MECHANISMS UNDERLYING CESSATION OF RABBIT SINOATRIAL NODE PACEMAKER ACTIVITY IN HIGH POTASSIUM SOLUTIONS

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Abstract To determine the effect of high extracellular potassium concentration ([K]o) on the membrane current of the sinoatrial node cell, voltage clamp experiments were conducted using the double micro-electrode technique. When depolarizing clamp pulses were applied, a transient inward current was followed by an outward current and an outward current tail flowed after the pulse. The amplitude of both the transient inward current and the outward current tail were markedly reduced with increasing [K]o, but the magnitude of the outward current during depolarization scarcely changed. The inward current during hyperpolarizing clamp pulses increased in magnitude at higher [K]o and the reversal potential for the inward current change decreased with increasing [K]o.

From these results it was concluded that the suppression of the sinoatrial node automaticity at higher [K]o was due to the decrease in magnitude of both the transient inward current and the outward current tail. As the cause of the depression of the transient inward current, its inactivation through depolarization, increased outward leak current and some direct inhibitory effect of K were proposed. The reduction of the outward current tail was attributed to the decrease in the driving force of the K current.

It is well known that raising the extracellular potassium concentration ([K]o) eventually suppresses the spontaneous activity of the sinoatrial node (S-A node). The mechanism of this negative chronotropic effect of K may be deduced from the theory of the spontaneous action potential discharge in the myocardium which is summarized as follows (McAllister et al., 1975; Brown et al., 1976; Noma and Irisawa, 1976a, b). The transient inward current responsible for the rising phase of the action potential is triggered by slow diastolic depolarization.

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which results from the combination of a relatively large background inward current with a slowly decaying outward current, which is activated by the preceding action potential and is chiefly carried by K ions.

If \( [K]_o \) is raised, the magnitude of the membrane currents will change. The transient inward current will be partially inactivated by the depolarization of the membrane. The slowly decaying outward current will also be reduced by the decrease of the driving force of the K current. Furthermore, the slope conductance of the S-A node cell markedly increases when \( [K]_o \) is raised (SEYAMA, 1976). The present experiments were intended to test whether the suppressive effect of high \( [K]_o \) on the S-A node automaticity can be explained by these changes in the membrane currents.

**MATERIALS AND METHODS**

Hearts were removed from rabbits weighing 1.5-2.0 kg, which were killed by a blow on the neck. The S-A node was dissected from the right atrium, placed in Tyrode solution and stored in an icebox (4-10°C). A manmade strand of the S-A node having the endocardium intact and 0.3-0.5 mm in width was prepared by cutting the nodal region perpendicularly to the crista terminalis before each experiment. This strand was further cut in the recording chamber into thinner strands of various lengths, 0.2-0.3 mm in both width and thickness. The bottom of the recording chamber was made of glass, upon the surface of which it was possible to dissect the tissue using a razor blade. A pacemaking portion, roughly determined by recording the action potentials within the strand, was electrically isolated from the remaining portion by ligating the strand with silk fibers at two points with an interval of 0.2-0.3 mm. The specimen was held in place by means of several fine tungsten needles. Two or three glass micro-electrodes (30-50 MΩ) were inserted intracellularly from the endocardial surface. The method of perfusing the tissue and the electrical recording apparatus were the same as reported elsewhere (NOMA and IRISAWA, 1976a). When the perfusate was switched from one solution to another, the membrane current in response to a constant clamp pulse was repeatedly recorded with an interval of about 30-50 sec. After the response reached a stable state following 2-3 min of perfusion, the current voltage relation was obtained.

The composition of the control Tyrode solution was as follows in mm: NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 0.63, and the pH was adjusted to 7.4 by adding Na₂HPO₄. Various concentrations of K were prepared by mixing the control Tyrode with 100 mM K solution, which contained NaCl 39.6, KCl 100.0, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 0.63, and Na₂HPO₄ 2.24 in mm. In experiments shown in Figs. 6 and 7 crystalline KCl was added to the control Tyrode to make solutions containing 15.0 and 22.7 mM K with normal [Na]o without adjusting osmolarity. Similar changes in osmolarity made by adding sucrose did not produce any significant effect on the spontaneous action potential of the S-A
node cell. The temperature of perfusates was maintained at 36–37°C.

