Effect of Clonidine on Potential-Dependent Sodium Currents in Sensory Neurons

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In experiments on rat pups we studied the effect of clonidine on potential-dependent Na^+ currents in dorsal root ganglia by the voltage clamp method. Clonidine decreased the amplitude of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium currents. The range of acting concentrations and the absence of modulatory effect of norepinephrine on the efficiency of clonidine-induced blockade of sodium currents suggest that this blockade results from a direct interaction of clonidine with sodium channels.

Key Words: *clonidine; adrenoreceptors; analgesia; sensory neurons; sodium channels*

Imidazoline derivatives (*e.g.* clonidine) are characterized by a wide spectrum of pharmacological activity. Apart from their basic hypotensive effect, these agents administered via different routes in concentrations of 1-10 nM induce analgesia [6,9,14]. The hypotensive effect of clonidine is related to activation of adrenergic and/or imidazoline receptors in the brain [10,12], while the mechanism of clonidine-induced analgesia is practically unknown.

Analgesia results from modulation of nociceptive signals in the spinal dorsal roots [6,13]. Irrespective to the primary target, this effect results from a decrease in the expression and/or functional activity of Na^+ channels. A peculiar role in the formation of impulse activity of nociceptive C-afferents is played by slow tetrodotoxin-resistant (TTXr) Na^+-channels [4, 13]. Under pathological conditions related to hyperalgesia and during the action of various hyperalgesic agents (including adrenergic substances), the number of TTXr-channels increases and their localization changes [5,13]. At the same time, the analgesic effect is always accompanied by a decrease in the number of functionally active TTXr-channels [5,7,11,13]. It can be hypothesized that this type of sodium channels is the ultimate target of analgesic drugs.

Ample experimental material showed that the interaction with α-adrenoceptors in spinal neurons is an important component of clonidine-induced analgesia [8,14]. The possibility of direct inhibitory effect of clonidine on Na^+ channels was little studied. The experiments showed that in invertebrates, this agent affects TTX-sensitive (TTXs) Na^+-channels only in very high concentrations [1].

Our aim was to study the effect of clonidine on parameters of Na^+-channels in rat spinal ganglia.

MATERIALS AND METHODS

Voltage-clamp experiments with whole-cell recording of potential-dependent Na^+-currents were carried out on neurons isolated from dorsal root ganglia of Wistar rat pups (n=50). The currents were measured with hardware and software complex based on an L/M EPC-7 amplifier [2,3].

The standard bathing solution contained (in mM): 65 NaCl, 90 choline chloride, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES Na, (pH 7.4). To block TTXs sodium current, TTX (0.3 μM) was added to the bathing solution [11].

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The standard solution in the pipette contained (in mM): 100 CsF, 10 NaCl, 40 CsCl, 2 $MgCl_2$, 10 HEPES Na (pH 7.2). Clonidine, norepinephrine, and phenylephrine were from Sigma.

The dose-dependent inhibitory effect (IC) of clonidine was described by Hill formula:

$$IC=1/(1+([C]/[IC_{50}])nH),$$

where *IC* is the level of inhibition at concentration [C], IC_{50} is the concentration producing 50% inhibition (in comparison with the control value), and nH is Hill coefficient.

