Effects of Sodium Pump Activity on Spontaneous Firing in Neurons of the Rat Suprachiasmatic Nucleus

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Wang, Yi-Chi and Rong-Chi Huang. Effects of sodium pump activity on spontaneous firing in neurons of the rat suprachiasmatic nucleus. J Neurophysiol 96: 109–118, 2006. First published February 8, 2006; doi:10.1152/jn.01369.2005. Cell-attached and whole cell recording techniques were used to study the effects of electrogenic sodium pump on the excitability of rat suprachiasmatic nucleus (SCN) neurons. Blocking the sodium pump with the cardiac steroid strophanthidin or zero K+ increased the spontaneous firing of SCN neurons to different degrees with different recording modes, whereas turning the sodium pump into a nonselective cation channel with the marine toxin palytoxin invariably increased the spontaneous firing to the point of total blockade. Current-clamp recordings indicated that strophanthidin increased the rate of membrane depolarization and reduced the peak afterhyperpolarization potential (AHP), whereas zero K+ also increased the rate of depolarization, but enhanced the peak AHP. The dual effect of zero K+ was reflected by the biphasic time course of voltage responses to zero K+: an inhibitory phase with enhanced peak AHP and slower firing, followed by a delayed excitatory phase with faster rate of membrane depolarization and faster firing. In the presence of strophanthidin to block the sodium pump, zero K+ consistently decreased firing by enhancing the peak AHP. Repetitive applications of K+-free solution gradually turned the biphasic inhibitory-followed-by-excitatory voltage response into a monophasic inhibitory response in cells recorded with the whole cell (but not the cell-attached) mode, suggesting rundown of sodium pump activity. Taken together, the results suggest that spontaneous firing of SCN neurons is regulated by sodium pump activity as well as the AHP, and that sodium pump activity is modulated by intracellular soluble substances subject to rundown under the whole cell conditions.

INTRODUCTION

The sodium pump, or Na+/K+-ATPase, is a P-type ATPase that becomes autophosphorylated on adenosine triphosphate (ATP) hydrolysis and is specifically targeted by the cardiac steroids and the marine toxin palytoxin (see Hilgemann 2003; Kaplan 2002). Under physiological conditions, the sodium pump is activated by both intracellular Na+ and extracellular K+ and uses the energy derived from ATP hydrolysis to transport three Na+ ions out of the cell in exchange for two K+ ions into the cell (see Glitsch 2001). As a result, the sodium pump maintains the K+ and Na+ gradients across the plasma membrane and powers a variety of secondary active transport processes. In addition, the electrogenic nature of the sodium pump should also contribute to regulate membrane potentials and neuronal firing. For example, activation of sodium pump currents have been shown to underlie the N-methyl-d-aspartate (NMDA)-induced burst firing in the midbrain dopamine neurons (Johnson et al. 1992) and the strychnine- and bicuculline-induced burst firing in the neonatal rat spinal cord (Ballerini et al. 1997), to mediate the prolonged hyperpolarization after glutamate-induced depolarization in hippocampal CA1 neurons (Thompson and Prince 1986), and to be involved in rhythm generation in cultured spinal networks (Darbon et al. 2003) and in burst dynamics in trigeminal motoneurons (Del Negro et al. 1999).

In neurons of the suprachiasmatic nucleus (SCN), which is the central circadian clock coordinating the peripheral oscillators in controlling biochemical, physiological, and behavioral rhythms in mammals (see Kleit et al. 1991; Okamura et al. 2002; Reppert and Weaver 2002; Yoo et al. 2004), sodium pump activity is up- and down-regulated at day and at night, respectively (Wang and Huang 2004). The diurnal rhythm in pump activity oscillates in phase with the firing rates (Green and Gillette 1982; Groos and Hendriks 1982; Inouye and Kawamura 1979; Shibata et al. 1982) and internal calcium concentrations (Colwell 2000; Ikeda et al. 2003), consistent with the metabolic roles of this enzyme in maintaining the Na+ and K+ gradients and homeostasis of intracellular Ca2+. However, the electrophysiological role of the sodium pump in excitability of these neurons remained undetermined. The purpose of this study was to investigate the effects of sodium pump activity on the membrane potentials and spontaneous firing rates in SCN neurons. The experiments were done using cell-attached voltage-clamp and whole cell current-clamp recording techniques. Our results showed that blocking the sodium pump with the cardiac steroid strophanthidin or zero K+ increased the spontaneous firing rate by increasing the rate of membrane depolarization. Modulation of pump activity by intracellular molecules was suggested by the observations of rundown of sodium pump activity in the whole cell conditions and the prolonged inhibition after zero K+-induced excitation in the cell-attached conditions.