*Spatial homogeneity of the membrane potential within the specimen.* The double microelectrode technique (Deck et al., 1964) has been shown to be useful in voltage clamp experiments of the S-A node (Noma and Irisawa, 1976a). Spatial voltage homogeneity within the small S-A node specimen was further tested by recording the membrane potential at a third point which was not included in the negative feedback circuit. Figure 1 shows two voltage traces and one current record during the clamping of the membrane potential to various levels in a specimen 0.3 mm in diameter. The third monitoring electrode was incompletely inserted intracellularly, but the two parallel traces of the membrane potential during the voltage clamps suggested the homogeneity of the membrane potential within the specimen. Figure 2 shows the result in a current clamp experiment using three microelectrodes inserted in another specimen (0.25 mm in diameter); current pulses were applied through one microelectrode and potentials at two sites were recorded with two other microelectrodes. The action potentials recorded at two different points within the specimen were similar. These results confirmed the spatial homogeneity within a small S-A node specimen about 0.2–0.3 mm in diameter.

![Figure 1](image.png)
Fig. 2. The spatial homogeneity under current clamp conditions. Two voltage traces and a current record were shown in each A–E. During the cathodal current application the amplitude of the action potential was reduced in B, and in A the action potential disappeared and the membrane potential was \(-17\) mV about 1 sec after the onset of the pulse. In response to anodal current pulses (C–E) the activity stopped and the membrane potential was gradually depolarized after the initial peak. The anodal current suppressed the action potential even during the rapid rising phase of the action potential.

RESULTS

Decrease of the spontaneous rate at higher \([K]\)_o

When \([K]\)_o was raised from 2.7 to 15.0 or 20.0 mM (Fig. 3A), the overshoot and the maximum diastolic potentials decreased and the frequency of the spontaneous action potential was reduced. At 25.0 mM \([K]\)_o the specimen became

Fig. 3. The decrease of the spontaneous frequency in higher \([K]\)_o solutions. A; the potential change in response to high \([K]\)_o solutions. Straight lines under the traces indicate the period of the test perfusion. B; the relationship between the spontaneous frequency and \([K]\)_o. Circles connected by lines indicate data from the same specimen.

quiescent about 20 sec after the onset of perfusion. Quiescent membrane potentials at the end of the perfusion of 20.0 and 25.0 mM \([K]\)_o solutions were \(-33.1\)
and $-32.2 \text{ mV}$, respectively. These values are evidently higher than the quiescent membrane potential of $-17.3 \text{ mV}$ obtained after blocking the spontaneous activity by applying depolarizing current as seen in Fig. 2. Thus, cessation of the activity at 25.0 mm $[K]_o$ may not be due solely to depolarization of the membrane. When the perfusate was returned to the control Tyrode, the maximum diastolic potential and the quiescent membrane potential increased in association with an increase of the spontaneous rate.

In Fig. 3B, the spontaneous rate about 2–3 min after the onset of perfusion of various $[K]_o$ solutions was plotted against $[K]_o$ from three experiments. In every specimen the frequency decreased with increasing $[K]_o$, and at 20 mm $[K]_o$ the spontaneous action potential disappeared within 3 min. This finding is consistent with the results obtained by TODA and WEST (1967) and by SANO et al. (1967) in the rabbit S-A node.

**Membrane currents in various $[K]_o$ solutions**

The magnitude of the recorded membrane current varied from specimen to specimen, probably due to the difference in the number of cells contained in different preparations. Therefore, effects of $[K]_o$ on the membrane current were studied by comparing currents between different $[K]_o$ in the same specimen. Figure 4 gives membrane currents in 2.7, 10.0, and 25.0 mm $[K]_o$ solutions. The holding potentials at 2.7, 10.0, and 25.0 mm $[K]_o$ were $-42$, $-40$, and $-32 \text{ mV}$,

Fig. 4. Membrane currents in various $[K]_o$ solutions in a S-A node specimen. The magnitude of the voltage step change was shown in mV in the left margin of each row of current traces, and for the last trace in C it was given above the trace. The membrane potential was held at $-42$, $-40$, and $-32 \text{ mV}$ where no net current flowed in the steady state. Three horizontal bars at the top of each column indicate the period of test pulses. The arrow on the third trace in C indicates the time of switching off the voltage clamp.
respectively, where the membrane current was negligibly small in the steady state. Voltage clamp pulses of 0.5 sec duration were superimposed upon each holding potential, thus bringing the membrane potential during the pulse to the sum of the holding potential and the pulse magnitude. The magnitude of the pulse was shown in mV in the left margin of each row of current traces.

