FOR PLATON KOSTYUK'S MEMORY

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Those who love us
Watch our steps.
But rock'n'roll is dead,
And we are not yet...

B.G.

Calcium channels in the somatic membrane of the rat dorsal root ganglion neurons, effect of cAMP

S.A. Fedulova, P.G. Kostyuk, N.S. Veselovsky
Brain Research (Volume 214, Issue 1, 9 June 1981, Pages 210-214)

Isolated rat dorsal root ganglion neurons have been perfused with potassium-free solutions containing cAMP, ATP and Mg^{2+} ions. In these conditions stable inward calcium currents can be recorded in the somatic membrane of all inves-

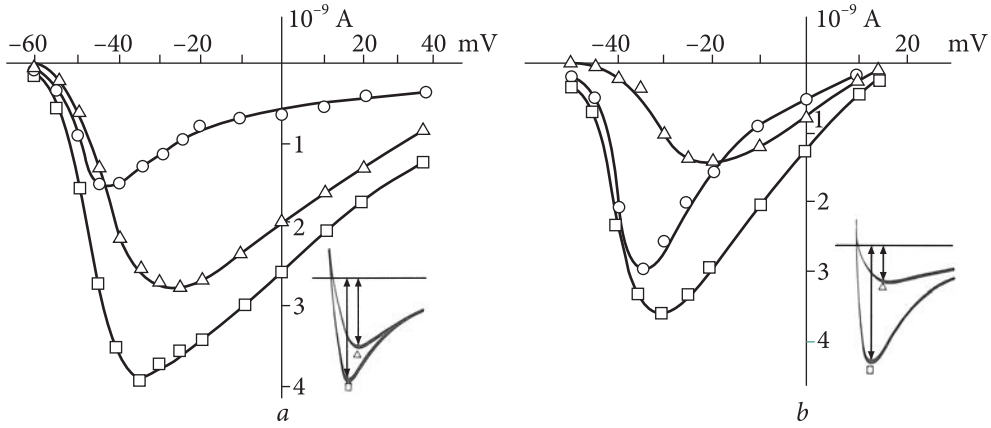

Fig. 1. Separation of fast and slow components of sodium inward current by tetrotoxin ($2 \times \times 10^{-7}$ g/ml) (*a*); Exampls of two cells with different ratios between fast and slow components of the inward sodium current (b). Squares: total current-voltage characteristics of the inward sodium current; triangles: current-voltage characteristics of the TTX-resistant sodium current; circles: current-voltage characteristics of TTX-sensitive sodium current obtained by substriction of the second curve from the first. Bottom, right: schematic representation of total sodium current and its TTX-resistant component

tigated cells. The kinetics of these currents can be approximated by a modified Hodgkin-Huxley equation using a square power of the m-variable; its inactivation is extremely slow. The corresponding channels pass Ba^{2+} ions about twice more effective than Ca^{2+}.

Ionic currents in the somatic membrane of rat dorsal root ganglion neurons-I. Sodium currents

P.G. Kostyuk, N.S. Veselovsky, A.Y. Tsyndrenko
Neuroscience (Volume 6, Issue 12, December 1981, Pages 2423-2430)

Measurements of sodium transmembrane ionic currents evoked by depolarizing shifts in membrane potential have been performed on isolated dorsal root ganglion neurons of 5-10-day-old rats. Potassium currents were eliminated by dialysing the neurons with potassium-free solutions. In 10-15% of investigated neurons a tetrodotoxin-resistant component has been revealed in the sodium inward current which differs in its potential-dependent and kinetic characteristics from the main tetrodotoxinsensitive one. The activation kinetics of the tetrodotoxin-sensitive sodium current could be described by the Hodgkin-Huxley model using the cubic power of the m-variable, whereas the activation kinetics of the tetrodotoxin-resistant one can be described using only the square power of m. The time constants of activation and inactivation of the tetrodotoxin-resistant current were about ten times longer than those of the tetrodotoxin-sensitive cur-

rent. The tetrodotoxin-resistant current was highly sensitive to all extracellular agents which are known as effective blockers of calcium channels (Co^{2+}, Mn^{2+}, Cd^{2+}, D-600 and its derivatives). At the same time, the selectivity of the corresponding channels did not differ significantly from the selectivity of the tetrodotoxin-sensitive sodium channels. The sequence of relative permeability for univalent cations was $P_{Na}: P_{Li}: P_{hydrazinium}: P_{NH4}: P_{hydroxylammonium}: P_K = 1.0:0.79:0.43:0.33:0.25:0.18$ for the tetrodotoxin-sensitive channels and 1.0:0.98:0.47:0.42:0.26:0.26 for the tetrodotoxin-resistant ones.

Thus, the tetrodotoxin-resistant sodium channels combine some features of sodium (selective filter) and calcium (gating mechanism and binding properties) channels.