METHODS

Reduced SCN preparations

All experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Chang Gung University School of Medicine. Sprague–Dawley rats (17–26 days old) were kept in a temperature-controlled room under a 12:12 light:dark cycle (light on 0700–1900 h). An animal was killed by decapitation, and the brain was put in ice-cold artificial cerebrospinal fluid (ACSF) prebubbled with 95% O2-5% CO2. The ACSF...
RESULTS

Strophanthidin effects

Figure 1 shows the effects of 30 μM strophanthidin on two representative SCN neurons recorded with cell-attached patch-clamp and whole cell current-clamp techniques. Strophanthidin at this concentration almost completely blocks the high-affinity sodium pump in these neurons (IC50 = 4 μM at day; Wang and Huang 2004). In the cell-attached mode, application of 30 μM strophanthidin increased the spontaneous firing rate, from 1.3 to 7.9 Hz for this particular cell (Fig. 1A1). The onset of the strophanthidin effect had a few seconds of delay (see Fig. 4C), but the washout was much slower, typically requiring >3–5 min (see Fig. 5, A2 and B2). The increase in firing rate was accompanied by a decrease in the peak-to-peak spike amplitude. Comparison of the current traces indicates that strophanthidin decreased the amplitudes of both the action-potential–like inward current and the afterhyperpolarization-potential (AHP)–like outward current (Fig. 1A2). On average, 30 μM strophanthidin increased the spontaneous firing rate from 2.4 ± 0.4 Hz (n = 15) to 6.5 ± 0.7 Hz (n = 15) (P < 0.01; paired t-test).

In the whole cell mode, the spontaneous firing rates of SCN neurons tended to be higher, even when the same cells were compared with both recording modes (data not shown). For most cells, only the first 10 min of recordings were used in this study because of the rundown of sodium pump activity over the course of experiments (see Fig. 7B). Figure 1B1 shows the effect of 30 μM strophanthidin on a cell recorded in the current-clamp mode. Although the spontaneous firing rate was higher in the whole cell mode, the response to application of strophanthidin was similar, with a few seconds of delay followed by an increase in firing rate and a decrease in the amplitude of action potentials and AHPs. On average, 30 μM strophanthidin increased the rate from 6.6 ± 0.9 Hz (n = 8) to 10.9 ± 1.4 Hz (n = 8) (P < 0.01; paired t-test). Superimposing the voltage traces indicates that strophanthidin increased the rate of membrane depolarizations, slightly raised the thresholds for action potentials, and reduced the amplitudes of action potentials and AHPs (Fig. 1, B2 and B3). The increase in the rate of membrane depolarization—i.e., a faster rate of membrane charging toward firing threshold—was apparently caused by the inward current induced by blocking the sodium pump with strophanthidin (Wang and Huang 2004). Because the inward current is present at a wide range of potentials with very negative reversal potentials (see Gadsby and Nakao 1989; Glitsch 2001; Glitsch and Tappe 1995), for quantitative purposes, we simply defined the rate of membrane depolarization as the slope of a straight line drawn between the peak AHP and the threshold. In this case, the rate was increased from 0.32 to 0.62 mV/ms (Fig. 1B3). On average, 30 μM strophanthidin increased the rate from 0.26 ± 0.02 mV/ms (n = 6) to 0.43 ± 0.02 mV/ms (n = 6) (P < 0.01; paired t-test). The increase in the threshold for action potentials can be better seen by comparing the digitized voltage points, with the threshold potential defined as the potential above which a runaway depolarization occurred to produce a spike potential. For a total of six cells, 30 μM strophanthidin raised the threshold potential by 2.1 ± 1.4 mV (n = 6) and reduced the peak AHP by 5.1 ± 2.0 mV.
(n = 6). The cause for the increase in the threshold potential, as well as the reduced spike amplitude and the peak AHP, is most likely a result of partial inactivation of sodium channels. Taken together, the results suggest that the blockade of the persistent outward pump current with 30 μM strophanthidin induced a persistent inward current (Wang and Huang 2004), leading to an increase in the rate of membrane depolarizations and thus an increase in spontaneous firing and, at the same time, partial inactivation of sodium channels raised the spike thresholds and reduced the spike amplitudes and peak AHPs.

