Sodium Channel Gating Modes During Redox Reaction

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Key Words

Redox • Hydrogen peroxide • Sodium channel • Cardiac myocyte • Patch-clamp techniques

Abstract

Background/Aims: Many studies have confirmed that persistent sodium current (I_{NaP}) is altered during a redox reaction, but little attention has been paid to transient sodium current (I_{NaT}) and its correlation with I_{NaP} during the redox reaction. The aim of the study was to investigate the effect of the redox states on the correlation between I_{NaT} and I_{NaP} in cardiomyocytes. **Methods:** I_{NaT} and I_{NaP} were recorded using whole-cell and cell-attached patch-clamp techniques in guinea pig ventricular myocytes. **Results:** In whole-cell recordings, dithiothreitol (DTT, 1 mM) simultaneously increased I_{NaT} and decreased I_{NaP} . Hydrogen peroxide (H_2O_2 , 0.3 mM) increased I_{NaP} and decreased I_{NaT} in a time-dependent manner, which were reversed by DTT (1 mM). In cell-attached recordings, the increasing of I_{NaP} and decreasing of I_{NaT} induced by H_2O_2 (0.3 mM) were similarly recovered by DTT (1 mM). H_2O_2 (0.3 mM) prolonged the action potential (AP) duration of ventricular papillary cells whereas decreased the AP amplitude and maximum rate of depolarization (V_{max})

in a time-dependent manner, which were reversed by DTT (1 mM). **Conclusion:** These results indicate that the redox states could modulate the sodium channel gating modes in guinea pig ventricular myocytes.

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Introduction

Sodium current is an important ion current in cardiac tissues. Transient sodium current (I_{NaT}) is responsible for the upstroke and conduction of the action potential (AP). Persistent sodium current (I_{NaP}) plays an important role in maintaining the plateau of the AP, determining AP duration (APD), transmural dispersion of repolarization and development of cardiac arrhythmias. According to recent reports, gradients of I_{NaT} and I_{NaP} across the left ventricular wall occur, which may play key roles in normal cardiac function as well as in susceptibility to arrhythmias [1-4]. Some studies reported that hypoxia can increase cardiomyocyte I_{NaP} and the potentiated I_{NaP} can induce intracellular Ca^{2+} overload and ischemic arrhythmias [5-7]. Previous studies suggested that I_{NaP} was produced by

a change in the gating mode of I_{NaT} , possibly because of a change in phosphorylation or protein structure, e.g. modification of inactivation gate [8]. It is generally speculated that I_{NaP} might result from multiple reopenings of a small fraction of I_{NaT} or that a naturally occurring isoform of I_{NaT} could account for I_{NaP} [9]. Literature reported that hypoxia could increase I_{NaP} [5] but decrease I_{NaT} [10]. Similarly, nitric oxide also enhances I_{NaP} [11] whereas inhibits I_{NaT} [12]. These results suggest that there may be some correlations between I_{NaT} and I_{NaP} during a redox reaction. But up to now little attention has been paid to this issue. A burst of hydrogen peroxide (H_2O_2), an important reactive oxygen species, is generated in the myocardium during ischemia and reperfusion [13–20]. The excessive amount of H_2O_2 leads to intracellular Ca^{2+} overload and cell damage [21, 22]. Thus, in the former article we researched the effect of H_2O_2 on I_{NaP} [23]. We found that H_2O_2 increased the amplitude of whole-cell I_{NaP} in a concentration-dependent manner and persistent sodium channel activity in cell-attached and inside-out patches whereas reducing agent glutathione (GSH) recovered these changes induced by H_2O_2 [23]. H_2O_2 is a cysteine-specific oxidizing agent and dithiothreitol (DTT) is a cysteine-specific reducing agent [24]. In the present study we used H_2O_2 and DTT as a redox system, made cysteine-specific residues of sodium channel in ventricular myocytes as target, and I_{NaP} , I_{NaT} together with AP as observational indexes to probe into the correlation between I_{NaP} and I_{NaT} . We found that the redox states could modulate the sodium channel gating modes in guinea pig ventricular myocytes.

