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Modulation by chloramine-T of 4-aminopyridine-sensitive transient outward current in rabbit atrial cells

Hideo Tanaka [a,*], Yoshizumi Habuchi [a], Manabu Nishio [b], Fumiaki Suto [c], Manabu Yoshimura [a]

[a] *Department of Laboratory Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan*
[b] *Department of Internal Medicine III, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan*
[c] *Department of Pediatrics, Children's Research Hospital, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan*

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Abstract

The effects of an oxidizing agent, chloramine-T, on the 4-aminopyridine-sensitive transient outward current (I_{TO}) were investigated in rabbit atrial myocytes by using patch–clamp techniques. Extracellular application of chloramine-T at 20 μM irreversibly slowed the time course of inactivation of the whole-cell I_{TO}, and increased the peak by 19.3% ($n = 19$) at +40 mV. At 100 μM, chloramine-T decreased the peak by 22.5% ($n = 9$) of the control, and subsequently induced a glibenclamide-sensitive time-independent outward K^+ current. Under superfusion with dithiothreitol (3 mM), chloramine-T (100 μM) produced no change in I_{TO}. The chloramine-T-induced slowing of I_{TO} inactivation was partially reversed by subsequent application of 3 mM dithiothreitol. In single-channel recordings with the cell-attached patch configuration, chloramine-T (20 μM) increased the open probability of the I_{TO} channel from 0.15 to 0.46 at a potential 100 mV positive to the resting potential, and the mean open lifetime from 5.1 ms to 7.0 ms ($n = 5$). The unitary current amplitude was not affected. As a result, chloramine-T increased the ensemble current in amplitude and slowed its decay. These results indicated that: (1) inactivation of the native A-type channels of rabbit heart is susceptible to oxidation; and (2) oxidation of I_{TO} channels may contribute to the genesis of arrhythmias.

Keywords: Transient outward current; Atrial myocyte: Chloramine-T; Oxidation; Rabbit

1. Introduction

Oxidation of sulfhydryl (SH–) amino acid residues in proteins by biological oxidants determines various pathophysiological conditions (Ziegler, 1985). Membrane proteins including ion channels have been considered to be targets for SH oxidation (Brodwick and Eaton, 1982; Wang, 1984; Marinov, 1991). Since the redox modulation of the transient outward A-type K^+ current (A-current) was demonstrated using the cloned channels from rat brain (Ruppersberg et al., 1991), many investigators have examined the effects of oxidizing agents on the A-current expressed by various cloned K^+ channels. Some reported a slowing of the fast inactivation (Ruppersberg et al., 1991; Vega-Saenz de Miera and Rudy, 1992; Duprat et al., 1995), and others showed a suppression of the current amplitude (Duprat et al., 1995; Schlief et al., 1996). The 4-aminopyridine-sensitive transient outward K^+ current (I_{TO}) in the heart, which is equivalent to the A-current in nerves, contributes to the initial repolarization phase of action potentials and thereby plays an important role in determining the refractoriness of the heart (for review, see Campbell et al., 1995). Since oxidation plays a key role in the development of arrhythmias during myocardial ischemia or reperfusion (Manning et al., 1984), it is important to elucidate whether and how cardiac I_{TO} is modified by oxidation. However, there have been no previous studies concerning the effects of oxidation on the native cardiac I_{TO}. Therefore, we examined the effects on the native rabbit atrial I_{TO} of a representative oxidizing agent chloramine-T (Shechter et al., 1975). This agent is known to modify the fast Na^+ current (I_{Na}) in toad myelinated fibers (Wang, 1984) and ventricular myocytes (Koumi et al., 1991), and the A-current in neuroblastoma cells

* Corresponding author. Tel.: +81-75-251-5659; Fax: +81-75-251-5687; E-mail: hideotan@koto.kpu-m.ac.jp

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(Rouzaire-Dubois and Dubois, 1990) and in rat central neurons (Huang, 1995).

2. Materials and methods

2.1. Animals

Male rabbits weighing 2–2.5 kg were used. The animals were treated in accordance with the *Guide for the care and use of laboratory animals* published by US National Institutes of Health, and with the approval of the Animal Care Committee at Kyoto Prefectural University of Medicine.

