The Effect of Stimulation Frequency on the Ionic Currents in Single Atrial Cells of the Rabbit[†]

Insuk So[*], Chae Hun Leem, Suk Ho Lee, Choon Ok Park
Won Kyung Ho, Yung E Earm and Woo Gyeum Kim

Department of Physiology, Seoul National University College of Medicine, Seoul 110-460 and
*Department of Physiology,
Inha Univercity, College of Medicine, Incheon 402-751, Korea

=Abstract=In single atrial cells isolated from rabbit hearts the calcium current and $[Ca]_i$-dependent transient outward current were recorded using the whole-cell clamp technique and the effect of stimulation frequency on these currents was investigated. Voltage dependent transient outward current, which contributes the initial, rapid repolarization phase of the action potential and is frequency-dependent, was also investigated. Increasing the stimulation frequency from 0. 025 Hz to 1 Hz had no effect on the calcium current and $[Ca]_i$-dependent transient outward current and greatly inhibited voltage-dependent transient outward current. The amplitude of voltage dependent transient outward current increased as the membrane potential became depolarized, its steady-state inactivation spans the voltage range -70 mV to -10 mV and steady-state activation curve -30 mV to 30 mV. Within the range of the resting membrane potential (at -70 mV), the voltage-dependent recovery time constant was 1. 3 s. The reversal potential was about -50 mV. Voltage-dependent transient outward current was inhibited by K-channel blockers and not inhibited by modulation of $[Ca]_i$. From the above findings, it is concluded that due to the amplitude and voltage-dependent recovery time constant which were the basic mechanisms for frequency-dependency, the voltage- dependent transient outward current contributes the initial, rapid repolarization phase and changed the action potential configuration according to stimulation frequency in the rabbit atrium.

Key Words: *Single atrial cell, Rapid repolarization, Transient outward current, Whole-cell clamp*

INTRODUCTION

Repolarization process occurs following

upstroke phase of action potential and the process results from a dynamic balance between inward currents i. e. Ca current, window current, Na-Ca exchange current, background current and outward current i. e. delayed K current, inward rectifier K current(Noble 1984; DiFrancesco and Noble 1985; Cohen *et al.* 1987). Ca current, delayed K current and Na-Ca exchange current are important in the repolarization process of the SA node or the AV node(Noble 1984; Ho *et al.* 1986). In Purkinje

fibre the repolarization process results not only from Ca current, Na-Ca exchange current, window current, delayed K current, inward rectifier K current and background current but also from transient outward current which participates in the repolarization process making rapid repolarization(phase 1) or notch following upstroke(DiFrancesco and Noble 1985)

The shape of the repolarization phase during action potential changed when the stimulation frequency increased and this change was closely related to the staircase phenomena in rabbit atrial cells(Hilgemann 1986; Park et al. 1989). Thus, it would be important to elucidate which ionic currents cause such changes. Preliminary results were presented in abstract form(So and Earm 1988)

MATERIALS AND METHODS

Single atrial cells of the rabbit were isolated by a method similar to that described by Earm et al. (1990). Briefly the heart was perfused with low Ca-Tyrode solution (30-50 μM Ca) containing collagenase (20 mg per 50 ml, Sigma, type I or Worthington, type II) for 10-15 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. During experiments cells were superfused (1 ml/min) at 35-37 ℃.

The solution used to superfuse atrial cells contained (in millimoles per litre): NaCl, 140; KCl, 5.4; CaCl$_2$, 1.8; MgCl$_2$, 1; NaH$_2$PO$_4$, 0.33; glucose, 5; HEPES, 5; adjusted to pH = 7.4 with NaOH. The internal solution of the patch electrode normally contained (in millimoles per litre) : K-aspartate, 110; Mg-ATP, 5; di-Tris-creatine phosphate, 5; MgCl$_2$, 1; KCl, 20; HEPES, 5; EGTA, 0.1 or 0.5; adjusted to pH = 7.4 with KOH.

Chemicals and drugs used in this study included; Ryanodine (Penick); all other chemicals were obtained from Sigma.

The cells were voltage clamped or current 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 (PCM) data recorder (NF, 880) for future analysis. Data were also displayed on a digital oscilloscope (Hitachi, 6041, or Nicolet, 9024) and pen recorder (Harvard oscillograph) and could then be directly reproduced onto an X-Y recorder (Graphtec, WX 2400).

RESULTS

The effect of stimulation frequency on action potential

Among the electrical properties of single atrial cells of the rabbit, resting membrane potential was -65 ~ -75 mV and the amplitude of action potential was about 110 mV which was larger than that recorded at multicellular preparations. The duration of action potential was 100 ~ 250 ms. There were two phases in repolarization of action potential when stimulated at 0.025 Hz. Initially there was a rapid phase of repolarization, then a late plateau phase which appeared slowly and at negative potential level. These properties of ac-
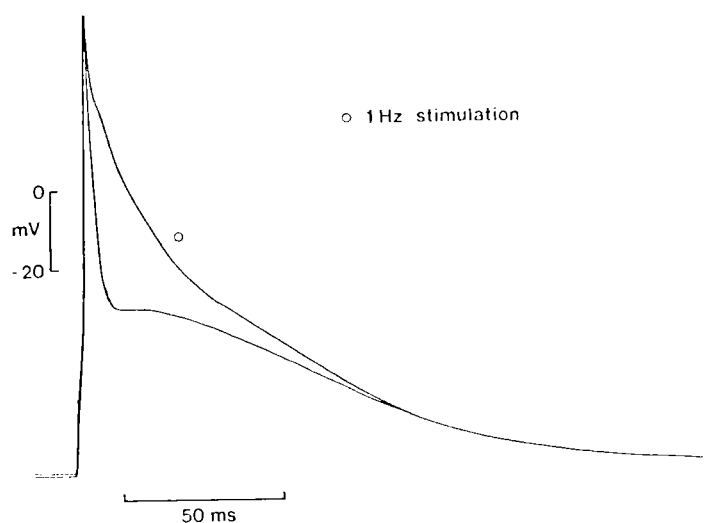

Fig. 1. The action potential in the atrial cell of the rabbit heart and the effect of stimulation frequency. Stimulated at 1 Hz (o), the initial, rapid repolarization was greatly inhibited and the action potential configuration changed.

tion potential were similar to the results of other investigators(Hilgemann 1986; Giles and Imaizumi 1988). Fig. 1 shows action potential recorded when stimulated at 1 Hz. Increase of stimulation frequency affected the rapid phase of repolarization and repolarization phase was not clearly distinguished as two phases.

The effects of stimulation frequency on the ionic currents

In order to see what caused the change of repolarization phase we initially investigated the
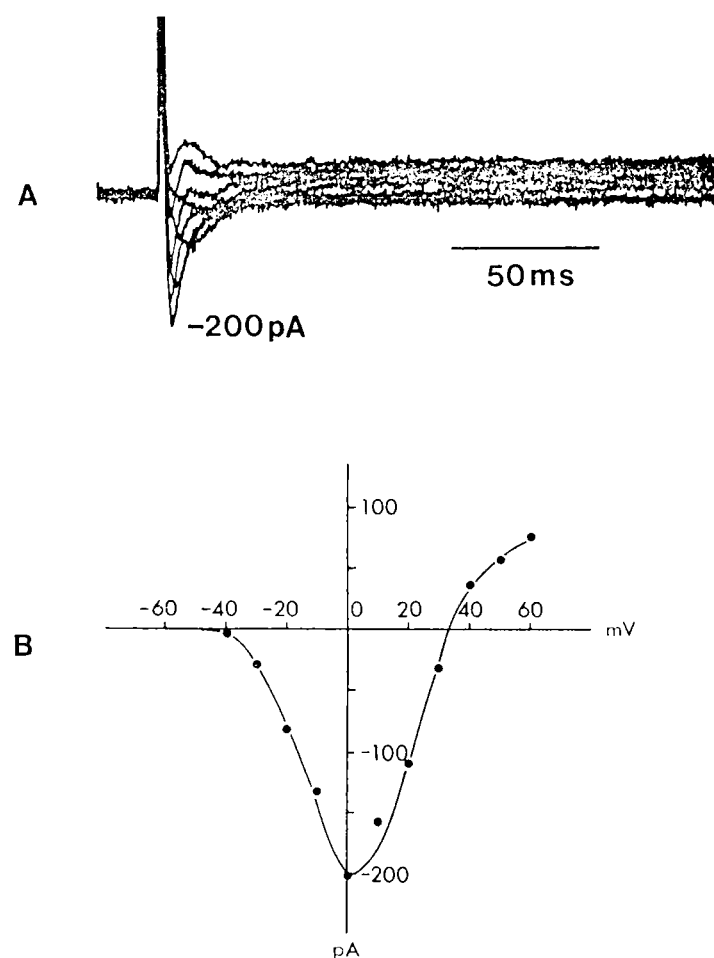
effect of stimulation frequency on calcium channel which is important in maintaining the plateau phase. Fig. 2A shows currents activated by 200 ms depolarizing pulses from holding potential -40 mV. Ca current was activated, then transient outward current was also activated from 10 mV. There were small delayed K currents and inward rectifier K currents. Ca current was activated around -30 mV and reached maximum value at 0 mV(Fig. 2B). Stimulated at 1 Hz, calcium current changed little(Fig. 3).

