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CHARACTERISTICS OF THE ANION CHANNEL IN THE SINO-ATRIAL NODE CELL OF THE RABBIT

By ISSEI SEYAMA

From the Department of Physiology, School of Medicine, Hiroshima University, Hiroshima 734, Japan

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SUMMARY

1. The anion permeability of the sino-atrial node cell membrane was determined by substituting various anions for Cl and observing the resultant transient changes in membrane potential. The permeability sequence was found to be in the following order: thiocyanate > NO_3 > I > Br > Cl > acetate.

2. The membrane resistance in Cl solution was compared with that in various anion solutions by the voltage-clamp method. The conductance sequence for the sino-atrial node cell membrane was observed to be the same as the permeability sequence.

3 The potential generated by the Na–K pump was partly short-circuited in normal bathing solution, and thus the pump could be responsible in part for the generation of the sino-atrial node resting membrane potential.

4 When Cl was replaced by acetate, a less permeable ion, the inward-going rectification disappeared. Thus, the inward-going rectification might be due partly to a time- and voltage-dependent Cl current.

INTRODUCTION

Since the pioneering work of Hutter & Noble (1961), the contribution of anions to the electrical activity of the myocardium has been extensively studied in Purkinje fibre. They found that the replacement of chloride by permeant anions caused either an arrest or an initial slowing of the rhythm followed by an acceleration. On the substitution of extracellular Cl by impermeant anions, the heart rate transiently increased and then eventually decreased to between 40 and 90 % of that found in Cl solution. These changes were explained by the passive movement of anion during the action potential, assuming that the equilibrium potential for chloride (E_{Cl}) was -50 mV. Carmeliet (1961) also supported the passive movement of Cl ion in Purkinje fibre and observed a contribution of Cl to the total membrane conductance.

Since the resting membrane potential of the sino-atrial node cell lies around -30 to -40 mV, the contribution of Cl ion to the diastolic depolarization of the pace-maker fibres may be pronounced in the sino-atrial node cell. Indeed, de Mello (1963) has found that Cl may constitute an appreciable fraction of the membrane current during the action potential. Moreover, two recent observations also support this possibility. First, 9 % of the total membrane conductance of the resting potential is

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due to Cl movement (Seyama, 1977). Secondly, since inward-going rectification develops at a voltage more positive than the potassium equilibrium potential (E_K), Cl-ion may be partly responsible for this rectification (Seyama, 1976; Noma & Irisawa, 1976*b*).

Development of the voltage-clamp experiment in rabbit sino-atrial node cells now makes possible a more quantitative study of anion permeability. By substituting Cl-with various anions of different sizes, the property of anion permeation through the membrane has been examined by observing changes in both membrane potential and membrane conductance. It has been found that sino-atrial node cell membrane seems to have a pore through which organic anions cannot pass and that inward-going rectification may be due partly to the flow of Cl^- ion.

METHODS

Preparations of sino-atrial node strands. The procedure employed in these experiments for making the sino-atrial node preparations was similar to that reported by Noma & Irisawa (1976*a*). The right atrium, including the sino-atrial node region, was removed from an albino rabbit anaesthetized with pentobarbitone (20 mg/kg). The sino-atrial node was then cut into two strands directed perpendicularly to the *crista terminalis*. After confirming that the strands were spontaneously beating, they were carefully trimmed with a fragment of a razor blade until they were about 300 μm wide. The trimming method was a deliberate, stepwise removal of tissue slivers, each about 50 μm in diameter. After each cut, the strands became quiescent. We waited until rhythmical activity was restored before continuing to trim. Finally, the epicardial side of these thin preparations was removed. Each strand was then ligated at two points, about 300 μm apart, with silk fibres. The result was a small sausage, 300 x 300 μm in the middle of the strand. About 90% of these sausage preparations began beating spontaneously within 10–60 min after immersion in normal Tyrode solution. The action potentials were similar to those of intact sino-atrial nodes.

These sausage preparations are particularly advantageous for studying the electrical activity of sino-atrial node cells for the following reasons. (1) Because of their small size, no appreciable conduction occurs, and the phenomenon of pacemaker shift can therefore be avoided. (2) The apparent lack of conduction also precludes the development of electrical interference from the asynchronous electrical activity of neighbouring cells. (3) Since two-thirds of the sino-atrial node wall from the epicardial surface is removed, exchange of solute with the external solution is mainly delayed by diffusion. (4) Micro-electrodes stayed within cells for as long as 1–2 hr, and during the first 10 min of penetration, both the input impedance of the cell and the amplitude of the action potential gradually increased. Apparently, the membrane seal around the micro-electrodes improved during this interval, a phenomenon never observed in a large sino-atrial node specimen.

The most serious problem under the voltage-clamp condition came from the non-homogeneity of the spatial control of the membrane potential. This problem was overcome successfully by using a small preparation (Noma & Irisawa, 1976*a*). In this experiment, a test on the spatial control of the membrane potential was carried out on a small quiescent preparation of the sino-atrial node by the current-clamp method. By using the bridge method, current was applied through one electrode which simultaneously monitored the membrane potential. The other electrode recorded the membrane potential change produced by the applied current. The resultant changes of the membrane potential at two electrodes from -40 mV to either -20 or -60 mV were compared. The difference between the recorded membrane potential at two micro-electrodes was within experimental error, being less than 1 mV. Because of the restriction of current supply through the bridge circuit, this method could check the condition of spatial homogeneity of the membrane potential only at the narrow range of membrane potential. The limitations of the voltage-clamp technique with two micro-electrodes have been discussed by Noma & Irisawa (1976*a*) and can be applied equally to the present experiment.

