Effects of Rb and Cs on the Electrogenic Na-pump in Rabbit Sinoatrial Node Cells

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Abstract The effects of Rb and Cs on the electrogenic Na-pump of the rabbit sinoatrial node cell were studied, using conventional microelectrode techniques. Hyperpolarizations induced by K or its substitutes were recorded from the preparations which were perfused previously with an extracellular K-free solution. The K-induced hyperpolarizations after K-depletion depended linearly on K-concentration in the range of 1.35 to 10.8 mM.

When 2 mM Rb was used as an activator, a subthreshold oscillation appeared and the amplitude of the spontaneous action potentials in the Rb solution was larger than with the standard solution. As the concentration of Rb increased, the Rb-activated hyperpolarization increased, but the amplitude of the action potential decreased. In the case of Cs, the induced hyperpolarization was smaller than with K or Rb, and a transient recovery of pacemaker activity and gradual hyperpolarization preceded the true recovery in the standard solution. Since these hyperpolarization effects were not observed after the application of ouabain-containing K-free solution, the hyperpolarizations were considered to be electrogenic. A small hyperpolarization induced by activation of the external site of the Na-pump appears to be necessary for the immediate recovery of automatism from arrest at low resting potentials.

Key Words: S-A node, Rb, Cs ion, electrogenic pump.

Although there are numerous references available on the electrogenic Na-K pump mechanisms in nerve and muscle cells (KERNAN, 1962; RANG and RITCHIE, 1968; BOLTON, 1971; THOMAS, 1972; DEN HERTOOG, 1973; HIRAOKA and HECHT, 1973; MORITA and KOKETSU, 1979), relatively few experiments have been performed on cardiac preparations. One early observation related to the electrogenic Na-pump was that cardiac arrest occurred on returning isolated frog heart to normal Ringer solution after perfusing with a K-free solution for several minutes (SURAWICZ and GETTES, 1963). The K-induced hyperpolarization after

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K-free incubation was used as a means of demonstrating the electrogenic Na-pump in various cardiac tissues (NOMA and IRISAWA, 1974, 1975; ELLIS, 1977; GADSBY and CRANFIELD, 1979).

Recently, the monovalent cations Rb and Cs have been employed instead of K to reactivate the Na-pump when examining transient increases of hyperpolarization in mammalian ventricular muscle and Purkinje fibers (EISNER and LEDERER, 1979, 1980). Hyperpolarization and recovery from arrest provide a good measure of the degree of reactivation of the external site of the Na-pump. The effect of Rb is quite similar to that of K, but not the same. Therefore, using Rb and Cs as substitutes for K, detailed observations were made in the present study of the external site of the Na-pump in sinoatrial (S-A) node cells as compared to the effects of K.

METHODS

Albino rabbits of either sex, weighing 1.5 to 2.5 kg, were anesthetized with ether. While being ventilated through an endotracheal tube, the thorax was opened and the heart was rapidly removed. A preparation was then made, including the sinus node area, the crista terminalis, and part of the atrial appendage, but without the right atrium. The preparation was trimmed to dimensions of 1×1 mm for recording the dominant pacemaker potential. It was mounted in a tissue bath of 1 ml volume with its endocardial surface on top. The superfusion fluid contained (in mM): NaCl, 133; KCl, 5.4; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.5; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.05; NaHCO_3 , 15; $\text{Na}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.2; and glucose, 5.5. The pH was maintained at 7.4 and the temperature, at $36 \pm 0.5^\circ\text{C}$. The fluid, oxygenated by gassing with a mixture containing 95% O_2 and 5% CO_2 , was led into the bottom of the tissue bath and was suctioned from the surface at a rate of 2 ml/min. Transmembrane potentials were recorded with conventional glass capillary microelectrodes filled with 3 M KCl, whose electrical resistances ranged from 30 to 50 M Ω . The electrodes were coupled *via* an Ag-AgCl wire to an amplifier having high input impedance and capacitance neutralization (MEZ 8261 Nihon-Koden). The output of the amplifier was displayed on an oscilloscope and a rectifier. A modified solution was administered by switching the superfusion fluid from the control to the modified solution. In describing the superfusing solutions, we specify only alternatives to the above standard solution. Thus, a solution described as 10 mM Rb, is a solution in which all the K has been removed and 10 mM Rb added. When more than 10 mM was added, the NaCl was reduced to maintain the tonicity. Control experiments demonstrated that increasing the NaCl by up to 10 mM exerted no effect on the membrane potential.

Ouabain (Merck) was made up as a 10 mM stock in ethanol. The highest concentration of ouabain used was 10^{-5} M, the ouabain containing 0.1% ethanol. We confirmed that this ethanol concentration had no significant effect on potential.