On depolarization a transient inward current was followed by a slowly increasing outward current. On repolarization, a sudden change in the current trace was observed and its magnitude increased with increasing $[K]_o$. The outward current tail on repolarization at 25 mM $[K]_o$ was smaller than those at 2.7 mM and 10 mM $[K]_o$. The magnitude of the inward current during hyperpolarization increased with time at 2.7 mM $[K]_o$. The inward current at 10 mM $[K]_o$ also increased with time during hyperpolarization of 24 mV, but a hyperpolarization of 42 mV reversed the direction of the current change. At 25 mM $[K]_o$ the inward current decreased with time during hyperpolarization of 24 mV. Decrease of the reversal potential for the current change from 10 to 25 mM $[K]_o$ is consistent with the hypothesis that the current change during hyperpolarization is partially attributable to declining K conductance (Vassalle, 1966).

**Current voltage relations at various $[K]_o$**

From the experiment shown in Fig. 4, the current voltage relations for the early current (Fig. 5A) and those for the delayed current (Fig. 5B) were drawn. The maximum magnitude of the transient inward current was obtained at about $-10$ mV at every $[K]_o$, but its value at 10 mM $[K]_o$ was smaller than that at 2.7 mM $[K]_o$ in spite of the similarity in the holding potential. In 25 mM $[K]_o$ solution, the peak value was considerably smaller than the other two values at lower $[K]_o$. The magnitude of the inward current on hyperpolarization measured 10 msec after the onset of the pulse increased as $[K]_o$ was increased. This fact is in good agreement with the increase of the membrane conductance with increasing $[K]_o$, which was previously demonstrated by the instantaneous current voltage relation obtained with the holding potential of 0 mV (Noma and Irisawa, 1976b).

The current voltage relation obtained 0.5 sec after the onset of the clamp pulse in 10 mM $[K]_o$ solution almost duplicated the curve at 2.7 mM $[K]_o$ (Fig. 5B). At 25 mM $[K]_o$, the magnitude of the inward current during hyperpolarization was larger than that at 2.7 or 10 mM $[K]_o$, but the curve for the outward current was similar to those at lower concentrations.

The decreased magnitude of the transient inward current (Fig. 5A) might partially be due to reduction of both the holding potential and $[Na]_o$ replaced by K, because the maximum rate of rise of the action potential is sensitive to these factors (Noma and Irisawa, 1974). To avoid these two effects on the transient inward current, experiments were carried out using a constant holding potential in solutions having normal $[Na]_o$ with higher $[K]_o$. Figure 6 shows the current voltage relations obtained in such experiments. When $[K]_o$ was raised, the in-
ward current during hyperpolarization increased, the transient inward current during depolarization decreased and the outward current on depolarization remained constant. These results are qualitatively similar to those in Fig. 5. This finding suggests that some mechanism is involved in the depressing effect of K in addition to the inactivation of the transient inward current.

![Graph A and B showing current-voltage relations](image)

**Fig. 5.** Current voltage relations at various $[K]_o$. In A, the peak amplitude of the transient inward current and the values measured about 10 msec after the onset of the hyperpolarizing pulses, and in B values about 0.5 sec after the onset of the pulse were plotted. Different symbols indicate data obtained in different $[K]_o$ solutions from the same specimen; closed circles 2.7 mM, open circles 10.0 mM, and triangles 25.0 mM. Data were obtained from the same specimen shown in Fig. 4.

![Graph A and B showing effect of $[K]_o$ on membrane current](image)

**Fig. 6.** Effect of $[K]_o$ on the membrane current. The $[K]_o$ was changed without replacing K with Na and the membrane potential was held at $-40$ mV. The current voltage relations were measured at 2.7 mM (closed circles), 15.0 mM (triangles) and 22.7 mM $[K]_o$ (open circles). The membrane current in A was measured in the same way as in Fig. 5, and in B it was measured at 0.28 sec.

