Transient Outward Current Carried by Potassium and Sodium in Quiescent Atrioventricular Node Cells of Rabbits

Toshio Nakayama and Hiroshi Irisawa
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SUMMARY. Single atrioventricular node cells were dispersed by treating the rabbit heart with collagenase. In Tyrode's solution, the cells became rounded, and about 20% of them showed spontaneous activity, whereas the rest remained quiescent. When those quiescent cells were whole-cell clamped, depolarizing clamp pulses from the holding potential of —83 mV induced an outward current which decayed quickly, with a time course similar to that of the transient outward current in the Purkinje fiber. The amplitude of the current became larger when progressively more positive clamp pulses were given from a very negative holding potential. The inactivation time course of this current consisted of two exponential components. Single-channel current recordings from those cells revealed a class of channels that activated more frequently during the initial part of depolarizing pulses. Summation of those unitary currents reproduced activation and inactivation time courses of the macroscopic current well, suggesting that this channel corresponds to the transient outward current. The current-voltage relationship of the channel was linear with the slope conductance of 19.9 ± 1.8 pS (n = 7), and the reversal potential was near the resting potential of the atrioventricular node cell with 5.4 mM potassium chloride and 134.6 mM sodium chloride in the pipette. The channel was passing mainly potassium ions, but sodium ions also seemed to carry a fraction of the current. The possible role of the transient outward current in the quiescent node cell is discussed. (Circ Res 57: 65-73, 1985)

THE presence of a transient outward current (also called an early outward current, or positive dynamic current) was demonstrated in the sheep Purkinje fiber as early as 1964 (Deck and Trautwein, 1964), and it was to this current that the initial phase of repolarization of the action potential was attributed. Although the voltage-dependent activation, inactivation, and recovery kinetics of the current were studied extensively in various cardiac cells (Fozzard and Hiraoka, 1973; Kenyon and Gibbons, 1979a, 1979b; Siegelbaum and Tsien, 1980; Boyett, 1981; Kukushkin et al., 1983; Josephson et al., 1984), the ionic nature of this current has been a matter of debate.

In the sheep Purkinje fiber, Cl~ ions were considered to be charge carriers of this current (Dudel et al., 1967; Reuter, 1968; Fozzard and Hiraoka, 1973), despite the fact that the reversal potential was not at the Cl~ equilibrium potential (Peper and Trautwein, 1968). Kenyon and Gibbons (1977), on the other hand, suggested that K~ ions might be carrying the current. To attempt to settle this controversy, it seemed appropriate to investigate the current with a single-channel recording technique.

Recently, we found a current very similar to the transient outward current in quiescent cells of the atrioventricular (AV) node of the rabbit. In the present study, we analyzed this current, using both the whole cell clamp and the patch clamp methods. We concluded that the transient outward current in the AV node cell was analogous to the one in the Purkinje fiber, and that K~ ions, as well as Na~ ions, were carrying the current.

Methods

Cell Isolation Technique

Rabbits (0.8–1.2 kg) were anesthetized with pentobarbital sodium (40 mg/kg, iv) while the blood was heparinized (300 l.U./kg, sodium salt). The enzymatic dispersion procedure for isolating single rabbit AV node cells has been described elsewhere (Nakayama et al., 1984). Briefly, using the Langendorff apparatus, we perfused an excised heart with 0.04% collagenase (type 1, Sigma) dissolved in nominally Ca~2+-free Tyrode's solution. The heart then was kept in the high-K~ solution (Taniguchi et al., 1981; Isenberg and Klockner, 1982). The central region of the AV node was identified from its anatomical location (Paes de Carvalho and Almeida, 1960; Kokubun et al., 1982). A small piece of nodal tissue (1 × 2 mm²) dissected from the underlying working muscle was gently teased in the recording chamber filled with Tyrode's solution. In Tyrode's solution, the cells from the node became round in shape, and about 20% of them started to beat spontaneously, whereas the rest remained quiescent (Nakayama et al., 1984). The intact quiescent cells, with a smooth surface and appearing transparent, were distinct from damaged cells. The size of the quiescent AV node cells was 42.1 ± 9.7 μm × 66.0 ± 21.5 μm (n = 32), and the resting membrane potential (RP) was —42.1 ± 10.0 mV (n
To measure the surface area, we assumed the shape of the cell to be a plane oblate spheroid (Nakayama et al., 1984).

