THE MEMBRANE TARGET FOR CIRCADIAN CLOCK RESPONSIBLE FOR CIRCADIAN MODULATION OF FIRING RATE IN SUPRACHIASMATIC NUCLEUS NEURONS

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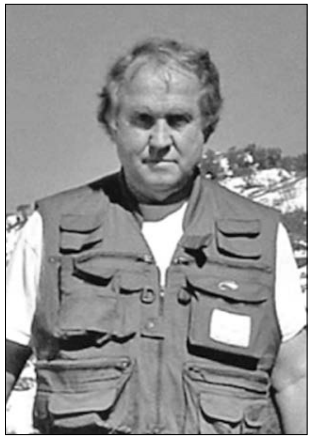

Mykola Kononenko *is a scientific researcher working in the Bogomoletz Institute of Physiology (Ph.D. 1976; supervisor Prof. Platon Kostyuk). His researches are focused on understanding the mechanisms of spontaneous activity in CNS neurons. In recent years (2000-2008), he studied membrane mechanisms responsible for circadian modulation of electrical firing in suprachiasmatic nuclei neurons in Colorado and Utah State Universities (Prof. F.E. Dudek's lab). Now he investigates calcium signaling in hippocampal neurons related to long-term depression. For this, Dr. M. Kononenko successfully utilizes different electrophysiological and fluorimetrical methods and approaches.*

Introduction

In mammals, the SCN of the hypothalamus contains a circadian (*circa day*) clock which regulates physiological functions with a period of approximately 24 h (Inouye and Kawamura, 1979; Meijer and Rietveld, 1989). One of the important findings was that the population of isolated and cultured SCN neurons exhibits a circadian rhythm of firing rate that lasts for many days (Welsh et al., 1995; Herzog et al., 1998; Honma et al., 1998), and thus, the circadian rhythms of the SCN neuronal population are a result of synchronized activity of many separate SCN neurons. The circadian clock in single neuron can be divided into three components: (a) an intracellular circadian clock based on transcription/translation mechanisms and studied thoroughly in the last decade (Reppert and Weaver, 2001; Reppert and Weaver, 2002); (b) cytoplasmic messenger(s) of signals between the intracellular circadian clock and the receptors located on the internal membrane surface, and (c) the receptor-regulated membrane channels which open and close to change the membrane potential and, correspondingly, the firing rate of SCN neurons from zero during the subjective night to a high frequency during the

subjective day. The precise nature of all of the cytoplasmic messenger(s) is unknown, and hypotheses regarding the membrane channels responsible for circadian modulation of firing rate are rather contradictory (Jiang et al., 1997; Pennartz et al., 2002; Cloues and Sather, 2003; Kuhlman and McMahon, 2004; Itri et al., 2005; Meredith et al., 2006; Pitts et al., 2006). In current work, I suggest hypothesis based on experimental data that SVC channels play a pivotal role in circadian modulation of firing rate in SCN neurons.

Effect of nifedipine and 4-aminopyridine

The aim of our initial experiments (Kononenko et al., 2008) was to test pharmacologically two the most popular hypotheses. One of them suggests that circadian modulation of L-type Ca^{2+} current(s) results in circadian modulation of the firing rate (Pennartz et al., 2002), while the other suggests that modulation of fast delayed rectifier (FDR) K^+ currents is responsible for the same phenomenon (Itri et al., 2005). For this, dissociated SCN cells from pups were pooled and cultured in the central area of MED. Generally, recordings from 30-40 neurons growing on the 64-electrode array demonstrated sufficient signal-to-noise ratio for action potential recording. About half the neurons exhibited variations of the firing rate, which could be considered as circadian after about 3 days of control recording. During drug application, nifedipine and 4-AP, complete medium change was done every second day. In our experiments, no clear suppression of the circadian changes in firing rate by nifedipine (Fig. 1, upper recording) and 4-aminopyridine (Fig. 1, lower recording) was found. Thus, the experiments with nifedipine and 4-aminopyridibe using prolonged MED recordings from single SCN neurons suggest that the corresponding Ca^{2+} and K^+ channels are not key modulators of firing rate during circadian cycles in SCN neurons. Noteworthily, the amplitude and shape of the averaged extracellular spike were unchanged during the circadian cycle (not shown here), even though the firing rate increased strongly (by ~120 times). This may suggest that the ionic channels directly involved in action-potential generation are not key membrane targets for circadian modulation of electrical firing in SCN neurons.