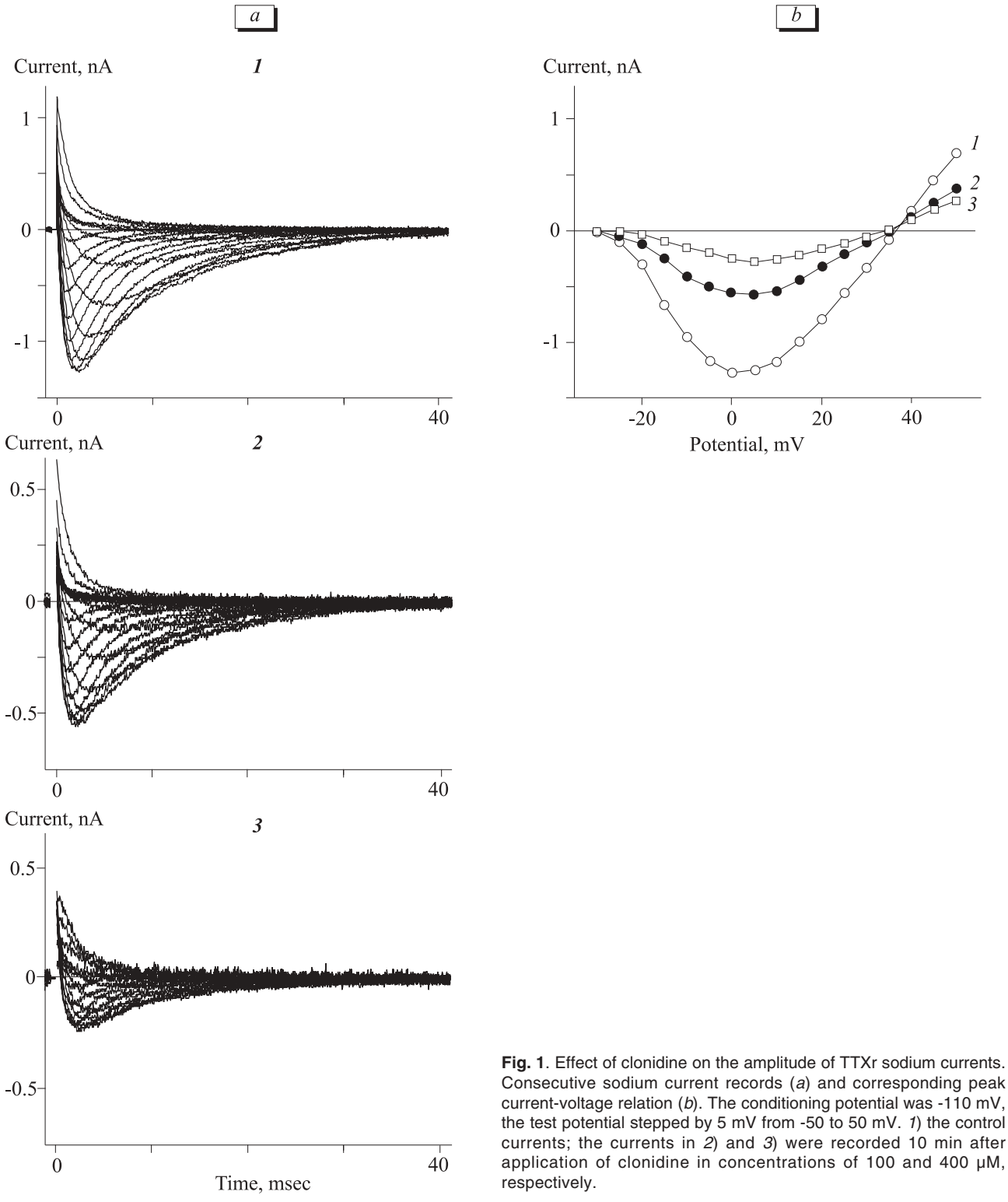

Fig. 1. Effect of clonidine on the amplitude of TTXr sodium currents. Consecutive sodium current records (*a*) and corresponding peak current-voltage relation (*b*). The conditioning potential was -110 mV, the test potential stepped by 5 mV from -50 to 50 mV. *1*) the control currents; the currents in *2*) and *3*) were recorded 10 min after application of clonidine in concentrations of 100 and 400 μM, respectively.

The means were calculated from 6-10 experiments.

RESULTS

Neurons of rat spinal ganglia usually contain both TTXs and TTXr potential-operated Na^+ channels. The relative contribution of each family into the total sodium conduction can vary (0.2-0.8). Two subfamilies of TTXr are known as fast and slow TTXr channels, which mainly differ by the rate of inactivation [11,13]. Slow TTXr channels are characterized by the time constant of inactivation of about 8 msec near 0 mV. In neurons examined in this study (n>25), inactivation kinetics of TTXr sodium currents was described by a single exponential function with the time constant of 4-10 msec within the potential range from -10 to +10 mV. Probably, in our experiments the slow TTXr channels (type Na1.8V) form a single or dominating pool of sodium channels, which made it possible to examine the effect of clonidine selectively on this type of Na^+ channels.