Solutions

The composition of the Tyrode's solution was (in mm): NaCl, 136.9; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; glucose, 5.5; NaH₂PO₄, 0.33; and 5 HEPES-NaOH buffer (pH = 7.4). The composition of the pipette solution for the whole cell clamp was (in mm): potassium aspartate, 110; KCl, 20; MgCl₂, 1; KH₂PO₄, 2; adenosine 5'-triphosphate (dipotassium salt, Sigma), 5; creatine phosphate (dipotassium salt, Calbiochem-Behring Corp.), 5; glycocholate (Calbiochem-Behring Corp.), 5; MgCl₂, 1; glucose, 10; HEPES, 5; and the pH was adjusted to 7.2 with KOH. The compositions of the pipette solution for the cell-attached patch clamp are given in Table 1. D600 (Knoll) or CoCl₂ was used to block the calcium current. To block the transient outward current, 4-aminopyridine (4AP, Sigma) or CsCl was simply added to the Tyrode's solution. Experiments were carried out at 32 -36°C.

Recording Techniques

The single-pipette whole-cell clamp and cell-attached patch clamp techniques were similar to those described by Hamill et al. (1981). The space constant of the cell was estimated as 0.19–0.29 mm, assuming an internal resistivity of 100–200 Ω·cm (Weidmann, 1952, Hume and Giles, 1981) and a specific resistance of 21.2–26.0 KΩ·cm² (Fig. 1, B and D). Thus a cell of 66 μm in diameter could be space-clamped adequately.

Glass pipette electrodes were pulled to have a tip diameter of 2–3 μm, and the resistance was 4–10 MΩ. The sealing resistance of 5–100 GΩ was established by applying a negative hydrostatic pressure less than 30 cm H₂O inside the pipette. For the patch clamp recording, we used an EPC-7 amplifier (List-Medical-Electicron), and for the whole cell voltage clamp recording, we used the same amplifier we have reported elsewhere (Nakayama et al., 1984). To reduce the capacitive current, a transient cancellation system of the List amplifier was used in the patch clamp modes. In the whole cell clamp mode, the electrode resistance in series to the cell membranes was compensated to minimize duration of the capacitive surge on the current trace. Voltage and current signals were displayed on an oscilloscope (Tektronix 5113) while being recorded simultaneously on the data recorder (TEAC, R-210A) for later analysis. After the recorded signals were low-pass filtered with a Bessel type active filter (48 db/oct., NF Circuit Design Block Co., FV-625A) at a frequency of 0.8–3.0 kHz, they were sampled with a 12-bit A/D converter at an interval of 0.1–5 msec and stored in the computer (Hitachi, E-600).

<table>
<thead>
<tr>
<th>Concentration</th>
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<th>K-aspartate</th>
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<tr>
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pH = 7.4 at 35°C.

To obtain a macroscopic current by summation, we reconstructed the single-channel current traces from original records. The beginning and the end points of the channel opening and the unitary amplitude were estimated by eye on the digitalized original records.

The electrode potential was adjusted to zero immediately before the cell was attached. The liquid junction potential between the Tyrode's solution and pipette solution (mainly potassium aspartate) was 7.8 ± 0.2 mV (n = 3, negative inside the pipette). The voltage axis was therefore shifted in the positive direction by 8 mV when plotting current-voltage (I-V) curves of the whole cell clamp experiments. In the cell-attached patch clamp, the liquid junction potential between the Tyrode's solution and pipette solutions containing 120 mm aspartate was 10.4 ± 0.7 mV (n = 3, negative inside the pipette). The voltage axis thus was shifted negatively by 10 mV (see Fig. 8) in the single-channel I-V curves. When the chloride-rich pipette solution was used, we did not correct the liquid junction potential (Table 1). The membrane potential of the cell-attached patch was described as the deviation from the holding potential, i.e., RP ± AV. Outward currents are displayed as an upward deflection, and all experimental values are given as mean ± SD.

