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Direct inhibition of the transient voltage-gated K^+ currents mediates the excitability of tetrodotoxin-resistant neonatal rat nodose ganglion neurons after ouabain application

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ABSTRACT

The purpose of the present study was to determine the relationship between the responses of transient and sustained K^+ currents, and action potentials to ouabain, and to compare the immunoreactive expression of alpha Na^+–K^+-ATPase isoforms (α_1, α_2 and α_3) in neonatal rat small-diameter nodose ganglion neurons. We used perforated patch-clamp techniques. We first confirmed that the neurons (n = 20) were insensitive to 0.5 μM tetrodotoxin (TTX). Application of 1 μM ouabain 1) decreased the transient K^+ currents in 60% of neurons and the sustained K^+ currents in 20%, 2) increased voltage-gated transient and sustained K^+ currents in 20% of neurons, and 3) had no effect on transient K^+ currents in 20% of neurons and on sustained K^+ currents in 60%. Thirteen of the neurons were of a rapidly adapting type, and the remaining 7 were of a slowly adapting type. In 6 rapidly adapting type neurons (46%), their activity was not significantly altered by ouabain application, but in 4 rapidly adapting type neurons, the activity increased. In the remaining 3 rapidly adapting type neurons, ouabain application hyperpolarized the resting membrane potential. The slowly adapting type 7 neurons each showed increased activity after 1 μM ouabain application. The α_1 isoform of Na^+–K^+-ATPase was identified as the predominant immunoreactive isoforms in small-diameter nodose ganglion neurons. These results suggest that the increased activity of small-diameter nodose ganglion neurons seen after application of 1 μM ouabain is mediated by direct inhibition of the transient K^+ current.

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1. Introduction

The Na^+–K^+-ATPase consists of a heterodimer of catalytic (α) and glycosylated (β) subunits. The α subunit controls the enzymatic and pumping activities, and the β subunit is required to induce the structural and functional maturation of the α subunit (Hasler et al., 1998; Abriel et al., 1999). The two subunits have several isoforms (α_1–α_4 and β_1–β_3), which are found in the peripheral and central neurons (Levenson, 1994) and which show species- and tissue-dependent patterns (Blanco and Mercer, 1998). Although human α_4 Na^+–K^+-ATPase has a physiological role in sperm motility (Hlivko et al., 2006; Sanchez et al., 2006), the function of this α isoform in other cell types has not been clarified.

Pulmonary vagal receptors are generally classified into three types of receptors on the basis of electrophysiological studies: namely slowly adapting pulmonary stretch receptors, and rapidly adapting pulmonary stretch receptors and vagal C-fiber receptors. Slowly adapting pulmonary stretch receptor structures identified by using an antibody against α_3 Na^+–K^+-ATPase in rabbits have multiple endings that form terminal knobs (Yu et al., 2003). Similar α_3 Na^+–K^+-ATPase-immunoreactive structures have been identified in slowly adapting pulmonary stretch receptors in rat nerve terminals; in contrast, α_1 Na^+–K^+-ATPase-immunoreactivity has not been detected in these receptors (Matsumoto et al., 2004).

Neurons in the nodose ganglia (*i.e.* the inferior ganglion of the vagus nerve) project to pulmonary, cardiovascular, and gastrointestinal afferent inputs. Ninety percent of these neurons have unmyelinated axons and the remainders have myelinated axons (Stansfeld and Wallis, 1985; Undem and Weinreich, 1993). Na^+–K^+-ATPases located in the plasma membrane exchange 3 Na^+ ions (outwards) for every 2 K^+ ions (inwards) during an active cycle (Skou, 1965), thereby producing an electrogenic current that is outward-hyperpolarizing, and resetting the resting membrane potential.

The cardioactive glycoside ouabain functions as an inhibitor of Na^+–K^+-ATPase. Application of ouabain at a low concentration (1 μM) in neonatal rats can depolarize the resting membrane potential, as well as enhances the afterhyperpolarization-activated current (I_h), in small-diameter (*i.e.* <30 μm in diameter) nodose ganglion neurons (Matsumoto et al., 2008); depolarization of the resting membrane potential is involved in the observed decrease in transient voltage-gated K^+ current. However, the exact mechanisms underlying the excitability neurons after application of ouabain at a low concentration (1 μM) have not yet been addressed.

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The purpose of the present study was to examine whether the responses of transient and sustained voltage-gated (transient and sustained) K^+ currents and action potential are altered by the application of 1 μM ouabain in neonatal rat small-diameter nodose ganglion neurons. We also examined which of the α Na^+–K^+-ATPase isoforms, containing α_1, α_2 or α_3 subunits, shows the predominant expression in these neurons. Our data support the idea that the reduction in transient K^+ current seen after the application of 1 μM ouabain is related to the excitability of small-diameter nodose ganglion neurons.

2. Materials and methods

All experiments were approved by the Animal Use and Care Committee of Nippon Dental University and were conducted in accordance with the ethical and animal welfare issues in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23).