Subthreshold voltage-dependent cation single channels

In acutely isolated SCN neurons, about 25% of cell-attached patches exhibited single-channel currents characterized below and defined as subthreshold, voltage-dependent cation (SVC) channels (Kononenko et al., 2004). A prominent feature of these channels, which were persistently active at resting potential, was their nonlinear voltage dependence. At resting membrane potential, SVC single-channel currents were inwardly directed (Fig. 2, *a*, *1*) and had amplitudes ranging from about 0 pA to 2 pA in different patches.

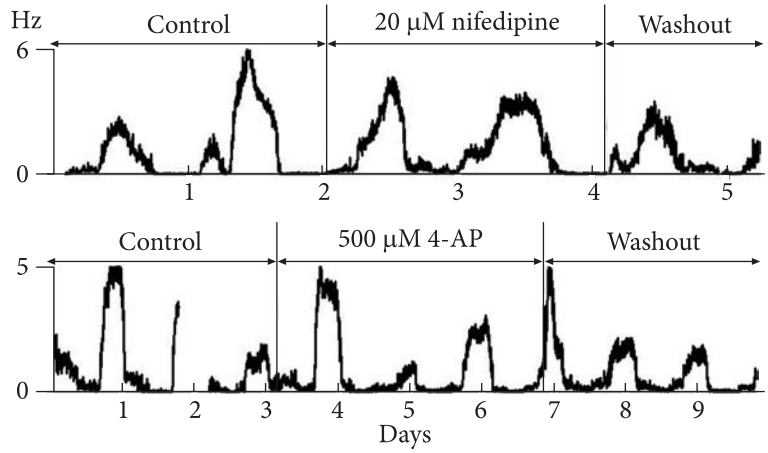

Fig. 1. Effect of 20 μM nifedipine and 500 μM 4-aminopyridine (4-AP) on circadian firing rhythms in two representative SCN neurons. The firing rate (Hz) shown on the ordinate was averaged over 100 periods. The missing recording near the middle of the second day in lower plot (Control) indicate an 8-h interval during which electric power in the building was switched off (From Kononenko et al., 2008 with modifications)

Alteration of the patch membrane potential using applications of voltage ramps from –45 to +55 mV resulted in a clear change in the channel activity and amplitudes. At hyperpolarized potentials, the channel was silent, while depolarization of the patch membrane progressively increased the frequency of channel openings (Fig. 2, *a*, *2*). The profile of the averaged cell-attached currents exhibited a negative-resistance region on the current-voltage (I—V) relation of the patch membrane (Fig. 2, *a*, *3*). The conductance of single channels recorded with Na^+ ions in the pipette was 55.2 ± 5.1 pS. A simple estimation suggests that an average SCN neuron contains about 75 SVC channels. To characterize the kinetic components responsible for the voltage-dependence of open probability, we analyzed the steady-state kinetics of a single channel in patches showing a single level of channel opening at different potentials (Fig. 2, *b*), and have found that depolarization of patch membrane induced a significant decrease of the exponential describing slow component of the channel closed-time, whereas the kinetics of the channel open-time and fast closed-time were not modified. In most patches, we observed spontaneous transient increases in the open probability at resting potential during prolonged recordings of single-channel activity. Frequently a positive correlation between the spontaneously increased open probability of these SVC channels and the acceleration of action potential firing was observed in isolated SCN neurons (Fig. 2, *c*). Such a correlation demonstrates the physiological significance of SVC channels.