In concentrations >20 μM, clonidine dose-dependently and reversibly decreased the amplitude of both (TTXs and TTXr) sodium channels, although the range of effective concentrations and the peculiarities of clonidine action on both fractions were different (Figs. 1-3).

The blocking effect of clonidine on TTXr currents was observed in a concentration range of 20-1000 μM (IC_{50}=101±9 μM). The steady-state level of this effect was usually attained 5-10 min after application of the substance, and was only partially reversible after long-term washout (10-15 min). Clonidine produced no effect on the reversal potential and kinetics of TTXr currents.

Fast and slow Na^+ channels are characterized by different sensitivity to pharmacological agents, including local anesthetics [7,11]. However, the agents

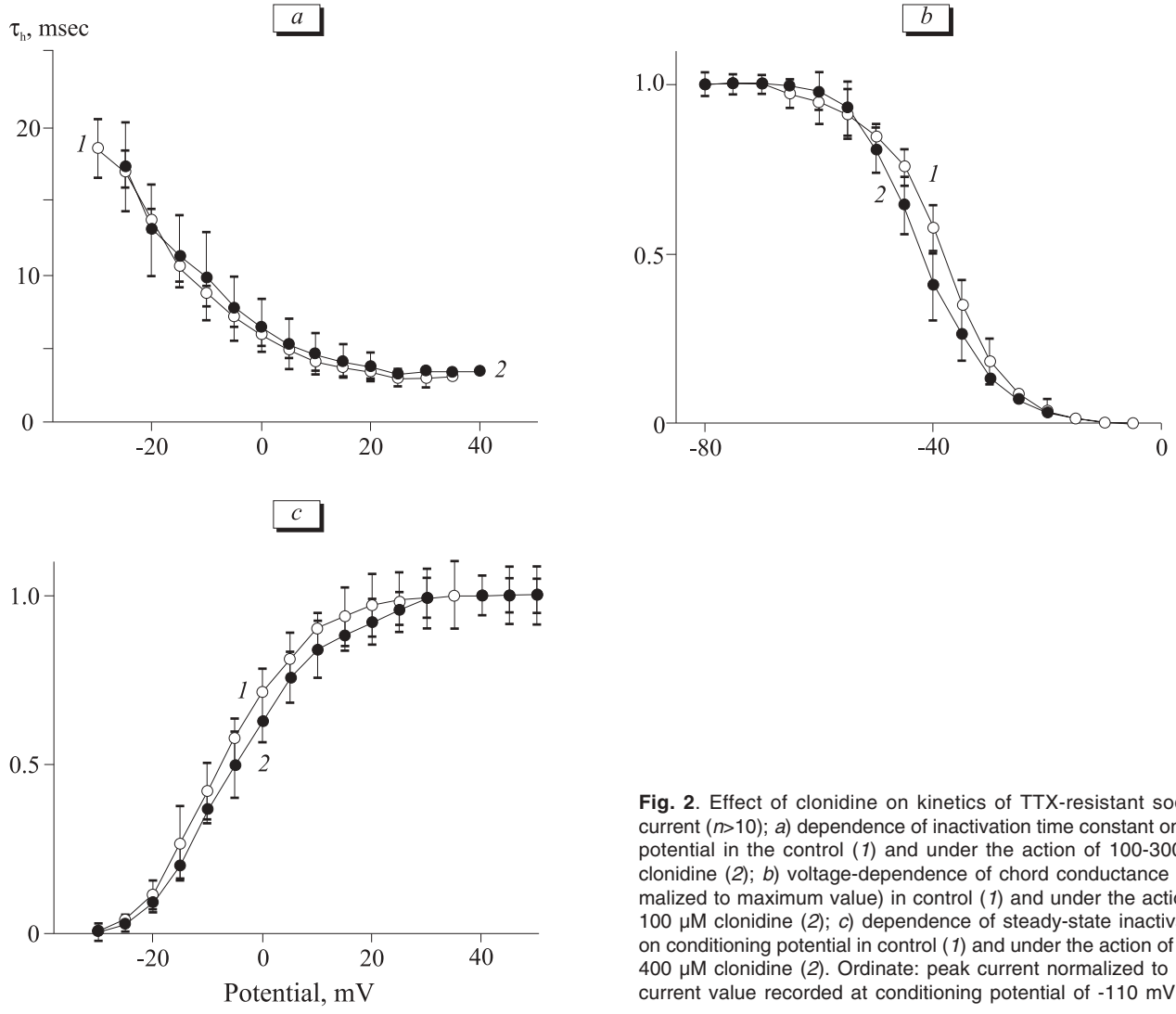

Fig. 2. Effect of clonidine on kinetics of TTX-resistant sodium current (n>10); *a*) dependence of inactivation time constant on test potential in the control (*1*) and under the action of 100-300 μM clonidine (*2*); *b*) voltage-dependence of chord conductance (normalized to maximum value) in control (*1*) and under the action of 100 μM clonidine (*2*); *c*) dependence of steady-state inactivation on conditioning potential in control (*1*) and under the action of 100-400 μM clonidine (*2*). Ordinate: peak current normalized to peak current value recorded at conditioning potential of -110 mV.

