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Inward rectifier and low-threshold calcium currents contribute to the spontaneous firing mechanism in neurons of the rat suprachiasmatic nucleus

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Abstract. Intracellular and voltage-clamp studies were carried out to clarify the mechanism for spontaneous firing activity in neurons of the suprachiasmatic nucleus (SCN) of rat hypothalamic brain slices in vitro. SCN neurons displayed spontaneously firing action potentials that were preceded by a depolarizing pre-potential and followed by a short spike after-hyperpolarization (AHP). Injection of inward current with a duration longer than 50 ms resulted in a depolarizing voltage "sag" on hyperpolarizing electrotonic potentials. The inward rectification was depressed by bath application of caesium (1 mM) but not by barium (500 μM). SCN neurons also showed a rebound depolarization associated with spike discharge (anodal break) in response to relaxation of hyper polarizing current injection. The rebound depolarization was reduced by nominally zero calcium. Cadmium (500 μM), cobalt (1 mM) or caesium (1 mM) but not nicardipine also depressed the rebound depolarization. Under voltage-clamp conditions, hyperpolarizing steps to membrane potentials negative to approximately −60 mV caused an inward rectifier current, probably H current (I_H), which showed no inactivation with time. Bath application of caesium (1–2 mM) suppressed I_H. Caesium (2 mM) depressed the slope of the depolarizing spike pre-potential, resulting in a prolongation of the interspike interval of tonic firing neurons. We conclude that both the inward rectifier current, I_H, and the low-threshold calcium current contribute to the spike pre-potential of spontaneous action potentials in firing neurons of the rat SCN.

Key words: Intracellular and voltage-clamp recordings – Suprachiasmatic nucleus – Low-threshold calcium current – Anomalous rectification – Pacemaker potential

Introduction

The suprachiasmatic nucleus (SCN) of the ventral hypothalamus of mammals has been strongly implicated as an important neuronal pacemaker component in regulation of circadian rhythmicity [11, 15, 17, 19, 20, 25, 29]. Earlier electrophysiological studies for the SCN using extracellular recordings of multiple- or single-unit activity demonstrated that specific firing patterns of neuronal activity are maintained in a brain slice preparation of the SCN [4, 7, 27] and even in an organotypic explant of SCN, which had been cultured for several weeks [1]. These results imply that the SCN neurons have an intrinsic mechanism for generating the rhythmic firing activity as a circadian pacemaker in mammals. Recent intracellular studies have shown the existence of spontaneously firing action potentials as well as excitatory and inhibitory postsynaptic potentials in rat SCN neurons [10, 23, 26]. However, the membrane properties of neurons and the endogenous mechanism underlying spontaneous firing of action potentials have not been fully studied.

In the present study, we have made intracellular and voltage-clamp recordings from neurons of the SCN using a rat brain slice preparation in vitro. The results suggest that activation of both inward rectifier current, probably H current (I_H), and low-threshold calcium current during spike after-hyperpolarization (AHP) results in a depolarizing voltage shift towards the threshold for action potential.

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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 day. Their brains were removed rapidly and immersed for 8–10 s in a cooled (4–6°C) artificial cerebrospinal fluid (ACSF) prebubbled with 95% O_2/5% CO_2. 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, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11. Calcium-free Krebs solution contained nominally zero calcium and 11 mM magnesium.

Glass micropipettes were filled with either 4 M potassium citrate or 3 M potassium chloride (90–120 MΩ). Recordings of membrane potential and current from SCN neurons were amplified with an Axoclamp 2A amplifier (Axon Instruments). A bridge-type circuit within the amplifier was used to inject constant direct current into SCN neurons. During single-electrode voltage clamp, the head-stage output was continuously monitored to ensure adequate settling time. Sampling frequencies were between 2 kHz and 3 kHz and the amplifier gain was around 0.8 nA/mV. The reliability of "space clamping" is unknown under our experimental conditions. However, neurons in the SCN are rather small in size (6–8 μm) [8, 16] and have a relatively high input membrane resistance (about 180 MΩ, see text). Furthermore, voltage traces of step commands that activate I_H showed no serious errors for the voltage clamp at a potential of −120 mV. A sufficient voltage clamp was, therefore, considered to be obtained from SCN neurons in the present experiments. The membrane potential and current signals were monitored continuously by a chart recorder (Nihon Kohden, RTA-1100) with a "cut-off" frequency of 2.5 kHz. These signals were also digitized and stored on magnetic tapes (TEAC, RD-110T) for later analysis. A Pclamp software program (Axon Instruments) operating on an IBM-AX computer (Sanyo) was used to analyse membrane currents. Data were expressed as means ± SE.