RESULTS

In order to observe the processes of recovery of automatism in the sinus node cell, we employed the K-depletion solution by which the automatism was decreased. Initially, we carried out a control experiment in which the electrogenic Na-pump was activated by adding K.

1. *K-induced hyperpolarization and its process of recovery in the S-A node cell*

It should be noted that the entire series in this experiment was run on the same cell. For recording, three kinds of paper speeds were used.

Immediately after K-depletion, the amplitude of the action potentials of the S-A node cell was increased by 4 mV for a few minutes, and gradually decreased thereafter. After 10 min, the action potentials almost disappeared, leaving only small oscillatory potentials (Fig. 1a and b). When standard Tyrode ($K=5.4$ mM) was reintroduced, the membrane hyperpolarized by 18 mV, followed by a gradual depolarization for 1.5 min. Complete recovery of spontaneous action potentials was observed within a few minutes. The K-induced hyperpolarization increased as the concentration of K in the recovery solution increased (Fig. 1b, c, d, and e), their relationship showing a hyperbolic curve (Fig. 7). This K-induced hyperpolarization confirmed the existence of the electrogenic Na-pump observed previously in S-A node cells (NOMA and IRISAWA, 1974, 1975) as well as in A-V node cells (KURACHI *et al.*, 1981).

2. *Rb-induced hyperpolarization and its process of recovery in the S-A node cell*

Rb has been found to substitute for K in the K sites of the Na-K pump in Purkinje fibers (EISNER and LEDERER, 1979, 1980). This property of Rb was examined in the S-A node cell. In Fig. 2a, b, and d, nodal cells were superfused for 10 min in K-free solution, and the 5.4 mM K, 2 and 4 mM Rb-induced hyperpolarizations were observed. In the same specimen, higher concentrations of Rb (10 and 20 mM Rb) were superfused after K-free perfusion (Fig. 3). The hyperpolarization increased as the concentration of Rb increased, and it appeared to approach a maximum amplitude (-20 mV) at concentrations greater than 10 mM Rb. The rate of recovery from transient hyperpolarization increased as the concentration of Rb increased. Spontaneous activities were recovered and maintained in the 2 to 10 mM Rb solutions, but they ceased within 4.5 min in the 20 mM Rb solution. This suggests that Rb can substitute for K within a certain concentration range. The spontaneous activities in the 2 mM Rb solution revealed a transiently larger amplitude than those in the standard solution (Fig. 2b). In 4 mM Rb (Fig. 2d), the transient oscillation was small and the spontaneous activities were almost the same as those for 5.4 mM K solution. The spontaneous activities in the 10 mM Rb solution were smaller than those for the standard solution.

Fig. 1. K-induced hyperpolarizations in the same sinus node cell after exchange of the K-free solution for the standard one, and different K-concentration Tyrode solutions. a: control of the action potential and depolarization after application of the K-free Tyrode solution. The uppermost panel shows the maximum rate of rise of action potentials (2 V/sec). The baseline of the lowermost trace shows the temperature, 35°C. Vertical bar: 1°C. b: 10 min after switching to the K-free solution, the automatic potential almost disappeared and the standard solution (5.4 mM) was perfused. c: 10 min after switching to the K-free solution, the potential decreased and the 1.35 mM KCl Tyrode solution was perfused. d: 10 min after switching to the K-free solution, the 2.7 mM KCl Tyrode was perfused. e: 10 min after switching to the K-free solution, the 10.8 mM KCl Tyrode was perfused. Calibration: 20 mV. Time scale: 0.5 and 5 sec for the fast record and 1 min for the slow one.

Fig. 2. Rb-induced hyperpolarizations after exchange of the K-free for the 2 and 4 mM Rb solution. a: at 5.4 mM, the perfusion was exchanged for the standard solution after K-depletion, as a control for Rb experiments. b: 10 min after application of the K-free solution, the 2 mM Rb solution was perfused at 2 mM Rb. After the exchange there were transient oscillation (triangle), disappearance, depolarization, and reappearance of the action potential. c: at 5.4 mM, the standard solution was perfused. Increased amplitude returned to a normal level before application of the standard solution. d: 10 min after application of the K-free solution, the 4 mM Rb was perfused. After switching to the 4 mM Rb, there occurred a small transient oscillation, disappearance, depolarization, and reappearance of action potential. e: at 5.4 mM, the standard solution was perfused. Amplitude did not change. Calibration: 20 mV. Time scale: 0.5, 1, and 5 sec for the fast record and 1 min for the slow one.

