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REGULATION OF SODIUM CURRENTS THROUGH OXIDATION AND REDUCTION OF THIOL RESIDUES

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Abstract—Changes in redox state are involved in several physiological and pathophysiological processes. Previous experiments have demonstrated that nitric oxide can function as a reactive oxygen species, inhibiting neuronal sodium currents by nitrosylation of thiol residues. We hypothesized that nitric oxide and thiol oxidizers similarly modulate voltage-dependent sodium currents. Voltage-dependent sodium currents were studied with the whole-cell patch-clamp technique in NB41A3 neuroblastoma cells. The nitric oxide donor 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine did not affect sodium currents. In contrast, the thiol oxidizers thimerosal and 4,4′-dithiopyridine significantly inhibited sodium currents. The effect of thimerosal persisted after washout, but could be fully reversed by the reducing agent dithiothreitol. Reduced glutathione did not restore the sodium current amplitude when given extracellularly, while intracellular glutathione prevented the inhibitory effect of thimerosal. Pretreatment with the alkylating agent *N*-ethylmaleimide blocked the inhibitory action of thimerosal. Thiol oxidation caused a shift in the voltage dependence of fast and slow inactivation to more hyperpolarized potentials without concomitant effects on the voltage dependence of activation. Mercaptoethanol and reduced glutathione enhanced sodium currents by shifting the voltage dependence of inactivation to depolarized potentials.

These results demonstrate that the oxidation and reduction of thiol residues alters the properties of voltage-sensitive sodium channels and may play an important role in the regulation of membrane excitability. © 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: neuroblastoma cells, thiol oxidation, slow inactivation.

Post-translational modification of ion channels plays an important role in fine-tuning cellular function of excitable and non-excitable cells. Phosphorylation of serine or threonine residues, as well as tyrosine phosphorylation, have been studied extensively.[5] The covalent modification of ion channels through protein kinases and phosphatases significantly alters their properties, thereby changing membrane function and excitability. More recently, several studies have demonstrated that changes in redox state may contribute to the control of cell function.[4,19,20] Oxidation of cysteine or methionine residues by reactive oxygen species affects ligand-gated and voltage-dependent ion channels in a variety of different cells.[1,8,10,11,18,25,26] Such reactive oxygen species are generated during inflammatory processes and reperfusion injury, and contribute to the structural and functional damage seen in these disorders.[2,6,7] In addition, changes in cellular redox state have been observed during signaling processes, suggesting a potential role of reactive oxygen species in second messenger cascades.[14,22]

We have recently demonstrated that nitric oxide (NO) inhibits voltage-dependent sodium currents in vagal sensory neurons.[3,19] NO caused this inhibition by functioning as a reactive oxygen species, because this effect was due to nitrosylation of cysteine residues and did not depend on the activation of guanylate cyclase. As nodose neurons express NO synthase, NO may function as an autocrine modulator of neuronal activity. Vagal sensory neurons from the nodose ganglion express at least two different sodium channels that differ in their biophysical and pharmacological properties. Both were equally affected by NO.[19] To identify whether NO alters sodium currents in another cell, we investigated the effects of NO in NB41A3 cells, a murine neuroblastoma cell line. Moreover, we hypothesized that oxidation of thiol groups similarly inhibits sodium currents. Previous studies have demonstrated that NB41A3 cells express tyrosine hydroxylase, a marker for cells of neuronal lineage.[13] Even in the absence of specific neurotrophic factors, rapidly activating voltage-dependent sodium currents can be recorded from cells that are round in appearance and do not have long processes.[27] Therefore, this murine cell line appeared to be an approprite model system for the study of ion channel modulation in neuronal cells.

EXPERIMENTAL PROCEDURES

Cell culture

We used the neuroblastoma cell line NB41A3 (CCl-147; American Type Culture Collection, Manasses, VA) for all experiments. The cells were cultured in Ham's F10 supplemented with 2 mM L-glutamine, 1.5 g/l $NaHCO_3$ and 10% horse serum. Cells were passaged in poly-L-lysine-coated flasks after five to seven days. Two days before performing the electrophysiological experiments, the cells were plated on to poly-L-lysine-coated coverslips. At the time of the experiment, most cells were round or triangular, with no or few short processes, minimizing space clamp problems that might interfere with the voltage control of the cells.

Electrophysiological recordings

The cells attached to the coverslips were transferred into a 0.5-ml recording chamber on the stage of an inverted microscope (Nikon). Sodium currents were recorded using the whole-cell patch-clamp technique with an Axopatch 200A amplifier (Axon Instruments, CA)

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Abbreviations: DTT, dithiothreitol; EGTA, ethyleneglycol-bis(β-aminoethyl ether)tetra-acetate; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; NEM, *N*-ethylmaleimide; NO, nitric oxide; papaNONOate, 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine; TTX, tetrodotoxin.