Kor J Physiol (1992) 26 (1), 27-35

Cl^--sensitive Component of Ca^{2+}-activated Tail Current in Rabbit Atrial Myocytes

Choon Ok Park*, Insuk So, Won Kyung Ho, Woo Gyeum Kim and Yung E Earm

Department of Physiology & the Heart Research Institute, Seoul National University College of Medicine, Seoul
**Department of Physiology & the Cardiovascular Research Institute, Gyeongsang National University, Chinju 660-280*

=ABSTRACT=

We used the whole-cell patch-clamp technique to examine the ionic basis for the tail current after depolarizing pulse in single atrial myocytes of the rabbit. We recorded the tail currents during various repolarizations after short depolarizing pulse from a holding potential of -70 mV. The potassium currents were blocked by external 4-aminopyridine and replacement of internal potassium with cesium. The current was reversed to the outward direction above +10 mV. High concentrations of intracellular calcium buffer inhibited the activation of the current. Diltiazem and ryanodine blocked it too. These data suggest that the current is activated by intracellular calcium released from sarcoplasmic reticulumn. When the internal chloride concentration was increased, the inward tail current was increased. The current was partially blocked by the anion transport blocker niflumic acid. The current-voltage curve of the niflumic acid-sensitive current component shows outward rectification and is well fitted to the current-voltage curve of the theoretically predicted chloride current calculated from the constant field equation. The currents recorded in rabbit atrial myocytes, with the method showing isolated outward Na-Ca exchange current in ventricular cells of the guinea-pig, suggested that chloride conductance could be activated with the activation of Na/Ca exchange current.

From the above results it is concluded that a chloride sensitive component which is activated by intracellular calcium contributes to tail currents in rabbit atrial cells.

Key Words: Ca^{2+} activated Cl^- current, Whole cell voltage clamp, Single atrial myocyte, Rabbit.

INTRODUCTION

Recent studies have shown the existence of several calcium-activated mechanisms. Internal calcium controls the opening of several classes of channels, which are respectively selective for K^+ ions(Meech, 1978), for monovalent cations(Coloquhoun et al, 1981; Ehara et al, 1988) and for Cl^- ions(Mieledi & Parker, 1984). The slow inward tail current following a brief depolarizing pulse has been identified as Na-Ca exchange current which is activated by intracellular calcium increase(Fedida et al, 1987; Earm et al, 1989; Earm et al, 1990). It has been known that Na-Ca exchange current in cardiac cells is electrogenic with exchange of 3 Na^+ with 1 Ca^{2+} (Philipson, 1985; Kimura et al,1986). Therefore the current plays a role to maintain the late low-voltage plateau of the action potential which is associated with Ca^{2+} efflux and contractile force caused by the free calcium transient (Mitchell et al, 1984, 1987; Hilgemann 1986; Hilgemann & Noble 1987). But it cannot be ruled out that other calcium-activated currents, besides Na-Ca exchange current, can occur as the tail current following a brief de-

polarization which brings to increase in calcium concentration.

It has been reported that calcium-activated chloride current is recorded following depolarization and shows very similar feature with Na-Ca exchange current in other preparations(Owen et al, 1984, in cultured mouse spinal neurones; Pacaud et al, 1989, in rat portal vein). Its fuctional roles are not clear yet. But it can be activated by inward calcium current and can modulate the action potential duration and is inwardly directed near the resting membrane potential in vascular smooth muscle cell(Pacaud et al, 1989). It may repolarize and stabilize the cell membrane at a level of subthreshold for generating action potential in mouse spinal neurones(Owen et al, 1984).

In rabbit ventricular cells it has been reported that calcium-activated chloride current is responsible for the calcium-sensitive transient outward current(Zygmunt & Gibbons, 1991). But such a current has not been identified in the atrial cells. In this study we tested the possibility that calcium-activated chloride current might exist and whether it contribute to the tail current after short depolarizing pulse which causes calcium transient increase in rabbit atrial myocytes.

METHODS

Single atrial cells of the rabbit were isolated by a method similar to that described by Earm et al.(1989, 1990). Briefly the heart was perfused with low Ca^{2+}-Tyrode solution (30-50 μM Ca^{2+}) containing collagenase (4 mg per 50 ml, Yakult) for 15~20 min by using a Langendorff perfusion system. Atrial tissue was dissected out and mechanically agitated to disperse the cells and then stored in low Cl^-, high K^+ medium in the refrigerator.