Results

The Transient Outward Current in the Whole Cell Clamp Experiment

The macroscopic currents induced by depolarizing clamp pulses were markedly different, depending on the holding potential in the quiescent AV node cell. Typical results were illustrated in Figure 1, where the holding potential was at −43 mV (Fig. 1A) and −83 mV (Fig. 1C). When the holding potential was −43 mV, the Ca⁺⁺ current and the delayed outward current were seen on depolarization, and the outward current tail was seen on repolarization. Those currents were quite similar to those in the pacemaking cells (Nakayama et al., 1984). The hyperpolarization-activated current was not found in this example, but was observed in 4 of 11 examples.

Depolarizations from −83 mV, however, induced a rapidly decaying outward current which could be seen to counterbalance the Na⁺ and Ca⁺⁺ currents. The current subsided almost completely during the depolarizing clamp pulses of 300 msec. The peak amplitude of the transient outward current in the AV node cell was 0.6 nA (21.4 μA/cm²) at −17 mV. A current-voltage (I-V) relation measured 5–10 msec after shifted in the positive direction by 8 mV when plotting current-voltage (I-V) curves of the whole cell clamp experiments. In the cell-attached patch clamp, the liquid junction potential between the Tyrode's solution and pipette solutions containing 120 mm aspartate was 10.4 ± 0.7 mV (n = 3, negative inside the pipette). The voltage axis thus was shifted negatively by 10 mV (see Fig. 8) in the single-channel I-V curves. When the chloride-rich pipette solution was used, we did not correct the liquid junction potential (Table 1). The membrane potential of the cell-attached patch was described as the deviation from the holding potential, i.e., RP ± AV. Outward currents are displayed as an upward deflection, and all experimental values are given as mean ± SD.

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**Table 1**

Pipette Solution for the Patch Clamp

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pH = 7.4 at 35°C.
Nakayama and Irisawa/Transient Outward Current in AV Node

FIGURE 1. Transient outward current recorded in the quiescent AV node cell. In panels A and C, the current (upper) and the voltage traces (lower) are shown. Panel A shows depolarizing test pulses of +10, +30, +50, and +70 mV, and a hyperpolarizing pulse of −20 mV from the holding potential (H.P.) of −43 mV. The Ca** current and the delayed outward current are seen. The fast inward sodium current is almost entirely inactivated at this holding potential. The current activated at the hyperpolarization voltage range (iK or iO) was not observed in this example but was observed in 4 out of 11 examples. Panel B: I-V relations of panel A. The peak amplitude of the Ca** current and the initial (10 msec after the onset of the pulse) current during hyperpolarizing clamp pulses are plotted (filled circles). The current amplitudes at 300 msec are also plotted (open circle). The current density of the Ca** current was estimated as 11.9 ± 3.6 nA/cm² (n = 3). In panel C, in response to depolarizing clamp pulses of +20, +40, +50, +60, and +70 mV from the H.P. of −83 mV, the transient outward current is activated at potentials positive to −40 mV. The peak of the current appears within 10 msec after the onset of the clamp pulse, and becomes larger with increasing depolarizations. The peak amplitude was 567 pA at −17 mV (21.4 nA/cm²). Panel D: I-V relations of panel C. The input resistances measured at −40 mV in panels B and D are 0.98 and 0.80 GΩ, and the specific membrane resistances are 26.0 and 21.2 kΩ/cm², respectively. The duration of the clamp pulses are 300 msec in panels A and C. Symbols in panel D are the same as in panel B. The size of the cell was 24 × 40 μm.

after the onset of the clamp pulse revealed a marked outward current (Fig. 1D) at potentials positive to −50 mV, in contrast to an inward current deflection, shown in Figure 1B. It should be noted that the slope conductance in the hyperpolarizing potential range was almost identical in panels B and D, even when the holding potential was varied (1020 pS in Fig. 1B and 1250 pS in Fig. 1D). Therefore, the marked outward current in Figure 1C seemed to be the activation of an additional outward current in the quiescent AV node cell, rather than deactivation of currents activated at the negative holding potential.

An activation phase of the transient outward current could be seen as in the inset of Figure 2, when the Ca** current was blocked by D600. It was distinct from the Na* current, since the activation and inactivation time courses of iNa, were less than 1 msec (Brown et al., 1981). In the presence of D600, the decay of inactivation was fitted to two exponential components, having a fast time constant (τ1) of 20 msec and a slow time constant (τ2) of 169 msec (Fig. 2). In another cell, τ1 was 41 msec and τ2 was 122 msec. The time course of the rapid inactivation was very similar to that of the transient outward current in the Purkinje fiber (Fozzard and Hiraoka, 1973). The slow phase might contain some other current such as the delayed outward current.