3. Cs-induced hyperpolarization and its process of recovery in the S-A node cell

Figure 4 shows Cs-induced hyperpolarizations after K-depletion, followed by their recovery. Two and 4 mM Cs solutions were superfused after K-free solution, small hyperpolarizations (3 and 4 mV, respectively) being observed (Fig. 4b and d). By reintroducing standard K-Tyrode solution (Fig. 4c and e),

Fig. 3. Ten and 20 mM Rb-induced hyperpolarizations and their recovery processes. a: 10 min after application of the K-free solution, the 10 mM Rb was perfused. After the exchange of solutions, there were large hyperpolarization, depolarization, and reappearance of action potential. The potential was smaller than that with the standard solution. b: at 5.4 mM, the standard solution was perfused and the amplitude increased. c: 10 min after application of the K-free solution, the 20 mM Rb was perfused. After the exchange of solutions, there occurred the largest hyperpolarization, depolarization, and reappearance of action potential. The potential grew smaller and disappeared in 3.5 min. At 5.4 mM, the standard solution was perfused. d: recovery process. Three kinds of time scale were used. Calibration: 20 mV. Time scale: 0.5, 1, and 5 sec for the fast record and 1 min for the slow one.

other small hyperpolarizations were seen, indicating that Cs could not completely substitute for K-induced hyperpolarization. The amplitude of the hyperpolarization increased as the concentration of Cs increased (Fig. 5a and c). In 2 and 4 mM Cs solutions (Fig. 4b and d), small oscillatory potential changes were noted during the period of recovery, whereas in 10 and 20 mM Cs (Fig. 5), the membrane hyperpolarized and full-blown spontaneous action potentials were observed which lasted 4–1.5 min. These spontaneous action potentials gradually faded away and the resting membrane appeared to be at a slightly depolarized level.

Fig. 4. Cs-induced hyperpolarizations after exchange of the K-free for the 2 and 4 mM Cs solution. a: at 5.4 mM, the perfusion was exchanged for the standard solution after the K-depletion, as a control for Cs experiments. b: 10 min after application of the K-free solution, the 2 mM Cs was perfused. After the exchange of solutions, there was a small hyperpolarization followed by a transient oscillation. c: at 5.4 mM (6 min after the exchange) the standard solution was perfused. d: 10 min after application of the K-free solution, the 4 mM Cs was perfused. After the exchange of solutions, there were small hyperpolarization and transient oscillation. e: at 5.4 mM the standard solution was perfused. A small hyperpolarization followed by recovery of the action potential was seen. Calibration: 20 mV. Time scale: 1 and 5 sec for the fast record and 1 min for the slow one.

The above experiments indicate that Cs can also be a substitute for K sites of Na-K pump activity in the membrane. The relation between the hyperpolarization and the concentration of Cs showed some differences from those observed in K and in Rb (Fig. 7A).

4. Effects of ouabain on Rb-activated hyperpolarization

Figure 6 illustrates the ouabain inhibition of 4 mM Rb-activated hyperpolarization in the sinus node cell. In Fig. 6a, application of ouabain K-free solution

Fig. 5. Ten and 20 mM Cs-induced hyperpolarizations and their recovery processes. a: 10 min after application of the K-free solution, the 10 mM Cs was perfused. After the exchange of solutions, there was a small hyperpolarization followed by spontaneous potentials arising from Cs. b: at 5.4 mM the standard solution was perfused. c: 10 min after application of the K-free solution, the 20 mM Cs was perfused. After the exchange of solutions, there was an abrupt hyperpolarization followed by full-blown spontaneous potentials. At 5.4 mM the standard solution was perfused. d: the process of recovery of the action potentials was seen soon after. Calibration: 20 mV. Time scale: 1 min.

after recording of the control pacemaker activity is shown. At 7 min after the application, this was exchanged for ouabain-free 4 mM Rb solution (b). There was no Rb-activated hyperpolarization (c). At 9 min after application of the 4 mM Rb, a small hyperpolarization appeared before the restart of spontaneous activity (d). Almost complete recovery was observed within 30 min, before application of the standard solution (f). It should be noted that little difference in appearance was discernible between the actions of K and Rb.

5. Relation between dose and response for K, Rb, and Cs

The diagrams in Fig. 7A and B were constructed from average values obtained from 6–10 experiments. The mean values of the hyperpolarization for 1.35, 2.7,

Fig. 6. Effects of ouabain on Rb-activated hyperpolarization. a: control of the pacemaker activity and Ou-KCl-F shows application of the ouabain K-free solution. b: at 4 mM Rb (7 min after application of ouabain K-free solution) the solution was exchanged for the 4 mM Rb. c: a quiescent and recovery process. d: a small hyperpolarization (3 mV) is shown. e, f: gradually increasing, and complete reappearance of, potentials in the standard solution (5.4 mM). Calibration: 20 mV. Time scale: 0.5 and 5 sec for the fast record and 1 min for the slow one.