selectively blocking TTXr channels are not known. Therefore, the effect of clonidine on TTXs currents was studied in experiments with total sodium currents, where the component currents were separated basing on different kinetics of inactivation. TTXs sodium currents are inactivated at more negative potentials (from -50 to -110 mV) [7,11,13]. Therefore, the steady-state curve inactivation of the total sodium current has two steps (Fig. 3, *b*): transitions in the potential ranges of -120 to -60 mV and -60 to -15 mV reflect inactivation of TTXs and TTXr channels regions, respectively.

Analysis of this curve allows evaluation of the contribution of each population of sodium channels into integral sodium permeability before and after application of the test agents. In the control solution, the amplitude of maximum current was 1200 pA, the amplitudes of TTXr- and TTXs-currents were about 800 pA and 400 pA, respectively (Fig. 3, *a*). After application of clonidine, the maximum current decreased to 500 pA (40% control value). In this case, the contribution of TTXr-current did not exceed 180 pA (<23% control value), while TTXs-current was no less than 300 pA (60% control value). The results of similar experiments performed with various concentrations of clonidine and different values of the test potential and stimulation rate showed that blockade of fast TTXs-currents is achieved at higher concentrations in comparison with slow TTXr-currents (IC_{50}=487±57 μM, nH=2.10±0.16, Fig. 3).