Ca current decreased rapidly at positive membrane potential in single atrial cells of the rabbit unlike in other myocytes(Fig. 2B) and this

A

50ms

-200pA

B

Fig. 2. The calcium current and transient outward current. In A the curents were activated by depolarizing pulse from holding potential (H. P.) -40 mV. From 0 mV the transient outward current appeared. In B the I-V relation curve is shown. Below 40 mV maximum inward current was plotted and above 40 mV maximum outward current was plotted. The calcium current was maximum at 0 mV and decreased more rapidly above 0 mV because of the transient outward current.

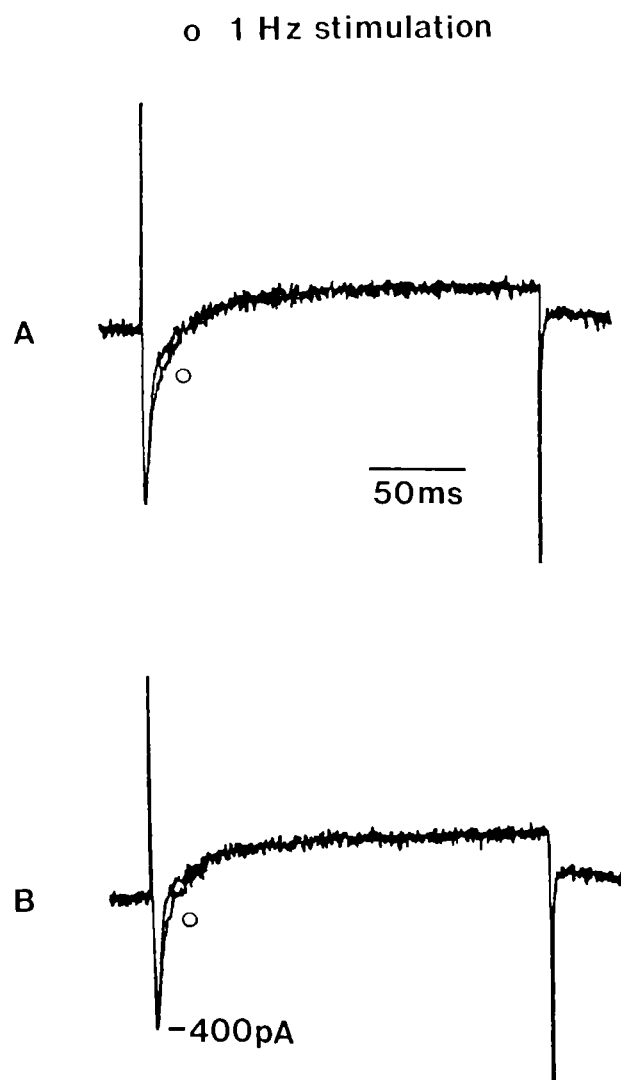### o 1 Hz stimulation

A

50ms

B

-400pA

Fig. 3. Effects of stimulation frequency on the calcium current. The calcium current was activated by depolarizing pulse to 10 mV(A) and 30 mV(B) from H. P. -40 mV. Stimulated at 1 Hz(o), the calcium current was little changed.

was due to the activation of transient outward current. Since the transient outward current was activated together with the Ca current by depolarizing pulses from holding potential -40 mV similar to calf Purkinje fibre(Siegelbaum and Tsien 1980), we investigated the effect of change of [Ca]i on current activation. First, we tested the effect of ryanodine on current activation. Ryanodine is known to inhibit Ca release from sarcoplasmic reticulum and to decrease slow inward tail current in rat ventricular cells(Mitchell *et al.* 1987). Ryanodine also inhibited the transient outward current in rabbit atrial cells(Fig. 4). Next we tested the effect of Ca buffer, i. e. EGTA, on current activation. The transient outward current was larger in amplitude at 0. 1 mM EGTA than 0. 5 mM EGTA

(Fig. 5). These results indicate that the transient outward current is [Ca]i-dependent type which was activated by Ca release from the sarcoplasmic reticulum. Stimulated at 1 Hz, the [Ca]i-dependent transient outward current changed little(Fig. 6).

0.5 EGTA

(A)

0.1 EGTA

(B)

200 pA

50 ms

Fig. 5. Effects of EGTA on the transient outward current. The transient outward current was elicited by depolarizing to 20 mV from H. P. -40 mV. The transient outward current was observed more frequently and was larger in amplitude at 0. 1 mM EGTA than at 0. 5 mM EGTA.

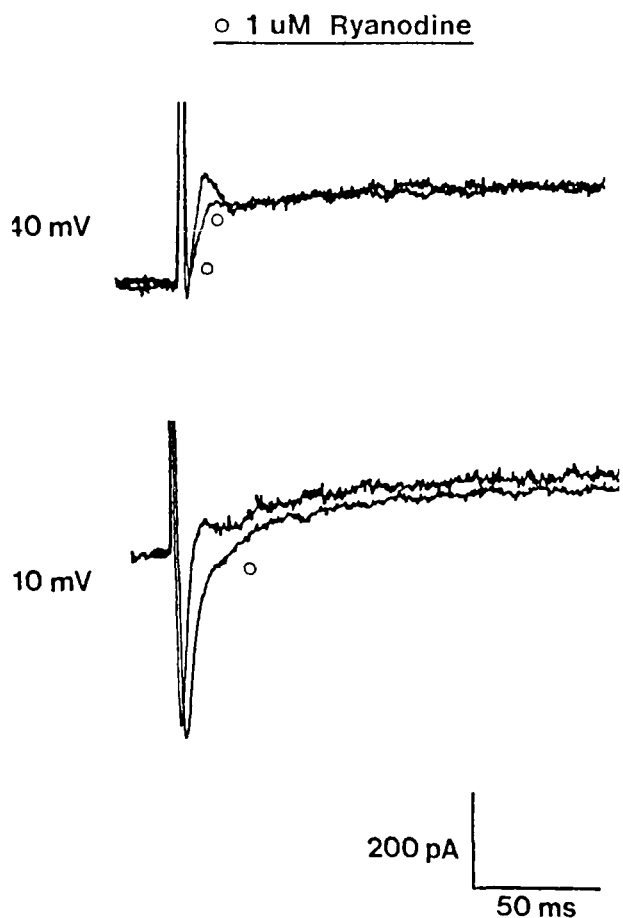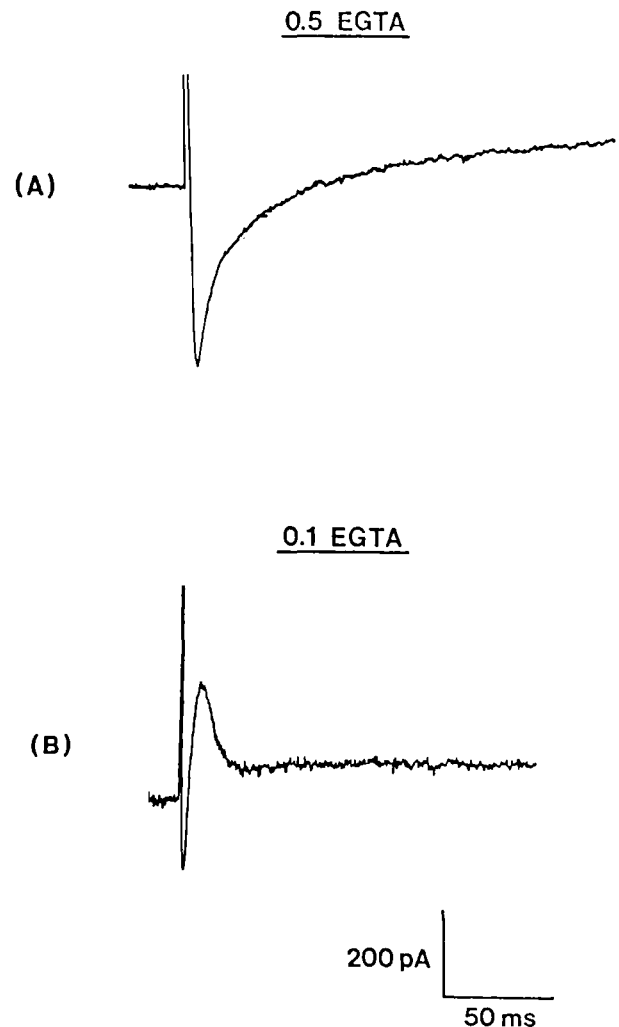**o 1 uM Ryanodine**