The solution used to superfuse atrial cells contained (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; NaH_2PO_4, 0.33; glucose, 5; HEPES, 5; adjusted to pH = 7.4 with NaOH. In experiments in which extracellular sodium was reduced, NaCl was replaced by equimolar LiCl or N-methylglucamine chloride. The internal solution of the patch electrode normally contained (in mM): K-aspartate, 110; Mg-ATP, 5; di-Tris-creatine phosphate, 5; $MgCl_2$, 1; KCl, 20; HEPES, 10; EGTA, 0.1; adjusted to pH = 7.4 with KOH. In experiments in which intracellular chloride concentration was high, K-aspartate was replaced by equimolar KCl. For a Cs^+-rich internal solution to block potassium currents, K-aspartate or KCl was replaced by equimolar Cs-aspartate or CsCl, 20 mM KCl was replaced by equimolar TEA-Cl, and the pH was adjusted to 7.4 by using CsOH. With the experiment to record isolated outward Na-Ca exchange outward current, the components of the internal soution were (in mM): Na-aspartate, 50; EGTA, 42; Mg-ATP 10, K_2CrP, 5; $MgCl_2$, 3; HEPES, 5; TEA-Cl, 10; $CaCl_2$, 18 and pH was adjusted to 7.4 with CsOH. As blockers in the external solution, ouabain (Sigma) (20 uM) was used to block the Na-K pump, $BaCl_2$ (1 mM) and CsCl (2 mM) to block K^+ channels and D600 (2 uM) to block Ca^{2+} channels. To activate the current, 2 mM $CaCl_2$ was added to the Ca^{2+}-free external solution. During experiments, cells were superfused (1 ml min^{-1}) at 37°C. Chemicals and drugs used in this study included; ryanodine(Penick), diltiazem (Tanabe); niflumic acid and all other chemicals obtained from Sigma.

The cells were voltage-clamped by using a whole-cell patch-clamp apparatus (List, EPC-7) according to the original technique developed by Hamill et al.(1981). Glass electrodes with resistances of 2-3 MΩ were used. The data were recorded on a pulse code modulator data recorder (Medical system, PCM-4/8) for future analysis. Data were also displayed on a digital oscilloscope (Hitachi, 6041, or Nicolet, 9024) and pen recorder (Gould, oscillograph 220) and could then be directly reproduced onto a XY plotter (Graphtec, MP 4100).

RESULTS

Using whole cell voltage clamp technique, we recorded the tail current during repolarizations

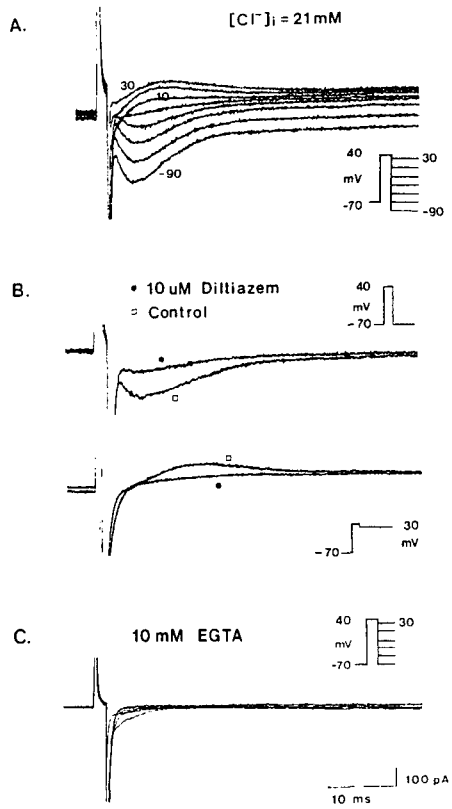

Fig 1. Recordings showing calcium dependence of the tail currents. The tail currents show larger magnitude in inward direction at more hyperpolarized membrane potentials and then it reverses at +10 mV (A). Both of inward and outward currents were abolished by 10 μM diltiazem (B) and by 10 mM EGTA in the pipette (C).

to various membrane potentials following a brief 2 ms depolarizing pulse to +40 mV from a holding potential of -70 mV (Fig. 1A). The tail current was reversed to the outward direction above +10 mV (Fig. 1A). In this experimental condition there was little Na^+ in internal pipette solution and voltage sensitive transient outward current was blocked by replacement of K^+ with Cs^+ and TEA^+. Therefore it seems unlikely that the current was pure Na-Ca exchange current described by Earm et al.(1989,1990). The current reversal shows the possibility of the existence of other current component.