Electrophysiological methods. The voltage-clamp system was essentially the same as that employed by Wang, Narahashi & Scuka (1972). The control amplifier was 603 K (Analog Device Co., Norwoods, Mass., U.S.A.). To obtain a high speed clamp, the ground partition was inserted between the two micro-electrodes. When the membrane potential alone was monitored, the external saline was clamped at the ground potential by a feed-back circuit (Hagiwara, Toyama & Hayashi, 1971). For the reference electrode, an Ag–AgCl wire was inserted into a glass pipette which was filled with 3 M-KCl agar. It was assumed that in this experiment the junction potential between the reference electrode and bathing solutions was always constant. To avoid the effect on the membrane potential of KCl which leaked into the bathing solution, the reference electrode was placed close to the outlet.

Solutions. A modified Tyrode solution having the following composition (mM) was used as a standard solution: NaCl 136·8, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 0·5 and HEPES 6·0. In preparing various foreign anion solutions, concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were held constant except in the thiocyanate and iodide solutions. Nitrate, bromide and acetate were 145·6 mM. Thiocyanate and iodide were 143·2 mM, because Mg salts for these anions were not available and were not added to these anion solutions. The high Ca solutions were obtained by simply adding appropriate amounts of Ca acetate. The Na-free acetate solution was preparared by titrating 142·8 mM-Tris(hydroxymethyl) aminomethane with glacial acetic acid to pH 7·4. The rest salts was contained in acetate form. In preparing the methanesulphonate solution, NaOH 136·8, KOH 2·7, $CaCl_2$ 2·07, $MgCl_2$ 0·5 and HEPES 6·0 (all mM) were titrated by methanesulphonic acid to pH 7·4. The temperature of the perfusate was maintained at 37 °C throughout the experiments. Unless otherwise stated, means ± S.E. of means are given in this paper.

RESULTS

The effect of chloride replacement on the action potential

When Cl^- was replaced by thiocyanate, within a few seconds the preparations ceased beating and the membrane transiently hyperpolarized and then gradually repolarized to a potential level slightly more negative than the original resting potential. The arrest persisted as long as thiocyanate solution was perfused. On re-admission of Cl solution, the membrane depolarized transiently, followed by a resumption of spontaneous activity. de Mello (1963) pointed out a fall in the maximum diastolic potential and in the slope of diastolic potential after 2 min in thiocyanate solution without cessation of beating. The difference between the two observations may be due to the size of the preparations. In this study the time course of the generation of the thiocyanate-replacement action was much shorter than that (2 min) seen by de Mallo (1963).

The substitution of Cl by NO_3, I and Br produced a slight decrease in maximum diastolic potential and in overshoot of the action potential. The frequency of the action potentials transiently decreased. de Mello (1963) also observed a similar change in the same medium.

The substitution of Cl by organic anions, such as acetate, glutamate, proprionate and gluconate, increased both the amplitude and maximum rate of rise of the action potential. Both the threshold potential and maximum diastolic potential were shifted by 20–30 mV in the hyperpolarizing direction, while the frequency and duration of the action potentials were reduced. Preparations which had been quiescent in Cl solution would often begin beating spontaneously in organic anion solution. Upon re-application of Cl solution, such preparations became hypodynamic again.

The changes of the action potential after exposure to inorganic anion solutions

could be explained by the hypothesis proposed by Hutter & Noble (1961). However, on replacement of Cl by organic anion, a sustained increase of action potential and bradycardia were observed. Both phenomena were different from the observation in Purkinje fibre (Hutter & Noble, 1961). This finding suggests that there may be a current system in the sino-atrial node cell carried by Cl ion.

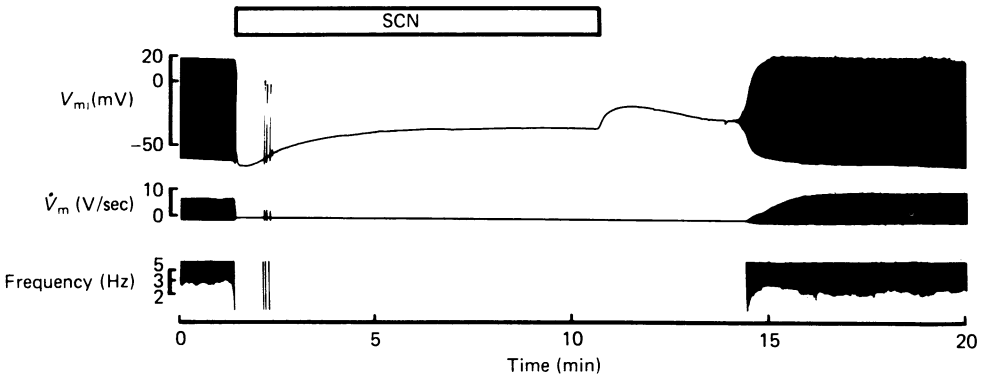

Fig. 1. Effect of replacement of Cl with SCN on the electrical activity of sino-atrial node cell. The uppermost panel shows the action potential of the sino-atrial node cell, the middle panel the maximum rate of rise of action potential and the lowermost panel cardiac frequency. The application of SCN ion is indicated by the open rectangle.