The drugs used in the present study were applied to the brain slices by bath superfusion. Tetrodotoxin was obtained from Wako Pure Chemicals. Tetraethylammonium chloride was from Tokyo Kasei. DL-2-Amino-5-phosphonopentanoic acid (AP5), nicardipine and 4-aminopyridine were purchased from Sigma (St. Louis, Mo., USA). ω-Conotoxin was from Peptide Institute (Osaka, Japan). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was from Tocris Neuramin Ltd. (England).

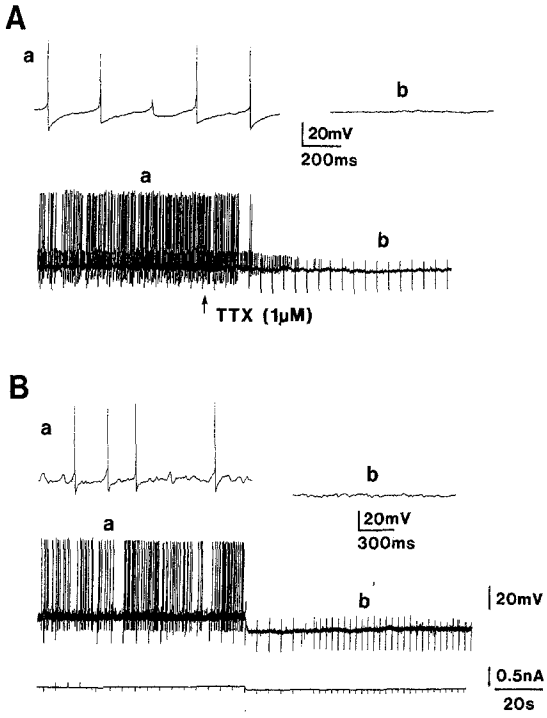

Fig. 1 A, B. Properties of spontaneously firing neurons in the suprachiasmatic nucleus (SCN). **A** Effects of tetrodotoxin (*TTX*) (1 μM) on the spontaneous action potentials. *Upper trace* is shown at a ten-times faster recording speed. Action potentials are truncated. **B** Effect of membrane hyperpolarization on the spontaneous action potentials. Hyperpolarization of SCN was brought about by injection of inward direct current (lowest trace) through a recording microelectrode. **A, B** Records *a* and *b* in the *upper traces* were obtained at the times marked by respective letters in the *lower* (**A**) and *middle* (**B**) *traces*

Results

Action potentials of spontaneously firing neurons

Intracellular recordings were made from 79 neurons in the entire area of the SCN. Most neurons (75%) displayed a spontaneous firing (0.5–10 Hz in frequency) of action potentials without any applied holding current (Fig. 1). Spontaneous action potentials were characterized by short spike duration (0.5–1 ms at half spike amplitude), shallow spike after-hyperpolarization (AHP) with a duration of 50–100 ms and a depolarizing spike pre-potential, which followed the AHP (Fig. 1 A). A subthreshold depolarizing response with a duration of 5–10 ms was also recorded, when the pre-potential did not reach the spike threshold. Tetrodotoxin (1 μM) blocked the spontaneous action potentials (Fig. 1 A). The resting membrane potential obtained in the presence of tetrodotoxin (1 μM) was −53 ± 6 mV (n = 40). The slope of the instantaneous voltage/current curve shows an input resistance of 183 ± 22 MΩ (n = 9). The spontaneous activities of SCN neurons were also eliminated by injection of hyperpolarizing current (0.05–0.1 nA) at potentials more negative than −60 mV (Fig. 1 B).

Focal stimulation of the lateral portion of the SCN produced graded depolarizing responses (excitatory postsynaptic potentials; EPSP) that reach action potential threshold with increasing stimulus intensity (Fig. 2 A). The EPSP was blocked in a solution containing tetrodotoxin (1 μM) (Fig. 2 A). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), a non-*N*-methyl-D-aspartate(NMDA) receptor antagonist partially reduced the EPSP. A residual component of the EPSP was blocked by AP5 (20 μM), an antagonist for the NMDA receptor type (Fig. 2 A). Figure 2 B shows examples of the effects of CNQX and AP5 on the spontaneous firing in SCN neurons. In six out of eight neurons, bath application of both CNQX (10 μM) and AP5 (20 μM) did not produced any significant change in the frequency of the spontaneous action potentials. The remaining two neurons showed about 30% depression of firing frequency during application of these agents. However, CNQX and AP5 did not block the spontaneous firing activity even in the latter neurons.

