PHYSIOLOGY

The Resistance of Cutaneous Feline Polymodal C-Fiber Units to Tetrodotoxin as Revealed by Mechanical and Thermal Stimulation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 369-371, October, 1997
Original article submitted May 27, 1996

Mechanical and thermal excitability of cutaneous feline polymodal C-fiber units is maintained under the action of subcutaneous tetrodotoxin injected in concentrations suppressing the mechanosensitivy of A_β-units. A number of features of inhibition and excitation of C-fiber polymodal sensory units can be explained by existence of tetrodotoxin-resistant Na channels in their termination.

Key Words: *nociception; skin; CMH units; tetrodotoxin; Na channels*

The cutaneous C-fiber mechano- and heat-sensitive (CMH) units are the primary elements of the nociceptive system in the warm-blooded animals [12]. The excitation of these sensors in humans evokes pain. The terminations of cutaneous CMH units are characterized not only by individual excitability, but also by the mode of inhibitory action of local anesthetics [2]. These features make it possible to distinguish in the structure of a CMH unit a particular subdivision, which differs from the proximal part of the axon by its reaction both to exciting and inhibiting procedures. This work studies the properties of sodium permeability of the CMH termination with the help of the fast sodium channel blocker tetrodotoxin (TTX).

MATERIALS AND METHODS

The responses of 7 CMH and 7 mechanosensitive A_β units were studied on 11 cats anesthetized with Chloralose (40 mg/kg) and Urethane (600 mg/kg). The signals of individual fibers in a fine strand of saphenous nerve in the hindleg were recorded by a standard technique [4]. The cutaneous sensitive terminations of both sensory types were found in the medial part of the hindleg. Mechanical stimuli were applied to CMH units manually with calibrated Frey hair and to A_β units with a soft brush. The thresholds in mechanical sensitivity of CMH units were 25-50 g/mm^2. The thermal ramp stimuli were applied to CMH units by a radiant heat of a projector lamp that was controlled by a feed-back signal from a thermocouple contacting the skin near the receptive site (Fig. 1). The parameters of thermal stimulus were controlled by a computer. The thermal threshold was 39-41°C.

For subcutaneous application of solutions the part of skin with a receptor was exfoliated. The control and test solutions were administered into this lumen (Fig. 1). The skin exfoliation and application of the control Ringer's solution did not modify the responses of CMH units. TTX was added to Ringer's solution (in mM): NaCl — 154, KCl — 5.6, $CaCl_2$ — 2.2, Tris-HCl — 5.0; 37°C, pH 7.3). To test the effect of TTX on the axons of the recorded CMH and A_β units, the test solutions were applied directly to the nerve trunk with opened perineurium sheath. The neurograms were transformed into the discharge

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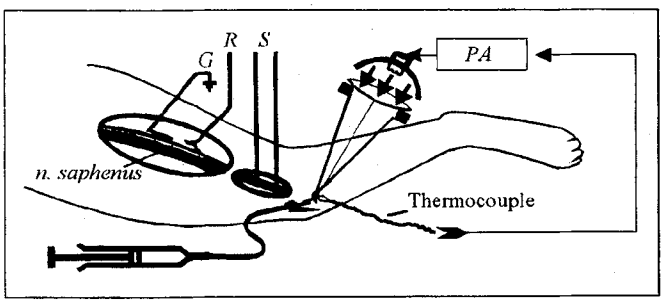

Fig. 1. A scheme of recording from individual nerve fibers, electrical stimulation of nerve trunk, radiant heat stimulation of the receptive sites of CMH units, and subcutaneous application of the control and test solutions. Shown are the recording (*R*), ground (*G*), and stimulating (*S*) electrodes. Radiant heat lamp was driven by a power amplifier (*PA*) under the control of the thermocouple signal.

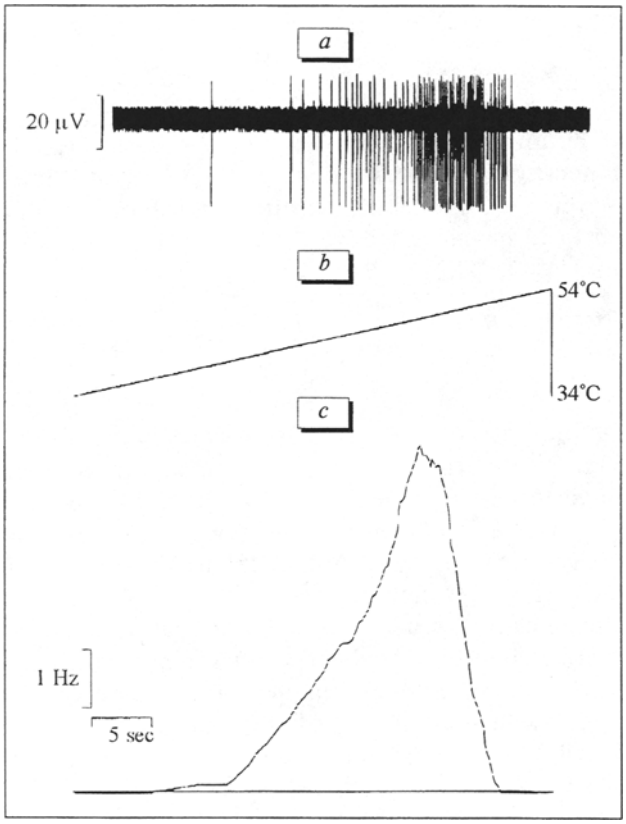

Fig. 2. Response of a CMH unit to a ramp heat pulse. *a*) neurogram, *b*) ramp heat stimulus, *c*) the discharge frequency curve.

frequency curves (Fig. 2) with the help of running average procedure. The stimulation, recording and data processing were made with the help of an ADC12m digitizer and Spike-96 software (Biola, Moscow).