40 mV

10 mV

200 pA

50 ms

Fig. 4. Effects of ryanodine on the transient outward current. The transient outward current was elicited by depolarizing pulse to 10 mV (lower trace) and 40 mV (upper trace) from H.P. -40 mV and inhibited by 1 μM ryanodine(o). This indicates that the transient outward current is intracellular Ca-dependent.

Since there was little effect on Ca current and [Ca]i-dependent transient outward current, we shifted holding potential from -40 mV to -70 mV and tested the effect of stimulation frequency on other current systems. Fig. 7 shows currents activated by depolarizing pulses from holding potential -70 mV. Another transient outward current was activated from -30 mV
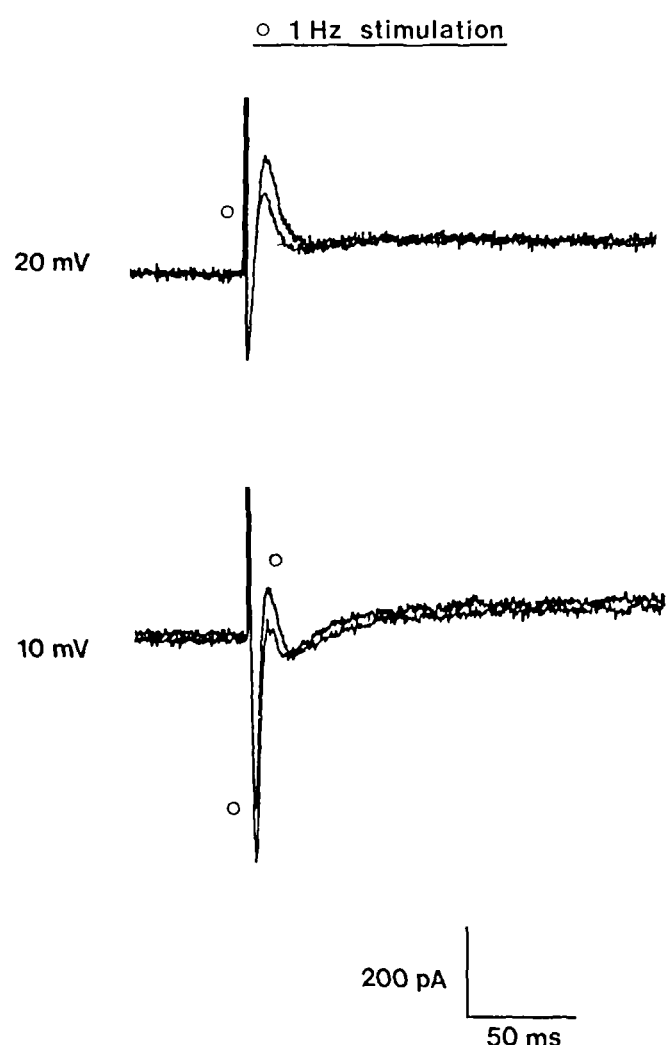
o 1 Hz stimulation

20 mV

10 mV

200 pA

50 ms

Fig. 6. Effects of stimulation frequency on the [Ca]$_i$-dependent transient outward current. The transient outward current was elicited by depolarizing pulse to 10 mV(lower trace) and to 20 mV(upper trace) from H. P. -40 mV. Stimulated at 1 Hz(o), the transient outward current was little changed.

following activation of Na current. In addition a transient inward current was activated and the transient inward current was developed by inward mode of Na-Ca exchange(Earm *et al*. 1989, 1990). Fig. 8 shows currents activated by 200 ms depolarizing pulses from holding potential -70 mV after blocking of Ca current and [Ca]$_i$-dependent transient outward current with 1 mM Cd. The transient outward current was voltage-dependent type because it was activated at -30 mV and increased as the membrane potential depolarized. Stimulated at 1 Hz, the voltage-dependent transient outward current was greatly inhibited(Fig. 9). These results
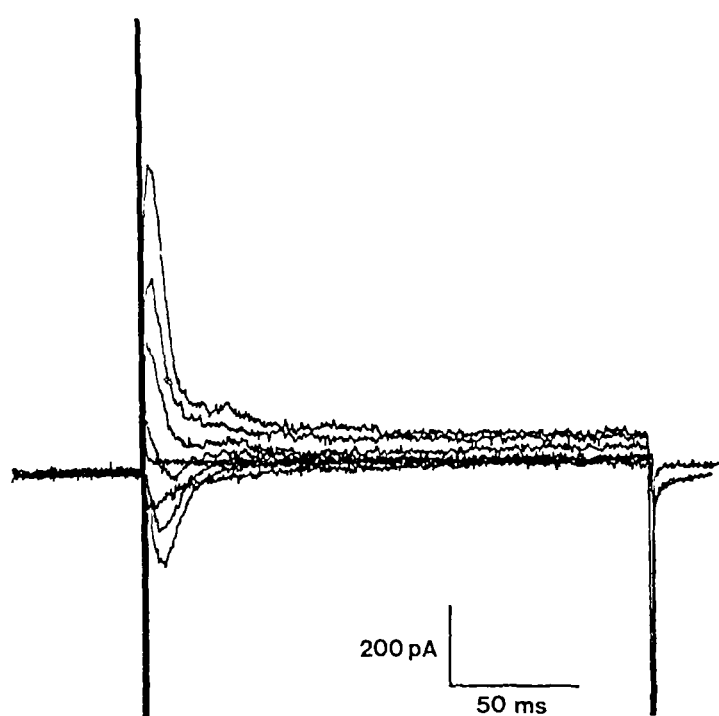
200 pA

50 ms

Fig. 7. The currents activated by depolarizing pulses from H. P. -70 mV. Na current, Na-Ca exchange current and transient outward current were activated.

indicate that since voltage-dependent transient outward current was activated to more than 1 nA, counteracted the action of Ca current stimulated at 0.025 Hz and greatly changed increasing stimulation frequency from 0.025 Hz to 1 Hz, the change of repolarization phase during action potential was almost due to the change in voltage-dependent transient outward current. Thus it would be important to elucidate the properties of voltage-dependent transient outward current.