**Time course of the membrane current change by increasing $[K]_o$.**

Membrane currents in response to constant clamp pulses were recorded repeatedly every 40–50 sec after changing the perfusate from 2.7 mM $[K]_o$ solution...
to 22.7 mM [K]_o solution at normal [Na]_o. To record the transient inward current more clearly than in Fig. 4, a high time base speed record was made as shown in Fig. 7. At the holding potential of -40 mV apparently no net current flowed in

![Figure 7](image-url)

**Fig. 7.** Time course of the membrane current change generated by increasing [K]_o. A shows the continuous record of the membrane potential before and after changing the perfusate from 2.7 mM [K]_o to 22.7 mM [K]_o solution. At different times shown by numerals the membrane potential was clamped. B shows the membrane current (upper trace) in response to constant clamp pulses from -40 to -15 mV for about 0.1 sec (lower trace). The number of the record corresponds to that in A. The dotted line in each record indicates the level of zero net membrane current.

2.7 mM [K]_o, but after changing to 22.7 mM [K]_o the membrane was depolarized and an inward current of 6 × 10^{-9} A flowed at -40 mV. The amplitude of the transient inward current was significantly reduced after 70 sec perfusion of 22.7 mM [K]_o solution. The amplitude of the outward current tail was also reduced 20 sec after the perfusion. All of these current changes were reversible and 2–3 min after re-perfusion of the control Tyrode the membrane currents returned to the control. These findings confirmed the idea that the transient inward current was depressed by increasing [K]_o.

**Decrease of the outward current tail at higher [K]_o.**

It has been suggested that the outward current tail is responsible for the slow diastolic depolarization of the S-A node cell (NOMA and IRISAWA, 1976b). Therefore, the effect of K on the outward current tail was investigated by plotting the magnitude of the outward current tail at 2.7, 10, and 25 mM [K]_o against the membrane potential during the preceding test pulses in Fig. 8. At 2.7 mM [K]_o the magnitude of the outward current tail increased over the range of the membrane potential from -40 to 10 mV and above this range it becomes saturated. The outward current tail obtained in 10 mM [K]_o solution increased in a manner similar to that at 2.7 mM [K]_o, but the peak magnitude was about 80% of the value obtained at 2.7 mM [K]_o. A slight decrease in the magnitude of the outward current tail was observed when the potential of the preceding pulse was 20–30 mV.
The outward current tail was considerably depressed by raising \([K]_o\) to 25 mM. Its peak amplitude at 25 mM \([K]_o\) was about 20\% of that obtained at 2.7 mM \([K]_o\).

**Decrease of the outward current tail at higher holding potential**

The decrease in the magnitude of the outward current tail by raising \([K]_o\) may be due to a reduction in the driving force for K, which is defined by the difference between the membrane potential \((E)\) and the K equilibrium potential \((E_K)\). To confirm these notions, the effect of reducing the driving force on the

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**Fig. 8.** The decrease in the magnitude of the outward current tail at higher \([K]_o\). Relationships were obtained from the experiment shown in Fig. 4. Different symbols indicate data obtained at different \([K]_o\) corresponding to those in Fig. 5.

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**Fig. 9.** Effect of the holding potential on the outward current tail. A; upper trace is the membrane current and the lower trace is the membrane potential. The numerals in A indicate the membrane potential in mV during the voltage clamp. In 2 and 3, anodal break excitation occurred at the break of the voltage clamp. The holding current was negligibly small at \(-44\) mV. B; open circles, triangles, and closed circles were obtained with the holding potential of \(-44, -60,\) and \(-75\) mV.
magnitude of the outward current tail was investigated by holding the membrane potential to higher levels at a constant $E_K$. Figure 9A gives the results of the voltage clamp experiment, in which test pulses were applied from holding potentials of $-44$, $-60$, and $-75$ mV. The sudden change in the current trace on repolarization became larger and the amplitude of the outward current tail decreased as the holding potential was shifted to a more negative level. When the magnitude of the outward current tail was plotted against the membrane potential during the preceding depolarizing test pulse (Fig. 9B), it was found that the magnitude of the outward current tail decreased at all membrane potentials as the holding potential was increased.

**DISCUSSION**

Deceleration of the spontaneous rate of the action potential discharge of the S-A node cell at higher $[K]_o$ may be attributable to the decrease in magnitude of both the outward current tail and the transient inward current. Because of the reduced outward current tail, the maximum diastolic potential may decrease, resulting in an incomplete removal of inactivation of the transient inward current during the diastolic period. When the amount of the available transient inward current is reduced, the threshold potential for the action potential may shift in a positive direction so that the diastolic interval is prolonged.