**Palytoxin effects**

Figure 2 shows the effects on the SCN neurons of palytoxin, a marine toxin that turns the sodium pump into a nonselective cation channel (Artigas and Gadsby 2003; Habermann 1989). We used a saturating concentration of 100 nM for experiments to speed up the rate of drug action (Artigas and Gadsby 2004). In the cell-attached mode, application of 100 nM palytoxin increased the spontaneous firing, but to the extent that palytoxin totally abolished the action potentials (Fig. 2A1). Comparison of the current traces indicates that palytoxin gradually decreased the amplitudes of both the action-potential–like inward current and the AHP-like outward current (Fig. 2A2), suggesting a gradual depolarization of membrane potential leading to depolarization block. The effect of palytoxin was virtually irreversible, with no sign of recovery after washout for ≤ 20 min.

The palytoxin-induced depolarization block could be demonstrated with a cell recorded in the whole cell mode (Fig. 2B1). As indicated, application of palytoxin gradually depolarized the membrane potential and reduced the action potentials, and eventually abolished the spontaneous firing within 20 s of drug application. Because the drug is sensitive to light and deteriorated during the course of experiments, the exact concentration of the drug at the time of application was undetermined. Nevertheless, all four cells treated with palytoxin responded similarly, with different latencies, ranging from 15 s to 2 min, to total elimination of action potentials, depending on the freshness of the drug. The result is consistent with the activation of nonselective cation conductance by palytoxin acting on the sodium pump (but see DISCUSSION).

**Zero K⁺ effects**

The requirement of external K⁺ for normal functioning of sodium pump suggests that eliminating external K⁺ should exert similar effects on the SCN neurons as did the application of 30 μM strophanthidin. For the experiment, external K⁺ was transiently eliminated by switching the bath solution from control (3.5 mM) to a K⁺-free solution (zero K⁺). Figure 3 shows the results of such experiments. As indicated in Fig. 3A1, zero K⁺ increased the spontaneous firing rate of a cell recorded in the cell-attached mode, from 2.4 to 5.5 Hz for this particular cell. On average, zero K⁺ increased the rate from 2.1 ± 0.2 Hz (n = 15) to 4.7 ± 0.5 Hz (n = 15) (P < 0.01; paired t-test). However, unlike the slow washout of the stro-
neurons recorded in the whole cell mode (Fig. 3, effect, zero K\(^+\)) also Fig. 7). Furthermore, contrary to the strophanthidin (rebound inhibition), the latter being the case for the cell (see strophanthidin effect, the effect of zero K\(^+\)) also Fig. 7). The zero K\(^+\)-induced increase in the rate of depolarization is consistent with the induction of an inward current caused by the blockade of sodium pump, whereas the increase of peak AHP suggests that zero K\(^+\) additionally potentiates the K\(^+\) currents responsible for generating the AHP in SCN neurons (Cloues and Sather 2003; Kim and Dudek 1993; Pennartz et al. 1998; Teshima et al. 2003; Thomson and West 1990; Wheal and Thomson 1984). The larger peak AHP in zero K\(^+\) might have prevented the partial inactivation of sodium channels that otherwise raised the firing thresholds and reduced the spike amplitudes as seen in the presence of 30 μM strophanthidin (Fig. 1B).