Action potentials were also evoked by injection of depolarizing current (Fig. 3 Aa). The threshold for directly evoked action potentials was −46 ± 4 mV (n = 10). The peak amplitude and duration of the AHP of single action potentials were −73 ± 8 mV (n = 8) and 122 ± 68 ms (n = 8) respectively. No long-lasting AHP could be recorded even when repetitive firing of action potentials was evoked by injection of a prolonged depolarizing current into SCN neurons (Fig. 3 Ab). Figure 3 B shows action potentials evoked by depolarizing outward current pulses with a duration of 400 ms. Most neurons (85%) showed a repetitive firing pattern of action potentials during the outward current, where no ob-

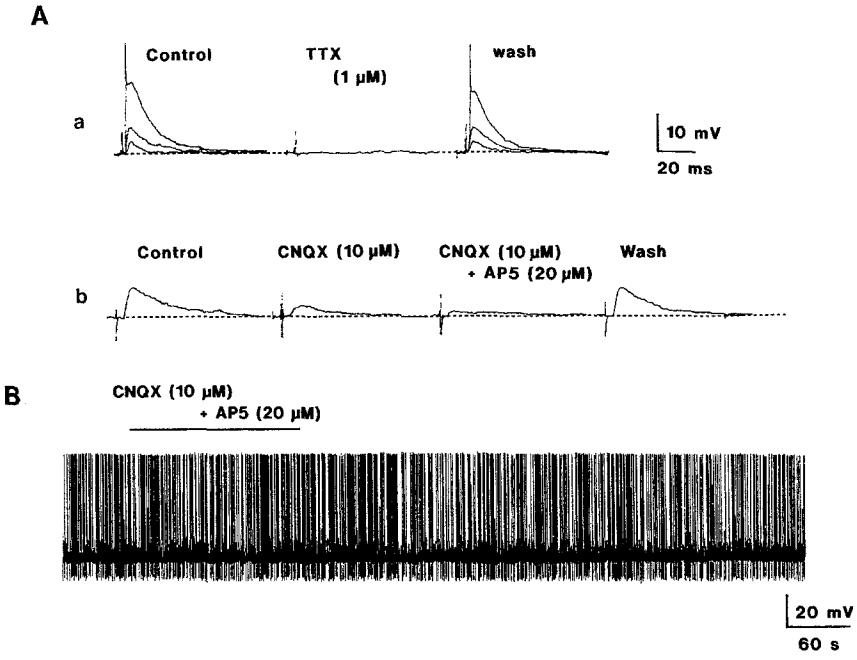

Fig. 2 A, B. Excitatory postsynaptic potentials (EPSP) of a neuron in the SCN evoked by stimulation of the lateral site of the SCN. The membrane potential was held at −60 mV by injection of anodal direct current. **A** *a*, The effect of TTX on the EPSP. TTX (1 μM) was applied to the superfusing solution for 5 min. *b*, The effects of 6-cyano-7-nitroquinoxaline-2,3-dione (*CNQX*) (10 μM) and AP5 (20 μM) on the EPSP. CNQX was applied to the ACSF for 5 min and then DL-2-amino-5-phosphopentanoic acid (*AP5*) was added to CNQX-containing solution for 5 min. **B** An example of the effect of CNQX (10 μM) and AP5 (20 μM) on the spontaneous action potentials in a SCN neuron. *Horizontal line* indicates the period of bath application of these drugs

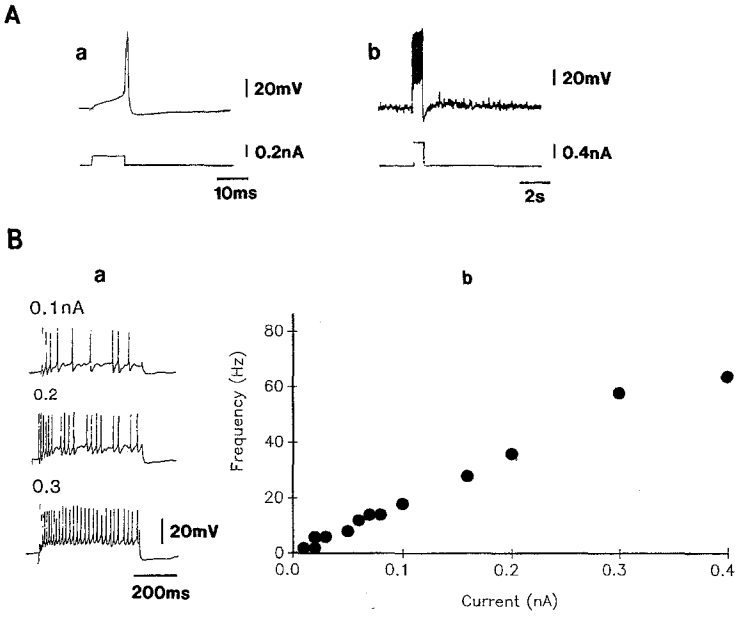

Fig. 3. A Action potentials evoked by depolarizing current pulses with duration of 10 ms (*a*) and 200 ms (*b*). *Upper* and *lower traces* indicate membrane potential and current respectively. **B** Frequency/current relation of a SCN neuron. The membrane was initially hyperpolarized at −60 mV. Spike discharges were evoked by depolarizing steps lasting 400 ms

vious accommodation was seen. The firing activity of neurons was enhanced by increasing the strength of injected current at potentials more positive than −60 mV (Fig. 3 B). The frequency (the reciprocal of the interspike interval) was obtained from five spikes at steady-state firing; an almost linear frequency/current relation was observed between 0.03 nA and 0.3 nA. SCN neurons showed, however, a saturation in firing frequency at 50−60 Hz. This particular neuron showed a repetitive firing pattern with a frequency of 62 Hz at the steady state, when a 0.4 nA current pulse was injected (Fig. 3 B).