Figure 1B shows the effect of the Ca-channel blocker, 10μM diltiazem. The inward tail current at -70 mV (Fig. 1B, upper panel) as well as the outward tail current at +30 mV (Fig. 1B, lower panel) were abolished by 10 μM diltiazem. And the currents were inhibited by ryanodine 1 μM which has been known to inhibit Ca^{2+} release from the sarcoplasmic reticulumn (Chamberlain et al, 1984; Sukto & Kenyon, 1983) (data not shown). High concentration of intracellular calcium buffer suppresses the increase of the cytoplasmic free calcium transient (Fedida et al, 1987). With 10 mM EGTA in the pipette solution the tail current was not recorded (Fig. 1C). These results showed that the tail current components following depolarizing pulse were activated by intracellular calcium.

Effect of intracellular chloride concentration on the inward tail current

In order to see whether calcium sensitive chloride current could contribute to the tail current, the effect of chloride concentration gradient was investigated. To minimize the changes in junction potential due to the changes of chloride ion concentration, we used 3M KCl^- agar bridge as reference electrode. Figure 2 shows the inward tail current which was recorded during repolarization following a brief 2 ms depolarizing pulse to +40 mV from a holding potential of -70 mV. In figure 2A, the chloride concentration of the internal solution of the patch electrode was 21 mM and that of figure 2B was 131 mM. The magnitude of the inward tail current was the largest when it was evoked by the stimulus of +110 mV from a holding potential -70 mV during 2 ms. Therefore the control stimulus of a 2 ms depolarizing pulse to +40 mV from a holding potential of -70 mV was given in every 30 sec in most experiments. In figure 2A, when the chloride concentration was 21 mM in the pipette the magnitude of the peak tail current at -70 mV was in the range of

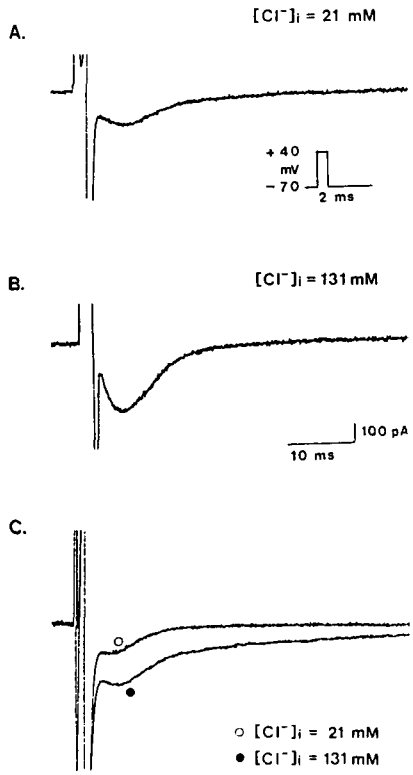

Fig 2. *Effects of changes in chloride concentration on inward tail currents. The magnitude of the peak inward tail current at -70 mV was in the range of -100 to -300 pA with 21 mM chloride (A) and -400 to -600 pA with 131 mM chloride (B) in the pipette. The tail current increases by the increase in chloride concentration in the pipette using intracellular perfusion technique (C).*