Material and Methods

Solutions and drugs

The Tyrode solution contained (in mM): NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.33, glucose 10, HEPES 10 (pH 7.4). The KB solution contained (in mM): KOH 70, taurine 20, glutamic acid 50, KCl 40, KH_2PO_4 20, $MgCl_2$ 3, EGTA 0.5, glucose 10, HEPES 10 (pH 7.4).

For whole-cell current recordings, the intracellular pipette solution contained (in mM): CsCl 120, $CaCl_2$ 1, $MgCl_2$ 5, Na_2ATP 5, tetraethylammonium (TEA) 10, EGTA 11, HEPES 10 (pH 7.3). In general, I_{NaT} was examined using a low-sodium bath solution [25] and I_{NaP} recorded using a high-sodium bath solution [1]. In this research, I_{NaT} and I_{NaP} must be recorded simultaneously to reveal the correlation between them. Therefore, low sodium solutions and several intermediate values up to 135 mM NaCl were tried. Finally we chose a modified Tyrode solution as a bath solution containing (in mM) NaCl 65, CsCl 70, $CaCl_2$ 1, $MgCl_2$ 1, $CdCl_2$ 0.05, glucose 5, HEPES 5 (pH 7.4).

For cell-attached, patch-clamp recordings, the pipette solution contained (in mM): NaCl 180, KCl 1.3, $CaCl_2$ 1.5, $MgCl_2$ 0.5, Na_2ATP 5, $CoCl_2$ 3, TEA 10, 4-aminopyridine 10, CsCl 10, glucose 5, HEPES 5 (pH 7.4). The cells were initially immersed in Tyrode solution. After obtaining the cell-attached configuration, a high-potassium extracellular solution (in mM, potassium acetate 140, NaCl 5, $MgCl_2$ 4, glucose 25, HEPES 10, pH 7.4) was superfused into the bath to bring the cell membrane potential (RP) close to 0 mV.

Collagenase type I and CsCl were obtained from Gibco (GIBCO TM, Invitrogen Co., Paisley, UK). Protease E, 4-aminopyridine, TEA, Na_2ATP , and EGTA were purchased from Sigma (Saint Louis, MO, USA). Bovine serum albumin (BSA), HEPES, DTT and taurine were obtained from Roche (Basel, Switzerland). Tetrodotoxin (TTX) was purchased from Hebei Fisheries Research Institute (Qinhuangdao, China). H_2O_2 was a production of Wuhan Zhongnan Chemical Reagent Co. (Wuhan, China). All other chemicals were purchased from Sigma. Stock solutions of drugs were prepared in water. Each of the stocks was diluted to the required concentration in the external recording solution immediately before use.

Isolation of myocytes

Single cardiac ventricular myocytes were enzymatically isolated from adult guinea pigs (250–300 g, of either sex, Grade II) as preciously described [23]. In brief, guinea pigs were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) 20 minutes after an intraperitoneal injection of 2000 units of heparin. Hearts were excised rapidly, and retrogradely perfused through the aorta using a modified Langendorff apparatus. The following solutions were applied: (1) Tyrode solution without added Ca^{2+} (' Ca^{2+} -free') for 5 minutes; (2) Ca^{2+} -free Tyrode solution containing 0.1 mg/ml collagenase type I; 0.01 mg/ml protease E; 0.5 mg/ml BSA for 8–10 minutes; and (3) a 'KB' solution for the final 5 minutes. These perfusates were bubbled with 100% O_2 and maintained at 37 °C. The ventricles were cut into small chunks and gently agitated in KB solution. The cells were filtered through nylon mesh and stored in KB solution at 4 °C until use. The study conforms to national Guide for the Care and Use of Laboratory Animals.