Inward (anomalous) rectification and rebound spike discharge in SCN neurons

Injection of inward current to SCN neurons produced a hyperpolarizing electrotonic potential associated with a depolarizing voltage "sag" that developed during a hyperpolarizing membrane current that lasted longer than 50 ms (Fig. 4 A). Although the magnitude of the time-dependent decrease in membrane resistance varied with the individual neurons, about 65% of discharge neurons showed a large sag during hyperpolarization. Figure 4 Ba shows the voltage dependence characteristic

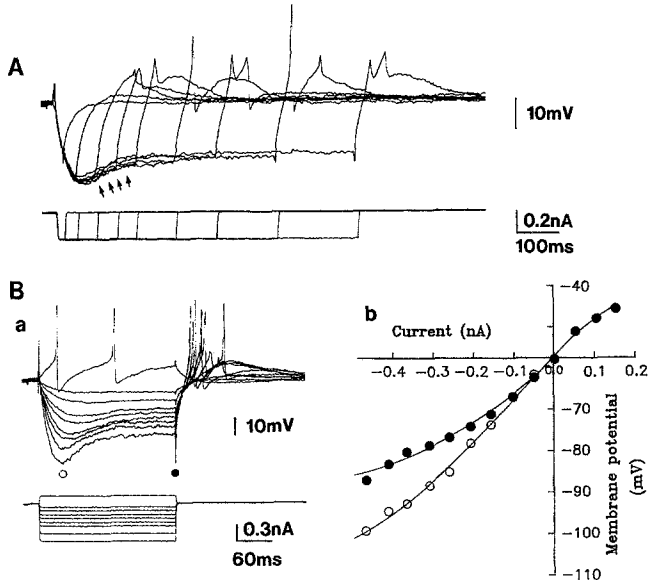

Fig. 4. A Depolarizing voltage "sag" during hyperpolarizing current and rebound depolarizations in SCN neurons (*arrows*). Hyperpolarizing electrotonic potentials were evoked by current pulses (0.2 nA) lasting from 25 ms to 750 ms. Initial membrane potential was −56 mV. **B** Voltage/current relationship of a firing SCN neuron. *a*, Sample recordings of electrotonic potentials produced by inward current pulses with duration of 250 ms. *b*, The membrane potential was initially held at −57 mV. The voltage responses were measured at its peak (●) and the end of current pulses (○)

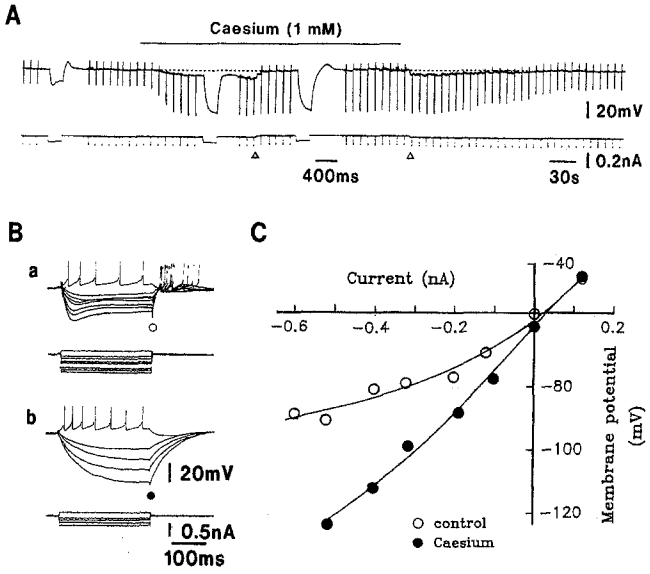

Fig. 5 A–C. Effect of caesium on inward rectification. **A** The period of bath application of caesium (1 mM) is indicated by a *horizontal line*. The neuron was initially hyperpolarized at −60 mV (····) to eliminate spontaneous firing of action potentials. Compensation of the caesium-induced hyperpolarization was by injection of depolarizing direct current between two *triangles*. Expanded records are shown in each trace. **B** Sample records of electrotonic potentials obtained before (*a*) and 5 min after (*b*) application of caesium (1 mM) at the resting membrane potential of −58 mV. **C** Effect of caesium (1 mM) on the voltage/current relationship; data were obtained from records **B**. Amplitudes of electrotonic potentials were measured at the end of pulses. ○, ●, As in **B**

of the voltage sag; this was enhanced by an increase in the membrane potential. The voltage/current relationship (*V/I* curve) obtained at the end of the rectangular current pulses with a duration of 250 ms showed an inward (anomalous) rectification at potentials more negative than −65 mV (Fig. 4 Bb). In contrast, it was almost linear when measured 50 ms after the beginning of the injected current pulses.

