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Effect of 8-bromo-cAMP on the tetrodotoxin-resistant sodium (Nav 1.8) current in small-diameter nodose ganglion neurons

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Abstract

We examined whether 8-bromo-cAMP (8-Br-cAMP)-induced modification of tetrodotoxin-resistant (TTX-R) sodium current in neonatal rat nodose ganglion neurons is mediated by the activation of protein kinase A (PKA) and/or protein kinase C (PKC). In 8-Br-cAMP applications ranging from 0.001 to 1.0 mM, 8-Br-cAMP at 0.1 mM showed a maximal increase in the peak TTX-R Na^+ (Nav1.8) current and produced a hyperpolarizing shift in the conductance–voltage ($G-V$) curve. The PKC inhibitor bisindolylmaleimide Ro-31-8425 (Ro-31-8425, 0.5 μM) decreased the peak Nav 1.8 current. The Ro-31-8425-induced modulation of the $G_{V1/2}$ baseline (a percent change in G at baseline V1/2) was not affected by additional 8-Br-cAMP application (0.1 mM). The maximal increase in Nav 1.8 currents was seen at 0.1 μM after the application of a PKC activator, phorbol 12-myristate 13-acetate (PMA) and forskolin. The PMA-induced increase in Nav 1.8 currents was not significantly affected by additional 0.1 mM 8-Br-cAMP application. Intracellular application of a PKA inhibitor, protein kinase inhibitor (PKI, 0.01 mM), inhibited the baseline Nav 1.8 current, significantly attenuated the 8-Br-cAMP-and PMA-induced increase in the peak Nav 1.8 current, and caused a significant increase in the slope factor of the inactivation curve. The PKI application at a higher concentration (0.5 mM) greatly inhibited the PMA (0.1 μM)-induced increase in the peak Nav 1.8 current amplitude and further enhanced the Ro-31-8425-induced decrease in the current. These results suggest that the 8-Br-cAMP-induced increase in Nav 1.8 currents may be mediated by activation of both PKA and PKC.

Keywords: Nodose ganglion; 8-Bromo-cyclic AMP; Tetrodotoxin-resistant sodium current; Protein kinase A; Protein kinase C; Internal application

1. Introduction

In electrophysiological studies of the Na^+ current of dorsal root ganglion (DRG) neurons, two different types of current have been identified: tetrodotoxin-sensitive (TTX-S) Na^+ currents that are blocked by nanomolar concentrations of TTX and TTX-resistant Na^+ (Nav 1.8) currents that are only partially blocked or unaffected by micromolar concentrations of TTX (Ogata and Tatebayashi, 1992; Elliott and Elliott, 1993). The Nav 1.8 current is preferentially expressed in capsaicin- and prostaglandin-sensitive neurons (Schuligoi et al., 1994; Gold et al., 1996), as compared with the Na^+ current of capsaicin-insensitive neurons (Pearce and Duchen, 1994; Arbuckle and Docherty, 1995). The Nav 1.8 channels have been cloned from rat DRG neurons and the expression of these channels is restricted to small cells in the DRG, which are sensitive to capsaicin (Akopian et al., 1996).

The majority of nodose ganglion (NG) neurons, which are projected from the cardiovascular, pulmonary and gastrointestinal

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afferent inputs, have unmyelinated axons (C-type neurons) and about 10% NG neurons have myelinated axons (A-type neurons) (Baccaglini and Cooper, 1982; Stansfeld and Wallis, 1985; Seagard et al., 1990). A-type neurons are completely blocked by TTX, but after application of micromolar concentrations of TTX, C-type neurons still generate action potentials (Baccaglini and Cooper, 1982; Stansfeld and Wallis, 1985; Ikeda and Schofield, 1987; Bielefeldt, 2000; Fazan et al., 2001). In DRG neurons insensitive to TTX, the effect of prostaglandin E_2 (PGE_2) on Nav 1.8 current is mimicked by the drugs that upregulate cAMP-dependent protein kinase A (PKA) phosphorylation of the channel (England et al., 1996; Gold et al., 1998). Using site-directed mutagenesis, five serines located within the intracellular I–II loop of sensory neuron-specific Nav 1.8 channels were identified as the major sites of PKA modification (Fitzgerald et al., 1999). Conversely, based on evidence that the inhibitors of protein kinase C (PKC) significantly attenuated PGE_2-induced modulation of the I_{NaR} obtained from adult DRG neurons or that PKA inhibitors had little or no effect on the PKC activator-induced increase in Nav 1.8 currents, Gold et al. (1998) suggested that PKC activity may play an important role in regulating the subsequent PKA-mediated modulation of Nav 1.8 currents. This suggestion is consistent with the observation demonstrating that PKC-induced phosphorylation of the channel protein at serine 1506 in the III–IV loop of the brain type II_A Na^+ channel is required to enable PKA-induced phosphorylation of other sites on the channel protein (Li et al., 1993). Nevertheless, the question arises as to how the activation of two protein kinases, PKA and PKC, is involved in the modulation of the Nav 1.8 currents obtained from neonatal NG neurons, particularly when the level of cAMP is increased after application of the drugs.