One of the causes for the reduction of the magnitude of the transient inward current is the depolarization of the membrane at higher $[K]_o$. The decrease of the maximum diastolic potential and of the resting membrane potential on raising $[K]_o$ was not so marked in the S-A node cell as compared to other kinds of myocardium, but the depolarization was consistently observed at higher $[K]_o$ (De Mello and Hoffman, 1960; Trautwein and Kassebaum, 1961; Noma and Irisawa, 1975). In agreement with this fact, the reference potential, defined by the potential at which no net membrane current flows at the steady state (Figs. 1–3), decreased in response to increasing $[K]_o$. It was suggested that the degree of inactivation of the transient inward current increased as the membrane became depolarized in the S-A node cell (Noma and Irisawa, 1974).

The magnitude of the transient inward current was reduced, however, when the effect of depolarization was removed by holding the membrane potential at a constant level at various $[K]_o$ (Figs. 6 and 7). This finding suggests that some mechanisms other than the inactivation are involved in the depressing effect of K on the transient inward current. It was demonstrated in the mammalian atrial fiber that the magnitude of the net slow inward current was decreased by an increased time-independent outward current under the effect of acetylcholine (Eick et al., 1976). The time-dependent outward current may not significantly change the magnitude of the net inward current, because it is activated relatively slowly compared to the time course of the transient inward current during depolarization. Similar mechanisms as in the atrial fibers may be involved in the
effect of high $[K]_o$ on the transient inward current of the S-A node cell. The increased time-independent leak current at higher $[K]_o$ was suggested by the finding that the membrane conductance for the early hyperpolarizing current increased at higher $[K]_o$ (Figs. 5A and 6A). To test this assumption further quantitative analysis of the membrane current during depolarization is necessary in the S-A node cell.

The large reduction of the transient inward current at 25 mm $[K]_o$ (Fig. 5) and at 22.7 mm $[K]_o$ (Fig. 7) cannot be explained by the neutralizing effect of the time-independent outward current. The magnitude of the outward current at the end of the clamp pulse is too small to counterbalance the inward current. Therefore, an unknown additional mechanism may be involved in the depressing effect of $K$.

The reduction in the magnitude of the outward current tail is due to a diminished driving force for the $K$ current at higher $[K]_o$. In the S-A node cell the outward current tail was attributed to a deactivating $K$ current on repolarization and its magnitude ($\Delta i$) was expressed by the equation,

$$\Delta i = \Delta p \cdot \bar{g}K (E - E_K),$$

where $\Delta p$ is the change in the gating variable $p$, and $\bar{g}K$ is the fully activated potassium conductance. This equation estimates about 50% decrease of the outward current tail produced by a constant clamp pulse if $\bar{g}K$ is constant and $E - E_K$ is 58 mV and 30 mV at 2.7 mm and 10 mm $[K]_o$, respectively (Noma and Irisawa, 1976b). However, the magnitude of the outward current tail at 10 mm $[K]_o$ decreased only by about 20% of that at 2.7 mm $[K]_o$. This fact suggests that the $\bar{g}K$ increased at higher $[K]_o$. The increase of the $K$ conductance at higher $[K]_o$ has been shown in Purkinje fibers (Carmeliet, 1961; Hall et al., 1963; Vassalle, 1965).

The fact that the slope of the diastolic depolarization decreases at higher $[K]_o$ (Lu, 1970) may be interpreted as follows. The diastolic depolarization is attributable to a slow decrease of the $K$ conductance from a high value at the end of the action potential to a diminished resting value (Dudel and Trautwein, 1958; Trautwein and Kassebaum, 1961; Deck and Trautwein, 1964; Vassalle, 1965; McAllister et al., 1975; Noma and Irisawa, 1976b). Correspondingly, the membrane potential approaches $E_K$ at the end of the action potential and then gradually returns toward the reference potential. Therefore, the slope of the diastolic depolarization is a function of the difference between $E_K$ and the reference potential as well as of the time constant for the decay of the outward current. However, changes in the former factor will mainly contribute to the effects of high $[K]_o$, since the time constant for the outward current tail does not change appreciably when $[K]_o$ was altered (Noma and Irisawa, 1976b). The value of $E_K$ decreases with increasing $[K]_o$. Furthermore, if $gK$ of the S-A node cell membrane increases relatively to other ionic conductances at higher $[K]_o$ in the same way as in Purkinje fibers (Vassalle, 1965), the reference potential of the S-A
node cell becomes near to $E_K$ with increasing $[K]_o$. Thus, the slope of the diastolic depolarization decreases with increasing $[K]_o$.

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