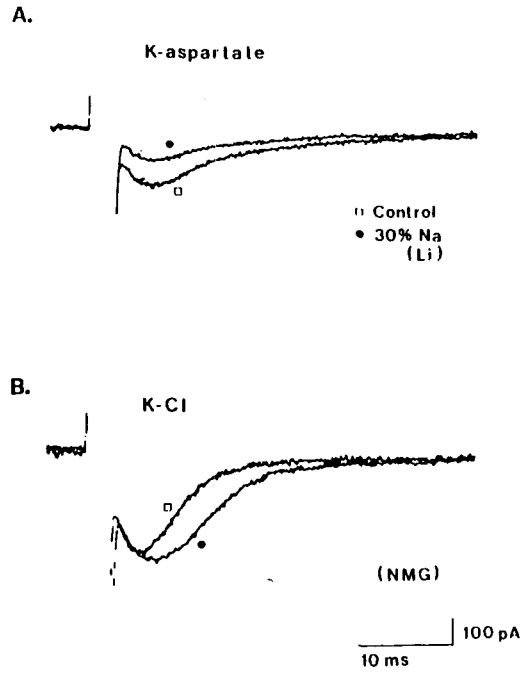

Fig 3. Effect of extracellular sodium reduction on the inward tail currents. Extracellular NaCl was substituted with LiCl or NMG-Cl. The current was recorded at -70 mV after depolarizing pulse to +40 mV for 2 ms. The inward current decreased when intracellular chloride concentration was 21 mM (A: K-aspartate pipette solution). But the inward current increased when intracellular chloride concentration was 131 mM (KCl pipette solution).

-100 to 300 pA. In 2B, when the chloride concentration in the pipette was 131 mM, the magnitude of tail current at -70 mV was in the range of -500 to -700 pA. We confirmed that the difference in the magnitude of the tail current is due to the difference in the chloride concentration by using the intracellular perfusion technique (Kimura et al. 1986; Earm & Irisawa 1986; Kimura et al, 1987). As shown 2C, the increase in chloride concentration increased the tail current.

Evidences of the exsistence of Ca^{2+}-dependent Cl^- current

Quantative studies on the Na-Ca exchange current in cardiac cells have been hindered by the absence of selective inhibitor (Mentard et al, 1984; Biellefeld et al, 1986). Therefore, to inhibit the component of the Na-Ca exchange current from the tail current following depolarization, extracellular sodium was substituted by equimolar lithium or N-

methylglucamine to reduce the electrochemical gradient as a driving force of Na-Ca exchange current (Fedida et al, 1987; Earm et al, 1990). Figure 3A shows the effect of extracellular sodium reduction from 147 mM to 42 mM by replacement of NaCl with LiCl. The inward current was recoreded at -70 mV was remarkably reduced. The current was also reduced when NaCl is replaced by NMG-Cl. When the chloride concentration was increased to 131 mM from 21 mM, the inward current was not decreased but increased by the extracellular sodium reduction by LiCl or NMG-Cl (Fig. 3B). And the time to peak and the decay time course were prolonged. In the case of high internal chloride concentration as in figure 3B, the current component which was increased by the extracellular sodium reduction can not be Na-Ca exchange current because its driving force was actually reduced. Therefore, we tried to see the effect of chloride channel blocker.

Niflumic acid has been known as an inhibitor to the anion carrier system (Aickin & Vermue, 1983; Gerstheimer et al, 1987) and voltage-dependent chloride current (Cousin & Motais, 1982; Inoue, 1985). To investigate the effect of niflumic acid in different membrane potentials, the tail currents were recorded at various levels of repolarizations. Voltage-dependent transient outward current was blocked by 2 mM 4-aminopyridine (Kenyon & Sutko, 1987). In figure 4A, the chloride concentration was 21 mM in the pipette. When we applied 10 μM niflumic acid, the tail currents were decreased. The effect of niflumic acid was larger in outward direction than in inward direction (Fig. 4B). To investigate the current-voltage relations of the niflumic acid sensitive current, the magnitudes of the difference currents before and after niflumic acid application were measured. When we plotted the current, as in figure 4C, it showed outward rectification and the reversal potential was at -50 mV. The dotted line is theoretical current-voltage curve of chloride current calculated by constant field equation (Hille, 1984). The experimental data were well fitted to the dotted line which was expected as chloride current. The same experiment was done in high concentration of the chloride in the pipette. In figure 5, the chloride concentration in the pipette was 131 mM. In this case, the internal chloride concentration was almost same as external chloride concentration. The tail currents were decreased by 10 μM niflumic acid (Fig. 5A & 5B). The current-voltage relationship of the niflumic acid sensitive current was, as shown in 5C, almost linear and the reversal potential was around 0 mV. Such a relationship was well fitted to the theoretically predicted current-voltage curve of the chloride current which was shown as dotted line in figure 5C. These results clearly show