Ionic currents in the somatic membrane of rat dorsal root ganglion neurons-II. Calcium currents

P.G. Kostyuk, N.S. Veselovsky, S.A. Fedulova
Neuroscience (*Volume 6, Issue 12, December 1981, Pages 2431-2437*)

The experiments have shown that the introduction of cyclic adenosine monophosphate, adenosine 5'-triphosphate and Mg^{2+} ions into dialysed isolated dorsal root ganglion neurons of 5-8-day-old rats not only prevents the rapid decline of calcium inward currents during the course of dialysis but restores to a considerable extent the calcium conductance. Introduction of adenosine 5'-triphosphate and Mg^{2+} has a much weaker stabilizing effect. This finding made it possible to separate and to investigate in detail the calcium current (I_{Ca}) in the somatic membrane of all investigated neurons. The maximal amplitude of I_{Ca} was proportional to the concentration of Ca^{2+} ions in the extracellular solution between 2 and 14 mM; with higher concentration a saturation effect was observed. Replacement of Ca^{2+} by Ba^{2+} caused about a two-fold increase in the maximal amplitude of inward currents. Addition of Co^{2+}, Mn^{2+}, verapamil, and related substances blocked the calcium current. The activation kinetics of I_{Ca} could be approximated by a modified Hodgkin-Huxley equation using a square power of the m-variable. The activation time constant τ_m changed in the range from 16 to 1.8 ms with testing potential change from −40 to +20 mV. The inactivation of I_{Ca} was extremely slow; the half value of steady-state inactivation was observed at holding potential about −60 mV. The potential-dependent and kinetic characteristics of the calcium currents obtained on several neurons from adult rats were similar to those for neurons of new-born ones.

It is concluded that the somatic membrane of the rat neurons has a system of electrically-operated selective calcium channels, the normal functioning of which is dependent on the intracellular cyclic nucleotide metabolism.

Ionic currents in the somatic membrane of rat dorsal root ganglion neurons-III. Potassium currents

P.G. Kostyuk, N.S. Veselovsky, S.A. Fedulova

Neuroscience (*Volume 6, Issue 12, December 1981, Pages 2439-2444*)

Potassium transmembrane currents induced by membrane depolarization have been studied on isolated dorsal root ganglion neruons of 5-10-day-old rats using the voltage-clamp technique. The neurons were intracellularly dialysed with solutions containing a fixed amount of K^+ ions, and the correspondence between the reversal potentials of the measured currents and the theoretical potassium equilibrium potential was determined. Sodium and calcium transmembrane currents were eliminated by replacement of Na^+ ions in the extracellular solution and by introduction of fluoride into the cell.

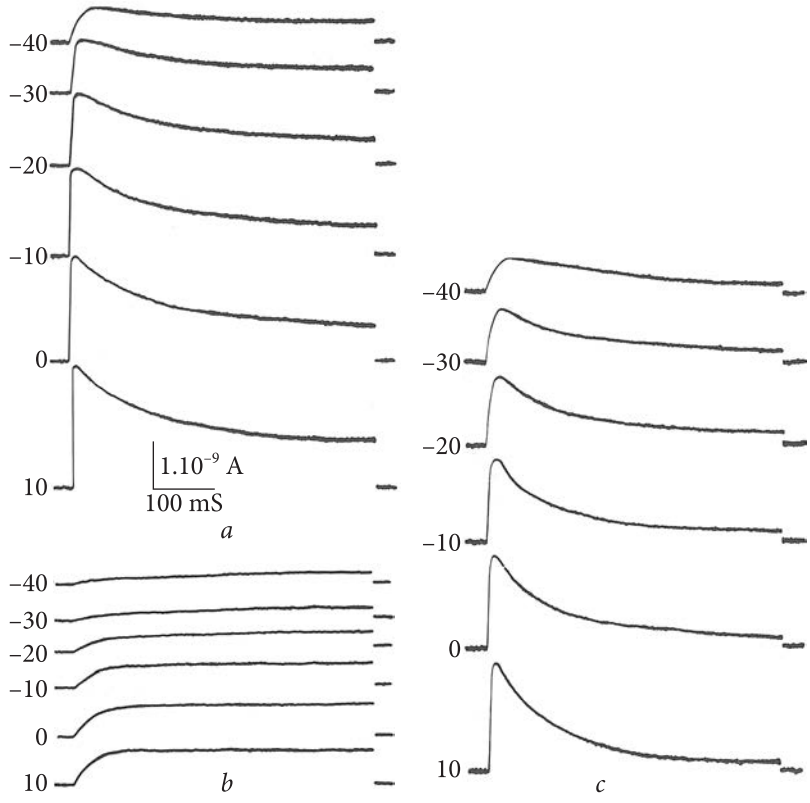

Fig. 2. Potassium outward currents in the somatic membrane of rat spinal ganglion neuron: *a* — Total outward current (holding potential –90 mV); *b* — slow outward current (holding potential –50 mV); *c* — fast outward current obtained by substriction of the records "b" from the corresponding records "a". Numbers near curves indicate testing potential in mV

In all cells studied, the total potassium current could be divided into two components, fast and slow (I_K^f and I_K^s), by changing the holding potential level. I_K^f was inactivated comparatively fast obeying the first-order kinetics. The dependence h_∞ (V) for this current was S-shaped with mean $V_{12} = -75\ mV$. Therefore, this current could be almost completely switched off at holding potentials more positive than −50 mV. On the contrary, the inactivation of I_K^s developed very slowly even at stronger depolarizing potential shifts. The mean activation time constants calculated on the basis of the Hodgkin-Huxley model for potassium currents were 0.5 ms at zero testing potential for I_K^f and 40 ms at +30 mV for I_K^s.