2.2. Isolation of cells

Single rabbit atrial myocytes were obtained by our standard dispersion method (Habuchi et al., 1996). Briefly, hearts were removed from rabbits under anesthesia induced by i.v. pentobarbital sodium (40 mg/kg body weight), and perfused on a Langendorff apparatus with Ca^{2+}-free phosphate-buffered solution composed of (in mM) NaCl 145, KCl 5.4, NaH_2PO_4 0.33, Na_2HPO_4 2.24, $MgCl_2$ 1, and glucose 5 (pH = 7.4). Following 10-min perfusion with the Ca^{2+}-free solution, the perfusate was switched to the Ca^{2+}-free solution containing 0.02 mg/ml collagenase (Yakult Pharmaceutical, Tokyo, Japan) and 0.01 mg/ml protease (Type III, Sigma, St. Louis, MO, USA). After treatment of the heart with the enzyme solution for 20 min, the pectinate muscle from the right atrium was incubated in another enzyme solution (200 μl) containing 1 mg/ml collagenase (Type H, Sigma) in a culture bottle. Cell suspensions collected every 2–3 min were stored in a storage solution composed of (in mM) K-glutamate 90, oxalate 10, taurine 10, KCl 25, $MgSO_4$ 1, NaOH 6, EGTA 0.5, HEPES 5, and glucose 10 (pH = 7.2 adjusted with KOH).

2.3. Electrical recordings

Voltage-clamp experiments were conducted with an Axopatch-1D amplifier (Axon Instruments, Foster City, USA). The whole-cell macroscopic transient outward current (I_{TO}) was recorded in HEPES-buffered Tyrode solution composed of (in mM) NaCl 135, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, HEPES 5, and glucose 10 (pH = 7.4 with NaOH). $CdCl_2$ (0.1 mM) was added to the solution to block the L-type Ca^{2+} current. The pipette solution for whole-cell recordings contained (in mM) K-aspartate 85, KCl 20, $MgCl_2$ 1, $CaCl_2$ 0.5, HEPES 5, Mg-ATP 5, Na_3-phosphocreatine 3, Na_3-GTP 0.1, EGTA 10, and $CaCl_2$ 0.5 (pH = 7.2 with KOH). The pipette had a tip resistance of 1.5–2.0 MΩ. Series resistance compensation was performed to minimize the capacitive surge. The liquid-junction potential of 10 mV between the pipette solution and the perfusate was corrected. The cell capacitance was 59.2 ± 1.5 pF ($n = 50$). Unless otherwise specified, I_{TO} was elicited by 500-ms depolarization to +40 mV from a holding potential of −90 mV every 20 s. For the ramp–pulse experiments, voltage ramp was applied between +30 mV and −110 mV in 1 s from a holding potential of −40 mV every 10 s. The single-channel recording was made in the cell-attached mode (Hamill et al., 1981) using a pipette with a resistance of 1.5–2.0 MΩ when filled with the pipette solution composed of (in mM) NaCl 144, KCl 5.4, HEPES 5, $CaCl_2$ 1, and $MgCl_2$ 1 (pH = 7.4 adjusted with NaOH). $CdCl_2$ (100 μM) and tetrodotoxin (50 μM) were added to the solution to block the L-type Ca^{2+} current and I_{Na}, respectively. The Tyrode solution described above was used for the bath-applied solution. Experiments were conducted at room temperature (22–24°C).

Macroscopic current signals, low-pass filtered at 5 kHz and digitized with a sampling time of 50 μs on a digital oscilloscope (Nicolet 310C, Madison, WI, USA), were analyzed on a computer using custom-made software. Single-channel signals, filtered at 1 kHz, were digitized at 3 kHz via Digidata 1200 interface for off-line analysis in the P-CLAMP system (Axon Instruments). The leakage current on voltage-step pulse was cancelled by subtracting the trace showing no channel opening (null sweep) from each trace. The half-amplitude threshold detection was used for calculation of open times with a bin width of 1 ms.

2.4. Chemicals and data analysis

Chloramine-T (*N*-chloro-*p*-toluenesulfonamide Na, Sigma) was freshly dissolved in distilled water at a stock concentration of 100 mM on the day it was used. 4-aminopyridine and dithiothreitol were from Wako (Osaka, Japan). Anthracene-9-carboxylic acid and glibenclamide were from Sigma. Data are expressed as means ± S.E.M. Statistical analyses of the data were performed by using Student's *t*-test, and P values less than 0.05 were considered significant.