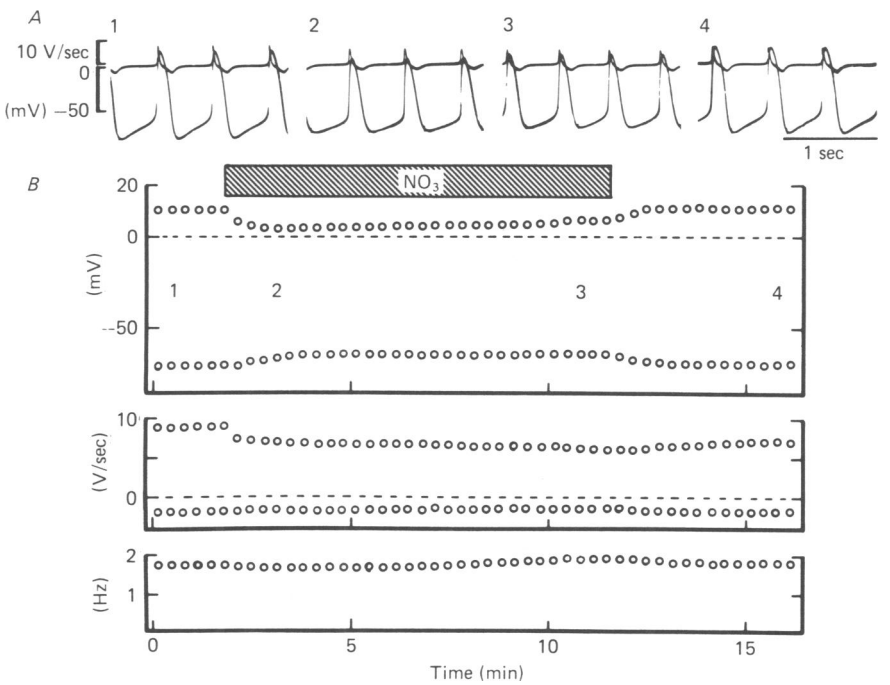

Fig. 2. Effect of replacement of Cl with NO_3 on the electrical activity of the sino-atrial node cell. In *A*, the action potentials recorded at fast time base are shown in mV. The zero base line applies to both the maximum rate of rise of action potential and zero membrane potential level. In *B*, the action potentials, maximum rate of rise and cardiac frequency were measured every 20 sec and plotted in the upper, middle and lower panels, respectively. The records in *A* were taken from those pictures of *B* shown by matching numbers 1–4.

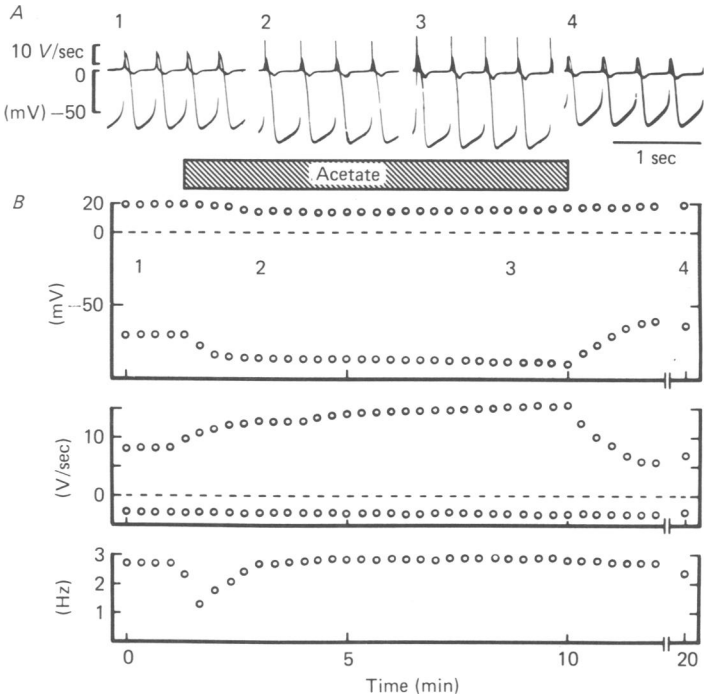

Fig. 3. Effect of replacement of Cl with acetate on the electrical activity of sino-atrial node cell. In *A*, the action potentials recorded at fast time base are shown in mV. The zero base line applies to both the maximum rate of rise of action potential and zero membrane potential level. In *B*, the action potentials, maximum rate of rise and cardiac frequency were measured every 20 sec and were plotted in the upper, middle and lower panels, respectively. The records in *A* were taken from those pictures of *B* shown by matching numbers 1–4.

Effect of Cl replacement on the resting membrane potential of quiescent cell

When treated with inorganic anion solutions, the membrane hyperpolarized transiently, followed by a recovery to a potential more hyperpolarized than the original resting level. The hyperpolarization of the membrane between the final and original resting level was 5 mV in thiocyanate, 4 mV in NO_3 and 3 mV in I. Changes of membrane potential by bromide substitution were barely detectable. By re-administration of normal bathing solution, the membrane showed a transient depolarization, followed by repolarization to the original resting potential level. The amplitude of transient change of the membrane potential caused by changing the anion in the external medium was measured as a difference between the original resting level and the peak of hyperpolarization or depolarization.

When the monovalent anion in the external medium was changed, a trans-membrane concentration difference, in anions only, was established perforce. At the beginning of perfusion of inorganic anion solution, cation is assumed not to move and the observed shifts in membrane potential must have been due primarily to the movement of anions through the membrane. Thus, changes in membrane potential can be interpreted as a measure of the difference of permeability between other anions and Cl^-. Since the counter-ion permeability is not negligible, these measured values only indicate the sequence of anion permeability relative to Cl^-.