These channels have conductance and kinetic properties similar to those of cyclic nucleotide-gated (CNG) channels (Kaupp and Seifert, 2002; Kononenko

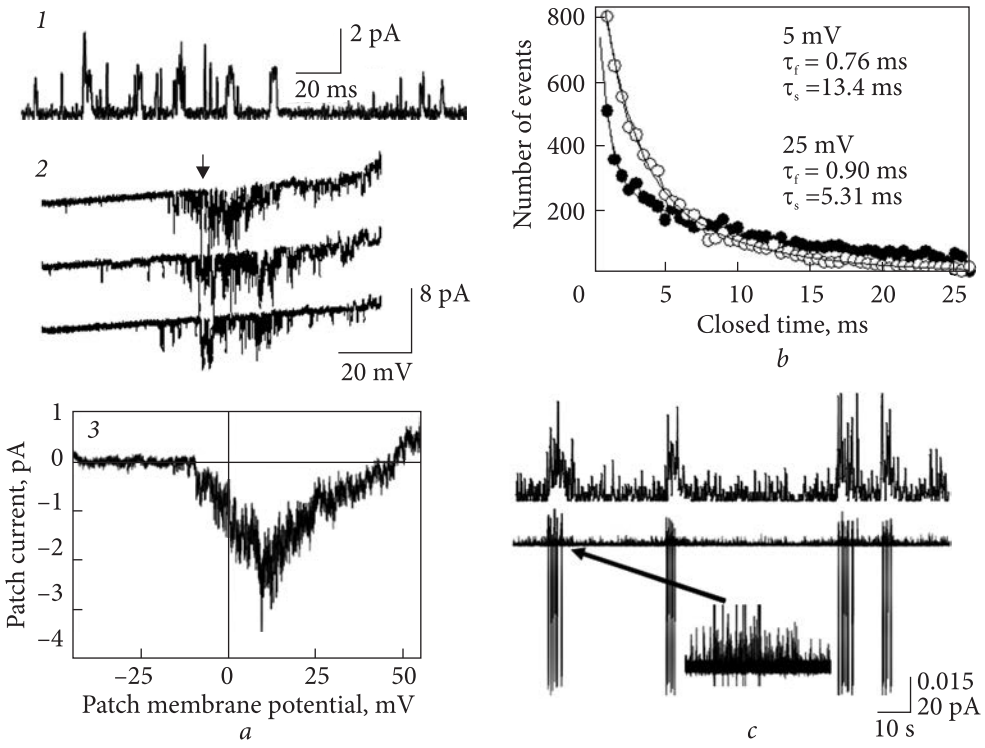

Fig. 2. Single-channel activity at resting potential (*a*, *1*) and three consecutive ramp (100 mV/s) I—V relationships (*a*, *2*) revealing voltage-activated channels. Arrow head shows bath pipette potential. Ten average consecutive I—V relationships (*a*, *3*). Fast and slow closed-time distribution for the single-channel activity at resting potential (closed circles) and at 20 mV depolarization (open circles), respectively (*b*). Distributions were fitted by two-exponential density (smooth line). Spontaneous transient increase in P_o of single-channel activity at resting potential correlated with bursting action-potential firing (*c*). Upper record, P_o of single-channel activity; lower record, cell-attached single-channel and action-potential activity. Inset: single-channel and action-potential activity recorded at expanded time and amplitude scale (Kononenko et al., 2004 with modifications)

and Dudek, 2006; Thompson 1997), raising the possibility for cyclic nucleotides to be messengers connecting the intracellular circadian-clock core with membrane effectors responsible for the circadian modulation of the firing rate in SCN neurons (O'Neill et al., 2008). Thus, features of these new channels make them a putative target for intracellular circadian clock to modulate the firing rate of SCN neurons.