Voltage-dependency of transient outward current

We investigated steady-state inactivation and activation to elucidate the voltage-dependency of transient outward current further. We used double-pulse protocol to obtain a steady-state inactivation curve. Saturation of transient outward current by post-pulse occurred when the prepulse was made more negative than -70 mV and the amplitude of transient outward current activated by post-pulse decreased as prepulse was depolarized(Fig. 10). Steady-state inactivation curve was well fitted to a
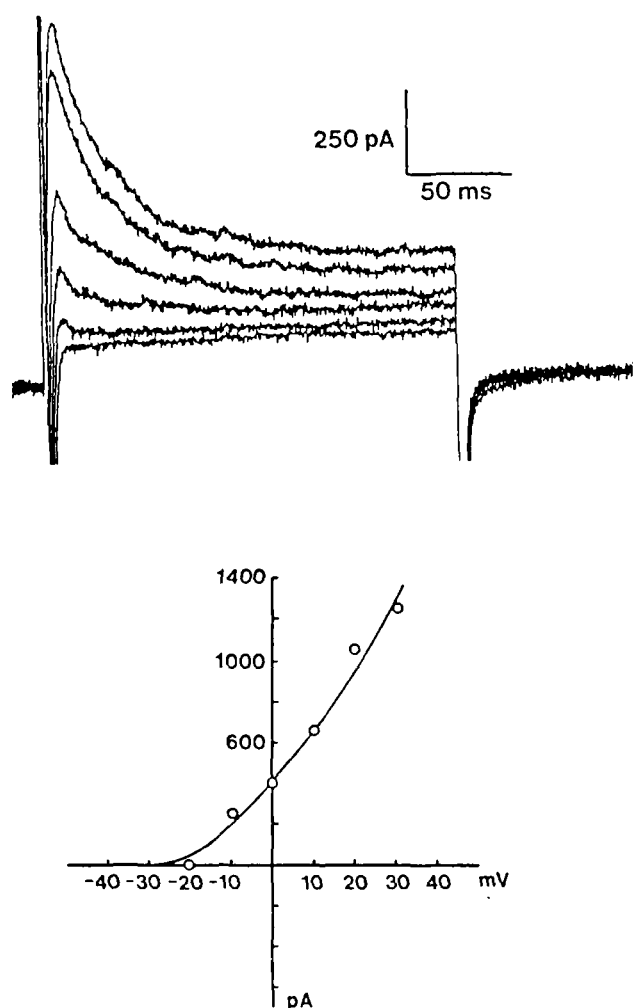
Fig. 8. The transient outward current activated by depolarizing pulses. 1 mM Cd was used to block Ca current, Na-Ca exchange current and [Ca]$_i$-dependent transient outward current. Larger depolarization induced greater transient outward current (upper panel). In the lower panel the I-V relation curve is shown and the transient outward current was activated at -30 mV and increased as the membrane potential depolarized.

Boltzmann distribution given by:

$$r(V) = 1 / \{ 1 + \exp(V-V_h)/h \}$$

where Vh was -30 mV and steepness of inactivation(h) was 7. 3. We used brief depolarizing pulses to obtain a steady-state activation curve(Fig. 11). We recorded the largest tail current by 2 ms depolarizing pulse to 40 mV and the amplitude of tail current decreased as the amplitude of the prepulse did. Steady-state activation curve was well fitted to a Boltzmann distribution given by:
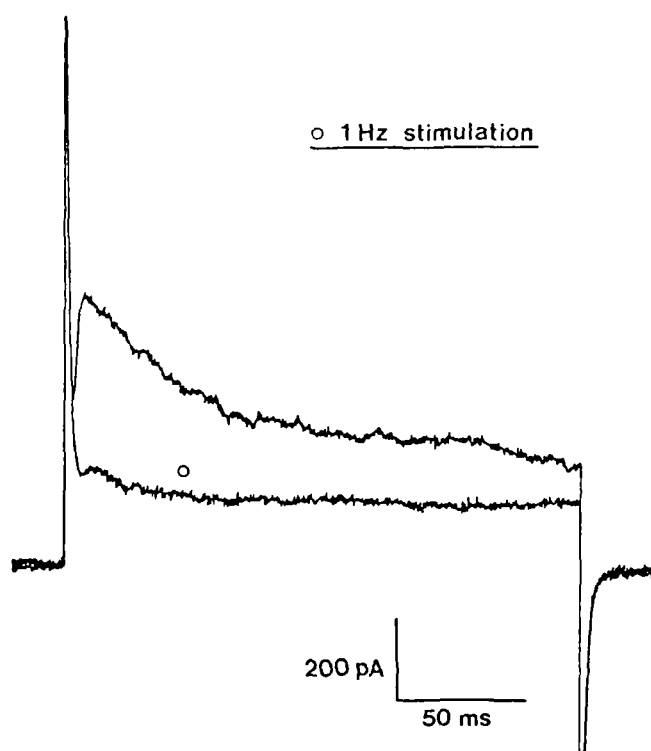

Fig. 9. Effects of stimulation frequency on the transient outward current. Stimulated at 1 Hz(o), the voltage-dependent transient outward current was greatly inhibited.

$$q(V) = 1 / \{ 1 + \exp(V-V_h)/h \}$$

where Vh = -7. 4 and h = - 10.3.

Because the time needed to reach peak after activation of transient outward current was within 10 ms, it could be sufficiently activated from deactivated state during 1 Hz stimulation. On the other hand the transient outward current was inactivated following activation, this portion was fitted to two exponential curves. Since fast time constant was 35 ms at 0 mV, 20 ms at 10 mV, 29 ms at 20 mV, 19 ms at 30 mV and 17 ms at 40 mV, it did not depend on membrane potential. Slow time constant was 320 ms at 0 mV, 270 ms at 10 mV, 480 ms at 20 mV, 400 ms at 30 mv and 200 ms at 40 mV. It did not also depend on membrane potential although it contaminated with other currents. The time course of transient outward current at negative membrane potentials which did not activate transient outward current was investigated by obtaining the recovery time constant. Fig. 12 shows the recovery of transient outward current. The recovery time constant
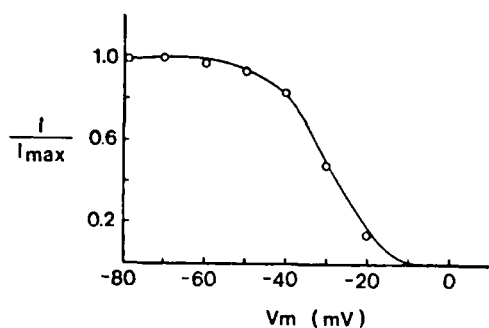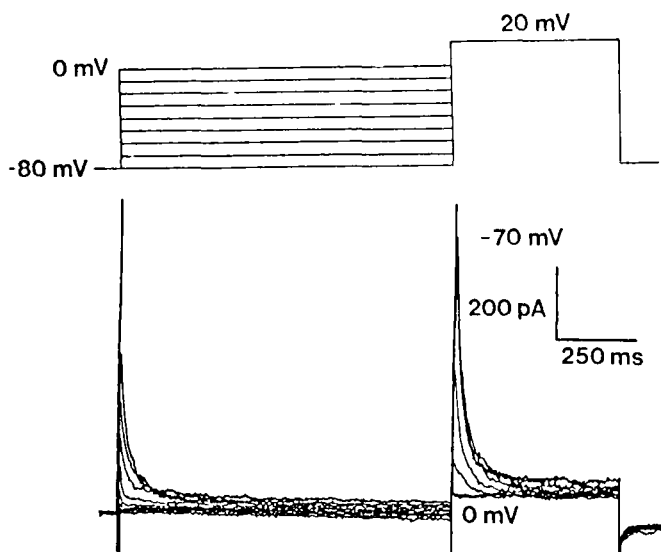
Fig. 10. The steady-state inactivation of transient outward current. In the upper panel the pulse protocol and current activation are shown. The pulse protocol consisted of variable prepulses lasting 1 second followed by a fixed post-pulse to 20 mV for 500 ms. Holding potential was -80 mV. Prepulse ranged from -80 mV to 0 mV. Saturation of transient outward current by post-pulse occurred when the prepulse was made more negative than about -70 mV. The lower panel shows the inactivation curve obtained by plotting the ratio of the current obtained when the post-pulse was preceded by more depolarized prepulse voltages to the post-pulse current at its maximum value. The curve fitted to a Boltzmann distribution given by:

$$r(V) = 1 / \{ 1 + exp(V+30)/7. 3 \}$$

was 660 ms at -90 mV, 730 ms at -80 mV, 1. 3 s at -70 mV, 2. 1 s at -60 mV and 2. 6 s at -50 mV, so it depended on membrane potentials. It was also likely to be affected by 1 Hz stimulation due to the recovery time constant unlike in rat
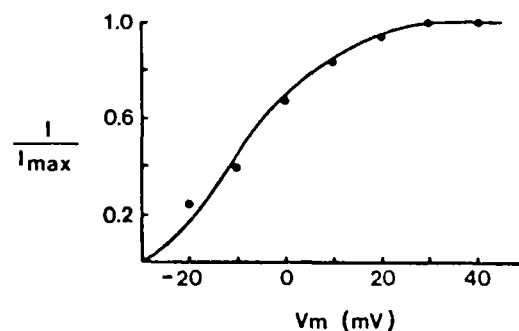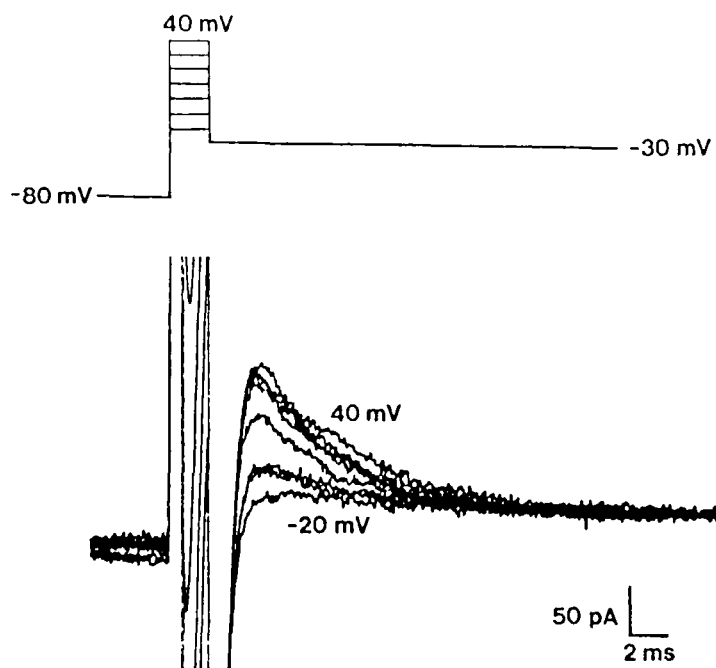
Fig. 11. The steady-state activation of transient outward current. In the upper panel the pulse protocol and current activation are shown. From a holding potential of -80 mV, graded 2 ms steps were applied from -20 mV to 40 mV. Each prepulse was sufficient to activate transient outward current and the relative amount of activation was measured from the initial amplitude of the current tail following return to a potential of -30 mV. The normalized amplitude was then plotted as a function of the prepulse potential yielding the activation curve shown in the lower panel. The curve fitted to a Boltzmann distribution given by:

$$q(V) = 1 / \{ 1 + exp(V+7. 4)/-10. 4 \}$$

ventricular cells (Josephson et al. 1984). Inactivation and reactivation processes were not reciprocal in rabbit atrial cells unlike in crist terminalis of the rabbit(Giles and van Ginneken
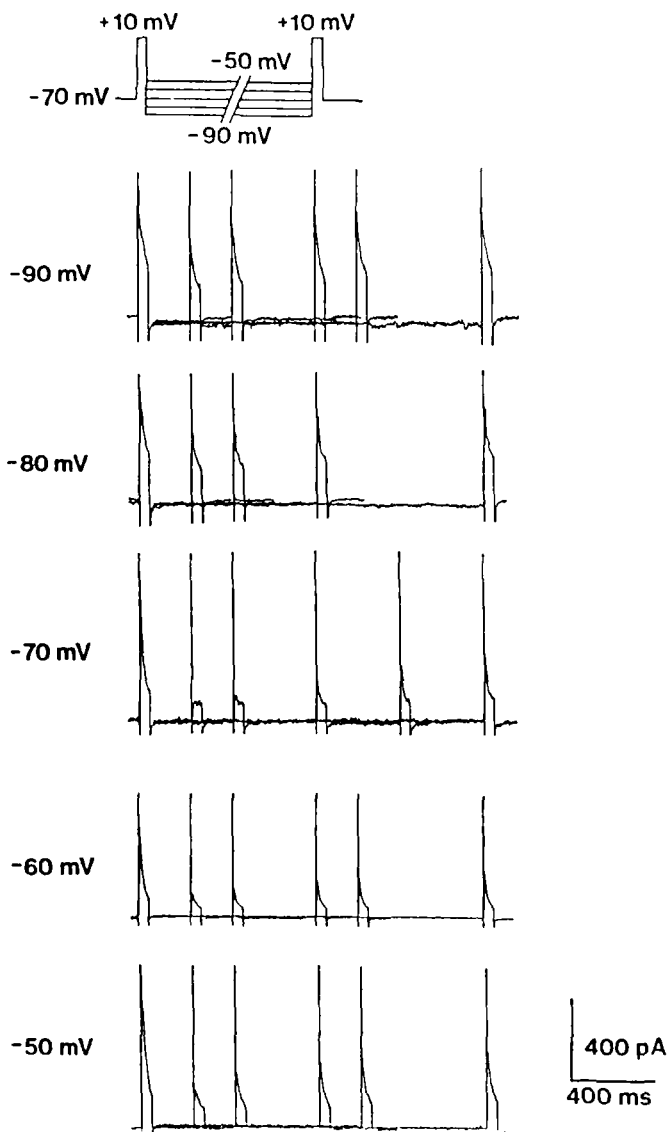
Fig. 12. Recovery of the transient outward current. Recovery time course at five different potentials (from -90 mV to - 50 mV) are shown. In these experiments, two identical pulses to 10 mV for 50 ms were used to activate the current, and the recovery time and interpulse potential were varied systematically.

1985). Since repriming process which means recovery from inactivated state was longer as membrane potential depolarized from -90 mV, voltage-dependent transient outward current was greatly affected by 1 Hz stimulation and this caused changes of shape in the repolarization phase during action potential.

Other properties of transient outward current

We measured reversal potential to see which ion causes voltage-dependent transient outward current(Fig. 13). Reversal potential was
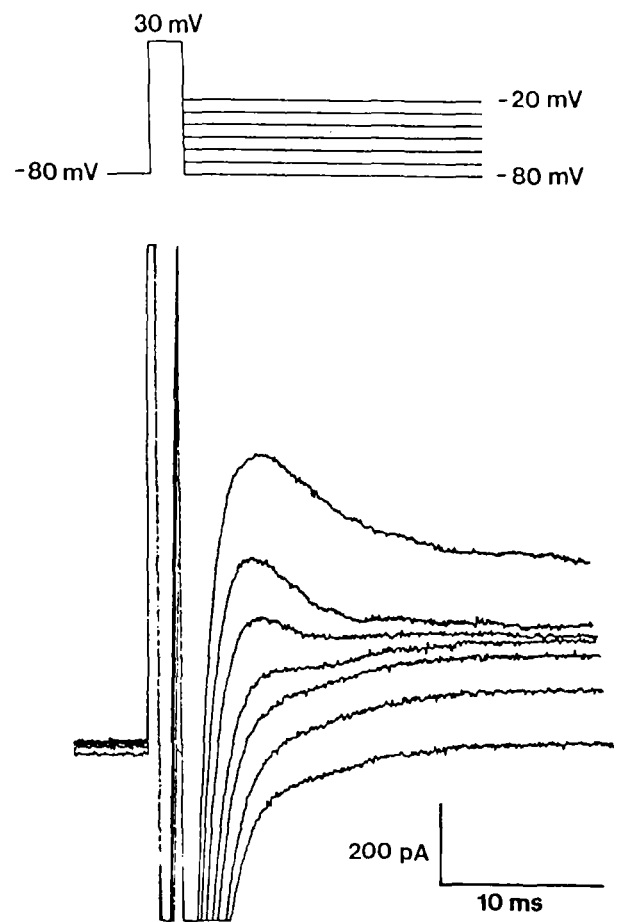

Fig. 13. Reversal potential of the transient outward current. A 2 ms depolarizing pulse from -80 mV to 30 mV was used to activate the current. Current tails were recorded after return to various potentials: -20 to -80 mV. Note reversal of tail currents between -40 and -50 mV.