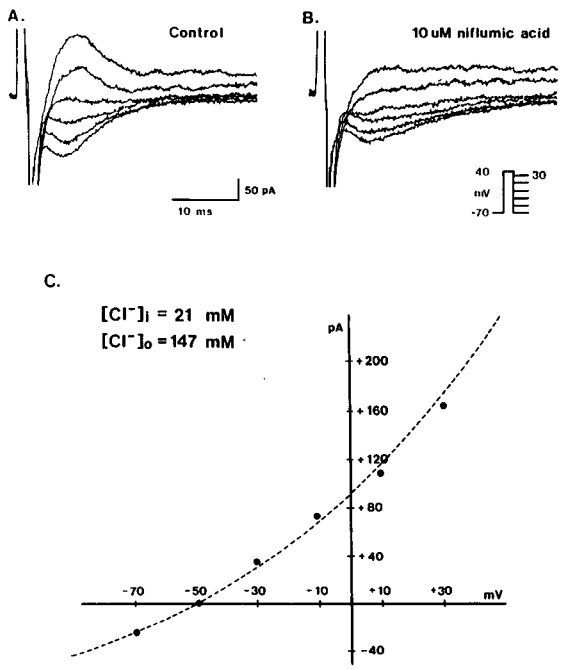

Fig 4. Effects of niflumic acid on the tail currents with 21 mM chloride in the pipette. Panel A and B shows the decrease in tail currents by 10 μM niflumic acid. The current-voltage curve of niflumic acid sensitive current shows outward rectification and the reversal potential at -50 mV. The dotted line is theoretical current-voltage curve of chloride current calculated by constant field equation.

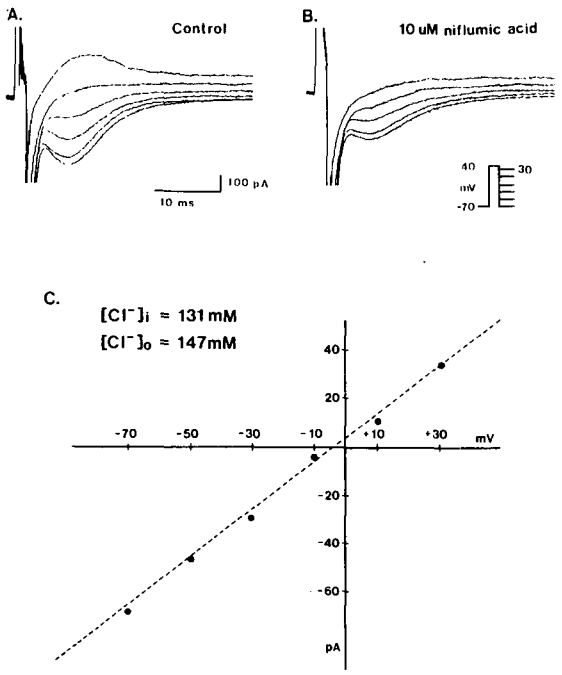

Fig 5. Effects of niflumic acid on the tail currents with 131 mM chloride in the pipette. The tail currents were decreased by 10 μM niflumc acid (A and B). The current-voltage relationship of the niflumic acid sensitive current shows almost linear and the reversal potential around 0 mV. Such a relationship is well fitted to the theoretically predicted chloride current.

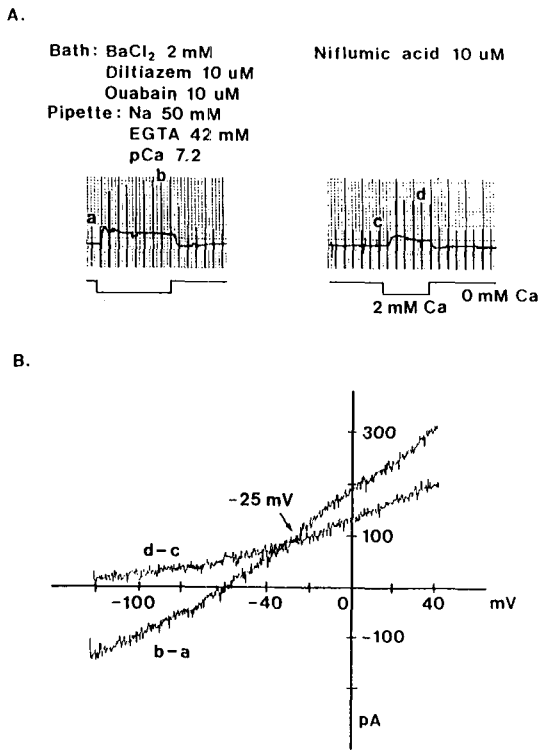

Fig 6. Effects of niflumic acid on the isolated Na-Ca exchange current. The currents was recorded by applying ramp pulse from -120 mV to +40 mV from a holding potential of -40 mV. The currents activated by 2 mM extracellular calcium was decreased by 10 μM niflumic acid (A). The current-voltage curves before and after niflumic acid application cross at about -25 mV which potential is well fitted to the calculated reversal potential of the chloride current (B).

that calcium sensitive chloride current contributes to the tail current.

