Abstract  Electrophysiological properties of the inward rectification of neurons in the rat suprachiasmatic nucleus (SCN) were examined by using the single-electrode voltage-clamp method, in vitro. Inward rectifier current ($I_h$) was produced by hyperpolarizing step command potentials to membrane potentials negative to approximately -60 mV in nominally zero-Ca$^{2+}$ Krebs solution containing tetrodotoxin (1 μM), tetraethylammonium (40 mM), Cd$^{2+}$ (500 μM) and 4-aminopyridine (1 mM). $I_h$ developed during the hyperpolarizing step command potential with a duration of up to 5 s showing no inactivation with time. $I_h$ was selectively blocked by extracellular Cs$^+$ (1 mM). The activation of the H-channel conductance ($G_H$) ranged between -55 and -120 mV. The $G_H$ was 80-150 pS (n=4) at the half-activation voltage of -84±7 mV (n=4). The reversal potential of $I_h$ obtained by instantaneous current voltage (I/V) relations was -41±6 mV (n=4); it shifted to -51±8 mV (n=3) in low-Na$^+$ (20 mM) solution and to -24±4 mV (n=4) in high-K$^+$ (20 mM) solution. Forskolin (1-10 μM) produced an inward current and increased the amplitude of $I_h$. Forskolin did not change the half-activation voltage of $G_H$. 8-Bromo-adenosine 3',5'-cyclic monophosphate (8-Br-cAMP, 0.1-1 mM) and dibutyryl-cAMP (0.1-1 mM) enhanced $I_h$. 3-Isobutyl-1-methylxanthine (IBMX, 1 mM) also enhanced $I_h$. The results suggest that the inward rectifier cation current is regulated by basal activity of adenylyl cyclase in neurons of the rat SCN. 

Key words  SCN · Voltage-clamp · Inward rectifier current ($I_h$) · cAMP · Forskolin

Introduction

Ample evidence has accumulated suggesting that the suprachiasmatic nucleus (SCN) of the ventral hypothalamus is a pacemaker component in the regulation of mammalian circadian rhythmicity [7, 8, 17, 23, 26, 30]. Entrainment of rhythms to the light dark cycle mediated through the photoreceptors in the retina may play an important role for adaptation of the mammals to the external environment [18]. An intrinsic mechanism has also been proposed for the circadian rhythmicity of the spike firing activity in SCN neurons which had been cultured for several weeks [2]. Although intracellular studies have demonstrated a firing activity in single neurons of the SCN [1, 25, 28], membrane properties of SCN neurons and the ionic basis for spontaneously occurring action potentials are not entirely clarified. Sugimori et al. [25] reported that the SCN neurons displayed low-threshold Ca$^{2+}$ spike potentials and time-dependent inward rectification that is produced by activation of a Ca$^{2+}$-dependent K$^+$ conductance. In contrast, no obvious inward rectifier properties were seen in the current/voltage (I/V) relationship in SCN neurons [28]. We suggested that the inward rectification and the low-threshold Ca$^{2+}$ spike are responsible for the generation of spontaneous firing activity in neurons of the rat SCN [1]. Recently, Kim and Dudek [13] reported that a subpopulation of the SCN neurons exhibited slight time-dependent inward rectification related to enhanced excitability and firing rate.

Since inward (anomalous) rectification was found in skeletal muscle [12] and cardiac muscle [9], at least three types of inward rectifier currents have been reported in vertebrate neurons. These are classical anomalous rectifier currents carried predominantly by K$^+$ [14, 32], $I_h$ and $I_q$ that are carried by multiple cations [13, 15, 16, 27, 29], and an inward rectification carried by voltage-dependent Cl$^-$ currents [3]. In the present study, we made voltage-clamp recordings from neurons of the rat SCN to investigate the electrophysiological properties of the inward rectifier current in detail. The results suggest that the inward rectifier current, $I_h$, is carried by non-selective cation currents and that $I_h$ is regulated by intracellular cAMP in SCN neurons. A part of this study has been published previously [1].
Materials and methods

Male Wistar-Kyoto rats weighing 200-300 g were maintained in a temperature-controlled room (22-25°C) and exposed to a 14/10 h light/dark cycle (light on at 7:00 a.m.) for more than 2 weeks. The rats were sacrificed by decapitation in the morning (after 9:00 a.m.) of the experimental day. Their brains were removed rapidly and immersed for 8–10 s in a cooled (4-6°C) artificial cerebrospinal fluid solution (ACSF) (Krebs solution) prebubbled with 95% O₂/5% CO₂. Coronal hypothalamic slices (500 μm in thickness) containing the entire SCN and the optic chiasm were cut with a Vibroslice (Campden Instruments, USA). Slices were submerged in a recording chamber and superfused at 32-34°C with ACSF (pH 7.4) of the following composition (mM): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, NaH₂PO₄ 1.2 and glucose 11. The properties of I₉ were studied in a nominally zero-Ca²⁺ solution (standard solution) with following composition (mM unless otherwise stated): NaCl 70, KCl 4.7, MgCl₂ 11, tetraethylammonium (TEA) 40, tetrodotoxin (TTX) 1 μM, Cd²⁺ 0.5, 4-aminopyridine (4-AP) 1, HEPES 4, TRIS 1, and glucose 11. In low-Na⁺ (50 mM) solution, NaCl (93 mM) was replaced with TEA-Cl. For high-K⁺ solution, 20 mM KCl was added to the standard solution, where NaCl (20 mM) was reduced.

Glass micropettes were filled with 4 M K-citrate (100–140 MΩ). The membrane voltage and current were amplified with an Axoclamp 2A (Axon Instruments). During single-electrode voltage-clamp, the head stage output was continuously monitored to ensure adequate setting time. Sample frequencies were between 2 and 3 kHz and the amplifier gain was 0.8 nA/mV. Reliability of “space clamp” is unknown under our experimental condition. However, neurons in the SCN are rather small in size (6-8 μm) [31]. Furthermore, voltage traces of step command potentials that activate I₉ showed no serious errors for the voltage-clamp at a potential of −120 mV. Sufficient voltage-clamp was considered, therefore, to be obtained from SCN neurons in the present experiments. Signals were monitored continuously by a chart recorder (Nihon Kohden, RTA-1100). These signals were also digitized and stored on magnetic tapes (TEAC, RD-110T) for later analysis. pClamp software (Axon Instruments) operating on an IBM-AX computer (Sanyo) was used to analyse membrane currents. Data are expressed as mean±SEM.

The drugs used in the present study were applied to the brain slices by bath perfusion. TTX was obtained from Wako Chemicals. TEA-Cl, forskolin, 1,9-dideoxyforskolin, 4-AR 8-bromoadenosine 3',5'-cyclic monophosphate (Na⁺ salt, 8-Br-cAMP), N₆,2'-O dibutyryladenosine 3',5'-cyclic monophosphate (Na⁺ salt), db-cAMP and cAMP (Na⁺ salt) were purchased from Sigma (St Louis, Mo., USA). 3-isobutyl-1-methylxanthine (IBMX) was from Aldrich.

Results

I₉ in SCN neurons

Membrane potentials and currents were recorded from 48 neurons in the entire area of the SCN. A majority of SCN neurons (67%, n=32) displayed a large time-dependent decrease in input resistance, a depolarizing voltage “sag”, when the neuronal membrane was hyperpolarized.