The dual effects of zero K\(^+\) on the peak AHP and the rate of depolarization are also reflected by the biphasic time course of voltage responses to the elimination of external K\(^+\) (Fig. 4). For better visualization, the voltage trace from Fig. 3B\(1\) is enlarged and its time course expanded in Fig. 4A. As indicated, the peak AHP increased almost immediately upon application of K\(^+\)-free solution, accompanied by a slight decrease in spontaneous firing (from 8 to 7 Hz), and then followed by a delayed increase in the firing rate (13 Hz). Figure 4B superimposes the voltage traces taken from three different time points before, during, and after the elimination of external K\(^+\). Compared with the voltage trace (#1) in control (0.21 mV/ms for the rate of depolarization), the voltage trace (#2) recorded in the middle of solution change showed an enhanced peak AHP, with a slight decrease in the rate of depolarization (0.18 mV/ms). Only after completion of the solution change and further increase in the peak AHP did the recorded voltage trace (#3) show an increase in the rate of depolarization (0.40 mV/ms) and thus spontaneous firing (Fig. 4, A and B). The time course of the zero K\(^+\)-induced increase in the peak AHP followed that of solution change, as determined by measuring the junction potential change (data not shown). In contrast to the rapid change in the peak AHP, the zero K\(^+\)-induced increase in the rate of depolarization and spontaneous firing proceeded with a delay, apparently resulting from a delayed increase in the inward current, by blocking the sodium pump of a slow turnover rate (see DISCUSSION). For comparison, Fig. 4C shows the time course of voltage responses to the application of 30 μM strophanthidin, indicating an even slower depolarizing response than to zero K\(^+\), consistent with the slow binding kinetics of strophanthidin to the sodium pump (see DISCUSSION).
Zero $K^+$ versus strophanthidin

Because zero $K^+$ enhanced the peak AHP, which prolonged the interspike interval ($\#2$, Fig. 4B), this effect should work in a direction opposite to that of the blockade of sodium pump, which increased the rate of depolarization and shortened the interspike interval ($\#3$, Fig. 4B), and so should diminish the effectiveness of zero $K^+$, compared with $30 \mu M$ strophanthidin, in increasing spontaneous firing. The results described so far, however, differed depending on the recording conditions. Specifically, when the cells were recorded in the cell-attached mode, the increase of spontaneous firing rates by zero $K^+$ (from $2.1 \pm 0.2$ to $4.7 \pm 0.5 Hz$) was less than that by $30 \mu M$ strophanthidin (from $2.4 \pm 0.4$ to $6.5 \pm 0.7 Hz$). On the contrary, when the cells were recorded in the whole cell mode, the increase of spontaneous firing rates was similar in zero $K^+$ (from $7.1 \pm 1.2$ to $10.4 \pm 1.4 Hz$) and in $30 \mu M$ strophanthidin (from $6.6 \pm 0.9$ to $10.9 \pm 1.4 Hz$). The conflicting results

FIG. 4. Dual effects of zero $K^+$ on the peak AHP and the rate of depolarization. A: expanded voltage traces to demonstrate the biphasic time course of voltage responses to zero $K^+$. Note the enhanced peak AHP and slower firing at the beginning of treatment ($\#2$) and the delayed increase in the spontaneous firing ($\#3$). B: comparison of action potentials in control ($\#1$) and during the application of $K^+$-free solution ($\#2$ and $\#3$). Voltage traces were taken from $A$ as marked by the arrows. C: time course of voltage responses to the application of $30 \mu M$ strophanthidin (STR). Note the increase in spontaneous firing near the end of voltage trace.

FIG. 3. Effects of zero $K^+$ on the SCN neurons. A1: elimination of external $K^+$ increased the spontaneous firing of a cell recorded with the cell-attached mode. Note the rebound from inhibition of spontaneous firing on return to control solution. A2: comparison of the action-potential-like current traces in control and in zero $K^+$, with the current traces taken from A1 as marked by the arrows. B1: in a different cell recorded with the current-clamp mode, zero $K^+$ enhanced the peak AHP but increased the spontaneous firing. B2: comparison of the action potentials in control (line) and in zero $K^+$, with the voltage traces taken from B1 as marked by the arrows. B3: superimposition of action potentials to indicate the zero $K^+$-induced increase in the peak AHP and in the rate of depolarization (lines connecting the peak AHP to the threshold). Horizontal broken lines were zero voltage levels (junction potential corrected).
A1a zero K+ 20 pA 5 s A1b 30 μM STR 20 pA 5 s

A2

B1a 20 mV 5 s B1b 30 μM STR 20 mV 5 s

B2

FIG. 5. Effects of 30 μM STR vs. zero K⁺ on the same cells recorded in the cell-attached (A) or the whole cell (B) mode. A1: in the cell-attached mode, zero K⁺ (A1a) was less effective than 30 μM STR (A1b) in increasing spontaneous firing. A2: time course of change in spontaneous firing rate in response to zero K⁺ and 30 μM STR. B1: in the whole cell mode, zero K⁺ (B1a) and 30 μM STR (B1b) were similar in their effectiveness in increasing spontaneous firing. B2: time course of change in spontaneous firing rate in response to zero K⁺ and 30 μM STR. Horizontal broken lines were zero voltage levels (junction potential corrected).