3. Results

3.1. Changes in the macroscopic I_{TO} produced by chloramine-T

Fig. 1 shows a representative example of the effects of chloramine-T on the whole-cell macroscopic I_{TO}. From a holding potential of −90 mV, depolarization to +40 mV elicited a rapidly activating transient outward current. During control, I_{TO} was almost completely inactivated at 200 ms during the depolarization (trace a in the upper panel). Bath application of chloramine-T at 20 μM slowed the

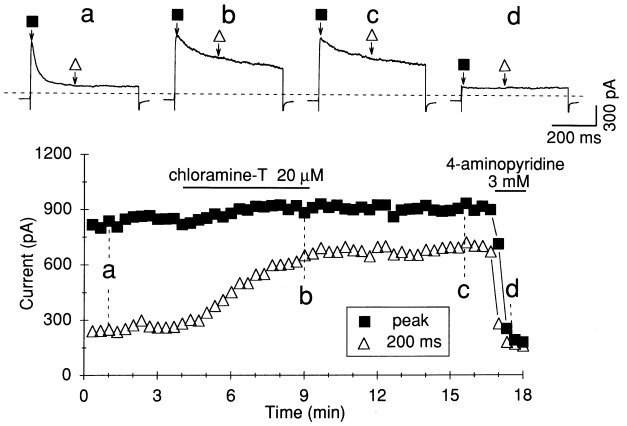

Fig. 1. Effects of chloramine-T (20 μM) on whole-cell macroscopic I_{TO}. I_{TO} was elicited by depolarization for 500 ms to +40 mV from a holding potential of −90 mV every 20 s. (Upper panel) Current traces of the depolarization before (a), during (b) application, and after washout (c) of chloramine-T. (d) denotes the current in the presence of 3 mM 4-aminopyridine. The horizontal dashed line indicates zero current level. (Lower panel) Time course of the changes in I_{TO} measured at peak (closed squares) and at 200-ms depolarization (open triangles). The current values corresponding to the traces in the upper panel are indicated by the same letters.

decay time course of I_{TO} and, as a result, increased the current at 200-ms depolarization (b). In the lower panel of Fig. 1, the currents at the peak (I_{peak}, closed squares) and at 200-ms depolarization (I_{200}, open triangles) were plotted against time. The ratio of these currents (I_{200}/I_{peak}) was markedly increased by 20 μM chloramine-T from 0.23 ± 0.02 to 0.65 ± 0.03 ($n = 19$, $P < 0.05$). The washout of chloramine-T did not reverse these changes (c). The chloramine-T-induced component was abolished by 4-aminopyridine (3 mM) (d), but was not affected by either anthracene-9-carboxylic acid (1 mM) or glibenclamide (10 μM), blockers of the chloride current (I_{Cl}) or ATP-sensitive K^+ current ($I_{K\text{-}ATP}$), respectively ($n = 3$ for each agent, data not shown).

Fig. 2A shows the concentration-dependent effects of chloramine-T on I_{TO}. At 1 μM, chloramine-T slightly slowed its inactivation time course without any significant changes in the peak amplitude (upper left). Chloramine-T at 20 μM increased the peak amplitude to $119.3 \pm 3.5\%$ ($n = 19$) of the control. A subsequent increase of the chloramine-T concentration to 100 μM did not retard the inactivation time course further, but decreased the peak to