When organic anions were substituted for Cl^-, the effect was qualitatively different from those of inorganic anions. On the replacement of Cl^- by organic anions, the membrane gradually hyperpolarized and stayed at that level. On re-admission of Cl solution the membrane depolarized to the original resting potential level. If acetate ion is less permeable than Cl ion, transient membrane depolarization due to

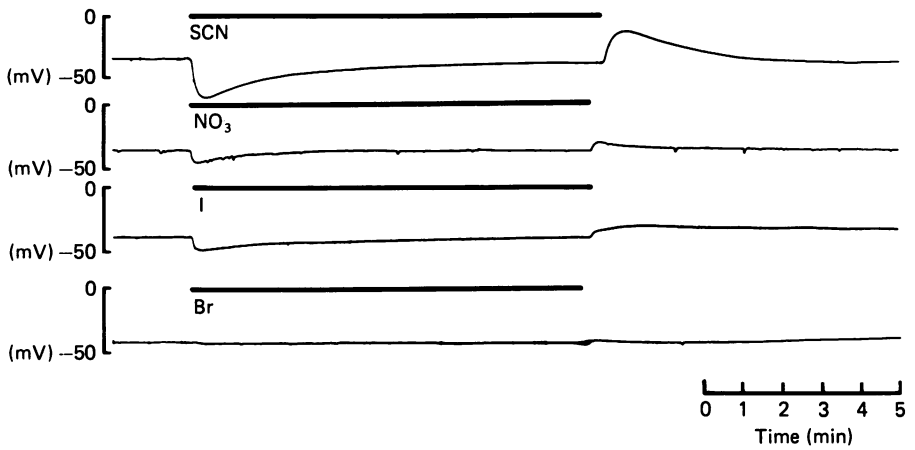

Fig. 4. Transient change of the resting membrane potential in quiescent sino-atrial node cell by replacing Cl with various anions. The thick lines in each panel indicate the application of Tyrode solution containing various anions.

TABLE 1. Amount of transient change in membrane potential caused by anion replacement

Ionic species	Experimental condition	Transient change in membrane potential (mV)	Experimental condition	Transient change in membrane potential (mV)
SCN	From Cl to SCN	$-27 \pm 13{\cdot}7$ ($n = 5$)	From SCN to Cl	$23 \pm 2{\cdot}2$ ($n = 5$)
NO_3	From Cl to NO_3	$-9 \pm 0{\cdot}6$ ($n = 11$)	From NO_3 to Cl	$7 \pm 0{\cdot}9$ ($n = 7$)
I	From Cl to I	$-8 \pm 0{\cdot}8$ ($n = 10$)	From I to Cl	$5 \pm 0{\cdot}8$ ($n = 5$)
Br	From Cl to Br	$-2 \pm 0{\cdot}7$ ($n = 5$)	From Br to Cl	$2 \pm 0{\cdot}8$ ($n = 3$)
In Na-free acetate	From Cl to acetate	$10 \pm 1{\cdot}2$ ($n = 7$)	From acetate to Cl	-8 ($n = 2$)

A negative sign indicates the amount of hyperpolarization from the original resting membrane potential and a positive sign indicates the amount of depolarization.

the efflux of Cl should follow with treatment of acetate solution. Conversely, upon re-perfusion with normal solution, transient hyperpolarization should occur due to the influx of Cl. This unexpected absence of depolarization in acetate solution (Fig. 5, upper panel) might be related to the decrease of intracellular Cl^- concentration during acetate perfusion, which in turn eliminates the short-circuiting effect of Cl on the Na–K pump (Noma & Irisawa, 1975*b*) and leads to membrane hyperpolarization. At the beginning of the substitution, the two opposing factors, Cl efflux and elimination of the short-circuit, counteract each other and are probably balanced. However, the hyperpolarizing effect should prevail since the action of the electrogenic Na-K pump continues. To test this possibility, three different methods of reducing the contribution of the Na-K pump to the membrane potential was employed. First, a similar experiment was done in Na-free medium to decrease the

amount of leaked-in Na ion which would control the activity level of Na-K pump. The lower panel of Fig. 5 shows how substitution of acetate for Cl caused the transient depolarization, and re-admission of Cl gave rise to transient hyperpolarization, as expected. The second method was to immerse the sino-atrial node cell in K-free medium in the presence of normal Na. When K ion was eliminated from the external

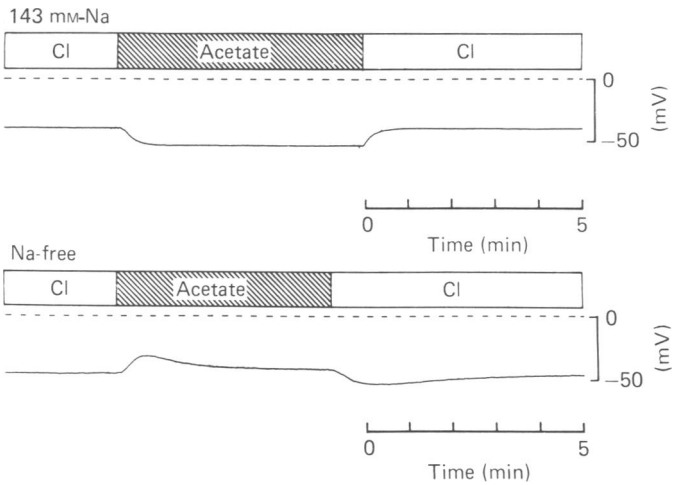

Fig. 5. Comparison of changes in resting membrane potential by replacing Cl with acetate in the presence of 143 mM-Na with that in the absence of Na.