Current recordings

Currents were recorded from isolated myocytes using an EPC 9 amplifier (Heka Electronic, Lambrecht, Pfalz, Germany) by means of either whole-cell or cell-attached configuration of the patch-clamp technique. The patch electrodes had resistances of 1–2 M Ω (whole cell) or 6–10 M Ω (cell attached) when filled with the pipette solutions. Capacitance and series resistances were adjusted to obtain minimal contribution of the capacitive transients. A 60% to 80% compensation of the series resistance was usually achieved without ringing. Currents were filtered at 2 kHz, digitized at 10 kHz and stored on a computer hard disk for analysis. Experiments were done at room temperature (22 ± 2 °C).

For the steady-state activation protocol, currents were elicited by a 500 msec depolarizing pulses applied at 0.5 Hz from a holding potential (HP) of -120 mV, in 10 mV increments between -110 and +70 mV. The steady-state inactivation

Fig. 1. Effects of TTX and DTT on I_{NaT} and I_{NaP} in guinea pig ventricular myocytes. Current were evoked by a voltage step to -30 mV from a holding potential of -120 mV. *A.* 1.5 μ M TTX abolished I_{NaP} , but had little effect on I_{NaT} . *B.* 1 mM DTT simultaneously increased I_{NaT} and decreased I_{NaP} . Left panels show representative example of current recordings before and after treatment. These two current tracks were superposed and displayed in right panels. Note the scale in insets.

protocol was from a HP of -120 mV; a 500 msec conditioning prepulse was applied in 10 mV increments between -130 and -30 mV followed by a 12 msec test pulse to -20 mV at 0.5 Hz. These measurements were made after a minimal time of 20 minutes after rupture of the patch to minimize the contribution of time-dependent shifts of steady-state gating parameter measurements.

Action potential recordings

Guinea pig ventricular papillary muscles were isolated as previously described [26]. The cardiac tissues were continuously superfused with oxygenated Tyrode solution that was equilibrated at 36-37 °C. They were stimulated with square pulses (basic cycle length [BCL]=2000 msec, 2 msec duration, 50% above the threshold voltage) using a stimulator with a stimulus isolation unit (RM6240C, Chengdu Instrument Co., Chengdu, China). After allowing 2 hours for stabilization, the APs were recorded with a 3 M KCl-filled conventional microelectrode (10-20 M Ω) connected to an amplifier (SWF-1B, Chengdu Instrument Co., Chengdu, China). The AP waveforms were stored to a computer hard disk for analysis.

Data analysis

Whole-cell recordings were analyzed using PulseFit (V8.74, HEKA). Current density was calculated by dividing the current amplitude by the cell capacitance. The amplitude of I_{NaP} was recorded at 200 msec of the pulse to eliminate the effect of I_{NaT} . Data from steady-state activation and inactivation relationship of I_{NaT} were fitted to the Boltzmann equation: $Y=1/[1+\exp((V_m - V_{1/2})/k)]$, where V_m is the membrane potential, $V_{1/2}$ is the half-activation or half-inactivation potential, and k is the slope factor. For steady-state activation curves, Y stands for

the relative conductance. The chord conductance was calculated using the ratio of the current to their electromotive for potential in individual current-voltage relationships. Then these conductances were normalized to their individual maximal conductance. For steady-state inactivation curves, Y represents the relative current ($I_{NaT}/I_{NaT,max}$).

Single-channel recordings were analyzed using TAC+TACFit (X4.0.9, Bruyton, Seattle, Washington, USA). Capacitance transients and leakage currents were nullified by off-line subtracting fits of average blunt traces. The channel activity after a 50 msec depolarization pulse was calculated as persistent sodium channel. Open probability was calculated from the total open times of at least 100 sweeps divided by the total sweep duration. Histograms of channel open time distribution were fitted to single exponentials using TACFit.

All data were expressed as mean $\pm$ SD. Statistical significance was estimated by Student's t test or the one-way analysis of variance (ANOVA), as appropriate. When the F -value allowed, Tukey multiple comparisons test was used to compare the groups *versus* control. $P<0.05$ was considered significant.

Results

Whole-cell currents

Isolated guinea pig ventricular myocytes were voltage-clamped using ruptured whole-cell patch-clamp technique. Currents were evoked by 500 msec voltage pulse to -30 mV from a HP of -120 mV.