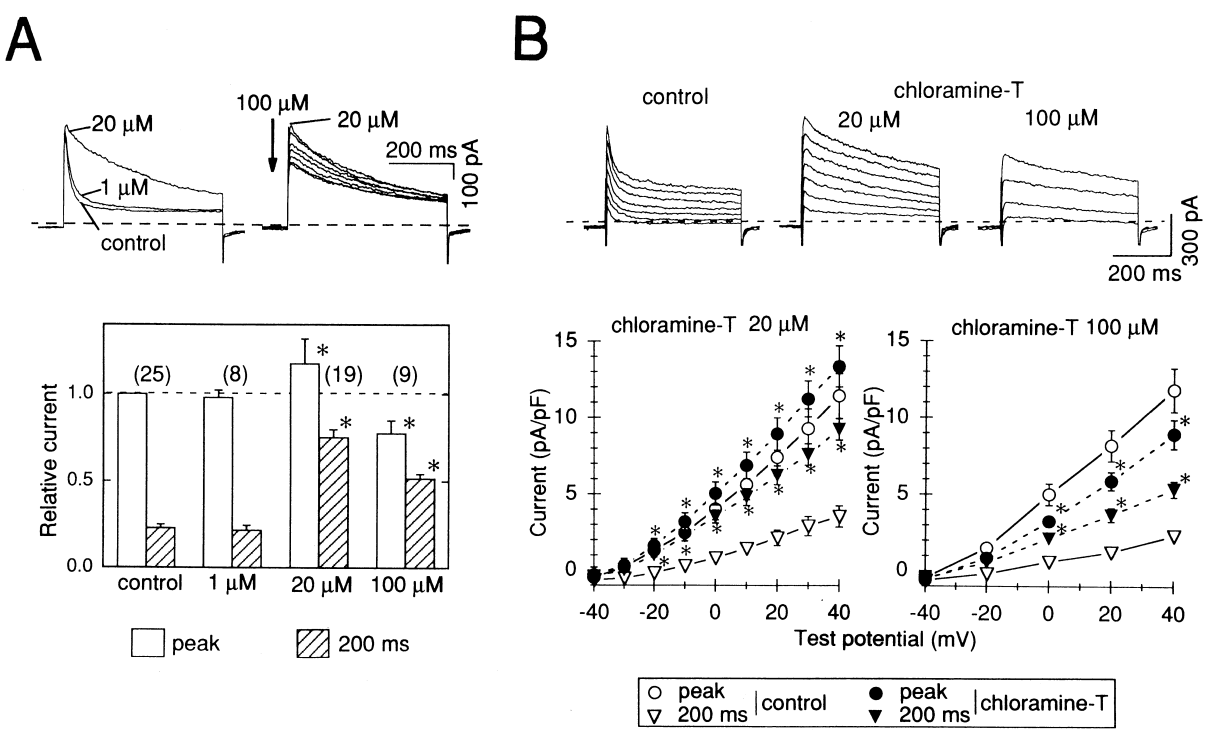

Fig. 2. (A) Dose-dependent changes in I_{TO} by chloramine-T. I_{TO} was elicited by 500-ms depolarization to +40 mV from a holding potential of −90 mV every 20 s. The upper left panel shows superimposed current traces before (control) and during application of 1 or 20 μM chloramine-T. The upper right panel shows a progressive decrease in I_{TO} by further application of 100 μM chloramine-T to the same cell used to generate the data shown in the left panel. The arrow indicates the direction of the change in the current. The lower panel summarizes the dose-dependent changes in I_{TO} measured at peak (open bars) and at 200-ms depolarization (hatched bars). Current values are normalized to those of the peak under control conditions. The numbers in parentheses indicate the number of cells examined. (B) Current–voltage relations for I_{TO}. I_{TO} was elicited by 500-ms depolarization from a holding potential of −90 mV. Top panel shows families of representative current traces (obtained from one cell) between −20 mV and +40 mV in 10-mV (left, middle) and 20-mV (right) voltage steps before (left) and during application of chloramine-T at 20 μM (middle) and 100 μM (right). The lower left panel shows the current–voltage plots for the currents at the peak and at 200-ms depolarization in the presence and absence of 20 μM chloramine-T for six different cells. The lower right panel shows the effects of 100 μM chloramine-T on the current–voltage plots for six different cells. * in (A) and (B) indicate the statistical difference ($P < 0.05$) as compared with the corresponding control values.

77.5 ± 7.4% ($n = 9$) of the control 3 min after application of the drug (upper right). These changes in I_{TO} are summarized in the lower panel of Fig. 2A, which shows the values of I_{peak} and I_{200} relative to the control I_{peak}. I_{peak} and I_{200} at 20 μM and 100 μM chloramine-T were significantly different from the corresponding control values. The ratio I_{200}/I_{peak} at 20 μM chloramine-T (0.65 ± 0.03, $n = 19$) was not different from that at 100 μM (0.67 ± 0.04, $n = 9$). With a longer (> 3 min) application of 100 μM chloramine-T, a time-independent outward current developed and hampered the measurement of I_{TO} in a steady state. This will be described in detail in the Section 3.3 (Fig. 4A).

The current–voltage relationships for I_{TO} showed that 20 μM chloramine-T enhanced currents at the peak (circles) and 200-ms depolarization (triangles) at all the potentials tested (Fig. 2B, lower left). The chloramine-T-induced current at 200-ms depolarization showed a voltage dependence similar to that of the peak current. The lower right panel of Fig. 2B shows the effects of 100 μM chloramine-T on the current–voltage relationships for I_{TO}. The data were obtained at five limited voltages because of the development of the time-independent outward current (see Fig. 4A). Chloramine-T at 100 μM reduced I_{TO} (both at the peak and 200-ms depolarization) without causing a voltage-shift in the current–voltage relationship.