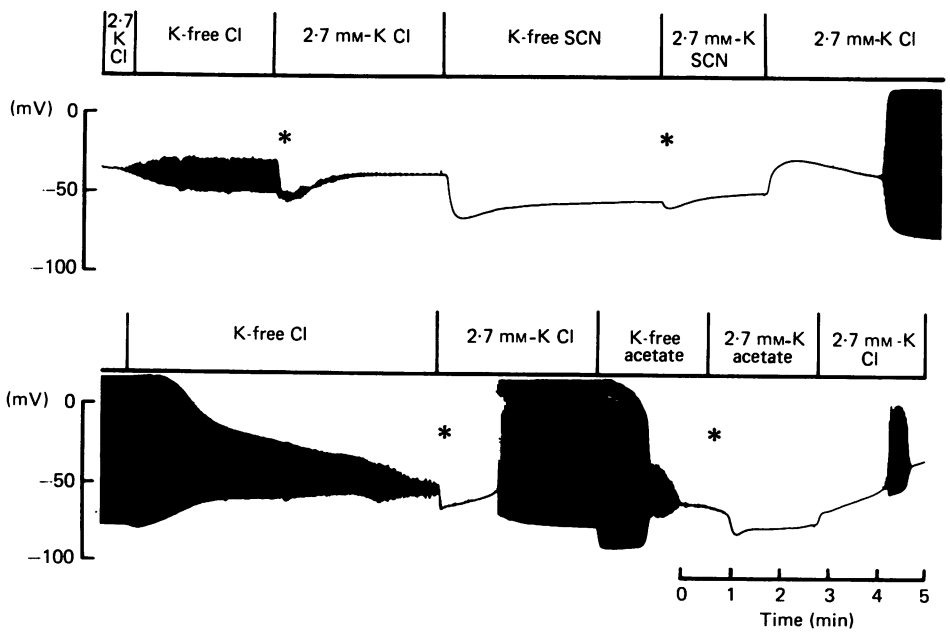

Fig. 6. Comparison of the amounts of transient hyperpolarization due to sudden activation of Na–K pump in solutions containing Cl, SCN and acetate. During the experiment, this particular quiescent sino-atrial node cell started to either oscillate or resume spontaneous beating, occasionally. Sequence of change of solutions is indicated in columns which show both K concentration and anion species. Asterisks show the timing of sudden activation of Na–K pump in various anion circumstances.

medium, the membrane potential did not change. This phenomenon was explained by the large contribution of Na conductance (Noma & Irisawa, 1975*a*; Seyama, 1977). Unexpectedly, either on replacement of Cl by acetate or on re-admission of Cl to acetate medium, changes in membrane potential were not observed. This result, different from that observed by the first method, may be due to the relatively large contribution of Na conductance (33 %) to the total membrane conductance, compared with that of Cl conductance (9 %) (Seyama, 1977). Hence, in K-free medium in the presence of normal Na ion, the change of membrane potential induced by Cl movement will be overcome by the movement of Na ion, so that change in the membrane potential on replacement of Cl by acetate would be null or extremely small. The third method was to apply ouabain (1×10^{-5} g/ml). Its application caused a positive inotropic effect which prohibited an extended recording of the micro-electrode.

Since the permeability ratio of various anions relative to Cl^- has been determined, it is possible to test the anion short-circuiting hypothesis mentioned above. When the electrogenic Na–K pump is forced to drive by switching K-free solution to 2·7 mM-K solution, the amount of transient hyperpolarization induced by the sudden activation of Na-K pump in the presence of thiocyanate, Cl^- and acetate ion could be compared. If the anion short-circuiting hypothesis is applicable, one could expect the magnitude of K-induced hyperpolarization to be in the order of thiocyanate < Cl < acetate. As shown in Fig. 6, the amount of transient hyperpolarization in thiocyanate, Cl and acetate solutions was estimated to be $2{\cdot}5 \pm 1{\cdot}1$ mV (five preparations), $14{\cdot}1 \pm 1{\cdot}2$ mV (ten preparations) and $22{\cdot}4 \pm 2{\cdot}8$ mV (five preparations), respectively. Moreover, in acetate solutions, hyperpolarization due to electrogenic Na-K pump was sustained, while in thiocyanate and Cl solutions, hyperpolarization was transient. Therefore, hyperpolarization in acetate solution is due to the electrogenicity produced by the Na–K pump.

Effect of Cl replacement on the membrane conductance

Conductance measurement is another quantitative method for examining the ease of anion permeation through the membrane. The conductance ratio of an anion is defined as the inverse of the ratio of its characteristic resistance relative to Cl. The procedure can be illustrated by an experiment in which thiocyanate is substituted for Cl. After the transient change of membrane potential subsided, the membrane was held at −35 mV and various depolarizing and hyperpolarizing pulses were applied. The resultant current–voltage relationship is shown in Fig. 7*B*. Since the current–voltage relation of the sino-atrial node cell is strongly non-linear above −35 mV and below −59 mV (Seyama, 1976) the characteristic resistance could only be measured under the voltage-clamp condition. The slope conductance was estimated from the current required for a 20 mV hyperpolarization from the holding potential. The characteristic conductance in normal bathing solution was estimated to be 2·5 μmho and that in thiocyanate solution to be 4·0 μmho. Thus, the conductance ratio of thiocyanate relative to Cl was obtained as 1·56. The same procedure for each NO_3, I and Br as that for thiocyanate was taken to obtain the conductance ratio. After substituting Cl^- with these anions, the membrane resistance decreased and the membrane property of the inward-going rectification was retained (Fig. 8, open squares). Since the difference of conductance among those anions was small,