Fig. 2. Effects of DTT on steady-state activation and inactivation curves for I_{NaT} . Panel *A* and *B*. Examples of I_{NaT} recordings elicited according to the activation and inactivation protocol, respectively. Panel *C* shows the steady activation and inactivation curves for I_{NaT} under control conditions and after application of DTT. Lines represent the fit of data to a Boltzmann distribution function.

Table 1. Effects of DTT and H_2O_2 on I_{NaT} and I_{NaP} in Guinea Pig Ventricular Myocytes. Values are expressed as mean $\pm$ SD. * $P < 0.05$; $^\dagger P < 0.01$ versus control group.

	I_{NaT} Current Density (pA/pF)	I_{NaP} Current Density (pA/pF)
Control	-47.2 ± 3.6	-0.39 ± 0.02
1 mM DTT	$-54.3 \pm 3.7^\dagger$ $n = 8$	$-0.22 \pm 0.03^\dagger$ $n = 8$
Control	-46.7 ± 4.9	-0.39 ± 0.03
0.3 mM H_2O_2 7 min	$-40.2 \pm 3.3^*$	$-0.57 \pm 0.05^\dagger$
0.3 mM H_2O_2 15 min	$-33.4 \pm 3.3^\dagger$	$-0.74 \pm 0.07^\dagger$
1 mM DTT	$-53.8 \pm 3.7^*$ $n = 7$	$-0.27 \pm 0.04^\dagger$ $n = 7$

Table 2. Effects of DTT and H_2O_2 on Steady-State Activation and Inactivation Parameters of I_{NaT} . Values are expressed as mean $\pm$ SD. * $P < 0.05$; $^\dagger P < 0.01$ versus control group.

	Steady-state activation		Steady-state inactivation	
	$V_{1/2}$ (mV)	k (mV)	$V_{1/2}$ (mV)	k (mV)
Control	-44.36 ± 0.79	6.34 ± 0.70	-88.30 ± 0.94	-7.18 ± 0.87
1 mM DTT	$-54.87 \pm 1.12^\dagger$ $n = 7$	6.76 ± 0.97 $n = 7$	-86.90 ± 1.64 $n = 6$	-7.62 ± 1.52 $n = 6$
Control	-44.30 ± 0.58	6.03 ± 0.51	-88.41 ± 0.52	-6.70 ± 0.48
0.3 mM H_2O_2	-45.94 ± 2.31	$8.47 \pm 2.17^*$	$-94.57 \pm 0.69^\dagger$	$-7.46 \pm 0.65^*$
1 mM DTT	$-54.30 \pm 1.11^\dagger$ $n = 7$	$6.92 \pm 0.97^*$ $n = 7$	-89.49 ± 1.82 $n = 6$	$-8.16 \pm 1.75^*$ $n = 6$

I_{NaP} is TTX-sensitive and 1.5 μM TTX can block it completely [1]. Thus we examined the effects of TTX on I_{NaT} and I_{NaP} . 1.5 μM TTX blocked I_{NaP} completely, whereas had little effect on I_{NaT} (Figure 1A).

The effects of reducing agent DTT on I_{NaT} and I_{NaP} were studied. 1 mM DTT simultaneously increased I_{NaT} and decreased I_{NaP} (Table 1, Figure 1B).

We further examined the effects of DTT on the

Fig. 3. Effects of H_2O_2 on I_{NaP} and I_{NaT} in guinea pig ventricular myocytes. *A.* H_2O_2 increased amplitude of I_{NaP} and decreased amplitude of I_{NaT} in a time-dependent manner. These effects were reversed by DTT. The traces show current recordings during control conditions, 7 min and 15 min after perfusion with 0.3 mM H_2O_2 , and after application of 1 mM DTT. *B.* The mean current densities of I_{NaP} and I_{NaT} under different conditions. $n=7$ cells. Mean $\pm$ SD, * $P<0.05$; † $P<0.01$ versus control group.

steady-state activation and inactivation curves for I_{NaT} . Figure 2A shows typical examples of I_{NaT} recordings obtained with incremental test pulses of the steady-state activation protocol. 1 mM DTT increased I_{NaT} . Furthermore, it induced a drastic leftward shift of the activation curve and the half-activation potential ($V_{1/2}$) was shifted -10.51 mV (Table 2, Figure 2C). Typical examples of I_{NaT} recordings obtained according to the steady-state inactivation protocol are shown in Figure 2B. DTT was devoid of significant effect on the inactivation process (Table 2, Figure 2C).

