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Vitamin C Pretreatment Attenuates Hypoxia-Induced Disturbance of Sodium Currents in Guinea Pig Ventricular Myocytes

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Abstract. As an important *in vivo* antioxidant, vitamin C is commonly used clinically to alleviate hypoxia-induced heart symptoms. To approach the protective mechanisms of vitamin C on hearts during hypoxia, we investigated the electrophysiological effects of vitamin C (1 mM, pretreated before hypoxia) on Na^+ currents (including transient and persistent Na^+ currents) in guinea pig ventricular myocytes during hypoxia by the whole-cell and single-channel patch-clamp techniques. Whole-cell recordings showed that the mean current density of I_{NaT} in the hypoxia group decreased from the control value of 40.2142 ± 1.7735 to 27.1663 ± 1.8441 pA/pF and current density of I_{NaP} increased from 0.3987 ± 0.0474 to 1.1854 ± 01994 pA/pF ($n = 9$, $P < 0.05$ *vs.* control) at 15 min. However, when vitamin C was administered before hypoxia as pretreatment, I_{NaT} and I_{NaP} varied moderately (mean current density of I_{NaT} decreasing from 41.6038 ± 2.9762 to 34.6341 ± 1.9651 pA/pF and current density of I_{NaP} increasing from 0.3843 ± 0.0636 to 0.6734 ± 0.1057 pA/pF; $n = 9$, $P < 0.05$ *vs.* hypoxia group). Single-channel recordings (cell-patched) showed that the mean open probability and open time of I_{NaP} increased significantly in both groups at hypoxia 15 min. However, the increased current values of the hypoxia group were still marked at hypoxia 15 min ($n = 9$, $P < 0.05$ *vs.* vitamin C + hypoxia group). Our results indicate that vitamin C can attenuate the disturbed effects of hypoxia on Na^+ currents (I_{NaT} and I_{NaP}) of cardiac myocytes in guinea pigs effectively.

Key words: Hypoxia — Vitamin C — Patch clamp — Transient Na^+ current — Persistent Na^+ current

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Introduction

Hypoxia injury, the major cause of ischemic heart disease, frequently results in mortality due to myocardial ischemia, circulatory shock, stroke, arrhythmia and myocardial infarction (Becker et al., 2000; Lefer & Lefer, 1993). It has become increasingly evident that reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated from metabolic processes during hypoxia play a central role in myocardial ischemic injury (Lefer & Granger, 2000; Dhalla, Temsah & Netticadan, 2000). They bring about oxidation of proteins, nucleic acids and lipids and can affect critical signal transduction pathways, resulting in alterations of cardiac function, cellular injury and death (Duranteau et al., 1998; Harsdorf, Li & Dietz, 1999; Hensley et al., 2000). In the heart, ROS-induced abnormalities can include cytotoxicity, cardiac stunning, arrhythmias, alterations of Ca^{2+} homeostasis and intracellular adenosine triphosphate (ATP) depletion (Lefer & Granger, 2000; Dhalla et al., 2000). In addition, hypoxia-induced injury influences myocardial Na^+ currents acutely, leading to significantly decreased transient Na^+ current (I_{NaT}) and increased persistent Na^+ current (I_{NaP}), which will further cause the degradation of excitability and conductibility in cardiac myocytes and ultimately induce reentry arrhythmia.

Critical in combating ROS-induced cell death and tissue damage, cellular antioxidants neutralize free radicals and minimize the oxidative stress resulting from a variety of insults, including ischemia/hypoxia injury (Das & Maulik, 1994; Hayes & McLellan, 1999; Wang et al., 2002; Bielefeldt et al., 1997). Previous work from our laboratories has shown that nitric oxide (NO) generated during hypoxia increases myocardial I_{NaP} significantly; on the contrary, the reducing agent dithiothreitol (DTT) blocks I_{NaP} entirely (Hammarstrom & Gage, 1999;

Bielefeldt et al., 1999; Wang & Ma, 2004). Moreover, H_2O_2 increased I_{NaP} in a concentration-dependent manner, while reduced glutathione (GSH) could reverse the effect of H_2O_2 on I_{NaP} (Barrington, Martin & Zhang, 1997; Luo & Ma, 2005). This means that oxidants enhance I_{NaP} yet reductants or antioxidants inhibit it.