prompted us to compare on the same cells the effects of zero K⁺ versus 30 μM strophanthidin. The results of such an experiment obtained from cells recorded in the cell-attached and whole cell modes are shown in Fig. 5, A and B, respectively. As indicated in Fig. 5A1, the cell under the cell-attached condition was stimulated to fire at a higher firing rate in 30 μM strophanthidin than in zero K⁺. For a total of 15 cells, 14 cells showed a higher firing rate in 30 μM strophanthidin (Fig. 5A1b) than in zero K⁺ (Fig. 5A1a). On average, the spontaneous firing rate was increased from 2.7 ± 0.2 Hz (n = 15) in control to 4.6 ± 0.4 Hz (n = 15) in zero K⁺ versus 6.3 ± 0.4 Hz (n = 15) in 30 μM strophanthidin. The time course of change in spontaneous firing is plotted in Fig. 5A2. Note a much slower recovery from strophanthidin effects over duration of several minutes.

Interestingly, when the cell was recorded in the whole cell mode, the averaged spontaneous firing rate was again increased to a similar extent in zero K⁺ and in 30 μM strophanthidin (Fig. 5B). However, the response of individual cells varied to some degree. For a total of six cells, three cells had a higher firing rate in zero K⁺ than in 30 μM strophanthidin, two cells had an opposite response, and one cell responded similarly to both treatments. The similar voltage responses of the latter cell to zero K⁺ versus 30 μM strophanthidin are shown in Fig. 5B1. On average, the spontaneous firing rate was 6.7 ± 1.4 Hz (n = 6) in control and was increased to 11.0 ± 1.1 Hz (n = 6) in zero K⁺ versus 10.1 ± 0.8 Hz (n = 6) in 30 μM strophanthidin. Figure 5B2 plots the time course of change in spontaneous firing rates. Note also a slow recovery from strophanthidin effects. Taken together, the results indicate that the cells responded differently to zero K⁺ and 30 μM strophanthidin, depending on the recording methods.

One reason for the inconsistent results may be that the cell had a higher firing rate to begin with, or simply being, under the whole cell condition, and may thus be easier to reach the maximum firing rates even in zero K⁺. A second reason may have something to do with the application order of drug treatments, i.e., zero K⁺ followed by 30 μM strophanthidin. This application order was chosen to minimize the effect of rundown of sodium pump activity on the results obtained from cells recorded in the whole cell mode. As indicated in Fig. 5, the washout of the strophanthidin effect was slow and required >3–5 min in both cell-attached (Fig. 5A2) and whole cell recordings (Fig. 5B2), and by this time rundown of sodium pump activity already occurred in the whole cell condition (see Fig. 7B). Therefore to compare the effects of zero K⁺ and 30 μM strophanthidin on the same cells, the drug treatment always began with zero K⁺. Run-down of sodium pump activity might account for the seemingly equal effectiveness of both treatments under the whole cell condition.

Nevertheless, the lower firing rate in zero K⁺ than that in 30 μM strophanthidin, as demonstrated with cell-attached recordings (Fig. 5A), is in accord with the idea that the enhancement of the peak AHP by zero K⁺ renders zero K⁺ less stimulatory than 30 μM strophanthidin. To test this idea, the experiment was done by first applying 30 μM strophanthidin to block the sodium pump, followed by further elimination of external K⁺ to enhance the peak AHP. The result of such an experiment is shown in Fig. 6. Indeed, in the presence of 30 μM strophanthidin, zero K⁺ reduced the firing from 6.0 ± 0.8 Hz (n = 7) to 4.8 ± 0.8 Hz (n = 7) (P < 0.01; paired t-test) and from 11.4 ± 1.5 Hz (n = 4) to 9.9 ± 1.7 Hz (n = 4) (P < 0.01; paired t-test) in cells recorded with cell-attached (Fig. 6A) and whole cell (Fig. 6B) modes, respectively, apparently by an increase in the peak AHP, with an average of 14.0 ± 1.6 mV (n = 4) (Fig. 6B). Taken together, the results indicate that zero K⁺ increased the peak AHP and reduced the spontaneous firing either in the absence (#2 of Fig. 4A) or in the presence (Fig. 6B).
of 30 µM strophanthidin, demonstrating a role of the AHP in regulating the spontaneous firing rate.