A rebound spike discharge (anodal break) associated with a slow depolarizing after-potential (rebound depolarization) was elicited upon repolarization of the membrane when a hyperpolarizing current injection was terminated. Conditioning hyperpolarizations for less than 50 ms resulted in little rebound depolarization. Further lengthening of the hyperpolarization increased the amplitude of the rebound depolarization, the growth of which was a function of the length of the conditioning hyperpolarization (Fig. 4 A). The rebound depolarization also increased in size when larger hyperpolarization steps were used (Fig. 4 Ba). The duration of the rebound depolarization produced by a hyperpolarizing current pulse (0.1 nA for 250 ms) was 80−250 ms ($n = 4$) in the presence of 1 μM tetrodotoxin.

Effects of caesium and barium on inward rectification

Bath application of caesium (1 mM), a blocker for inward rectifier currents [6, 13, 21, 28], produced a hyperpolarizing response associated with increased input membrane resistance at a membrane potential of −60 mV (Fig. 5 A). Caesium (1 mM) completely depressed the voltage sag of the electrotonic potential in SCN neurons at all hyperpolarizing membrane potentials (Fig. 5 B). The *V/I* curve obtained at the end of the inward current pulses (250 ms in duration) became linear in the presence of 1 mM caesium ions (Fig. 5 C). These results suggest that caesium depresses the inward rectification in SCN neurons. Addition of barium (500 μM), a selective blocker for potassium-selective inward rectifier current [2, 6, 30], to the external solution depolarized the membrane of SCN neurons and increase the input membrane resistance. However, it did not depress the depolarizing voltage sag and inward rectification produced by injection of hyperpolarizing current with a duration of 250 ms ($n = 4$) (Fig. 6 A). The slope of the *V/I* curve obtained at the peak of the electrotonic potentials (about 50 ms after the onset of the inward current pulse) increased under the effect of barium and the curve intersected with the control curve at −92 mV (Fig. 6 B) indicating that barium depressed a potassium conductance independently of inward rectification. Neither tetraethylammonium (5 mM), cadmium (200 μM) nor cobalt (1−2 mM) depressed the inward rectification of SCN neurons.

Properties of the rebound depolarization

The rebound depolarization was produced by a relaxation of hyperpolarizing stimulus to a holding potential

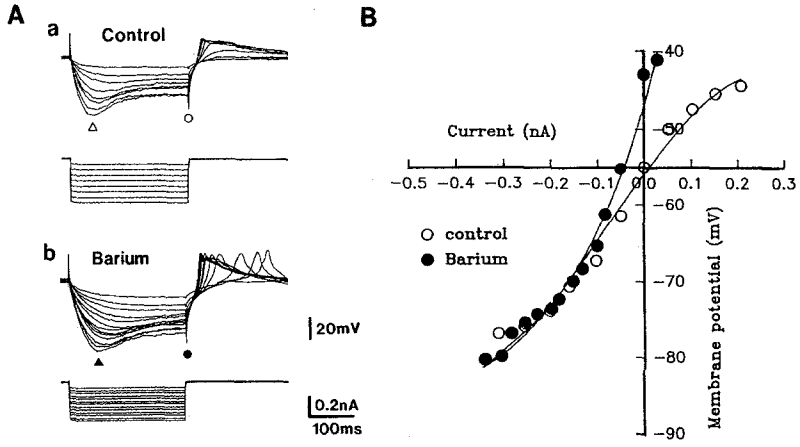

Fig. 6 A, B. Effect of barium on the inward rectification. **A** Sample records of electrotonic potentials obtained before (*a*) and 5 min after barium application (*b*). All records were taken from the same cell treated with TTX (1 μM). **B** Voltage/current relationships obtained at the end of current pulses (*a*) and the peak of electrotonic potentials (*b*). Data were from records in **A**