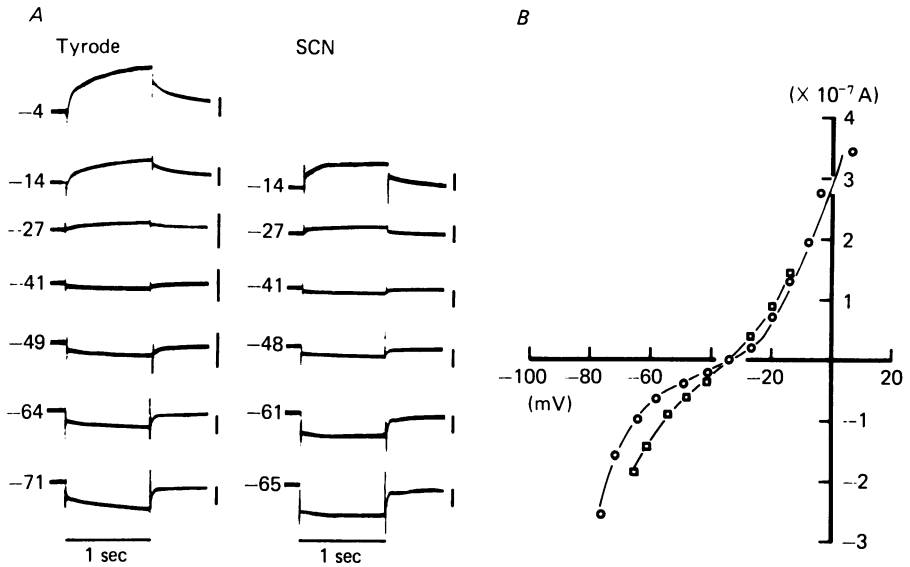

Fig. 7. Current–voltage relationship before and after replacement of Cl with SCN. Holding potential was −35 mV. In *A*, the numbers at the left side of the current record are the level of the membrane potential during the test pulses. Vertical bars at the right side of the current record indicate 1×10^{-7} A. In *B*, amount of current at the end of 1 sec pulse was plotted. Open circles show the data that were obtained in normal bathing solution and open squares that in SCN bathing solution.

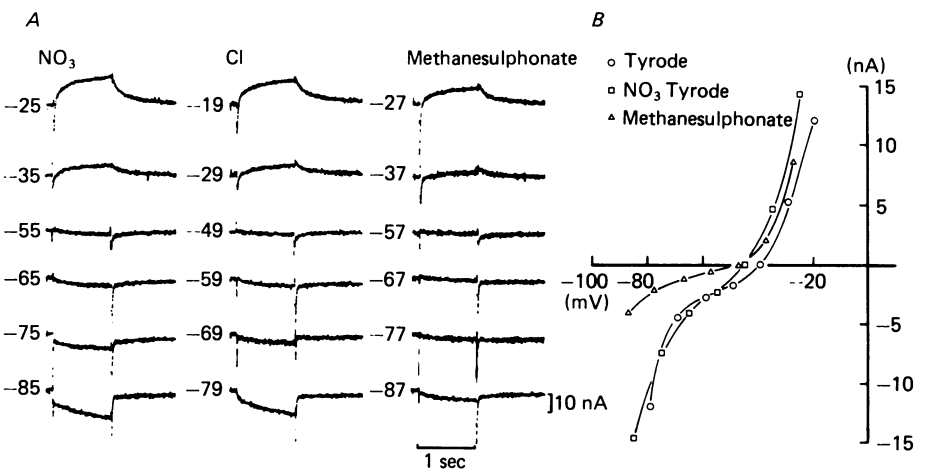

Fig 8. Current–voltage relationships in Cl, NO_3 and methanesulphonate solutions. The membrane was held at −39 mV in Cl, −45 mV in NO_3 and −47 mV in methanesulphonate. From these holding potentials, several depolarizing and hyperpolarizing test pulses of 10 mV steps were applied. The numbers at the left side of current records in each anion medium indicate the level of the membrane potential during the test pulses. Open circles represent the amount of current in normal bathing solution at the end of a 1 sec pulse, open squares that in NO_3 solution and open triangles that in methanesulphonate solution.

three series of experiments were performed in which the same cell was subjected to NO_3, Br and I solutions consecutively. Care was taken in these experiments to take measurements about 10 min after changing from one solution to another and to perfuse the preparation with normal bathing solution for 10 min between each anion test so as to restore the normal intracellular ionic composition. The order of conductance among No_3, Br and I was determined to be NO_3, I and Br.

TABLE 2. Membrane conductance in various anion solutions relative to that in normal Tyrode solution

Ionic species	Relative membrane conductance
SCN	$2{\cdot}16 \pm 0{\cdot}29$ ($n = 4$)
NO_3	$1{\cdot}50 \pm 0{\cdot}11$ ($n = 6$)
I	$1{\cdot}15 \pm 0{\cdot}06$ ($n = 4$)
Br	$1{\cdot}07 \pm 0{\cdot}02$ ($n = 5$)
Acetate	$0{\cdot}57 \pm 0{\cdot}14$ ($n = 5$)