The effects of H_2O_2 on I_{NaT} and I_{NaP} were studied. The administration of 0.3 mM H_2O_2 resulted in decreased amplitudes of I_{NaT} and increased amplitudes of I_{NaP} . These actions progressed with the increment in action time. After application of 1 mM DTT, those changes described above were recovered (Table 1, Figure 3). We found that the changes of I_{NaT} in either direction were accompanied by the contrary changes of I_{NaP} , that is, when I_{NaT} was increased, I_{NaP} was decreased and vice versa.

Figure 4 shows the effects of H_2O_2 on the steady-state activation and inactivation curves for I_{NaT} . 0.3 mM H_2O_2 decreased I_{NaT} gradually, whereas 1 mM DTT reversed it (Figure 4A). H_2O_2 was devoid of significant effect on steady-state activation curve whereas it was shifted toward more negative membrane potentials after application of DTT (Table 2, Figure 4C). H_2O_2 induced a drastic leftward shift of the inactivation curve, which suggests that inactivation process was augmented, whereas DTT recovered them (Table 2, Figure 4C).

Cell-attached, single-channel currents

To further confirm the findings in whole-cell recording, experiments were carried out in cell-attached configuration of the patch-clamp technique. In cell-attached recordings, currents were activated by 700 msec voltage pulse to -50 mV from a HP of -120 mV.

The representative traces are shown in Figure 5. Under control conditions, persistent sodium channel activity was very low or often absent, with multiple

Fig. 4. Effects of H_2O_2 on steady-state activation and inactivation curves for I_{NaT} . Panel A and panel B show representative I_{NaT} recordings elicited according to the activation and inactivation protocol, respectively. Panel C shows the steady-state activation and inactivation curves for I_{NaT} under different conditions.

openings of transient sodium channel being seen (Figure 5A). After 10 minutes perfusion with 0.3 mM H_2O_2 , persistent sodium channel activity increased remarkably, and the current type was changed from original background currents to burst currents gradually. Meanwhile, the transient sodium channel activity was decreased and the multiple openings disappeared (Figure 5B). After 20 minutes perfusion, the persistent sodium channel activity increased, the current amplitudes augmented remarkably and multiple openings appeared. The transient sodium channel activity continued to decrease (Figure 5C). After application of 1 mM DTT, persistent and transient sodium channel activities were recovered (Figure 5D). The ensemble average currents were similar to the currents recorded in whole-cell configuration (Figure 5E).

H_2O_2 (0.3 mM) 20 minutes increased the mean open probability and mean open time of persistent sodium

channel from control value 0.018 ± 0.002 and 0.694 ± 0.079 msec to 0.204 ± 0.030 and 1.565 ± 0.243 msec, respectively ($n=6$, both $P < 0.01$ versus control). They were decreased to 0.027 ± 0.004 and 0.767 ± 0.080 msec after application of DTT (1 mM), respectively ($n=6$, both $P > 0.05$ versus control). Figure 6 gives an example of corresponding all-point histograms and mean open-time histograms from another cell-attached patch.