We tried the different method, which was known to record the isolated Na-Ca exchange current (Fig. 6). All the known currents were blocked by diltiazem 10 μM, ouabain 10 μM, and $BaCl_2$ 2mM in the bath solution. In the pipette, sodium concentration was 50 mM and pCa was 7.2 with 42 mM EGTA (free Ca^{2+} 67 nM). The concentration of free internal Ca^{2+} was calculated by using Fabiato & Fabiato's equations (1979) with a correction by Tsien & Rink (1980). In figure 6A, the traces showed current records obtained by applying ramp pulse from -120 mV to +40 mV from a holding potential of -40 mV in every 30 sec. When the solution was changed from 0 mM to 2 mM calcium, the current was activated mainly in outward direction. This is the method that Kiumra et al. (1987) have done to show outward Na-Ca exchange current in ventricular cells of the guinea-pig. When we tried this method in rabbit atrial myocytes, the outward component activated by extracellular calcium was increased gradually in early phase of current activation (Fig. 6A, left panel). When we

plotted the activated component against voltage (Fig. 6B, b-a), the current-voltage curve reverses at about -60 mV. It seemed less likely that this current-voltage relationship was that of pure Na-Ca exchange current. Because according to our experimental condition, the reversal potential of Na/Ca exchange current was -119 mV ($E_{Na/Ca} = 3E_{Na}-2E_{Ca}$; $[Na^+]_o$ = 140 mM, $[Na^+]_i$ = 50 mM, $[Ca^{2+}]_o$ = 2 mM, $[Ca^{2+}]_i$ = 67 nM). Therefore, we applied niflumic acid (Fig 6A, right panel). The current activation by extracelluar calcium was smaller than in control condition. And the current increase in early phase of the current activation by extracellular calcium shown in control could not be seen. The current-voltage relationship in this condition was mainly outward and almost exponential (Fig. 6B, d-c). This current seemed to be more likely Na-Ca exchange current previously reported (Kimura et al, 1987). And we found that these curves crossed at about -25 mV. This membrane potential was well fitted to the calculated reversal potential of the chloride current. Therefore, it could be said that niflumic acid-sensitive current was chloride currnt and the current increase in early phase of the current activation by calcium application was due to this chloride current. These findings suggest the possibility that calcium activated chloride conductance can be activated with Na-Ca exchange mechanism.

DISCUSSION

In the present study we tested the possibility of other components are mixed in the tail current besides Na/Ca exchange current in rabbit atrial cells. In mammalian heart cells, the first evidence that the slow inward tail current is carried by an intracellular Ca^{2+} activated mechanism which requires extracellular Na^+ was obtained by Mitchell et al.(1984,1987). They showed that this tail current is responsible for maintaining the late low-voltage plateau in isolated rat ventricular cells. Fedida et al.(1987) showed that slow inward tail current displays kinetic and other properties that clearly distinguish it from Ca-current in guinea-pig ventricular cells. Mullins (1979, 1981) proposed the hypothesis slow inward current tails, that occur following repolarization from potentials at which Ca-current is activated, are carried by Na-Ca exchange current. Recently this hypothesis has become established experimentally. Earm et al. (1989,1990) isolated the slow inward tail current by recording the current during repolarization after depolarizing pulse and they concluded that it was Na-Ca exchange current. However, it is not clear whether other calcium activated currents could be activated in the tail current.

Current records after short depolarizing pulse showed reversal to the outward direction above + 10 mV. This seems unlikly that the current was pure Na-Ca exchange current. Because according to the current-voltage relationship of the Na-Ca exchange current (Kimura et al, 1987; Earm et al, 1990), it shows exponential curve with steeper voltage dependence at more hyperpolarized potentials and hardly reversed in this experimental condition with little Na^+ in internal pipette solution (Earm et al, 1990). Potassium component can be excluded because voltage-sensitive transient outward current was blocked by 4-aminopyridine (4-AP) and the current was still present after potassium was replaced by cesium and TEA in the pipette. Calcium-activated potassium channels may be present in rabbit atrial cells, but they seem to contribute little to this outward tail current which we have examined. Recently calcium-sensitive transient outward current has been proved to be calcium activated chloride current in rabbit ventricular cells (Zygmunt & Gibbon, 1991). Also calcium activated chloride currents was reported in other preparations (Owen et al, 1984; Pacaud et al, 1989) and showed very similar feature to the tail current in cardiac cells. Therefore we investigated the chloride effect on the tail currenet as a possible mixed component.