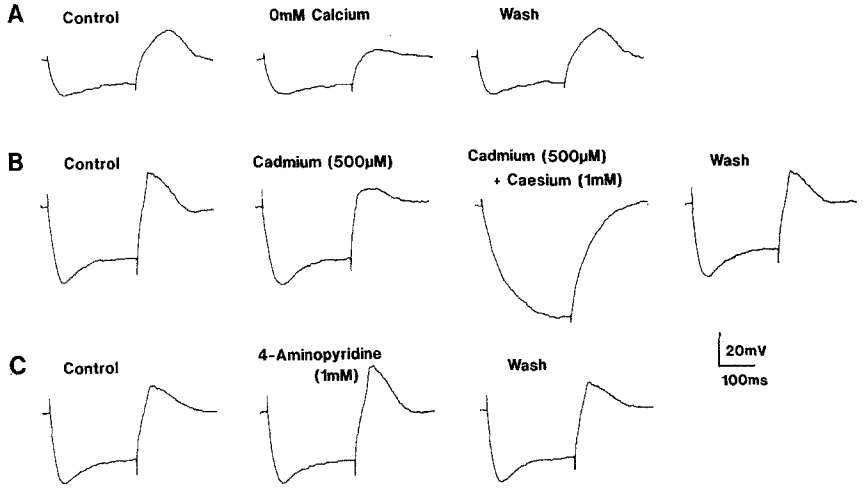

Fig. 7. A Effect of removal of external calcium on the rebound depolarization in SCN neurons treated with TTX (1 μM). Electrotonic responses were produced by injection of inward current pulse (0.3 nA for 250 ms). Calcium-free solution contained nominally zero calcium and 11 mM magnesium. **B** Effects of cadmium and caesium on the rebound depolarizations. Cadmium (500 μM) was first applied to an SCN neuron for 5 min (*second trace*) and then caesium (1 mM) was added to a solution containing 500 μM cadmium (*third trace*). **C** Effect of 4-aminopyridine (1 mM) on the rebound depolarization. **A, B, C** were obtained from three different neurons. Each trace is average of five consecutive recordings of electrotonic potentials

just below spike threshold. SCN neurons were superfused with a Krebs solution containing tetrodotoxin (1 μM). The depolarizing voltage sag on electrotonic potentials was not changed in nominally zero calcium (with 11 mM magnesium) solution ($n = 5$) (Fig. 7 A). In contrast, the rebound depolarization was reduced to 22 ± 4% ($n = 4$) of the control amplitude in calcium-free solution (Fig. 7 A). Simple addition of 11 mM magnesium ions to the Krebs solution containing 1 μM tetrodotoxin produced no significant effect on the voltage sag and the rebound depolarization ($n = 3$). Cadmium (500 μM) also produced 41 ± 6% ($n = 4$) depression of the amplitude of the rebound depolarization ($n = 4$) (Fig. 7 B). Similar results were obtained with 500 μM cobalt ($n = 5$). However, these calcium channel blockers did not produce the complete inhibition of the rebound depolarization. When caesium (2 mM) was added to a solution containing cadmium (500 μM), the residual component of the rebound depolarization was completely depressed (Fig. 7 B). Nicardipine (5 μM) and ω-conotoxin (1 μM), potent antagonists for voltage-dependent high-threshold calcium currents, had no inhibitory effect on the rebound depolarization ($n = 4$). The calcium-dependent component of the rebound depolarization obtained in the presence of caesium (1 mM) was abolished when the membrane potential was hyperpolarized to potentials more negative than −60 mV (see Fig. 5 A). Addition of 4-aminopyridine (1 mM), a selective blocker for the A current [18], to the external solution produced no effect on the sag of the hyperpolarizing electrotonic potential but largely enhanced (about 70%, $n = 3$) the rebound depolarization (Fig. 7 C).

Inward rectifier current (I_H) in SCN neurons

Neurons having depolarizing voltage sag were voltage-clamped at a holding potential of −65 mV. Figure 8 shows an example of slow inward relaxation obtained in a Krebs solution containing nominally zero calcium, tetrodotoxin (1 μM), tetraethylammonium (30 mM), 4-aminopyridine (1 mM) and cadmium (500 μM). Hyperpolarizing voltage steps resulted in a time-dependent increase in membrane conductance (Fig. 8 A). The instantaneous current obtained at the end of the hyperpolarizing step was larger than that measured at the beginning of the step, indicating that it represented the activation of an inward current, rather than deactivation of an outward current during hyperpolarizing commands.

Bath application of caesium (1−2 mM) produced an outward shift (0.1−0.2 nA) of the holding current at −65 mV. Caesium strongly reduced the current relax-