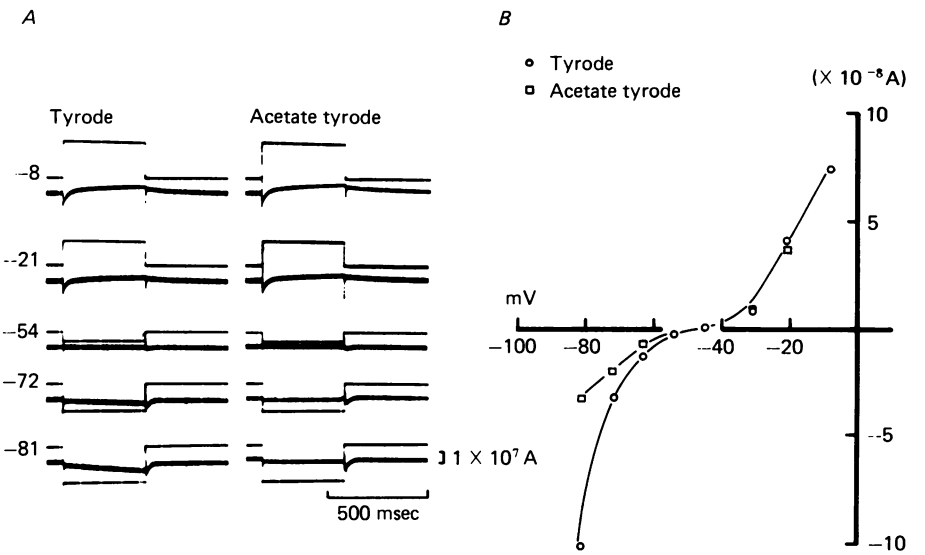

Fig. 9. Current–voltage relationship before and after replacement of Cl with acetate. The membrane was held at −45 mV in both Cl and acetate media. The records of both membrane potential and current during the voltage clamp are shown. The numbers at the left side of the voltage-clamp records indicate the level of the membrane potential during the test pulses. Open circles indicate the amount of current in normal bathing solution at the end of 400 msec pulse under the voltage clamp and open squares those in acetate bathing solution.

The membrane was held at −45 mV before and after substituting Cl by acetate. The several test pulses to either depolarizing and hyperpolarizing directions were applied and resultant currents at 400 msec were plotted on the current–voltage diagram (Fig. 9). The replacement of Cl with acetate not only decreased the membrane conductance but also markedly reduced the inward-going rectification. The membrane potentials at which rectifications occurred were unaffected. Since acetate ion has the lowest permeability among anion species explored, the difference between

the current–voltage relationship in Cl and in acetate solutions could be ascribed to the current carried by Cl ion. Therefore, the time- and voltage-dependent Cl current may be activated at a more hyperpolarized voltage than the resting potential. The order of conductance relative to Cl was thiocyanate > NO_3 > I > Br > Cl > acetate, which agrees with the sequence obtained from membrane potential measurements.

Effects of chloride replacement with organic anions under high Ca concentration on the electrical activity of the sino-atrial node cell

Recently, Kenyon & Gibbons (1977) have reported that the replacement of Cl^- with organic anions reduces the activity of Ca^{2+} ion. Since Ca^{2+} controls the ion permeation through the membrane, experiments were undertaken in acetate solution in which the extracellular Ca^{2+} concentration was 3·6 mM, which was twice that in the control, and in methanesulphonate solution in which the activity of Ca^{2+} ion was kept the same as that in the control (Kenyon & Gibbons, 1977). On replacement of Cl with acetate, a change in action potential was observed, similar to that in the medium of external Ca^{2+} concentration 1·8 mM, accompanied by a decrease in membrane conductance and disappearance of inward-going rectification. When Cl was substituted with methanesulphonate, in one quiescent preparation the membrane showed a 20 mV sustained hyperpolarization from −39 mV in Cl medium. During perfusion of methanesulphonate solution, a similar change in the action potential shown in Fig. 3 was observed. The membranes were held by the voltage-clamp method at the membrane potential where no holding current was necessary, referred to as the reference potential (Noma & Irisawa, 1975*a*), and then were raised to different levels of membrane potential in 10 mV steps. The currents were measured at the end of a 1 sec pulse and were plotted against voltage (Fig. 8, open triangles). The membrane conductance decreased to 0·42 of that in Cl medium (0·37 ± 0·05, three preparations) and the inward-going rectification was also eliminated.

DISCUSSION

In the sino-atrial node cell, the sequence obtained from membrane potential measurements for various anions agrees with that of conductance, being thiocyanate > NO_3 > I > Br > Cl > acetate. This order coincides with sequence I in the classification of Wright & Diamond (1977) and partly with the sequence in the Purkinje fibre (Hutter & Noble, 1961; Carmeliet, 1961) as well as in the rabbit sino-atrial node cell (de Mello, 1963).

Replacement of Cl with more permeable anions

In Purkinje fibre, substitution of Cl^- by the most permeable anion, I^-, eliminated spontaneous beating. The arrest persisted during the perfusion of I solution, but on admission of Cl solution spontaneous beating resumed. On replacement of moderately permeable anions, such as NO_3 and Br, the amplitude of the action potential was reduced (Hutter & Noble, 1961). In the sino-atrial node cell, a very similar change was observed. Substitution of Cl^- with the most permeable anion, thiocyanate, abolished spontaenous beating. However, transient hyperpolarization

accompanied with cessation of beating in the sino-atrial node cell was not observed in Purkinje fibre. This transient hyperpolarization may come from the relatively large contribution of anion conductance to the total membrane conductance in the sino-atrial node cell (Seyama, 1977) compared with that in Purkinje fibre. On replacement of Cl by moderately permeable anions, such as I, NO_3 and Br, a slight reduction in the amplitude of the action potential of the sino-atrial node cell developed. These observations in the sino-atrial node cell in a solution containing anions more permeable than Cl^- could similarly be explained by the hypothesis proposed by Hutter & Noble (1961) and Carmeliet (1961). During action potential, the slope of the slow diastolic depolarization might be accelerated and the height of the action potential might be suppressed because of the passive movement of Cl ion which has an equilibrium potential around -50 mV.