-50 mV and voltage-dependent transient outward current had permeability for not only K but Na like AV node(Nakayama and Irisawa 1984).

We studied the effects of K channel blockers on voltage-dependent transient outward current. Ba completely inhibited the current (Fig. 14). 10 mM TEA(tetraethylammonium) little inhibited the current and even 20 mM TEA partially inhibited the current(Fig. 15). When we activated voltage-dependent transient outward current by depolarizing pulses from holding potential -70 mV, the current was inhibited by 2 mM 4-aminopyridine and net inward current was recorded(Fig. 16). We applied 1 mM Cd to see the effect of 4-aminopyridine on only voltage-dependent transient outward current. 2 mM
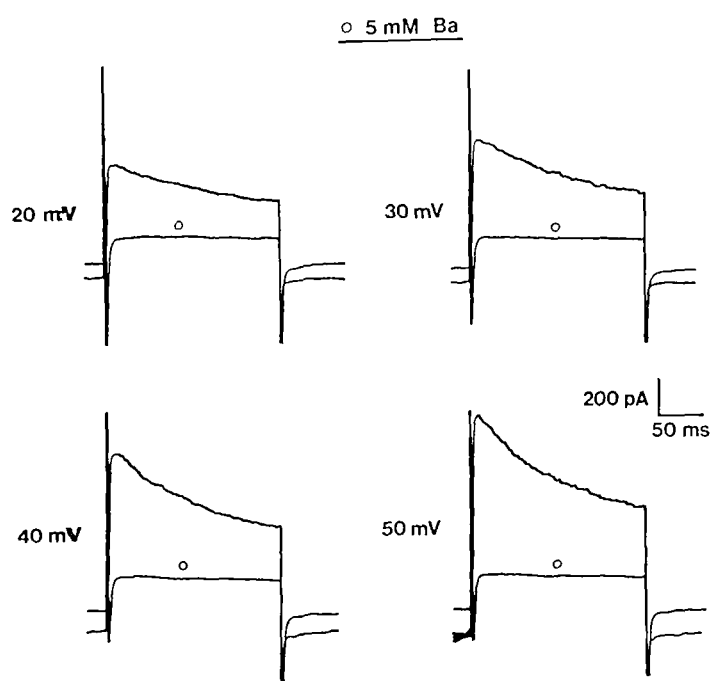
o 5 mM Ba

TEA

Fig. 14. Effects of Ba on the transient outward current. Transient outward currents were elicited by depolarizing pulses (20 to 50 mV) from H. P. of -80 mV and inhibited by Ba(o).

4-aminopyridine completely inhibited the current(Fig. 17). 4-Aminopyridine caused the change of shape in the action potential similar to that induced by 1 Hz stimulation.

We tested the effect of intracellular Ca on voltage-dependent transient outward current. The current was little affected by ryanodine and caffeine which are known to affect intracellular Ca by effects on the sarcoplasmic reticulum (Fig. 19). The voltage-dependent transient outward current was also activated by depolarizing pulses after the blocking of Ca channel with Cd. The amplitude of transient outward current was decreased and inactivation time course was slower by 5 mM quinidine which was known to be used in the treatment of arrhythmia on the basis of prolongation of the repolarization phase (Fig. 20).

DISCUSSION

The effect of change of stimulation frequency on the ionic currents

Our results showed that change of stimulation frequency from 0. 025 Hz to 1 Hz little af-
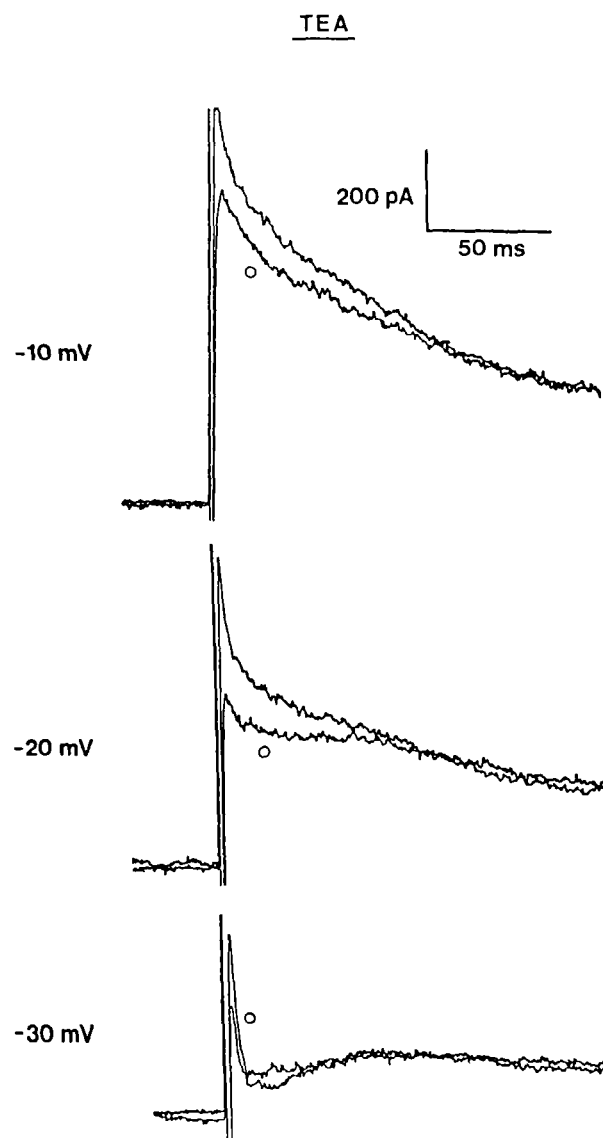
Fig. 15. Effects of TEA on the transient outward current. Transient outward currents were elicited by depolarizing pulses (-30 to -10 mV) from H. P. of -70 mV and partially inhibited by TEA(o).

fected ionic currents other than voltage-dependent transient outward current in rabbit atrial cells. Since the amplitude of voltage-dependent transient outward current at 40 mV, i. e. at the peak of action potential, was more than 1 nA which was much larger than that of Ca current (see Fig. 2 and 8), voltage-dependent transient outward current greatly repolarized the action potential counteracting the depolarizing action of Ca current. As a result the current induced activation of Na-Ca exchange and formed a low level plateau at negative membrane potential which was closely related to negative staircase phenomenon at low frequency of stimu-
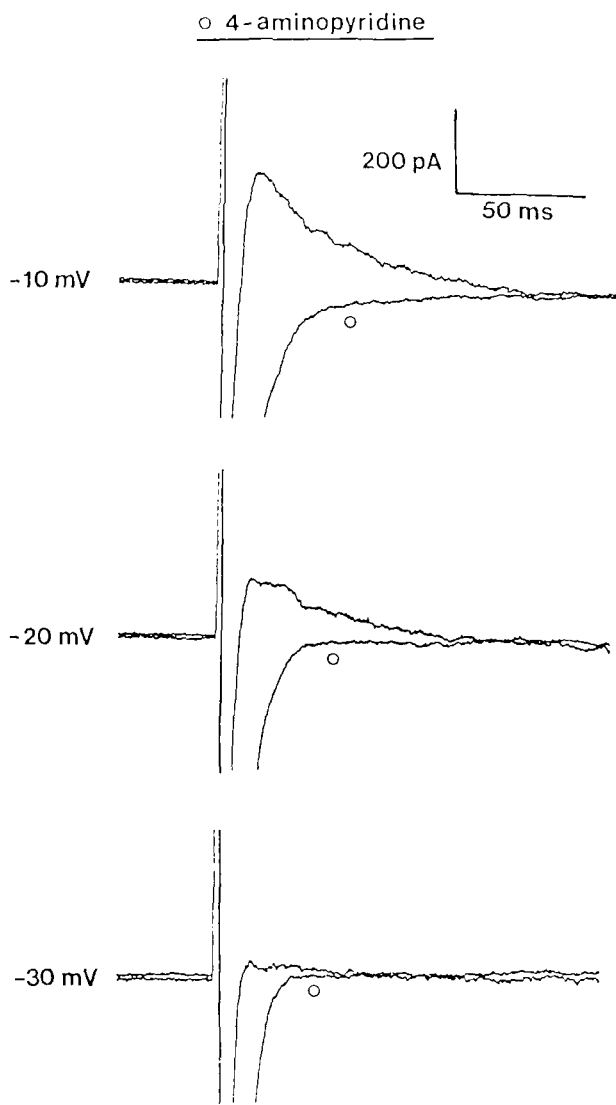
o 4-aminopyridine