**Rundown of sodium pump activity**

As demonstrated in Fig. 4A, the dual effects of zero K⁺ were reflected by the biphasic time course of voltage responses to zero K⁺ (i.e., an early inhibitory phase with enhanced AHP and slower firing), followed by a delayed excitatory phase with an increase in the rate of depolarization and faster firing. The continuous rundown of sodium pump activity in the course of whole cell, but not cell-attached, recordings should tip the balance of these two processes in shaping the firing patterns in response to zero K⁺, and might provide a way of looking into the two opposing forces in action to affect spontaneous firing.

For the experiments, external K⁺ was transiently eliminated for a duration of 30 s every 2 min, and the spontaneous firing was recorded (Fig. 7). Figure 7A shows the result obtained from a cell recorded in the cell-attached mode. As indicated in Fig. 7A1, zero K⁺ consistently stimulated the cell to fire action potentials at a higher rate, with no sign of rundown for as long as 30 min, when the experiment was terminated. Note the pronounced rebound inhibition on each return from K⁺-free to the control solution. Figure 7A2 shows the similar responses to the first three applications of K⁺-free solution, indicating that on each application of K⁺-free solution, the cell showed a typical biphasic inhibitory-followed-by-excitatory response to zero K⁺.

In sharp contrast to the lack of rundown in cell-attached modes, the excitatory, but not the inhibitory, effect of zero K⁺ gradually diminished in the first 10 min after breaking into the whole cell condition (Fig. 7B). Figure 7B1 shows the time course of changing responses to zero K⁺ of a cell recorded in the whole cell mode. Note that the zero K⁺ effects changed from being excitatory to being inhibitory during the course of experiment. The reversal of zero K⁺ effects could be clearly seen by comparing the voltage responses to the first three applications of K⁺-free solution (Fig. 7B2). As indicated, the biphasic inhibitory-followed-by-excitatory response to zero K⁺ (Fig. 7B, 2a and 2b) had changed to a monophasic inhibitory response (Fig. 7B2c), suggesting rundown of sodium pump activity. The dotted line beneath the voltage traces suggests that the peak AHP was enhanced by zero K⁺ to similar extents.
DISCUSSION

Effects of sodium pump activity on spontaneous firing

We previously reported that the SCN neurons in reduced preparations exhibit diurnal rhythms in the spontaneous firing rate and in sodium pump activity, both being higher during the day than at night (Wang and Huang 2004). In this study we used three different treatments to study the effects of sodium pump activity on excitability of SCN neurons. The cardiac steroid strophanthidin inhibits the sodium pump by locking the enzyme in its E2P·2Na⁺ form (Heyse et al. 1994), and at a concentration of 30 μM nearly completely blocks the sodium pump in SCN neurons (IC₅₀ = 4 μM at day; Wang and Huang 2004). We have chosen this concentration to compare with the results obtained with zero K⁺, which blocks the enzyme by arresting the sodium pump in E₂P form. The marine toxin palytoxin also appears to stop the enzyme in E₂P form and turns the sodium pump into a nonselective cation channel (Artigas and Gadsby 2003; Horisberger 2004).

Blocking the sodium pump with strophanthidin or zero K⁺ stimulated the SCN neurons to fire action potentials at higher rates in both cell-attached and whole cell modes (Figs. 1 and 3). Both treatments increased the rate of membrane depolarization, an effect consistent with the blockade of the sodium pump and induction of an inward current, leading to an increase in the speed of charging membrane toward firing threshold. As a result, the interspike interval shortened and the firing rate increased. However, strophanthidin and zero K⁺ differed in their actions on the afterhyperpolarization potential (AHP), with strophanthidin reducing the peak AHP amplitude (by about 5 mV) and zero K⁺ enhancing it (by about 6 mV). The difference in the peak AHP amplitude could account for the higher firing rate in 30 μM strophanthidin than in zero K⁺ as demonstrated with the results obtained from cell-attached recordings (Fig. 5A).