The reversal potential for I_K^f determined from instantaneous current-voltage characteristics was close to the equilibrium potential for a potassium electrode. The reversal potential for I_K^s was shifted in the depolarizing direction by about 25 mV indicating lower selectivity of the corresponding channels.

Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurons

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Ca^{2+} inward currents evoked by membrane depolarization have been studied by the intracellular dialysis technique in the somatic membrane of isolated dorsal root ganglion neurones of new-born rats. In about 20% of the investigated cells a

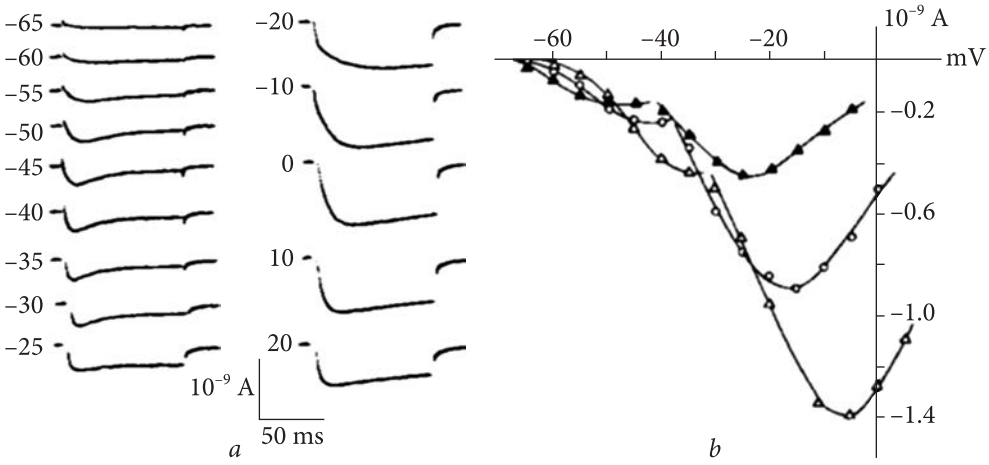

Fig. 3. Inward Ca^{2+} currents in the somatic membrane of rat dorsal root ganglion neurones. Intracellular dialysis by Tris phosphate (150 mM). Holding potential –90 mV: *a* — experimental traces of inward Ca^{2+} currents in Na^+-free outside solution with 14*8 тм-Ca^{2+}. Numbers near current curves indicate membrane potential in mV; *b* — ecurrent-voltage characteristics for the somatic membrane of one of the investigated neurones obtained in solutions with different external Ca^{2+} concentration

hump has been detected on the descending branch of the current-voltage curve, indicating the presence of two populations of Ca^{24}" channels differing in their potential-dependent characteristics.

An initial less regular component of the Ca^{2+} current was activated at membrane potentials from −75 to −70 mV. Its amplitude reached 0*2-0*9 nA at 14*6 тм- extracellular Ca^{2+}. The activation kinetics of this component could be approximated by the Hodgkin-Huxley equation using the square of the *m* variable. r_w varied in the range from 8 to 1 ms at potentials between −60 and −25 mV ("fast" Ca^{2+} current).

The second component of the Ca^{2+} current was activated at membrane depolarizations between −55 and −50 mV. It could be recorded in all cells investigated and reached a maximum value of 1-7 nA at the same extracellular Ca^{2+} concentration. This component decreased rapidly during cell dialysis with saline solutions. The decrease could be slowed down by cooling and accelerated by warming the extracellular solution. Intracellular introduction of 3',5'-cAMP together with ATP and Mg^{2+} not only prevented the decrease but often restored the maximal current amplitude to its initial level. The activation kinetics of this component could also be approximated by a square function, r_m being in the range 16-2*5 ms at membrane potentials between −20 and +3 mV ("slow" Ca^{2+} current).

The fast Ca^{2+} current inactivated exponentially at sustained depolarizations in a potential-dependent manner, T_h varying from 76 to 35 ms at potentials between −50 and −30 mV. The inactivation of the slow Ca^{2-1}" current studied in double-pulse experiments was current-dependent and developed very slowly (time constant of several hundreds of milliseconds). It slowed down even more at low temperature or after substitution of Ba^{2+} for Ca^{2+} in the extracellular solution.

Both currents could also be carried by Ba^{2+} and Sr^{2+}, although the ion-selecting properties of the two types of channels showed quantitative differences. Specific blockers of Ca^{2+} channels (Co^{2+}, Mn^{2-l}", Cd^{2+}, Ni^{2+} or verapamil) exerted similar effects on them.

The existence of metabolically dependent and metabolically independent Ca^{2+} channels in the neuronal membrane and their possible functional role are discussed.