Action potential recordings

APs were recorded using standard intracellular micropipette techniques in guinea pig papillary muscular cells. 1 mM DTT increased the AP amplitude (APA) and maximum rate of depolarization (V_{max}) whereas shortened AP duration at 90 % repolarization (APD_{90}) under control conditions (Table 3, Figure 7A). TTX shortened APD_{90} in concentration-dependent manner under control condition and APD_{90} came to a minimum

Fig. 5. Effect of DTT on persistent and transient sodium channel activities induced by H_2O_2 . The individual current trace was evoked by a voltage step to -50 mV from a holding potential of -120 mV in a cell-attached patch. The upper four traces in each of panels A-D are original current records whereas the fifth trace in each panel shows the average of 180, 150, 150, and 160 sweeps, respectively. A. Control. B. 10 min after perfusion with 0.3 mM H_2O_2 . C. 20 min after perfusion with 0.3 mM H_2O_2 . D. 0.3 mM H_2O_2 + 1 mM DTT. E. The fifth traces in panels A,B and panels C,D are superposed and displayed in left, right part of panel E, respectively. Note the scale.

when the concentration of TTX reached 1.5 μM (Table 3, Figure 7B). 0.3 mM H_2O_2 prolonged the APD_{90} but decreased APA and V_{max} in a time-dependent manner, reaching stable at approximately 15 minutes (Table 3, Figure 7C). In two additional experiments, 1.5 μM TTX and 1 mM DTT were applied respectively after 15 minutes perfusion with 0.3 mM H_2O_2 . TTX reversed the APD_{90} , whereas had no effect on the decrease of APA and V_{max} (Table 3, Figure 7D). However, DTT reversed the changes of APD_{90} , APA, and V_{max} induced by H_2O_2 (Table 3, Figure 7E).

Discussion

In this study, the effects of H_2O_2 on I_{NaT} and I_{NaP} were studied in guinea pig ventricular myocytes. In whole-cell patch-clamp recordings, DTT simultaneously increased I_{NaT} and decreased I_{NaP} . The steady-state activation curves were shifted toward more negative membrane potentials. And H_2O_2 increased I_{NaP} in a time-dependent manner, which was consistent with our previous report that H_2O_2 increased I_{NaP} in a concentration-dependent manner [23]. In the present study, H_2O_2 also decreased I_{NaT} simultaneously and DTT

Fig. 6. Effect of DTT on mean open time and mean open probability of persistent sodium channels enhanced by H_2O_2 . Panels A,D; B,E; and C,F come from 180, 160, and 180 individual current traces in a cell-attached patch, respectively. Panels A-C show the mean open time histograms, and panels D-F show the all-point histograms. A, D Control, B, E 0.3 mM H_2O_2 20 min, and C, F 0.3 mM H_2O_2 + 1 mM DTT. The fitted mean open times were 0.643 msec, 1.586 msec, and 0.738 msec in A-C, respectively.

reversed these effects induced by H_2O_2 . The changes of I_{NaT} were accompanied by the opposite changes of I_{NaP} , that is, when I_{NaT} was increased, I_{NaP} was decreased, vice versa. This suggests that I_{NaP} is associated with I_{NaT} . Thus we further studied the steady-state activation and inactivation relationships of I_{NaT} . The activation process of I_{NaT} was not affected by H_2O_2 , but the inactivation curve was shifted toward more negative membrane potentials, indicating the augmentation of inactivation process (Figure 4). DTT-induced augmentation of I_{NaT} is the result of a drastic leftward shift of the activation curve (Figure 4).

To further support the finding above, cell-attached single-channel recordings were made. H_2O_2 gradually increased I_{NaP} and decreased I_{NaT} with time, whereas DTT recovered these effects of H_2O_2 on persistent and transient sodium channel (Figure 5). These results were consistent with our recordings under the whole-cell recording configuration and confirmed our finding that I_{NaP} is associated with I_{NaT} and they could experience an

interconversion in contrary direction during a redox reaction. At present some researchers hold that I_{NaT} and I_{NaP} come from two different channel subtypes [27]. Others maintain that they arise from different modulation of channel gating within a single subtype [28-32]. Recently a study in a canine heart failure model and from a failing human heart shows that I_{NaT} is decreased, I_{NaP} is increased. Neither alpha subunit isoform switching nor altered beta subunit expression assumes responsibility for increased late I_{Na} [33]. According to previous reports and our data, we presume that I_{NaT} and I_{NaP} may be two different gating modes of a channel and could lead to interconversion modulated by the redox states. Due to the invariability of the sum of transient sodium channels, many I_{NaT} -charged gates may be switched to I_{NaP} -charged gates during application of H_2O_2 , resulting in more I_{NaP} gating modes and less I_{NaT} gating modes. Therefore, I_{NaP} was increased while I_{NaT} was decreased compared with normal conditions; on the contrary, after exposure to DTT many I_{NaP} -charged gates may be transited to I_{NaT} -charged