The current magnitude in atrial cells was increased when intracellular chloride concentration was elevated. These findings suggest two possibile mechanisms. One is that intracellular chloride could modulate Na-Ca exchange

mechanism, the other is that calcium activated chloride component exists in tail current. The possibility of the pH change could be ruled out because in our experiments pH of the bath solution and pipett solution was titrated to 7.4 containing 10 mM HEPES buffer and there was no HCO_3^- component in these experiments which might affect HCO_3^-/Cl^- exchange mechanism (Vaughan-Jones, 1979a; 1979b). The former hypothesis could be ruled out by the experiment of extracellular sodium reduction. When the chloride concentration in the pipette solution was elevated, the inward current was not decreased but increased by the extracellular sodium reduction and the time course of the current was prolonged. Because the driving force of the Na-Ca exchange current was decreased, the current increase must be due to the other component than Na-Ca exchange current. This current component was blocked by 10 μM niflumic acid. Niflumic acid is known to block anion transport systems (Aickin & Vermue, 1983; Cousin & Motais, 1982; Inoue, 1985). When we plotted the current-voltage curve of the niflumic acid-sensitive current, it is well fitted to the current-voltage relationship of the theoretically predicted chloride current calculated by constant field equation (Hille, 1984) and the reversal potential shifted as expected by the chloride equilibrium potential. From these results we can conclude that chloride is the prinicipal charge carrier of the niflumic acid-senitive component of the tail current.

Kimura et al. (1987) recorded the isolated Na-Ca exchange current in ventricular cells of the guinea-pig. In rabbit atrial myocytes we recorded Na-Ca exchange current with their method. Some fraction of outward component activated by extracellular calcium was gradually increased. This component was inhibited by niflumic acid. From the current-voltage curve of niflumic acid-sensitive component, we can conclude that it is a chloride current which was activated by internal calcium.

From the above results it is concluded that a chloride sensitive component, which is activated by intracellular calcium, contributes to the tail current in rabbit atrial myocytes. It might play a role to repolarize the cell from depolarization. Because when the chloride concentration in the pipette was low in physiologic condition, the current-voltage relationship of the niflumic acid-sensitive current shows outward rectification.

REFERENCES

Aickin CC & Vermuë NA (1983) Microelectrode measurement of intracellular chloride activity in smooth muscle cells of guinea-pig vas deferens. *Pflügers Arch* **397**, 587-606

Bielefeld DR, Hadley RW, Vassilev PM & Hume JR (1986) Membrane electrical properties of vesicular Na-Ca exchange inhibitors in single atrial myocytes. *Circ Res* **59**, 381-389

Chamberlain BK, Volpe P & Fleischer S (1984) Calcium- induced calcium release from purified cardiac sarcoplasmic reticulumn vesicles: general characteristics. *J Biol Chem* **259**, 7540-7546

Colquhoun D, Neher E, Reuter H & Stevens CF (1981) Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature* **294**, 752-754

Cousin JL & Motais R (1982) Inhibition of anion transport in the red blood cell by anionic amphiphilic compounds. II. Chemical properties of the flufenamate-binding site on the band 3 protein. *Biochim Biophys Acta* **687**, 156-164

Earm YE & Irisawa H (1986) Effects of pH on the Na-Ca exchange current in single ventricular cells of the guinea-pig. *Jap Heart J* **27** (suppl. I) 153-158

Earm YE, Ho WK & So IS (1989) An inward current activated during late low-level plateau phase of the action potential in rabbit atrial cells. *J Physiol* 1989 410:64P

Earm YE, Ho WK & So IS (1990) Inward current generated by Na-Ca exchange during the action potential in single atrial cells of the rabbit. *Proc R Soc Lond B* **240**, 61-81

Ehara T, Noma A & Ono K (1988) Calcium-activated non selective cation channel in ventricular cells isolated from adult guinea-pig hearts. *J Physiol* **403**, 117-133