RESULTS

Subcutaneous TTX (3×10^{-7} and 3×10^{-6} M) did not inhibit excitation of CMH units by thermal and mechanical stimuli (Table 1). An increase in the TTX concentration to 3×10^{-5} M resulted in suppression of excitability in 3 out of 7 CMH units. Up to this concentration, TTX virtually did not modify the discharge pattern of CMH units stimulated by a ramp heat (Fig. 3). By contrast, the subcutaneous application of TTX totally suppressed the mechanical sensitivity of A_β units (Table 1). According to χ^2 test, the terminations of A_β and CMH units differed significantly in the sensitivity to TTX in the concentration range 3×10^{-7}-3×10^{-6} M (Table 1). When TTX was applied in the minimal concentration (3×10^{-7} M) directly to the nerve trunk, it caused total conduction block in A_β- and C-axons of the examined units.

Suppression of mechanosensitive A_β units in our experiments is consistent with the views on relative resistance of the receptor potential of the myelinated mechanosensitive units to TTX [6]. The observed suppression can be explained by TTX action on the Ranvier nodes that are close to the terminations of A_β units.

By contrast, both thermal and mechanical excitability of CMH units were not disturbed by subcutaneous TTX (3×10^{-7} and 3×10^{-6} M). Stability of discharge patterns of CMH units under the action of TTX (Fig. 3) indicates that sodium permeability in their termination is TTX-resistant. The resistance of feline cutaneous nociceptive (CMH) units to the blocking effect of TTX agrees with the finding [10], that the resistance of mechanical sensitivity to TTX is confined to part of C-fiber units in the rat cutaneous *in vitro* preparation. The data on resistance of CMH unit termination to TTX suggest that similar to dorsal root ganglion C-neuron somatic membrane [9,11], this distal part of CMH unit also contains the TTX-resistant Na channels. This hypothesis is supported by the fact that the TTX-resistant Na channels in C-neurons are blocked by local anesthetics in a use-dependent manner at the same low frequencies (beginning from units of Hz [14]) as CMH unit termination [2]. At the same time, use-dependent inhibition of C-axons, which are sensitive to

TABLE 1. Effect of TTX on CMH and A_β-units

TTX concentration, M	Number			
	CMH units		A_β mechanosensitive units	
	tested	inhibited	tested	inhibited
3×10^{-7}*	7	0	7	7
3×10^{-6}*	7	1	7	7
3×10^{-5}	7	3	7	7

Note. *The difference in sensitivity to TTX is significant at fiducial level $p<0.01$ by χ^2 test with Yates' correction for continuity [5].

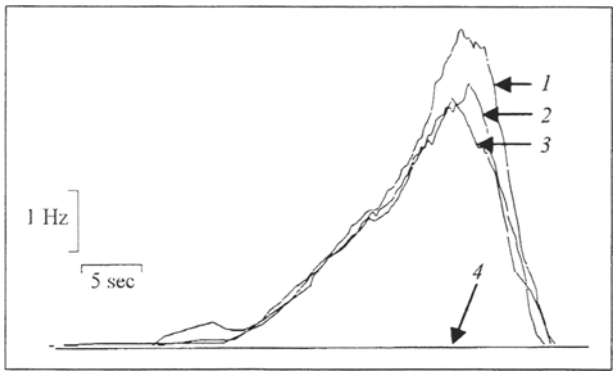

Fig. 3. The discharge rate of the CMH unit response to ramp heat stimulus in control solution (*1*) and under the action of TTX in concentrations: 2) 3×10^{-7} M, 3) 3×10^{-6} M, and 4) 3×10^{-5} M.

TTX [7], is produced by local anesthetics only at rather high (>40 Hz) frequencies [8].

The kinetic features of TTX-resistant Na channels can explain the character of excitation of CMH unit termination. Along with slow kinetics of activation and inactivation [11,14,15], these channels have the second, or slow inactivation [13], which is necessary to form the C-fiber discharge patterns when they are stimulated with a steady stimulus [3]. Under these conditions the discharge frequency in C-afferents is graded to the intensity of a stimulus [3], being far less than the maximum frequency, which can be elicited in C-fibers by electric stimulation. Thus, slow inactivation of TTX-resistant Na channels suggests the participation of these channels in the frequency encoding of stimulus intensity in C-afferents.

Does a certain location of TTX-resistant Na channels in the CMH unit termination respect to this supposed functional role? By analogy with myelinated mechanosensitive afferents, in which the site of frequency encoding is the Ranvier node nearest to the receptive ending, which is known to have the particular features of ionic channels [1], it seems possible that the frequency encoding site with TTX-resistant Na channels is also located proximal to the sensitive ending but distal to the TTX-sensitive axonal part of CMH unit. Morphological studies with the use of labeled TTX may be useful for determination of the proximal boundary of the CMH unit termination.

Supported by the Russian Academy of Medical Sciences (grant No. 4840F).

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