Modeling the circadian modulation of firing rate

Finally, with computational modeling based on experimental results from on-cell recording of SVC channel openings in acutely isolated SCN neurons and long-term continuous recording of activity from dispersed SCN neurons in MED, we attempt-

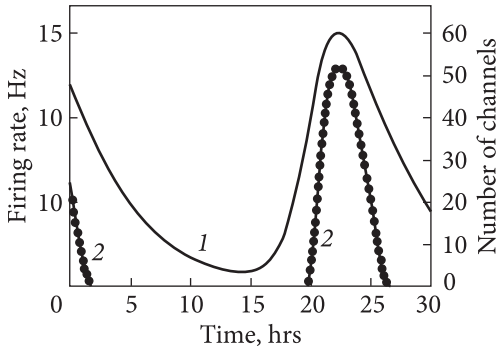

Fig. 3. Circadian modulation of the number of single channels produced modulation of the action potential firing rate. Plot of the number of single channels during the circadian cycle (plot 1, right ordinate) and corresponding circadian modulation of firing rate (plot 2, left ordinate; compare with experimental pattern in Fig. 1). Maximal number of single channels in this particular model neuron was 60, which is close to experimental 75 channels in averaged SCN neuron (see above)

ed to elucidate whether a reasonable number of SVC channels alone could provide smooth changes in firing rate of the model silent neuron (Kononenko and Berezetska, submitted). A basic conception of the model is that randomly and independently opening SVC channels, whose number changes in a circadian manner are incorporated into the silent neuron ("subjective-night" regime of SCN neuron) constructed in accordance with the Hodgkin-Huxley formalism (Fig. 3).

The model reproduced the circadian behavior if the number of SVC channels was modulated in accordance with the protein concentration in model of intracellular clock (Scheper et al., 1999). Such modulation changed the average firing rate of the model neuron from zero ("subjective-night" silence) up to 18 Hz ("subjective-day" peak). Furthermore, the variability of interspike intervals (ISI, not shown here) and the circadian pattern of firing rate (i.e. silence-to-activity ratio and shape of circadian peaks) are in reasonable agreement with experimental data obtained in dispersed SCN neurons in MED. These results suggest that the variability of ISI in intact SCN neurons is mostly due to stochastic single-channel openings, and that the circadian pattern of firing rate is specified by threshold properties of the dependence of spontaneous firing rate on the number of single channels. This plausible mathematical modeling supports the hypothesis that SVC channels could be a critical element in circadian modulation of the firing rate in SCN neurons.

Discussion

In this publication, we present hypothesis that voltage-dependent cation channels, which are persistently active in SCN neurons at resting potential and generate inward (i.e. depolarizing) current, could be a potential target for circadian modulation of action potential frequency. Why just these channels? Mainly, because we did not detect a suppressing effect of nifedipine and 4-AP on the circadian rhythms of firing rate (Fig. 1), and moreover, the amplitude and shape of the action potentials recorded from silent night and active day SCN neurons have not found significant differences (Kononenko et al., 2008). On the other hand, de-

scribed properties of the SVC channels (Fig. 2), as was said above, make them a prospective membrane target for the intracellular circadian clock to regulate the activity of SCN neurons. SVC channels share many properties of the CNG channels, including, selectivity, conductance and kinetics. Interestingly, openings of the SVC channels are strongly voltage dependent because of the voltage dependence of their slow closed-time (Fig. 2, *a*, *2*, *3*, *b*). In contrast, CNG channels show very slight voltage dependence (Thompson, 1997) and contribute only a linear leakage to the I—V relationship of the membrane, despite their belonging to the superfamily of voltage-gated ion channels (Kaupp and Seifert, 2002). The plausible reason for this is that the S4 segment of CNG channel differs from those for voltage-gated channels because it has only three or four regularly spaced positively charged Arg or Lys residues compared with five to seven Arg or Lys in the S4 segment of voltage-gated channels (Kaupp and Seifert, 2002). Thus, we hypothesize that intracellular cyclic nucleotides activate SVC channels, and thus they are likely CNG channels, while the structure of the putative S4 segment of SVC channel is close to those of voltage-gated channels. This suggestion is promising in the light of data demonstrating the facilitating effect of dibutyryl-cGMP on suprachiasmatic neuronal activity (Kononenko and Dudek, 2006), and role of cAMP-signaling in SCN pacemaking (O'Neill et al., 2008). Further work is required to examine the possible role of CNG channels in firing in SCN neurons. With this aim, it will be important to study the effect of CNG-channel blockers, e.g. L-*cis*-diltiazem, on spontaneous firing itself, in general, and circadian peaks of the firing rate, in particular, in these neurons.

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