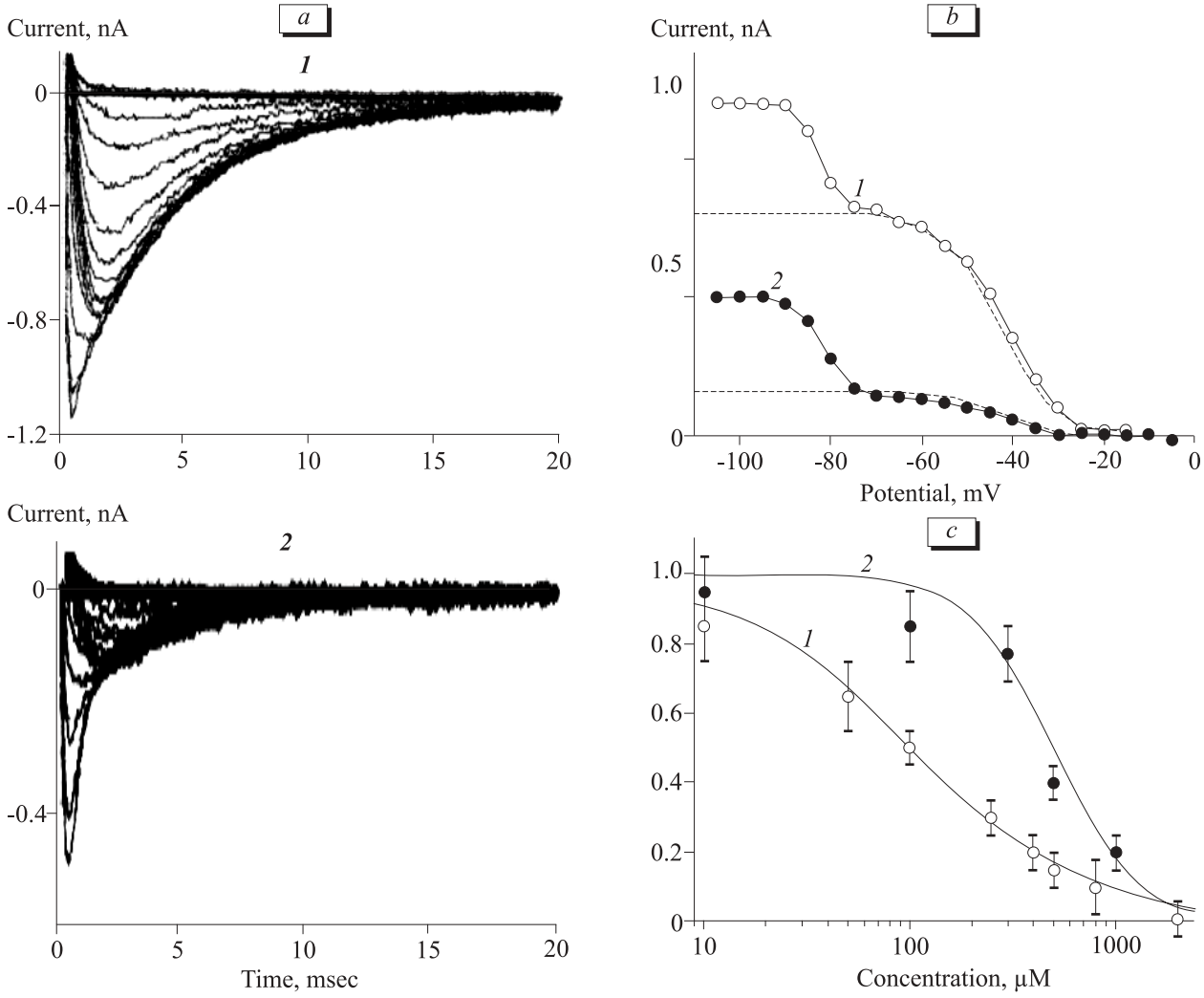

Fig. 3. Effect of clonidine on total sodium current. *a*) sodium currents recorded at the same test potential of -10 mV applied after various conditioning stimuli ranged from -110 mV to -10 mV with the step of 5 mV in control (*1*) and after the effect of 300 μM clonidine (*2*); *b*) voltage-dependence of normalized peaks of sodium currents on conditioning potential (steady-state inactivation) in control (*1*) and after application of clonidine (*2*). Calculated from data in *a*. Dashed lines show region of inactivation of TTXr currents; *c*) dose-dependence of clonidine blocking effect on TTXr (*1*) and TTXs (*2*) sodium currents. The data were fitted by Hill equation (solid lines): IC_{50}=101±9, nH=1.00±0.06 (*1*) and IC_{50}=487±57, nH=2.1±0.5 (*2*). Ordinate: ratio of maximum sodium conductance measured after clonidine application to control value.

Norepinephrine and phenylephrine in concentrations of 10-1000 μM had no inhibitory effect on sodium channels and did not modulate the blocking action of clonidine. It could be concluded that the inhibitory effect of clonidine is not mediated by α-adrenoreceptors, but results from its direct blocking action on the potential-operated Na^+ channels of sensory neurons, which can be a component of the analgesic effect of this agent. It should be noted that by the range of efficient concentrations and by the character of action on both types of sodium channels, clonidine is similar to typical local anesthetics [7,11]. However, in contrast to most local anesthetics, clonidine is more efficient in blocking TTXr sodium channels.

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