2.1. Cell culture

Acute dissociation of neonatal rat nodose ganglion neurons was performed by a technique described previously (Matsumoto et al., 2005; Ikeda et al., 2006; Matsumoto et al., 2007, 2010). In brief, 40 neonatal Wistar rats (6–11 days old, 14–26 g body weight) were deeply anesthetized with pentobarbital sodium (50–60 mg/kg, administered by intraperitoneal injection). The nodose ganglia were identified, dissected from the vagus nerve trunks and placed in a modified Hanks balanced salt solution, containing 130 mM NaCl, 5 mM KCl, 0.3 mM KH_2PO_4, 4 mM $NaHCO_3$ 0.3 mM Na_2HPO_4, 5.6 mM glucose, and 10 mM HEPES. The dissected nodose ganglia were transferred to modified Hanks balanced solution containing collagenase type XI (1.0 mg/ml, Sigma-Aldrich, St. Louis, MO, USA) and type II (1.0 mg/ml, Sigma-Aldrich). Single cells were obtained by triturating the suspension through a wide-pore Pasteur pipette and were subsequently placed onto glass cover slips pretreated with poly-L-lysine in a 35 mm dish. The plating medium contained Leibovitz's L-15 solution (Invitrogen Corp, Tokyo, Japan) supplemented with 10% newborn calf serum and 50 U/ml penicillin–streptomycin (Invitrogen Corp). The cells were maintained in 5% CO_2 at 37 °C and were used in experiments between 2 and 10 h after plating. The recording chamber (volume, 0.5 ml) was mounted on an inverted microscope (Nikon, Tokyo, Japan) equipped with phase-contrast filters, a video camera, and two micromanipulators. The chamber was perfused under gravity with standard external solutions (Table 1) at a rate of approximately 0.5 ml/min.

Table 1
Composition of extra- and intracellular solution.

	V-clamp (mM)		I-clamp (mM)
Extracellular solution			
Choline-Cl	150	NaCl	155
HEPES	10	HEPES	10
KCl	3	KCl	3
Mg-Cl2	1	CaCl2	1
Glucose	20	MgCl2	1
Adjusted to pH = 7.4 with KOH		Glucose	20
		Adjusted to pH = 7.3 with NaOH	
Patch pipette solution			
Potassium methanesulfonate			130
KCl			20
EGTA			2
HEPES			7.5
Adjusted to pH = 7.3 with KOH			

2.2. Recording solutions and drugs

Current clamp (I-clamp) experiments were performed to measure action potentials and voltage clamp (V-clamp) experiments were conducted to measure transient and sustained K^+ currents. The compositions of the different external solutions used in the I-clamp and V-clamp experiments are listed in Table 1. The same patch pipette solution was used in both systems, and its composition is listed in Table 1. In the experiment using the fluorescent Ca^{2+} indicator fura-2-based ratiometric Ca^{2+} imaging, the average value for internal Ca^{2+} concentration ($[Ca^{2+}]_i$) was over 55 nM in isolated adult rat NG and jugular ganglion neurons (Gu et al., 2007).

Tetrodotoxin (TTX) at a concentration of 0.5 μM was added to the external solution in the I-clamp experiments. Ouabain was obtained from Funakoshi Co. Ld. (Tokyo, Japan). A stock solution of ouabain was prepared by dissolving100 mg ouabain in 20 ml distilled water. Prior to the experiments, the stock solution of ouabain was added to the external solution to give a final concentration of 1 μM ouabain. All experiments were performed at room temperature (24–26 °C).

2.3. Electrophysiology

Whole-cell patch recordings were conducted by using a rapid perforated-patch clamp technique (Rae et al., 1991; Takeda et al., 2004; Kadoi et al., 2007). The fire-polished patch-pipette (resistance, 2–4 MΩ) was filled with an internal solution (Table 1), containing amphotericin B (100 μg/ml) and lucifer yellow dipotassium salt (0.1%, Sigma-Aldrich). After seal formation and membrane perforation, leakage and capacitance transients were canceled by the analog circuit. Series resistance compensation (>80%) was employed. V-clamp and I-clamp recordings were conducted with an Axopatch 200B amplifier (Axon Instruments, Forster City, CA, USA). Signals were low-pass filtered at 1 or 5 kHz with a four-pole Bessel filter and digitized at 10 kHz.

Nodose ganglion neurons (<30 μm in diameter) were bathed in a flowing stream of the external solution except during the application of drugs. External solutions were applied via a linear array of 7 polyethylene tubes (280 μm in diameter) positioned close to the cell bodies (approximately 200 μm), as described in previous studies (Ikeda et al., 2006; Matsumoto et al., 2005, 2007, 2010).

None of the tested cells showed lucifer yellow fluorescence even when a low series resistance, resulting from patch perforation, was established. But the fluorescence could be observed when the membrane was ruptured by applying negative pressure, and under such circumstances the resistance dropped to a lower value (usually <8 MΩ). All recordings were performed at room temperature (22–24 °C). Data were stored on a computer disk for off-line analysis.

2.4. I-clamp recording

We first confirmed that the nodose ganglion neurons recorded were insensitive to 0.5 μM TTX. The threshold that evoked one action potential (1 T) was defined as the current that elicited a depolarizing single pulse (50–100 pA, 1 s). The number of action potentials was measured during a current injection of 3 times T (3 T) in the presence of 0.5 μM TTX. Neurons were accepted for study, only if they showed a stable resting membrane potential more negative than −40 mV, an action potential overshoot more than +20 mV, and a whole cell capacitance less than 30 pF, throughout the experiments. We classified the neurons into two different categories, rapidly and slowly adapting type nodose ganglion neurons, as described in previous studies (Mo and Davis, 1998; Yoshida and Matsumoto, 2005). During the current injection at 3 T, the rapidly adapting type neurons fired one or two action potentials, but the slowly adapting type neurons fired multiple action potentials. The afterhyperpolarization amplitude was measured from the peak of the maximum

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