5.4, and 10.8 mM KCl were 8, 12, 18, and 20 mV, respectively. In Rb, the values at 2, 4, 10, and 20 mM (6 experiments) were 10, 14, 20, and 24 mV, respectively. In Cs, the values at 2, 4, 10, and 20 mM (6 experiments) were 3, 4, 7, and 20 mV, respectively. If the degree of hyperpolarization reflects the activity of the Na-pump at various external K, Rb, and Cs concentrations, the kinetics of pump activation by extracellular K, Rb, and Cs ions should be obtainable from Lineweaver-Burk plots of the above data. The relation between dose and response appears to be expressed by a hyperbolic curve (Fig. 7A). From these values, the Lineweaver-Burk plots give a straight line, except with Cs. The responses of K and Rb have a common regression line, but Cs yields a rather different one (Fig. 7B).

DISCUSSION

In Purkinje fibers, based on the ionic theory of membrane potential changes, the mechanism of automatism has been suggested to involve a decrease in the permeability of membrane to K^+ , an increase in the permeability of membrane to Na^+ and a reduction in the activity of the electrogenic Na-pump (WEIDMANN,

Fig. 7. Relation between dose and response for K, Rb, and Cs. Diagram A was constructed from average values obtained from 6–10 experiments. A appears to be expressed by a hyperbolic curve in K (○) and Rb (▲). From these values a reciprocal plot B shows a straight line, with the exception of Cs (■). The line of K resembles the Rb line very closely.

1956; BROOKS and LU, 1972). It has been shown recently that the current system i_f present during the pacemaker depolarization phase in the rabbit sinoatrial node appears as a large inward current slowly yielding hyperpolarizations below -50 to -60 mV (BROWN and DIFRANCESCO, 1980). The latter suggested a possible role of i_f in the phase 4 depolarization which is known to overlap the pacemaker range (-65 to -45 mV). However, the possible linkage between i_f and the electrogenic pump current has still not been elucidated. EISNER and LEDERER (1980) demonstrated a relationship between the membrane potential and outward current of the electrogenic pump in the Purkinje fiber where 4 mM KCl was added to the solution bathing a fiber which had previously been exposed to a K-free solution. The membrane was hyperpolarized abruptly from -40 to -65 mV in 1 min and gradually depolarized to a somewhat lower level than the original within 5 min. Simultaneously, a transient peak of outward current was observed within

60 sec, and it then attained an almost steady value by 2.5 min. However, the current did not decrease further. This indicates that there must have been some other background current in this range. In other words, the time course of the current does not parallel the change of voltage.

In our 5.4 mM KCl experiment (Fig. 1b), the hyperpolarization occurred within 20 sec, and the depolarizing time was 1.5 min. The time course depends on the rate of exchange of solution in the bath, but our data were almost compatible with those of Eisner and Lederer.

On the other hand, they reported that the membrane potential in their 20 mM Rb experiment changed from -45 to -80 mV within 15 sec, and that the outward current reached a peak within 30 sec and promptly decreased in 2–3 min. In our experiment using 20 mM Rb, the peak of the hyperpolarization potential appeared within 15 sec, and changed from -45 to a maximum of -70 mV. The spontaneous action potentials in the Rb solution decreased in 3.5 min.

The Rb-induced hyperpolarization and its time course observed in our experiments appear to originate from the outward current of the Na-pump, since they occur in parallel with the change in outward current mentioned. In order to continue the automatism in the presence of K, Rb, or Cs after the K-free perfusion, the membrane must retain a certain amount of inward current after the peak of the outward current. Too much inward current such as that after 20 mM Rb, leads to a decrease of repetitive excitation. In 5.4 mM K solution, the outward and inward currents are appropriately balanced.

Cs shows some differences from Rb and K. The dose-response curve for Cs is not hyperbolic and the double-reciprocal plot is not a straight line, in contrast to those for K and Rb (Fig. 7B). In this case, the Cs-induced hyperpolarization was very small and there was a subthreshold oscillation arising from Cs. Furthermore, K-induced hyperpolarization was noted even after application of normal solution.

According to ISENBERG (1976), Cs acts as a factor blocking the inward rectifying potassium current of the Purkinje fiber. OJEDA *et al.* (1981) stated that 20 mM Cs ions reduced the background current and abolished the K^+ depletion process, probably as a result of elimination of i_{k1} . The specific action of the Cs ion based on our data appears to be correlated with the background current. In order to continue repetitive excitation of the pacemaker cell, the resting potential must remain at a certain level so that the outward current is sufficiently maintained, followed by some inward current—not too much, but one which can retain only a subthreshold level. In conclusion, we suggest that a small hyperpolarization followed by some depolarization is necessary for the recovery of automatism in the case of monovalent perfusion which can be correlated with the external site of the Na-pump.

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