3.2. Oxidation mediates the effects of chloramine-T on I_{TO}

Chloramine-T oxidizes SH-containing amino acid residues of the channel proteins (Shechter et al., 1975). To test whether the observed chloramine-T-induced changes in I_{TO} were due to oxidation, we examined the effects of chloramine-T on I_{TO} in the presence of dithiothreitol, a specific reducing agent for the SH groups of amino acids (Clenand, 1963). As shown in Fig. 3A, dithiothreitol at 3

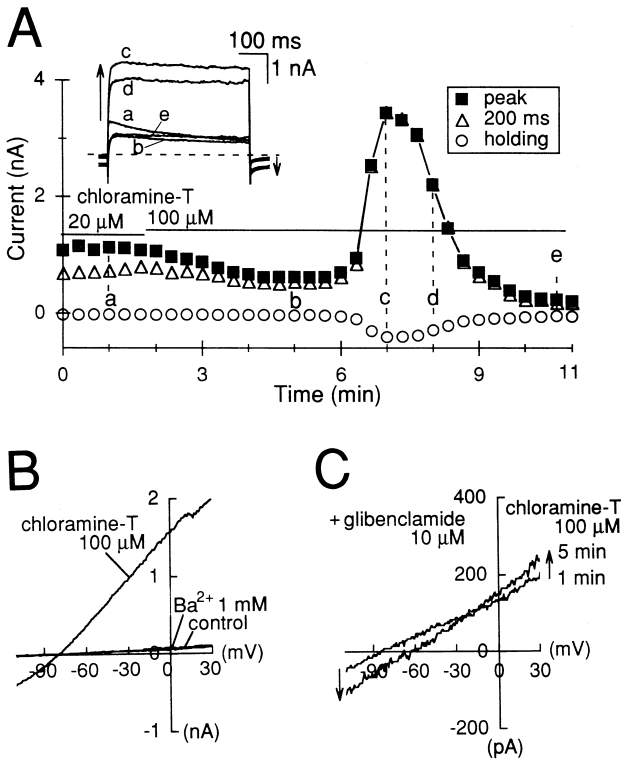

Fig. 4. (A) Activation of the time-independent current by long-term exposure to chloramine-T at 100 μM. The cell was depolarized to +40 mV from a holding potential of −90 mV every 20 s. The currents at the peak (closed squares) and at 200-ms depolarization (open triangles), and those at −90 mV (open circles) are plotted against time. Inset shows the current traces corresponding to those indicated (a–e) in the diagram. Arrows indicate directions of the change in the current from (b) to (c). (B) Current–voltage relationships for the quasi steady-state currents before (control) and during activation of the background current by 100 μM chloramine-T, and after application of 1 mM Ba^{2+}. The current was elicited by ramp pulses between +30 mV and −110 mV in 1 s with 3 mM 4-aminopyridine added. (C) Effect of 100 μM chloramine-T on the current–voltage relationship in the presence of 10 μM glibenclamide. The currents at 1 min and 5 min after commencement of chloramine-T application are shown. Arrows indicate directions of the changes in the current. The ramp-pulse protocol was the same as that in (B).

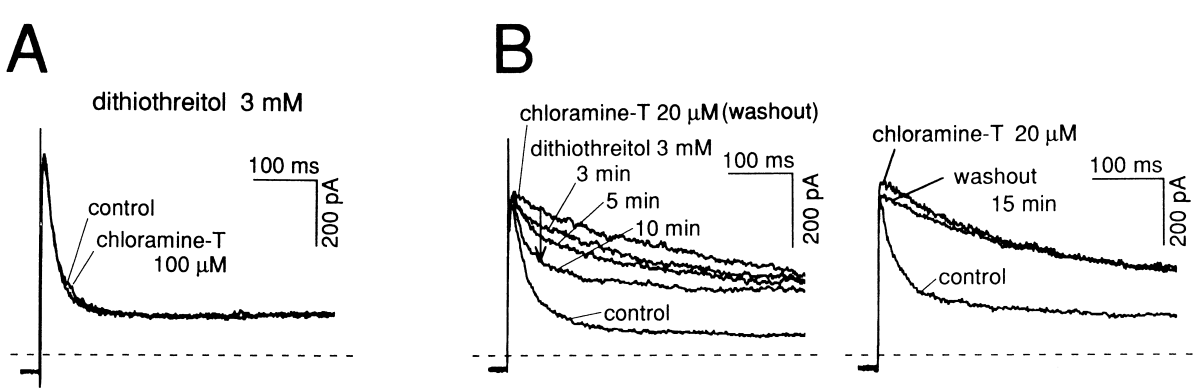

Fig. 3. (A) Absence of changes in I_{TO} produced by chloramine-T (100 μM) in the presence of 3 mM dithiothreitol in the superfusate. The traces before and after addition of chloramine-T are shown. (B, left) Partial reversal of the effects of chloramine-T on I_{TO} by dithiothreitol. Current traces before application (control) and 3 min after washout (washout) of 20 μM chloramine-T, and subsequent application of 3 mM dithiothretol (3, 5, 10 min) are superimposed. The arrow indicates the direction of the changes in I_{TO} after application of dithiothreitol. (B, right) Absence of changes in the time course of I_{TO} inactivation 15 min after washout of chloramine-T. The current traces in (A) and (B) were elicited by depolarizing pulses from −90 mV to +40 mV.