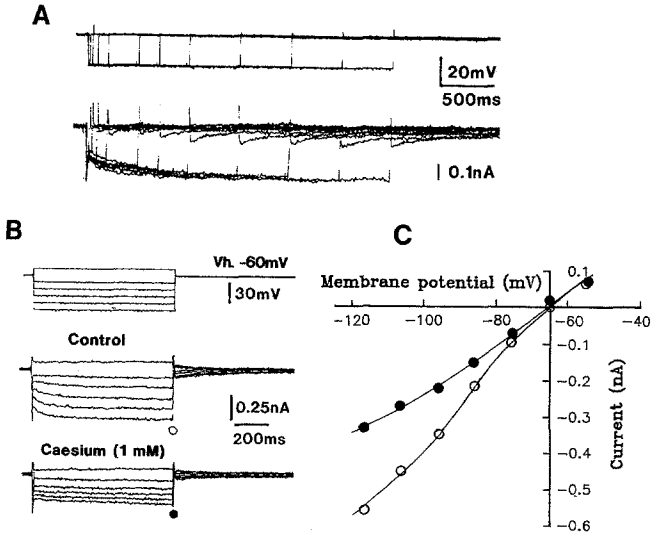

Fig. 8. **A** Time-dependent increase in membrane conductance produced by a hyperpolarizing voltage step lasting between 50 ms and 3 s. **B** Effect of caesium (1 mM) on I_H. I_H was taken after block of delayed rectifier potassium current with tetraethylammonium (30 mM), transient outward current with 4-aminopyridine (1 mM), voltage-dependent sodium current with TTX (1 μM) and calcium current with cadmium (500 μM). **C** Effect of caesium on *I/V* relation obtained from data in **B**. The holding potential was −65 mV. Results were obtained (○) before and (●) 5 min after application of caesium

ation activated ($n = 4$) (Fig. 8 B). The current/voltage relationship (*I/V* curve) became almost linear at all hyperpolarized potentials in the presence of 1 mM caesium ($n = 8$) (Fig. 8 C). This is consistent with caesium almost completely blocking the depolarizing voltage sag in current clamp (Fig. 5). In contrast, barium (500 μM) had no significant effect on I_H (not shown). These results suggest that the hyperpolarization-activated current in SCN neurons is most likely a non-selective cation current, which is found in many different cell types [6, 13, 28].

Frequency of spontaneous action potentials

We examined, in current clamp, the possible contribution of I_H to the spontaneous discharge of action potentials as a factor that would determine the interspike interval in SCN neurons. Figure 9 A shows an example of the effect of caesium (1 mM) on the spontaneous action potentials recorded from a single SCN neuron. Bath application of caesium (1−2 mM) prolonged the spike interval. In this particular neuron, the spontaneous action potential decreased in frequency from 4.4 Hz to 3.0 Hz in the presence of caesium (Fig. 9). Caesium did not significantly change the configuration of spike potentials ($n = 4$) but reduced the slope of the pre-potential of spontaneous action potentials and thereby prolonged the duration of the spike AHP (Fig. 9 B).

Discussion

A majority of SCN neurons are characterized by a spontaneous action potential associated with (a) a short spike duration, (b) shallow and short spike AHP and (c) a depolarizing pre-potential. No obvious accommodation occurred during a depolarizing current step with a duration of 400 ms. Spontaneous action potentials were blocked by application of tetrodotoxin (1 μM) and by membrane hyperpolarization to potentials more negative than −60 mV. The present study also showed that the EPSP was evoked by stimulation of the lateral site of the SCN in rat brain slice preparations. Although CNQX (10 μM) and AP5 (20 μM) each produced only a partial inhibition of the amplitude of the EPSP, the combined application of these agents completely reduced the EPSP. A recent study, using intracellular recording techniques, has suggested that the EPSP is mediated by NMDA and non-NMDA receptors in SCN neurons [10]. CNQX (10 μM) and AP5 (20 μM) receptors did not block the firing activity of SCN neurons, although these drugs decreased the rate of spontaneous action potentials in some neurons. These results suggest that an intrinsic mechanism exists for the spontaneous action potential in neurons of the SCN.

Inward rectification in SCN neurons

SCN neurons displayed a type of time- and voltage-dependent inward (anomalous) rectification activated at hyperpolarized membrane potentials. Voltage-clamp studies clearly showed that hyperpolarization produced an inward current relaxation associated with a time-dependent increase in membrane conductance at potentials of −60 mV to −120 mV. Bath application of caesium (1−2 mM), a selective blocker for the hyperpolarization-activated non-selective cation current (I_H) [6, 13, 21, 28], strongly reduced both the depolarizing voltage sag and the inward rectifier current in SCN neurons. In contrast, barium (500 μM), a blocker of the potassium-selective inward rectification [2, 6, 30], did not alter the expression of the inward rectification. Although the ionic mechanism is not fully clarified at present, the inward rectifier current of SCN neurons may be the I_H.