Fabiato A & Fabiato F (1979) Calculator programs

for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J de Physiol* **74,** 463-505

Fedida D, Noble D, Shimoni Y & Spindler AJ (1987) Inward current related to contraction in guinea-pig ventricular myocytes. *J Physiol* **385,** 565-589

Gerstheimer FP, Muhleisen M, Nehring D & Kreye VAW (1987) A chloride bicarbonate exchanging anion carrier in vascular smooth muscle of the rabbit. *Pflügers Arch* **409,** 60-66

Hamill OP, Marty A, Neher E, Sakmann B & Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflüger Archiv* **395,** 6-18

Hilgemann DW (1986) Extracelluar calcium transients and action potential configuration changes related to post-stimulatory potentiation in rabbit atrium. *J Gen Physiol* **87,** 675-706

Hilgemann DW & Noble D (1987) Excitation-contraction coupling and extracellular calcium transients in rabbit atrium; reconstruction of basic cellular mechanisms. *Proc R Soc Lond B* **230,** 163-205

Hille B (1984) Ionic channels of excitable membranes. Sinauer, Sunderland, p230-237

Inoue I (1985) Voltage dependent chloride conductance of the squid axon membrane and its blockade by some disulfonic stilbene derivatives. *J Gen Physiol* **85,** 519-537

Kenyon JL & Sutko JL (1987) Calcium- and voltage-activated plateau currents of cardiac Purkinje fiers. *J Gen Physiol* **89,** 921-958

Kimura J, Noma A & Irisawa H (1986) Na-Ca exchange current in mammalian heart cells. *Nature* **319,** 596-597

Kimura J, Miyamae S & Noma A (1987) Identification of sodium-calcium exchange current in single ventricular cells of guinea-pig. *J Physiol* **384,** 199-222

Meech RW (1978) Calcium-dependent potassium activation in nervous tissues. *Annu Rev Biphys Bioengin* **7,** 1-18

Mentrard D, Vassort G & Fischimeister R (1984) Calcium- mediated inactivation of the calcium conductance in cescium-loaded frog heart cells. *J Gen Physiol* **89,** 481-501

Miledi R & Parker I (1984) Chloride current induced by injection of calcium into Xenopus oocytes. *J Physiol* **357,** 173-183

Mitchell MR, Powell T, Terrar DA & Twist (1984) Possible association between an inward current and the late plateau of action potentials in ventricular cells isolated from rat heart. *J Physiol* 351,40P

Mitchell MR, Powell T, Terrar DA & Twist (1987) Calcium- activated inward current and contraction in rat and guinea-pig ventricular myocytes. *J Physiol* **391,** 545-560

Mullins LJ (1979) The generation of electric currents in cardiac fibers by Na/Ca exchange. *Am J Physiol* **236,** C103-110

Mullins LJ (1981) Ion transport in heart. Raven Press, New York

Owen DG, Segal M & Barker JL (1984) A Ca-dependent Cl^- conductance in cultured mouse spinal neurones. *Nature* **311,** 567-570

Pacaud P, Loirand G, Lavie JL, Mironneau C & Mironneau J (1989) Calcium-activated chloride current in rat vascular smooth muscle cells in short-term primary culture. *Pflügers Arch* **413:** 629-636.

Philipson KD (1985) Sodium-calcium exchange in plasma membrane vesicle. *Ann Rev Physiol* **47:** 561-571

Sutko JL & Kenyon JL(1983). Ryanodine modification of cardiac muscle reponses to potassium-free solutions. Evidence for inhibition of sarcoplasmic reticulum calcium release. *J Gen Physiol* **82,** 385-404

Tsien RY & Rink TJ (1980) Neutral carrier ion-selective microelectrodes for measurement of intracellular free calcium. *Biochimica et biophysica acta* **599,** 623-638

Vaughan-Jones RD (1979a) Non-passive chloride distribution in mammalian heart muscle: Microelectrode measurement of the intracellular chloride activity. *J Physiol* **295,** 83-109

Vaughan-Jones RD (1979b) Regulation of chloride in quiescent sheep-heart purkinje fibres studied using intracellular chloride and pH-sensitive micro-electrodes. *J Physiol* **295,** 111-137

Zygmunt AC & Gibbons WR (1991) Calcium-activated chloride current in rabbit ventricular myocytes. *Circ Res* **68** (2),424-437