mM prevented 100 μM chloramine-T from producing any changes in I_{TO} in all five cells examined. As described above (Fig. 1), the washout of chloramine-T did not reverse its effect on the inactivation time course. However, the chloramine-T (20 μM)-induced slowing of I_{TO} inactivation was gradually reversed by subsequent application of 3 mM dithiothreitol after washout of chloramine-T (Fig. 3B left). The ratio I_{200}/I_{peak} of 0.66 ± 0.01 during superfusion with chloramine-T was significantly reduced to 0.46 ± 0.03 10 min after addition of dithiothreitol ($n = 6$, $P < 0.05$). In sham experiments, the washout of chloramine-T alone (without dithiothreitol) did not reduce the I_{200}/I_{peak} value. It was 0.69 ± 0.03 during superfusion with 20 μM chloramine-T vs. 0.70 ± 0.04 15 min after the washout of chloramine-T (Fig. 3B right, $n = 6$).

3.3. Induction of time-independent currents by chloramine-T

When the cell was exposed to a higher concentration of chloramine-T (100 μM) for more than 3 min, a time-independent outward current developed rapidly and subsequently declined, as shown by the changes in the current elicited by 500-ms depolarization to +40 mV from −90 mV (Fig. 4A, traces c and d). An inward shift of the holding current (open circles) was also observed. The outward current began to develop 229 ± 22 s after the application of 100 μM chloramine-T and reached the maximal amplitude of 50.4 ± 8.0 pA/pF at +40 mV in 1–2 min ($n = 9$). The ramp–pulse experiments (Fig. 4B) in the presence of 3 mM 4-aminopyridine revealed that 100 μM chloramine-T produced a marked increase in the quasi steady-state current, showing a linear current–voltage relation. The chloramine-T-induced current showed a reversal potential of -79.6 ± 1.5 mV ($n = 5$) and was quickly (within 40 s) abolished by 1 mM Ba^{2+} (Fig. 4B, $n = 4$), suggesting that this current was a K^+ current. In addition, this current was sensitive to glibenclamide. As exemplified in Fig. 4C glibenclamide at 10 μM almost entirely prevented the activation of the chloramine-T-induced K^+ current in six of seven cells. Instead, 100 μM chloramine-T produced a slight increase in the background current with a reversal potential of -15.2 ± 4.8 mV and