Recovery from inactivation was examined by applying a pair of depolarizing pulses separated by a various period of time at the holding potential of −88 mV. Interpulse interval varied from 10 msec to 1 sec (Fig. 3, A–D). Figure 3E gives the time course of recovery from inactivation obtained by the whole cell clamp. The lines in Figure 3E were drawn using the equation,

\[
\frac{\Delta I_{\text{max}} - \Delta I}{\Delta I_{\text{max}}} = \exp (-t/\tau),
\]

where \(\Delta I_{\text{max}}\) was the peak amplitude of the current in response to the first pulse, whereas \(\Delta I\) was the peak amplitude during the second pulse. The recovery from inactivation was exponential, with a time constant of 806 msec at the holding potential of −88 mV, and 558 msec at −108 mV. In two other examples, it was 2308 msec at −80 mV and 3580 msec
FIGURE 3. Recovery from inactivation of the transient outward current in the whole cell clamp. Twin pulses 500 msec in duration are from the holding potential of -88 mV to +2 mV. From panels A-D, the interpulse interval varied from 10 msec to 1 sec. The time course of the recovery from inactivation is shown in panel E. The time constants of inactivation are 806 msec and 558 msec at the holding potential of -88 and -108 mV, respectively. Dotted lines indicate the zero current level.

The transient outward current was seen in 11 out of 24 cells, but the rest of them did not seem to possess this current. In two of three examples, the current was not observed until +30 mV, probably due to overlap of the Ca ++ current. After inhibiting the Ca ++ current with 10 μM D600 (n = 2) or 2 mM Co ++ (n = 3), the transient outward current was clearly disclosed in five out of five examples.

As in the Purkinje fiber (Kenyon and Gibbons, 1979b; Isenberg, 1976), the transient outward current in the AV node was partially blocked by 4AP (not shown), but was not affected by Cs+ (2 mM) from the outside of the membrane.

From the above findings, it was concluded that the transient outward current in the quiescent AV node cells was analogous to that in the Purkinje fiber.

To investigate the ionic selectivity directly, we attempted a single-channel recording of the transient outward current. The gigapohm seal was established on the cell membrane with the pipette filled with 5.4 mM KCl and 134.6 mM NaCl (Table 1). When the pipette potential was set at the resting potential, no channel activity was observed during depolarizing pulses of 70 mV applied for 300 msec with an interval of 12 seconds. When the holding potential was increased by 34 mV (RP-34 mV), 100-mV damp pulses (RP + 66 mV) induced a class of single-channel currents. Figure 5 shows four traces of the single-channel currents recorded from the same patch. In this example, the maximum number of simultaneous openings of the channel was two, observed in 31 of 40 traces. Simultaneous openings always occurred within 50 msec after the onset of the clamp pulse, except in two traces. The amplitude of the single-channel current was 1.5 pA. The duration of the channel opening was 11.5 ± 10.9 msec.

at -70 mV. Thus, the recovery from inactivation was time and voltage dependent.

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FIGURE 5. Single-channel recording of the transient outward current. Four traces of single-channel currents recorded from a cell. The patch pipette was filled with the pipette solution containing 5.4 KCl and 134.6 NaCl (see Table 1). Membrane was depolarized from RP-34 mV, i.e., 34 mV negative to the resting potential, to RP + 66 mV. Currents were low-pass filtered at 1 kHz, and the capacitive and leak currents were subtracted. Size of the cell was 28 × 51 µm and the surface area was calculated as 3887 µm².

(n = 429), and it did not change between the former and the latter half of the clamp pulse. This channel was observed in 18 of 128 cells (11%) with 5.4 mM KCl, 134.6 mM NaCl in the pipette, and the simultaneous openings of the two channels were recorded in three cells.

To determine whether this channel was responsible for the transient outward current, the kinetics and the current amplitude were compared between the whole cell clamp and the channel recording in the following sections.

