanted in the aorta, the thermodilution curve was recorded, and on that basis cardiac output was calculated. BP was recorded by means of a "Statham 23D" pressure transducer through a catheter implanted in the abdominal aorta through the femoral artery. The parameters were recorded on a "Nihon Kohden" polygraph (Japan). On the basis of the values obtained for cardiac output, heart rate (HR), and BP, values of CI, the stroke blood volume (SV), and TPVR were calculated. Parameters of the systemic hemodynamics were measured in conscious animals 2 days after the operation. For this purpose, the thermodilution curve and BP were recorded three times at intervals of 5 min. The values of BP, CI, TPVR, HR, and SV thus obtained were averaged. Next, diabetes was induced in the animal by a single injection of streptozotocin (STZ) (from "Upjohn," USA) in a dose of 50 mg/kg through a venous catheter (the solvent was citrate buffer, pH 4.5). The glucose and ketone

Laboratory of Pathophysiology, Research Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow. Department of Human and Animal Physiology, Faculty of Biology, Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 250-252, March, 1992. Original article submitted July 15, 1991.

 0007-4888/92/0003-0320$12.50