200 pA

50 ms

-10 mV

o

-20 mV

o

-30 mV

o

Fig. 16. Effects of 4-aminopyridine on the transient outward current. Transient outward currents were elicited by depolarizing pulses (-30 to -10 mV) from H. P. of -70 mV. It was inhibited by 2 mM 4-aminopyridine (o) and an inward current appeared.

lation(Hilgemann 1986; Park *et al.* 1989; Earm *et al.* 1990). On the other hand, since voltage-dependent transient outward current was frequency-dependent i. e. recovery time constant was longer as membrane potential depolarized (see Fig. 12), it was greatly affected by change of stimulation frequency, and relative increase of the amplitude of other currents changed the shape of action potential at high frequency of stimulation related to positive staircase phenomenon(Hilgemann 1986; Park *et al.* 1989).

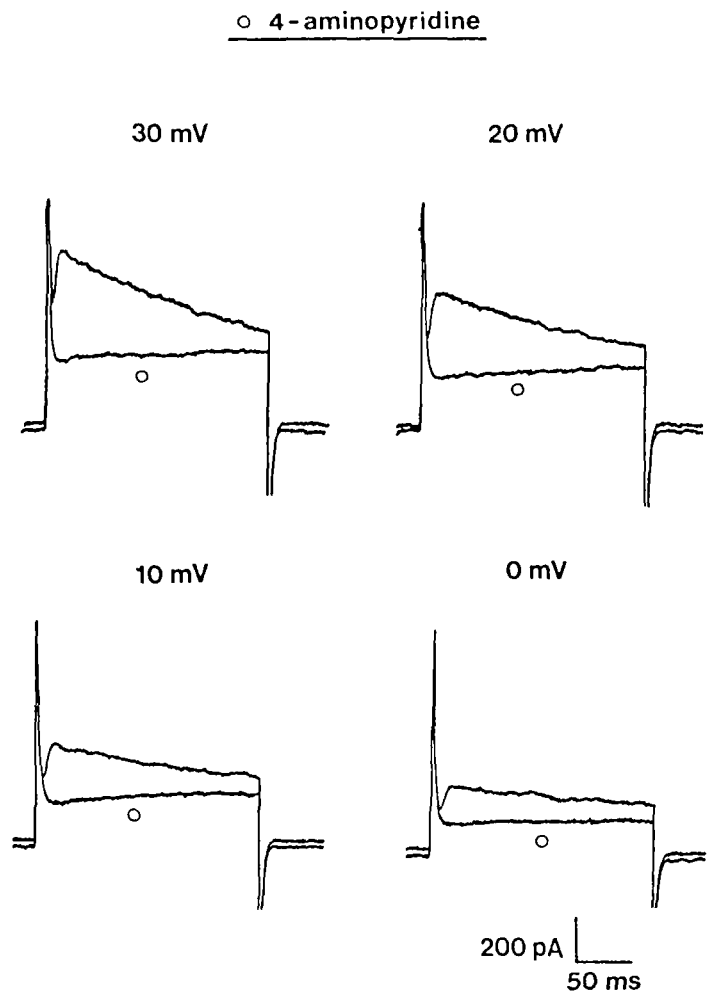Since the recovery time constant of transient outward current was shorter than those of

o **4-aminopyridine**

30 mV   20 mV

o   o

10 mV   0 mV

o   o

200 pA

50 ms

Fig. 17. Specific effects of 4-aminopyridine on the transient outward current. 1 mM Cd was used to block calcium current and Na-Ca exchange current. Transient outward currents were elicited by depolarizing pulses (0 to 30 mV) from H. P. of -40 mV and specifically inhibited by 4-aminopyridine(o).
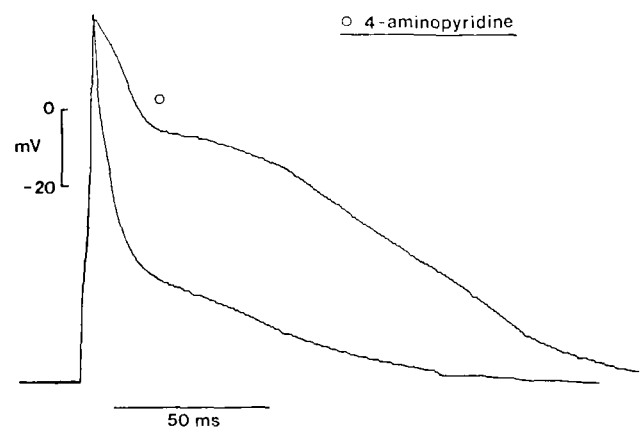
o 4-aminopyridine

0
mV

-20

o

50 ms

Fig. 18. Effects of 4-aminopyridine on the action potential. When 4-aminopyridine was applied the action potential configuration changed greatly, the plateau was increased and initial rapid repolarization greatly inhibited(o). It was similar to 1 Hz stimulation.

o 5 mM Caffeine

(A)

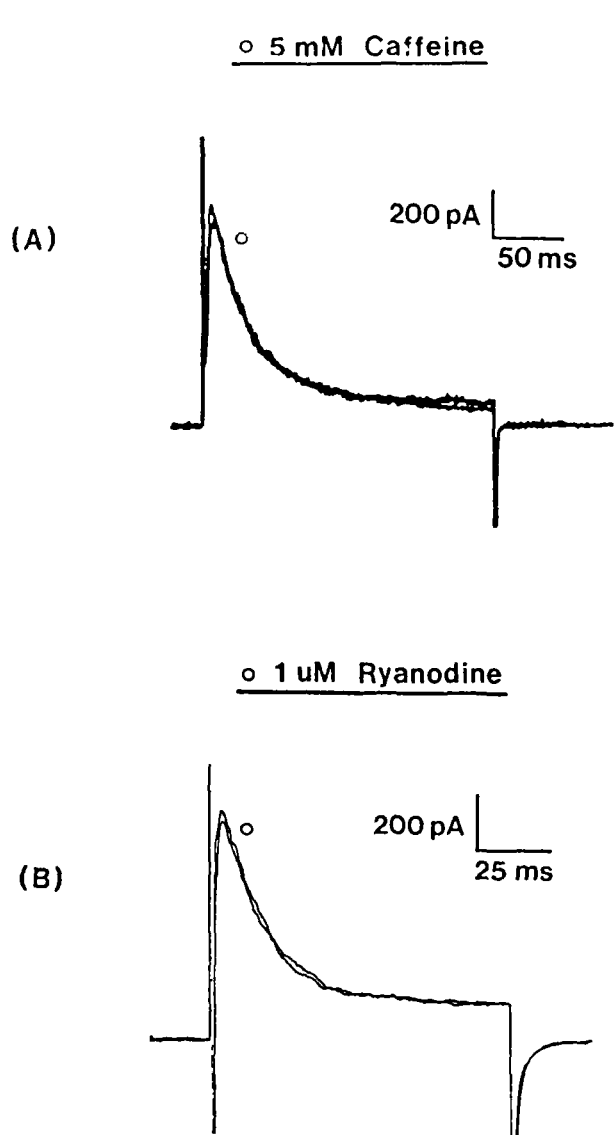200 pA

50 ms

o 1 uM Ryanodine

(B)