Kinetic Properties of the Unitary Channel Current

From the experiment shown in Figure 5, approximately 200 channels were open per one cell, assuming the area of the patch as 3.1 µm² and the cell surface area as 3887 µm². Since the unit amplitude of the channel was 1.5 pA at the membrane potential of −17 mV, the peak amplitude of the current in one cell would be about 300 pA. This value coincided well with the peak amplitude obtained in the whole-cell clamp experiment (see Fig. 1).

Figure 6A shows a summated current of 37 reconstructed single-channel traces. Summation yielded an outward current that activated almost instantaneously to reach its peak amplitude, 2.4 pA, at 6 msec; it inactivated first rapidly, and then slowly. The time course of the inactivation consisted of two components: τf = 33 msec for the fast component and τs = 525 msec for the slow one. In two other examples, τf = 29, 32 msec, and τs = 152, 797 msec, respectively. The time constant of the fast component was very similar to that of the macroscopic current.

The frequency- and voltage-dependent properties of the current were examined in the single-channel level (Fig. 7) by using a double-pulse protocol. The duration of each pulse was 300 msec, and the interpulse interval was varied between 100 and 800 msec. The averaged current decay obtained by summing the reconstructed single channel traces could be fitted by two exponential components. The semilogarithmic plots in Figure 7D was obtained by using the same equation as in Figure 3. The recovery of this summated current from inactivation was exponential with a time constant of 835 msec, which coincided strikingly well with the whole-cell-clamp experiment in Figure 3. The time constant of the recovery was faster, when the holding potential was more negative (figure not shown). The summated current and the macroscopic current obtained by the whole-cell-clamp method were very similar in both kinetics and amplitude. Thus, we concluded that this channel was responsible for the transient outward current.
FIGURE 7. Recovery from inactivation of the reconstructed transient outward current. The patch membrane was depolarized with a pair of clamp pulses from the holding potential of RP - 34 mV to RP + 36 mV. The pulse duration was 300 msec and the interpulse interval was varied between 100 and 800 msec. In the averaged trace, the difference between the peak current and that at the end of the pulse was denoted as $\Delta I_{max}$ during the first pulse and $\Delta I$ during the second one. $n$ is the number of traces summated. Solid curves superposed on the reconstructed traces were fitted by two exponential components consisting of two different time constants. During the first pulse, the fast time constants, $\tau_f$, of panels A, B, and C are 22, 29, and 49 msec, and the slow ones, $\tau_s$, are 939, 649, and 731 msec, respectively. In the second pulse, $\tau_f$ are 17, 46, and 41 msec, and $\tau_s$ are 210, 686, and 878 msec for 100, 300, and 800 msec, respectively. The time course of the recovery from inactivation was also exponential (from the straight line in panel D) with a time constant of 835 msec.

Ionic Selectivity of the Transient Outward Current

The ionic nature of this current was examined by the cell-attached patch clamp method. Figure 8A shows the single-channel current records in response to depolarizing pulses from the holding potential of RP - 34 mV to the voltage range between RP + 106 mV and RP + 26 mV with 5.4 mM KCl and 134.6 mM NaCl in the pipette. The amplitude and the frequency of the unitary current were increased as the test pulse became progressively more positive. The I-V relations recorded from seven cell-attached patches were linear, having the slope conductance of 19.9 ± 1.8 pS and the reversal potential at RP - 0.6 ± 5.7 mV (Fig. 8B), i.e., the resting potential of this cell. This reversal potential corresponded well with that measured by the current tail in the whole cell clamp experiment. As the pipette contained both KCl and NaCl, a charge carrier of this current might be either Cl⁻ alone, because the reversal potential for Cl⁻ was supposed to be near -40 mV, or a mixture of ions, presumably Na⁺ and K⁺. We thus examined these two possibilities.