8-Bromo-cAMP (8-Br-cAMP), one of the membrane-permeable cAMP analogues, is considered to appear relatively less efficient than forskolin because this substrate is hydrolyzed by a variety of phosphodiesterases in intact cells (Sandberg et al., 1991). However, there is evidence that the similarity between the effects of the membrane-permeable cAMP analogue, dibutyryl cAMP (db-cAMP) and the direct activator of adenyl cyclase, forskolin, or prostaglandin E_2 (PGE_2) on the Nav 1.8 current characteristics was found in neonatal DRG neurons (England et al., 1996). Recent evidence has demonstrated that the basal Nav 1.8 current obtained from neonatal NG neurons may be regulated by the level of PKC activity (Ikeda et al., 2005). To test the hypothesis that both PKA and PKC activities appear as the effects on the 8-Br-cAMP-modulation of Nav 1.8 currents, we investigated the effects of PKC and PKA modulators on the Na^+ current properties in small-diameter (<28 μm) neonatal rat NG neurons and also examined whether the 8-Br-cAMP-induced modulation of the Na^+ current is mainly mediated through both PKA and PKC signal transduction mechanisms. In another series of experiments, expression of the Nav 1.8 and Nav 1.9 channel proteins in the neonatal rat NG was examined by means of reverse transcription-polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Cell culture

Primary cultures of dissociated neonatal NG neurons were prepared as described previously (Ikeda and Matsumoto, 2003; Matsumoto et al., 2005). Wistar rats (6–11 days, 14–26 g) were deeply anesthetized with pentobarbital sodium (50–60 mg/kg, i.p.). The nodose ganglia were identified, dissected from the vagus nerve trunks and placed in modified Hank's balanced salt solution (HBSS) containing (in mM) 130 NaCl, 5 KCl, 0.3 KH_2PO_4, 4 $NaHCO_3$, 0.3 Na_2HPO_4, 5.6 glucose and 10 HEPES. Then the dissected nodose ganglia were transferred to HBSS containing 20 U/ml of papain (Worthington Biochemical, New Jersey) and incubated for 15–25 min at 37 °C. Single cells were obtained by triturating the suspension through a wide-pore Pasteur pipette, and subsequently were plated onto the glass cover slips pretreated with poly-L-lysine in a 35 mm dish. The plating medium contained Leibovitz's L-15 solution (Invitrogen Corp, Carlsbad, CA) supplemented with 10% newborn calf serum, 50 U/ml penicillin–streptomycin (Invitrogen Corp), 26 mM $NaHCO_3$ and 30 mM glucose. The cells were maintained in 5% CO_2 at 37 °C and used for recording between 2 and 10 h after plating.

2.2. Recording solutions and drugs

The internal solution for electrodes consisted of (in mM): 10 NaCl, 100 CsF, 40 CsCl, 2 $MgCl_2$, 1 $CaCl_2$, 2 Mg-ATP, 10 HEPES, 14 Na_2 creatine-phosphate and 11 EGTA; pH was adjusted to 7.2 with CsOH. The external solution consisted of (in mM): 30 NaCl, 80 choline-Cl, 40 tetrathylammonium (TEA)-Cl, 3 $MgCl_2$, 10 glucose and 10 HEPES; pH was adjusted to 7.4 with TEA-OH and the Ca^{2+} concentration in the pipette solution ranged 20–40 nM. In voltage-clamp mode, the solutions designed for the isolation of Na^+ currents were as follows: (1) the external solution was Ca^{2+} free to prevent contamination of Na^+ currents from voltage-gated Ca^{2+} currents; (2) the external solution contained 30 mM NaCl to obtain the accuracy of Na^+ currents and to improve the fidelity of the voltage-clamp; (3) in voltage-clamp experiments, tetrodotoxin (TTX, Sigma, St. Louis, MO) was added to the external solution and its concentration was adjusted to 1 μM. As suggested by Saab et al. (2003), a rapid increase in the I_{NaR} amplitude occurred after rupture of the membrane and at 5 min after 1 μM TTX application, the stabilization of the Nav 1.8 current was verified by no detectable changes in the series resistance as well as in the I_{NaR} amplitude at a −10 mV step pulse. After establishing a stable condition on the Nav 1.8 current amplitude, we started the experiments. We used the membrane-permeable cAMP analog 8-bromo-cAMP (8-Br-cAMP), a protein kinase C (PKC) activator phorbol-12-myristate 13-acetate (PMA), and a PKC inhibitor, bisindolylmaleimide Ro-31-8425 (Ro-31-8425), and a direct activator of adenyl cyclase, forskolin. These drugs were obtained from Sigma and dissolved in distilled water or in DMSO to form stock solutions (10–100 times more concentrated than the final concentration used). In some TTX-R NG neurons, no significant changes in the Nav 1.8 current were obtained in a Ca^{2+}-free solution. Stock solutions were stored at −20 °C and diluted in the external solution before use. The protein kinase A (PKA) inhibitor (protein kinase inhibitor, PKI, Sigma) used was the same as described in a previous study (Cheng et al., 1986), and was dissolved in the pipette solution. One microgram of the PKA inhibitor was able to inhibit approximately 2000 phosphorylating units of cAMP-dependent protein kinase.

2.3. Electrophysiological recording and pulse protocol

Recordings were performed by means of the whole-cell configuration of the patch-clamp technique. For whole-cell voltage-clamp experiments, glass pipettes with a low resistance between 2 and 5 MΩ were used. Isolated cells on the glass cover slip were placed in a recording chamber and visualized under phase contrast on an inverted microscope (Nikon, Tokyo, Japan). The signal was measured with an Axopatch-1D patch-clamp amplifier (Axon Instruments, Forster City, CA). Data were low-pass-filtered at 5–10 kHz with a four-pole Bessel filter and digitally sampled at 25–100 kHz. After seal formation and membrane disruption, the whole cell capacitance (10–20 pF) was cancelled and series resistance (>80%) was compensated. External solutions were applied via a linear array of seven polyethylene tubes (280 μm in diameter)