The enhancing effect of zero K⁺ on the peak AHP suggests that zero K⁺ enhanced the potassium currents that contribute to the AHP. In other words, zero K⁺ exerted dual actions: blocking the sodium pump and enhancing the potassium currents. Three lines of evidence support the idea. First, the time course of voltage responses to zero K⁺ proceeded with two distinct phases (Fig. 4A and B), with an early inhibitory phase of enhancing the peak AHP and slowing the firing rate, followed by a delayed increase in the rate of membrane depolarization and a faster firing rate. The early inhibitory phase paralleled the solution change as determined by measuring the junction potential, suggesting that the zero K⁺ effect on the peak AHP, presumably mediated by a change in the driving force for the potassium currents, was rate limited by the solution change. On the contrary, the delayed increase in the rate of membrane depolarization was likely rate limited by the blockade of sodium pump, resulting from a slow turnover rate of 80–200 s⁻¹ for the enzyme (see Glitsch 2001). Along this line of thinking, the even slower rate of strophanthidin binding to the enzyme may account for the even slower action of strophanthidin in increasing the firing rate (Fig. 4C). Strophanthidin binds to the sodium pump with a first-order rate constant of 2×10⁻⁶ M⁻¹ s⁻¹ (Heyse et al. 1994) and, even ignoring the off rate (0.01 s⁻¹), the rate of blocking the sodium pump by 30 μM strophanthidin would be slower than 60 s⁻¹. Second, in the presence of 30 μM strophanthidin to block the sodium pump, zero K⁺ exhibited only an inhibitory effect by enhancing the peak AHP, resulting in slower firing (Fig. 6). Third, rundown of sodium pump activity selectively diminished the delayed excitatory phase of zero K⁺ response, leaving only the inhibitory response phase (Fig. 7B).

Effects of the AHP on spontaneous firing

The potentiation of the AHP by zero K⁺ appeared to slow firing rate in three different situations: in the absence (#2, Fig. 4A) and in the presence (Fig. 6) of 30 μM strophanthidin, and after rundown of sodium pump activity (Fig. 7B), suggesting a role of AHP in regulating spontaneous firing rate of SCN neurons. Although the nature of the current that is potentiated by zero K⁺ is not known, the calcium-dependent potassium current is likely involved. In the cluster I neurons of the SCN (Pennartz et al. 1998), at least three subtypes of Ca²⁺-activated K⁺ channels—apamin-sensitive channels, iberiotoxin-sensitive channels, and channels insensitive to both antagonists—contribute to the monophasic AHP (Cloues and Sather 2003). Similarly, the apamine-sensitive small-conductance Ca²⁺-activated K⁺ channel contributes to the “train” afterhyperpolarization after a train of action potentials in response to a depolarizing current (Teshima et al. 2003). Experiments are being performed to determine the zero K⁺-potentiated current that resulted in an increase in the peak AHP.

Palytoxin effects

Palytoxin transforms the sodium pump into a nonselective cation channel and has been shown to increase firing in various tissues (Frelin and van Renterghem 1995; Kockskämper et al. 2004; Sheridan et al. 2005). This is also the case for SCN neurons as recorded with both cell-attached and whole cell modes (Fig. 2). However, compared with the effects of strophanthidin or zero K⁺, the palytoxin-induced increase in spontaneous firing was transient, only to be followed by a total elimination of spiking, which is virtually irreversible. The virtually irreversible nature of the palytoxin action is consistent with its extremely slow off-rate of about 1 day⁻¹ in the absence of external K⁺ (Artigas and Gadby 2004). Depolarization block might be brought about to explain the phenomenon of spike elimination after a short period of increasing spiking because palytoxin gradually depolarized the membrane potential and decreased the spike amplitude, in a way similar to, but to a larger extent than, the effects of strophanthidin. However, it appeared that the machinery for generating the action potentials was also affected by palytoxin in this study because transient hyperpolarization could not totally relieve the SCN neurons from depolarization block (data not shown). Further study is needed to better determine this effect.

Modulation of sodium pump activity

Two lines of evidence suggest that in the SCN neurons sodium pump activity is modulated by intracellular molecules. One is the possible potentiation of sodium pump activity, presumably by the accumulation of intracellular Na⁺. As shown in Figs. 3A1 and 7A1, the spontaneous firing was increased by switching the bath solution from a control (3.5 mM K⁺) to a K⁺-free solution and, on its return to the control solution, was inhibited to a lower level for tens of seconds.