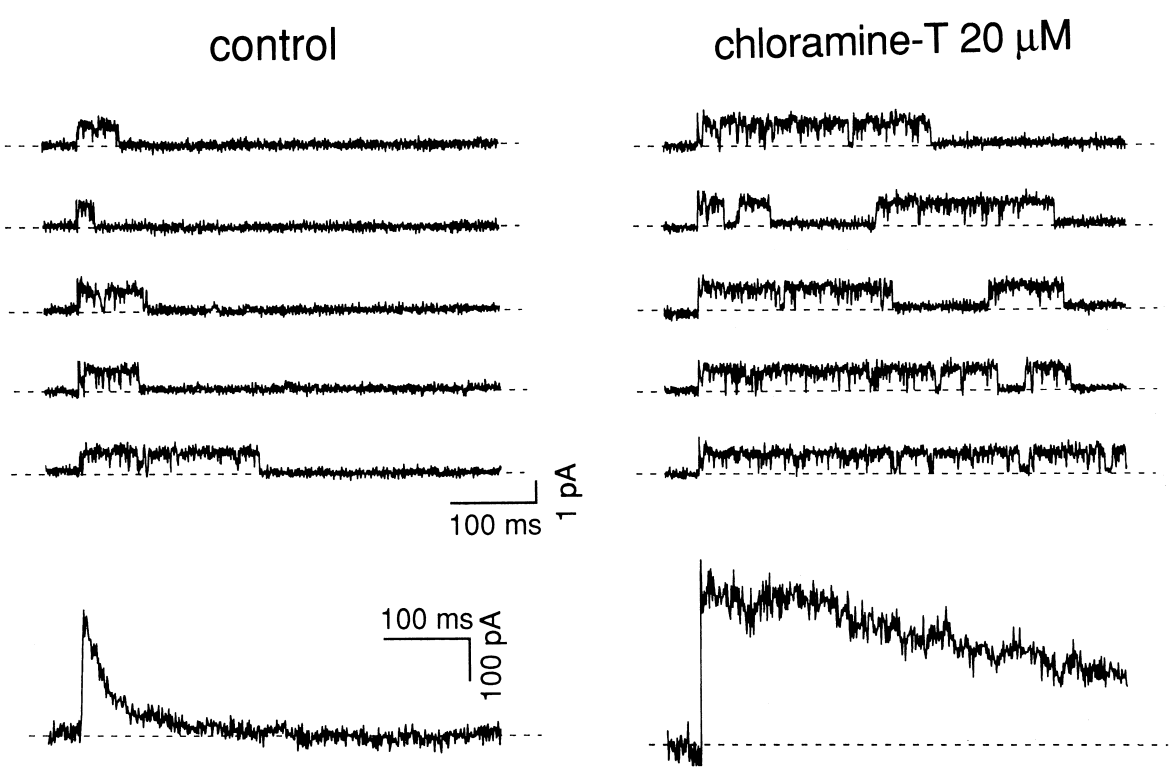

Fig. 5. Effects of chloramine-T on single I_{TO} channel activity in the cell-attached configuration before (left) and during superfusion (right) of 20 μM chloramine-T. From a holding potential of −50 mV with respect to the resting potential, a 500-ms depolarizing pulse was applied to +100 mV from the resting potential every 10 s. Five representative traces obtained from one patch are shown for each condition in the upper panel. Horizontal dashed lines through the traces correspond to the closed level. The lower panel shows the corresponding ensemble currents obtained from 120 consecutive sweeps. Horizontal dashed lines indicate the zero current level.

an amplitude of 1.2 ± 0.3 pA/pF at $+40$ mV (Fig. 4C, $n = 5$).

3.4. Effects of chloramine-T on single I_{TO} channels

To clarify the mechanism underlying the slowing of inactivation and the increase in I_{TO} by chloramine-T, single-channel analyses were conducted in the cell-attached patch mode. Fig. 5 shows typical traces before (left) and during bath application (right) of chloramine-T at 20 μM. Recordings which showed only one active channel per patch were used for analysis. Each trace was obtained with +150-mV depolarization for 500 ms from a holding potential at −50 mV with respect to the resting potential. Under control conditions, most of the open events occurred within the first 200 ms during depolarization. In contrast, chloramine-T at 20 μM produced multiple reopenings or longer 'bursts' during the entire period of depolarization. In a total of 5 cells, chloramine-T at 20 μM increased the probability of the channel being open from 0.15 ± 0.03 to 0.46 ± 0.04 ($P < 0.05$), and the mean open lifetime from 5.1 ± 0.51 ms to 7.0 ± 0.64 ms ($P < 0.05$), while the unitary current amplitude was unchanged (1.2 ± 0.06 pA during control vs. 1.2 ± 0.07 pA in the presence of chloramine-T). These changes resulted in both slowing of inactivation and an increase in the peak of I_{TO} in the ensemble currents (bottom traces). At a higher concentration of 100 μM, the channel activities were obscured due to instability of the current traces even in the presence of glibenclamide ($n = 3$, not shown), which suggests impairment of the GΩ seal of the patch. This instability of the traces prevented us from collecting sufficient data for quantitative analysis of the effects of chloramine-T at 100 μM.

4. Discussion

In the present study, we demonstrated that the oxidizing agent chloramine-T slowed the fast inactivation time course of native I_{TO} in rabbit atrial myocytes. The apparent slowing of inactivation was not due to enhancement of the background currents such as I_{Cl} and $I_{K\text{-}ATP}$, but was due to removal of the inactivation process. The oxidation of the channel proteins at SH-containing amino acid(s) appeared to be involved in the slowing of I_{TO} inactivation because chloramine-T had no effect on I_{TO} in the presence of dithiothreitol (Fig. 3A), and the chloramine-T-induced slowing of I_{TO} inactivation was partially reversed by dithiothreitol (Fig. 3B). These results were in good agreement with those of a recent study on Shaker K^+ channels, which showed that oxidation of methionine residues is responsible for the chloramine-T-induced slowing of K^+ channel inactivation (Ciorba et al., 1997). Single-channel analysis revealed that chloramine-T at 20 μM produced an increase in the probability of the channel being open and a prolongation of the mean open lifetime with the unit amplitude unchanged (Fig. 5). These alterations of single channel behavior explain the enhancement of the peak and the slowing of inactivation of I_{TO}. Similar chloramine-T-induced changes in single channel behavior were previously reported for Na^+ channels in neuroblastoma cells (Nagy, 1988; Niemann et al., 1991).