Calcium-dependent rebound depolarization

SCN neurons also displayed a rebound depolarization associated with spike discharges following relaxation of hyperpolarizing current pulses. The rebound depolarization appeared at a holding potential more positive than −65 mV. Removal of extracellular calcium or application of cadmium strongly reduced the rebound depolarization, suggesting that a voltage-dependent calcium conductance is activated during the rebound depolarization. Since extracellularly applied caesium (2 mM) also partially reduced the rebound depolarization, both the calcium current and I_H contribute to this depolarization in SCN neurons. The calcium-dependent component of the rebound depolarization obtained in the presence of caesium was abolished when the neuron was hyperpolarized to −60 mV. Furthermore, nicardipine, a selective blocker for high-threshold calcium current, did not de-

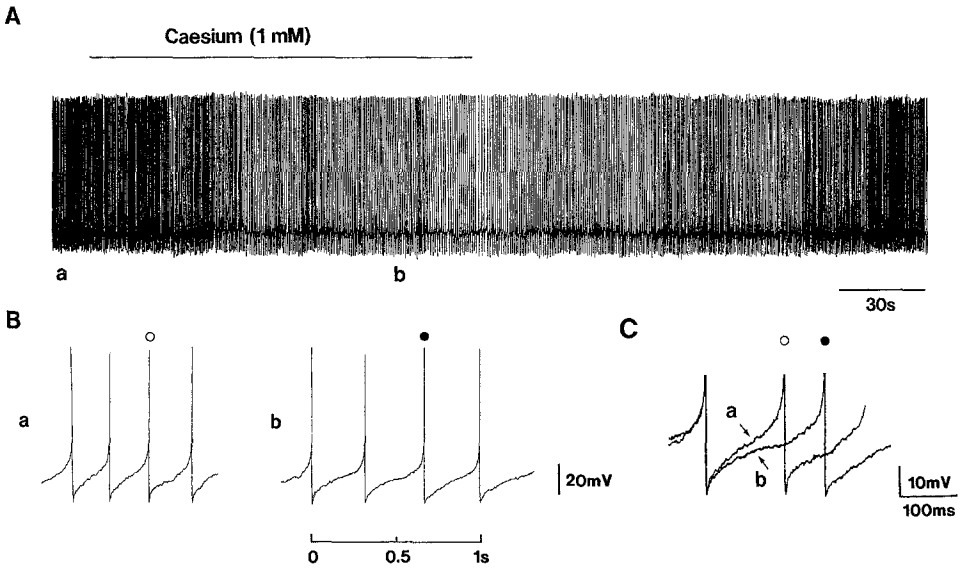

Fig. 9 A, B. Effect of caesium (1 mM) on spontaneously firing action potentials recorded from a SCN neuron. **A** A consecutive recording of membrane potential from a spontaneously firing neuron. Caesium (1 mM) was applied to the superfusing solution. *Horizontal line* indicates the period of caesium application. **B** Expanded record of spontaneous action potentials obtained before (*a*) and during (*b*) application of caesium. The resting membrane potential was −54 mV. *a*, *b*, Obtained from **A**. **C** Spontaneous spikes obtained before (○) and during application (●) of caesium were superimposed. Action potentials were partially truncated. These records were taken at the times marked with the respective symbols in **B**. Note that caesium suppressed the slope of spike pre-potential (*arrows*) and lengthened the spike interval

press the rebound depolarization. These results suggest that voltage-dependent, low-threshold calcium current is mainly responsible for the rebound depolarization [3, 5, 9, 24, 31]. Hyperpolarization of the membrane removes inactivation of the calcium channels resulting in a calcium spike in response to the return of the membrane potential toward rest. 4-Aminopyridine, an antagonist for an A current, which is a voltage-dependent transient potassium current activated at hyperpolarizing membrane potentials, enhanced the amplitude of rebound depolarization. A current would be involved in the rebound depolarization as a counter-current of I_H in SCN neurons.

Role of spike after-hyperpolarization (AHP) on firing activity

It has been reported that prolonged activation of a calcium-dependent conductance contributes to spike frequency adaptation producing large, long-lasting AHP that follow burst firing in hippocampal pyramidal neurons [12, 22]. However, a majority of neurons in the SCN displayed no event comparable to the long-lasting calcium-dependent AHP but only the fast AHP [6, 14, 26]. The inward rectification has been suggested to play a potential role in contributing to the firing pattern of CNS neurons, acting as a pacemaker current [13]. Griffith [6] has suggested that the I_H contributes to the fast spike AHP in spontaneously firing neurons of guinea-pig forebrain, which are characterized by a pronounced depolarizing sag of the membrane potential developed during the current pulse. In the present study, extracellular caesium (1−2 mM) depressed the depolarizing "pre-potential" of the action potential and thereby prolonged the spike AHP, resulting in a depression of the rate of spontaneous firing of action potentials. These results suggest that I_H is involved in the later component of a spike AHP and/or the spike pre-potential in SCN neurons. Previously we have demonstrated that activation of adenylyl cyclase potentiates the I_H in vertrebrate neurons [28]. If this is the case in the rat SCN, spontaneous firing activity would be expected to be modified when the intracellular levels of cyclic AMP are changed.

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