	RP (mV)	APA (mV)	V _{max} (V/s)	APD ₉₀ (ms)
Control	-86.5 ± 2.4	107.9 ± 4.4	162.1 ± 15.2	253.7 ± 8.4
1 mM DTT	-86.5 ± 2.5	118.0 ± 5.8*	197.5 ± 19.1 [†]	230.1 ± 12.3 [†]
	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5
Control	-86.3 ± 2.5	115.0 ± 4.1	167.3 ± 12.5	300.7 ± 10.0
0.5 μM TTX	-85.9 ± 2.5	110.3 ± 5.1	165.3 ± 12.3	273.7 ± 11.4 [†]
1.5 μM TTX	-85.7 ± 2.4	109.0 ± 5.3	162.8 ± 12.2	242.3 ± 7.3 [†]
3.0 μM TTX	-85.6 ± 2.2	108.4 ± 5.3	161.8 ± 12.2	240.2 ± 8.7 [†]
	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5	<i>n</i> = 5
Control	-86.0 ± 5.9	105.8 ± 6.2	163.0 ± 15.8	249.4 ± 13.8
0.3 mM H ₂ O ₂ 7 min	-85.5 ± 5.6	97.4 ± 7.1*	140.2 ± 12.9 [†]	304.5 ± 15.1 [†]
0.3 mM H ₂ O ₂ 15 min	-85.1 ± 5.7	89.9 ± 6.6 [†]	114.1 ± 12.0 [†]	349.8 ± 14.9 [†]
0.3 mM H ₂ O ₂ 30 min	-84.6 ± 5.7	88.7 ± 6.6 [†]	111.8 ± 11.7 [†]	355.1 ± 14.1 [†]
	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 8
Control	85.7 ± 6.0	107.6 ± 6.8	167.0 ± 13.3	278.1 ± 15.0
0.3 mM H ₂ O ₂ 15 min	-85.4 ± 5.9	96.0 ± 4.9 [†]	111.9 ± 13.2 [†]	372.5 ± 17.7 [†]
1.5 μM TTX 5 min	-85.0 ± 5.8	95.1 ± 4.5 [†]	109.3 ± 12.7 [†]	229.7 ± 14.6 [†]
	<i>n</i> = 7	<i>n</i> = 7	<i>n</i> = 7	<i>n</i> = 7
Control	-86.4 ± 5.4	111.8 ± 4.3	169.2 ± 12.9	269.0 ± 11.8
0.3 mM H ₂ O ₂ 15 min	-85.8 ± 6.0	91.8 ± 5.1 [†]	117.1 ± 11.5 [†]	360.7 ± 18.5 [†]
1 mM DTT 5 min	-86.0 ± 5.7	118.7 ± 5.1*	192.9 ± 14.5 [†]	242.7 ± 17.0 [†]
	<i>n</i> = 7	<i>n</i> = 7	<i>n</i> = 7	<i>n</i> = 7

Table 3. Effects of H₂O₂ on Action Potential Waveforms in Guinea Pig Papillary Muscle Cells. RP = resting membrane potential; APA = action potential amplitude; V_{max} = maximum rate of depolarization; APD₉₀ = action potential duration at 90 % repolarization. Values are expressed as mean ± SD. **P*<0.05; [†]*P*<0.01 versus control group.

gates, leading to more gating modes of I_{NaT} and less gating modes of I_{NaP}. Thus I_{NaT} was enhanced while I_{NaP} was decreased compared with during administration of H₂O₂. The enhancement of I_{NaP} is associated with the inactivation of I_{NaT}. H₂O₂ increases the number of I_{NaP} gating modes, therefore shifts inactivation curve toward more negative membrane potentials (Figure 4D). Considering the leftward shift of the activation curve induced by DTT, which also could result in the enhancement of I_{NaT}, we cannot conclude that the enhancement of I_{NaT} produced by DTT is consequentially result from the transition of some sodium channels from I_{NaP} gating modes to I_{NaT} gating modes. But the change tendency of I_{NaT} induced by DTT is agree with our hypothesis.