Replacement of Cl with less permeable anions

On replacement of Cl by large anions, Purkinje fibre exhibited a transient acceleration followed by deceleration of rhythm, but no significant change in action potential was observed (Hutter & Noble, 1961). These findings can be explained well by assuming the passive movement of anion as mentioned before. On the other hand, the sino-atrial node cell showed either a monotonous or transient reduction of rhythm, accompanied by an increase in action potential amplitude. The latter increase was mainly caused by the shift of maximum diastolic potential to a more negative potential (Fig. 3). Because of these differences, the effect of large anions on the sino-atrial node cell may be difficult to explain only from the hypothesis of passive distribution of anions. However, in the sino-atrial node cell, there is a current system showing inward-going rectification around the corresponding membrane potential and this current disappears after the administration of acetate solution. The shift of membrane potential to a more negative potential can be explained by the elimination of this current through the application of acetate solution. The current responsible for this inward-going rectification which was only activated by a membrane potential more negative than -60 mV is in essence an inward current, so that the slow diastolic depolarization may be generated partly by this current. Although the outward delayed current activated by the action potential forces the membrane to repolarize to an E_K of -100 mV (Noma & Irisawa, 1976*b*), this inward current component counteracts the action of the outward delayed current and may prevent the membrane potential from reaching E_K. Hence, the presence of this current may be one of the reasons why the sino-atrial node cell maintains a higher frequency than any other part of the heart. After eliminating this current component, it is reasonable that both the reduction in frequency of sino-atrial node rhythm and the increase in the amplitude of the action potential would be observed. The presence of a similar current system which is carried by Cl ion and also activated at a membrane potential more negative than the resting potential has been assumed in frog sinus venosus (Brown, Giles & Noble, 1977).

There is a striking difference in the electrical response of the sino-atrial node cell between the replacement of Cl^- by anions more permeable and that by anions less permeable than Cl^-. The experiment shown in Fig. 4 for the permeant anions and that shown in the lower panel of Fig. 5 for the non-permeant anions clearly indicate the existence of a sieve-like structure having a certain diameter. Thus, on replace-

ment of Cl^- by large anions, the membrane depolarized transiently because these anions could not pass through the membrane and only intracellular Cl^- could leave the cell.

The order among permeable anions (thiocyanate > NO_3 > I > Br > Cl) was inversely proportional to the naked radius of these ions and was not correlated with the magnitude of their hydrated radius. Hence, the sino-atrial node cell membrane may not discriminate permeable anions by the size of hydrated ion radius but may do so by the difference in the free energy between the binding site and the hydration of ions, as predicted by Eisenman (1961) and Diamond & Wright (1969).

Recently, Kenyon & Gibbons (1977) have reported that replacement of Cl^- with large anions, such as propionate, acetylglycinate, methylsulphonate or methanesulphonate, reduces Ca^{2+} ion activity in the external medium. On immersing in acetate solution containing twice as much Ca ion as the control or in methanesulphonate solution with Ca^{2+} activity adjusted to be the same as the control, sino-atrial node cell membrane showed a very similar change in the electrical activity to that in acetate solution containing 1·8 mM external Ca^{2+}. Therefore, changes in the electrical activity on substitution of Cl^- with large anions are not due to reduction of Ca ion activity but are due to elimination of Cl^- contribution to the electrical activity of the sino-atrial node cell.

Carmeliet & Verdonck (1977) observed a reduction of K permeability by measuring the K efflux in the resting Purkinje and ventricular fibres, when extracellular Cl^- was replaced with impermeant anions. Such a mechanism could partly explain the reduction of membrane conductance of the sino-atrial node cell after replacement of Cl with acetate. In the present experiment, however, changes in membrane potential could have occurred transiently. Furthermore, the replacement of Cl with acetate ion only reduced the time- and voltage-dependent current activated at the hyperpolarization voltage range beyond -60 mV and did not affect the delayed rectification. Therefore, the major changes in the membrane potential observed in the present experiment may be due to both the passive movement of anion through the membrane and the elimination of inward-going rectifying current.

When quiescent sino-atrial node cells were bathed in various permeable anion solutions, the resting membrane remained more hyperpolarized than the original resting potential (Fig. 4). The magnitude of this small hyperpolarization in various anion solutions is proportional to the order of permeability as shown in Table 1. As the anion permeability increases, the anion may distribute passively and the contribution of the anion concentration cell to the resting membrane potential will decrease. Especially in thiocyanate solution the membrane potential might be maintained mostly by Na and K concentration cells. Thus, E_{Cl} could be at a more depolarized level from the resting potential (de Mello, 1963) and the internal Cl^- concentration is higher than would be expected on the basis of a passive distribution. Therefore, there may be an active transport system of Cl ion in the sino-atrial node cell. The calculated E_{Cl} in rabbit sino-atrial node cell of -37 mV (de Mello, 1963), which is the middle point of the action potential, was in accord with this hypothesis. A similar mechanism seen in Purkinje fibre by substituting Cl with permeable anions could be involved in the change in the electrical activity of the sino-atrial node cell, besides the inward-going rectifier. This hypothesis is also compatible with the observations that the internal Cl^- concentration in various cells, such as squid axon (Russel,

1976), frog atrium (Ladle & Walker, 1975) and smooth muscle (Casteels, 1971) is greater than expected if Cl were passively distributed. Even, in skeletal muscle, which is highly permeable to Cl^-, the internal concentration is higher than that calculated from the Donnan ratio under conditions where Cl conductance is suppressed in acid solution (Bolton & Vaughan-Jones, 1977).

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