200 pA

25 ms

Fig. 19. Effects of intracellular Ca on the voltage-dependent transient outward current. 5 mM caffeine (upper panel: o) and 1 μM ryanodine (lower panel: o), which influence intracellular Ca, had no effect on the current activated by depolarizing pulses to 30 mV.

other currents in rat ventricular cells contrary to rabbit atrial cells, increase of heart rate caused the duration of action potential to shorten i. e. shortened the refractory period and lowered the level of the plateau phase below 0 mV which decreased Ca influx into the cells(Josephson et al. 1984). Because voltage-dependent transient outward current increased with age and changed the shape of the action potential in human atrial cells, it caused the possibility of arrhythmia to increase(Escande et al. 1985). Voltage-dependent transient outward current also played a role in inhibiting pacemaker
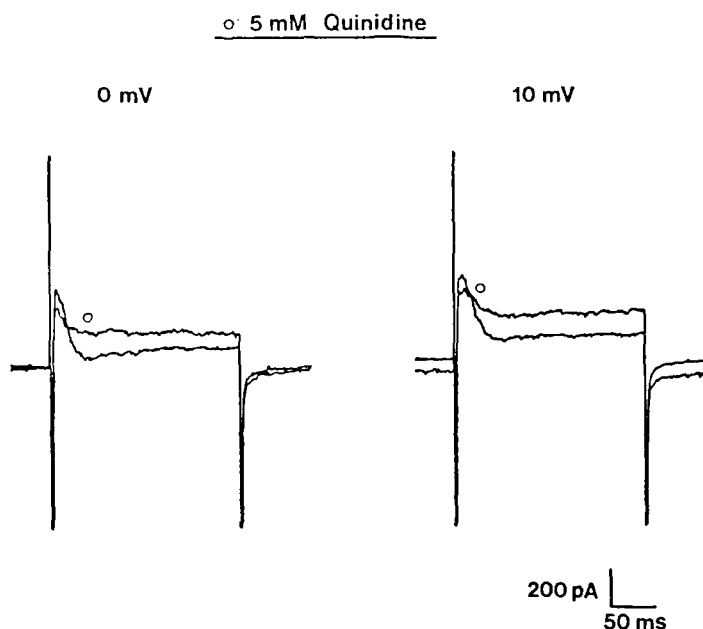
0 mV   10 mV

200 pA

50 ms

Fig. 20. Effects of quinidine on the transient outward current. Transient outward currents were elicited by depolarizing pulses to 0 mV and 10 mV from H. P. of -70 mV and partially inhibited by 5 μM quinidine(o).

activity of AV node in the rabbit(Nakayama and Irisawa 1985). In addition the fact that voltage-dependent transient outward current was inhibited by quinidine suggests it might be involved in the generation of arrhythmia(see Fig 20 and also Giles and Imaizumi 1987; Imaizumi and Giles 1987). Thus voltage-dependent transient outward current was closely related to staircase phenomena due to frequency-dependency, and it might play a significant role in generating arrhythmia.

Two types of transient outward current

There are two types of transient outward current i. e. [Ca]$_i$-dependent(Kenyon and Gibbons 1977, 1979a; Siegelbaum and Tsien 1980) and voltage-dependent type. Two types of transient outward current exist in neurons and they are very important in regulating the bursting activity of neurons. Voltage-dependent type called A-current(Conner and Stevens 1971; Neher 1971) induced repolarization of the action potential during bursting activity and [Ca]$_i$-dependent type induced repolarization of bursting activity i. e. tended to stop bursting activity.

In the heart, transient outward current was first observed in calf Purkinje fibre(Dudel et al. 1967). Fozzard and Hiraoka(1973) reported its kinetics and voltage-dependency and insisted that it was Cl-current. Kenyon and Gibbons (1977, 1979a) and Siegelbaum and Tsien(1980), however, showed it was K-current activated by [Ca]$_i$. Siegelbaum and Tsien(1980) also showed it was closely related to contraction in calf Purkinje fibre which represents [Ca]$_i$. Recently Zygmunt and Gibbons(1991) and Park et al. (1991) insisted once again that this current is Cl-current in rabbit ventricular and atrial cells respectively. Kenyon and Gibbons(1979b) and Coraboeuf and Carmeliet(1982) found another transient outward current which was not affected by [Ca]$_i$ and inhibited by 4-aminopyridine in calf Purkinje fibre. It was also found in rabbit papillary muscle(Kukushkin et al. 1983), rat ventricular cells(Josephson et al. 1984; Mitchell et al. 1984), crista terminalis of the rabbit(Giles and van Ginneken 1985), rabbit atrial cells (Giles and Imaizumi 1987) and human atrial cells(Escande et al. 1987; Shibata et al. 1986). After Coraboeuf and Carmeliet(1982) showed the existence of two types of transient outward current, there were also reports of the existence of two types of transient outward current in human atrial cells(Escande et al. 1987; Shibata et al. 1986) and rabbit atrial cells(Giles and Imaizumi 1988). Our results also showed there were two types of transient outward current in rabbit atrial cells like the results of Giles and Imaizumi(1988). We used EGTA buffering [Ca]$_i$ or ryanodine affecting release of sarcoplasmic reticulum to isolate [Ca]$_i$-dependent type instead of using Sr(Giles and Imaizumi 1988) or caffeine(Escande et al. 1987). Voltage-dependent transient outward current was not affected by caffeine or ryanodine and was selectively inhibited by 4-aminopyridine as other experimenters have claimed.

Properties of voltage-dependent transient outward current

Our results showed that inactivation was fully removed at potentials negative to -70 mV

and it was complete near -10 mV. Steady-state inactivations of calf Purkinje fibre(Fozzard and Hiraoka 1977) and rat ventricular cells (Josephson et al. 1984) shifted to more depolarized potential than that of the rabbit cell while steady-state inactivation of human atrial cells shifted to a more hyperpolarized potential. The threshold for activation of transient outward current was near -20 mV and it was fully activated at +30 mV in our result. It was very similar to steady-state activation of crista terminalis of the rabbit(Giles and van Ginneken 1985). The steady-state inactivation and activation were little affected by temperature(see Clark et al. 1988).

Time course of activation was around 10 ms and was very fast. Time course of inactivation was fitted to a sum of two exponential functions in our experiments. Time course of inactivation was also fitted to a sum of two exponential functions in calf Purkinje fibre (Fozzard and Hiraoka 1973; Coraboeuf and Carmeliet 1982) and in the AV node of the rabbit(Nakayama and Irisawa 1985). However it was fitted to a single exponential function in rat ventricular cells(Josephson et al. 1984), human atrial cells (Escande et al. 1987) and crista terminalis of the rabbit(Giles and van Ginneken 1985). The experiments mentioned above showed that the time course of inactivation in membrane potentials positive to 0 mV depended little on membrane potential as in our results. On the other hand, recovery time constant was voltage-dependent and frequency-dependent(see Fig 12), so there are differences of inactivation time course from recovery time course and voltage-dependency of inactivation from reactivation unlike those of crista terminalis of the rabbit(Giles and van Ginneken 1985). These results suggest that activation and inactivation processes of voltage- dependent transient outward current do not follow the classical Hodgkin-Huxley model. Clark et al. (1988) recorded single channel activities and suggested a more complex model. But even this model could not explain why inactivation and reactivation processes are not reciprocal. These

points still need to be explored.

We measured reversal potential of voltage-dependent transient outward current to see which ion passes through the channel. Transient outward current is known to be K channel in calf Purkinje fibre and a mixed channel in the AV node of the rabbit which means it permeates Na and K. Our results showed its reversal potential was -50 mV and it was partially inhibited by 20 mM TEA. This suggests that it is not K channel but a mixed channel like the AV node of the rabbit.

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