In the present study, we found that the redox states can modulate the activity of the sodium channel. However, the mechanism underlying that is unclear. Since both H₂O₂ and DTT are cysteine-specific reagent [24], we assume that they could work by modulating the redox status of

the cellular cysteine residues. It is known that there are 42 cysteine residues in the alpha subunit of the human cardiac voltage-gated sodium channel hNav1.5. They distribute in the four S5–S6 linkers, intracellular linkers between domains I and II, II and III, and other regions of the channel. Thus H₂O₂ and DTT could change the gating mode of the sodium channel by directly act on one or more cysteine residues in the sodium channel. In addition, protein kinase C (PKC) are susceptible to redox modification [34, 35], which can be activated by oxidative stress and inhibited by antioxidants [35–37, 35]. H₂O₂ can directly activate PKC by modifying the redox states of the critical cysteine residues [34, 38]. And it is well known that the phosphorylation of the cardiac sodium channel during activation of protein kinase C (PKC) can modulate sodium current [39–41]. Thus, H₂O₂ and DTT also could indirectly change sodium channel gating by modulating the activity of PKC. In summary, we suppose that the redox states could adjust the activity of the sodium channel by acting on the cysteine residues of the cellular sodium

Fig. 7. Effect of DTT on action potential waveforms in guinea pig papillary cells induced by H_2O_2 . Effects of DTT, TTX, and H_2O_2 on action potential waveforms are displayed in panel A–C, respectively. Panel D,E show effects of TTX and DTT on action potential waveforms induced by H_2O_2 , respectively.

channel or other protein. However, the definite mechanism remains to be elucidated.

In our experiment, H_2O_2 prolonged APD_{90} of guinea pig papillary muscle cells in a time-dependent manner (Figure 7C), which is consistent with most of the previous researches [41–44] but different from report of Choi *et al.* [45]. The disparity may be attributed to species differences. Moreover, H_2O_2 also decreased the APA and V_{max} . The prolongation of APD_{90} and the reduction of APA and V_{max} could induce cardiac arrhythmias. Therefore, H_2O_2 could play an important role in generation of arrhythmias during ischemia and reperfusion. After 5 min application of 1 mM DTT, those changes of AP induced by H_2O_2 all were recovered. These results were consistent with our recordings under the whole-cell and cell-attached and further indicated that the redox states could modulate the sodium channel gating modes.

Class I anti-arrhythmias agents are widely used in the management of ventricular arrhythmias. Their antiarrhythmic effects possibly result from depression of I_{NaP} , which is more sensitive than I_{NaT} to class I anti-arrhythmias agents [46]. These drugs block I_{NaP} but have little effect on I_{NaT} at proper concentration. Thus they can eliminate arrhythmias. At higher concentrations, they also block I_{NaT} to some extent, which could induce

generation of arrhythmias. Compared with these drugs, DTT can recover I_{NaT} in addition to depression of I_{NaP} . Thus, we speculate that reducing reagents could have better anti-arrhythmias perspective than class I anti-arrhythmias agents.

Previous reports have confirmed that I_{NaP} is more sensitive than I_{NaT} to TTX and class I anti-arrhythmias agents like lidocaine. Nevertheless, the mechanism underlying that is unclear yet. In this study, our hypothesis is that I_{NaT} and I_{NaP} may arise from two different gating of a sodium channel. Therefore, we assume that the sensitive to TTX and class I agents is correlate with the configuration of the sodium channel. The configuration of I_{NaP} gating mode is prone to block by those agents.

In summary, our present results indicate that the redox states could modulate the sodium channel gating modes in guinea pig ventricular myocytes.

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