Insensitivity of the Transient Outward Current to Cl⁻

When 120 mM Cl⁻ within the pipette was replaced by equimolar aspartate (Fig. 9A, right), the single channels of the transient outward current remained unchanged and their activity seemed quite similar to that in 140 mM Cl⁻ (Fig. 9A, left). The I-V relations in Figure 9B revealed that the reversal potentials of the control and in low Cl⁻ solution were +2 mV and -11 mV, and the slope conductances were 20.1 pS and 20.4 pS, respectively. Similar results were obtained in two other examples. If the transient outward current were carried by Cl⁻ ions, the reversal potential should have shifted positively 51.5 mV according to the Nernst equation by assuming intracellular Cl⁻ as 30 mM. Thereby, we excluded the possibility of Cl⁻ ions carrying this outward current. The result was consistent with the whole cell exper-
Effects of Na\(^+\) and K\(^+\) on the Single-Channel Current

Since Cl\(^-\) did not seem to carry this current, the remaining possibility was a mixture of Na\(^+\) and K\(^+\). This hypothesis was tested by removing Na\(^+\) from the pipette and substituting isomolar Tris. The results are shown in Figure 10. When Na\(^+\) was removed from the pipette (5.4 mM K\(^+\)), the reversal potential of the channel was shifted negatively by —23 mV and the slope conductance was reduced from 20 pS to 17 pS; suggesting that Na\(^+\) is at least partly responsible for this current.

The equilibrium potential of K\(^+\) on the quiescent AV node cell was estimated by measuring the acetylcholine (ACh)-activated K\(^+\) channel at different membrane potentials. The ACh-activated K\(^+\) current was elicited by using 0.1 \(\mu\)M ACh inside the pipette. The pipette contained 0.1 \(\mu\)M ACh, 85 mM KCl, 5 mM HEPES, and 3 mM CaCl\(_2\). The slope conductance was 49.3 ± 1.3 pS (n = 3), which was equivalent to those reported by Sakmann et al. (1983) and Soejima and Noma (1984). The reversal potential was —1.3 ± 3.1 mV (n = 3) under these conditions. The calculated intracellular K\(^+\) concentration was therefore 91 ± 16 mM, which was very similar to that of 85 mM, reported by Grant and Strauss (1982).

As the K\(^+\) concentration in the pipette was increased from 5.4 mM to 20 and 80 mM, the probability of the channel opening increased. The average slope conductances in 5.4 (n = 4), 20 (n = 3), and 80 mM K\(^+\) (n = 1) were 16.6 ± 0.9, 23.3 ± 2.5, and 30 pS, respectively, and the respective reversal potentials were —26.3 ± 9.3, +18.3 ± 3.2, and +48 mV from the resting potentials. The difference of the reversal potential between 5.4 and 20 mM K\(^+\) was 35 mV, and that between 5.4 and 80 mM K\(^+\) was 74 mV. These values are in good agreement with the difference between the reversal potential calculated from the Nernst equation (35 and 71 mV, respectively). These results strongly suggested that the major part of the transient outward current was carried by K\(^+\) ions.

Discussion

The Transient Outward Current in Quiescent AV Node Cells and in Other Tissues

Our experiments demonstrated the presence of a transient outward current in the quiescent cells of the rabbit AV node. This outward current was qualitatively similar in the activation, inactivation, and recovery kinetics to those observed in the sheep Purkinje fibers (Peper and Trautwein, 1968; Fozzard and Hiraoka, 1973; Kenyon and Gibbons, 1979a; Boyett, 1981) and also to an outward current known as \(i_A\) in various excitable cells (Hagiwara, 1983). Thus this current seems to be distributed widely among different tissues of various animals.

In the Purkinje fiber, inactivation of the positive dynamic current was reported to have double-exponential components (Fozzard and Hiraoka, 1973; Coraboeuf and Carmeliet, 1982). In the present study, two components were seen in the inactivation process, both in the whole cell clamp and the single channel analysis, and the values between the two methods were in good agreement. The fast inactivation component in the AV node cell also resembled that of the rat ventricular cell (Josephson et al., 1984).

Ionic Selectivity

We could exclude Cl\(^-\) as a charge carrier, since the amplitude of the current was unaffected by Cl\(^-\)-free solution substituted by aspartate. This finding was consistent with Kenyon and Gibbons' result (1979b), that the current was unchanged in low Cl\(^-\) solution in the multicellular preparations of the sheep Purkinje fiber. When we used propionate, i.e., a substitute for Cl\(^-\) often used by previous workers (Fozzard and Hiraoka, 1973; Peper and Trautwein, 1968; Dudel et al., 1967), the transient outward current was decreased partially at the potentials between —30 and +10 mV. Thus, possibly a part of the discrepancy can be attributed to the substitute for Cl\(^-\). Siegelbaum and Tsien (1980) found Ca\(^{2+}\)-
activated transient outward current in calf cardiac Purkinje fibers, but ranges of the activation voltage and the sensitivity to the intracellular Ca\(^{2+}\) appeared to be different between Ca\(^{2+}\)-activated transient outward current and the transient outward current in this experiment. Intracellular injection of EGTA reduced the magnitude of the Ca\(^{2+}\)-activated transient outward current, but the transient outward current studied in this paper remained unchanged in spite of using 1 mM EGTA in the pipette.