At a higher concentration of 100 μM, chloramine-T inhibited I_{TO} (Fig. 2). The lack of effect of 100 μM chloramine-T on I_{TO} in the presence of dithiothreitol (Fig. 3A) indicated that the inhibitory action of chloramine-T was also mediated by oxidation. Schlief et al. (1996) reported that chloramine-T at 100 μM or higher inhibited the A-current expressed by the Shaker K^+ channel mutant. This inhibition was demonstrated to be due to oxidation of a methionine residue within the channel pore. Thus, higher concentrations of chloramine-T could affect the open probability or the conductance of the single I_{TO} channels by modifying the channel pore structure. However, we could not clarify the effects of high concentrations of chloramine-T on single I_{TO} channels because of the instability of the recordings. There is one previous report on the effects of oxidation on single K^+ channels. Cai and Sauvé (1997) demonstrated that the SH-oxidizing agent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) inhibited the Ca^{2+}-activated K^+ channels with no effect on the single channel conductance. A possible mechanism for the observed inhibition of I_{TO} is a change in the voltage dependence of activation, as demonstrated in neuroblastoma cells by Rouzaire-Dubois and Dubois (1990), who found that inhibition of the A-current by chloramine-T (500 μM) resulted from a shift in the voltage-dependence to more positive potentials. In the present study, however, no voltage-dependent shift was observed in the current–voltage relationship for I_{TO} with 100 μM chloramine-T (Fig. 2B). Another mechanism for the inhibition of I_{TO} was suggested by Huang (1995), namely, that the chloramine-T (500 μM-1 mM)-induced inhibition of the A-current in rat central neurons was due to deterioration of the condition of the cell. The cardiac cells may well have deteriorated as a result of oxidation by chloramine-T. In this regard, Timerman et al. (1990) reported that oxidation of glutathione in the sarcolemma by externally applied oxidants led to irreversible cell damage, causing hypercontracture of the contractile elements and disruption of sarcolemmal integrity.

We found that the chloramine-T-induced slowing of I_{TO} was reversed by dithiothreitol (Fig. 3A). This indicated that native I_{TO} can be reversibly modulated by altered redox states of the SH-containing channel protein. Our results are in good agreement with those of Stephens et al. (1996), who demonstrated that chloramine-T-induced slowing of the inactivation of the A-current (Kv 1.4 channels) expressed in human embryonic kidney (HEK-293) cells was reversed by dithiothreitol. Also, Ruppersberg et al. (1991) showed a reversal by dithiothreitol of the slow-

ing of the fast inactivation of the A-current (Kv 1.4 channels) in inside-out patches of oocyte membranes. However, in contrast to the slow and partial recovery observed by us, these reports showed rapid and total recovery of the inactivation time course: within 3 min in HEK-293 cells and within seconds in the oocyte membrane patches. Although the different accessibility of dithiothreitol to the target SH group may be due to differences in the properties of channels or membranes used, the very quick effect of dithiothreitol in excised patches (Ruppersberg et al., 1991) would suggest that the oxidized SH group is located close to the cytoplasmic membrane surface.

Besides the effects on I_{TO}, chloramine-T at 100 μM activated a glibenclamide-sensitive K^+ current, i.e., $I_{K\text{-}ATP}$. The activation of this current appeared to be mediated by oxidation because the current was not activated in the presence of dithiothreitol (Fig. 3A). These results are relevant to the previous observations by Ichinari et al. (1996) and Tokube et al. (1996). They demonstrated that oxidants activated ATP-sensitive K^+ channels in ventricular myocytes. However, inhibition of ATP-sensitive K^+ channels by SH oxidation was reported by Coetzee et al. (1996) and Han et al. (1996) in ventricular myocytes. The gradual run-down of the time-independent current after maximal activation (Fig. 4A) may indicate such an inhibitory action. In the presence of glibenclamide chloramine-T induced a small background current that had a reversal potential of approximately −15 mV (Fig. 4C) suggesting that the current might be a chloride current or a non-specific cation current. Since investigation of these background currents was not our major goal, we did not examine the properties of the background currents any further.

Cardiac I_{TO} is an important determinant of repolarization (Campbell et al., 1995). If I_{TO} channels are oxidized in situ, the current would be modified as observed in the present study. The slowing of inactivation and enhancement of I_{TO} would shorten action potentials, while depression of I_{TO} would prolong them. Such changes in the action potentials may produce inhomogeneity in refractoriness, and may thereby contribute to the genesis of arrhythmias when redox states are altered, e.g., by ischemia and/or by reperfusion injury. At present, it is uncertain to what extent I_{TO} channels are oxidized under such pathophysiological conditions. The significance of the redox modulation of cardiac I_{TO} in situ should be investigated further in future studies.

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