We base our belief that the transient outward current was carried by K\(^+\) and Na\(^+\) in the AV node cell, on two different grounds. One is the shift of the reversal potential when eliminating Na\(^+\) from outside the membrane. When external Na\(^+\) was 134.6 mM and K\(^+\) 5.4 mM, and the resting potential was assumed to be -42 mV, the reversal potential of this current was -43 mV (RP-0.6 mV), whereas, in the Na\(^+\)-free solution, it was -68 mV (RP-26 mV). From this difference of the reversal potential, we estimated P_{Na}/P_{K} from the Goldman equation.

\[
E_{rev} = \frac{(P_{Na} \times E_{Na} + P_{K} \times E_{K})}{(P_{Na} + P_{K})}.
\]

Assuming the intracellular Na\(^+\) concentration to be 6.4 mM (Sheu and Fozzard, 1982), E_{Na} would be 81 mV. E_K estimated by recording the ACh-activated K\(^+\) channel was -75 mV. The P_{Na}/P_{K} was calculated as 0.26.

The second is the estimation of the slope conductance. With 134.6 mM Na\(^+\) and 5.4 mM K\(^+\) outside of the membrane, the slope conductance was 19.9 pS, whereas, without Na\(^+\), it was 16.6 pS. Assuming that Tris did not modify this channel, the difference between the currents in the presence and absence of Na\(^+\) outside the membrane (3.3 pS) would be a Na\(^+\) component. The P_{Na}/P_{K} estimated from the slope conductance study was 0.19, which resembled the value estimated above from the Goldman equation.

Figure 11 shows a possible scheme for the single channel I-V relations of the transient outward current components. The I-V relation of the K\(^+\) component crossed the voltage axis at -68 mV and that of the Na\(^+\) + K\(^+\) component crossed at -43 mV. Assuming the linear I-V relationship to be near the reversal potential, the Na\(^+\) component crossed the voltage axis at 87 mV, which coincided with the reversal potential of Na\(^+\). The unit amplitude of the Na\(^+\) component crossed at -43 mV (RP-0.6 mV), whereas, without Na\(^+\), it was -42 mV. Assuming that Tris did not modify this channel, the difference between the currents in the presence and absence of Na\(^+\) outside the membrane (3.3 pS) would be a Na\(^+\) component. The P_{Na}/P_{K} estimated from the slope conductance study was 0.19, which resembled the value estimated above from the Goldman equation.

The physiological significance of the transient outward current is necessary to clarify the role of this current. The role of this current has been interpreted to serve for the phase 1 of the action potential in the Purkinje fiber (Peper and Trautwein, 1968), for the formation of a short action potential duration in the rat ventricle (Josephson et al., 1984), and for changing an action potential duration when the interbeat interval was varied in the rabbit papillary muscle (Kukushkin et al., 1983). In the rabbit AV node, this current may help hyperpolarize the cell membrane shortly after the depolarization, when the resting potential of the AV node was negative. When Kokubun et al. (1982) studied the membrane currents of the AV node using multicellular specimens, they did not record the transient outward current, since their holding potential was not negative enough to activate this current. The amplitude of the transient outward current in the pacemaker cell of the AV node was smaller than those observed in the quiescent cells (unpublished data). These facts suggest that this current may contribute to the latent pacemaker activity of the AV node cell. Further investigation is necessary to clarify the role of this current in the AV node.


**Figure 11.** Schematic I-V relations of the ionic components of the single-channel current. KCl + Tris-CI indicate the K\(^+\) component, KCl + NaCl indicates the single-channel current of the normal composition, or, in other words, a mixture of Na\(^+\) and K\(^+\) components. The difference between those two I-V relations gives the dotted line which possibly indicates the Na\(^+\) component. The Na\(^+\) component reverses at 87 mV and has a slope conductance of 3.3 pS.

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