Vitamin C is a potent, nutritionally derived antioxidant that quenches ROS by donating two electrons and becoming oxidized itself to dehydroascorbic acid (DHA). Together with glutathione, vitamin C constitutes the primary line of defense against ROS and participates in the recycling of other antioxidants such as vitamin E (Guaiquil, Vera & Golde, 2001). Vitamin C and glutathione function independently and in concert to protect cells from death induced by oxidative challenge with hydrogen peroxide (Grech, Jackson & Ramsdale, 1995). Many clinical studies have reported that physicians often use vitamin C as an effective cardioprotectant to improve cardiac and endothelial function in coronary artery disease (CAD) by reducing elevated levels of ROS (Molyneux, Glyn & Ward, 2002; Gao et al., 2002), to restore coronary microcirculatory function and impaired coronary flow velocity reserve (CFVR) against oxidative stress in smokers and to inhibit myocardial damage as well as ischemic arrhythmia (Guaiquil et al., 2004; Teramoto et al., 2004; Erbs et al., 2003; Molyneux et al., 2002).

Although there is a great deal of evidence indicating that vitamin C has a protective effect on ischemic heart disease (Guaiquil et al., 2004; Senthil et al., 2004; Molyneux et al., 2002), there have been very few reports about the electrophysiological effect of vitamin C on cadiocytes during hypoxia. Furthermore, since almost no literature about the effects of vitamin C on I_{NaT} and I_{NaP} of cardiocytes has been found up to now, the purpose of this research was to probe further the protective mechanism of vitamin C on cardiocytes against hypoxia. Therefore, we studied the effects of hypoxia and vitamin C + hypoxia on I_{NaT} and I_{NaP} of ventricular myocytes in guinea pigs through the whole-cell and single-channel patch-clamp techniques, which may have never been researched before.

Materials and Methods

Animals used in these studies were cared for according to the Hubei (P.R. China) provincial government's *Guidelines for the Care and Use of Animals*. All experimental procedures were subject to prior approval of the Wuhan University of Science and Technology Animal Ethics Committee.

Isolation of Cells

Adult guinea pigs (250–300 g, either sex) were anesthetized with pentobarbital sodium (30 mg/kg, ip) 20 min after an ip injection of 2,000 units of heparin. Hearts were excised rapidly and perfused retrogradely on a Langendorff apparatus (with Ca^{2+}-free Tyrode's solution for 5 min), before the perfusate was switched to an enzyme-containing solution (0.1 g/liter collagenase type I, 0.01 g/liter protease E, 0.5 g/liter bovine serum albumin [BSA] in the same solution) for 8–10 min. The perfusate was finally changed to KB solution containing (mM): KOH 70, taurine 20, glutamic acid 50, KCl 40, KH_2PO_4 20, $MgCl_2$ 3, egtazic acid 0.5, 4–(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10 and glucose 10 (pH 7.4) for a 5-min period. These perfusates were bubbled with 100% O_2 and maintained at 37°C. Ventricles were cut into small chunks and gently agitated in KB solution. Cells were filtered through nylon mesh and stored in KB solution at 4°C.

Protocol of Hypoxia and Vitamin C + Hypoxia Treatments

The examined cell was continuously perfused with extracellular solution flowing through a small plastic tube from a test tube. Hypoxia was achieved by bubbling the perfuse solution (normal Tyrode's solution without glucose) in this test tube with 100% N_2 and saturating for at least 50 min. The cells were perfused at a constant flow rate (2 ml/min) for at least 20 min after the currents were stable; meanwhile, the perfusing bath was covered by a relatively tight covering and bubbled with 100% N_2 to prevent the oxygen in the air diffusing into the perfuse solution. The oxygen tension in the bath could be reduced to about 6 Kpa (45 mm Hg) in 3–5 min and was monitored by an ISO_2 isolated dissolved oxygen meter (World Precision Instruments Inc. Sarasota, FL. USA). The oxygen tension at the outlet of the perfusion tube would have been lower than this as the oxygen electrode sampled the perfusion solution after mixing with bath solution. For vitamin C + hypoxia experiments, vitamin C (1.0 mM) was added 15 min before hypoxia as a method of pretreatment. The concentration of vitamin C was based on our experimental results, the lower concentration 0.5 mM did not have a significant effect on I_{NaT} and I_{NaP} during hypoxia (mean current density of I_{NaT} decreased from the control value 40.7136 ± 0.4456 to 28.5437 ± 1.5809 pA/pF at hypoxia 15 min, $n = 6$, $P > 0.05$ *vs.* hypoxia group; while I_{NaP} increased from 0.3816 ± 0.0063 to 0.9603 ± 0.0794 pA/pF, $n = 6$, $P > 0.05$ *vs.* hypoxia group). All control values were recorded under normoxic conditions (before hypoxia).

Electrophysiological Recordings

Myocytes were transferred to a chamber mounted on the mechanical stage of an inverted microscope and perfused with normal Tyrode's solution containing (mmol/liter) NaCl 135, KCl 5.4, $CaCl_2$ 1.8, MgCl 1, NaH_2PO_4 0.33 and HEPES 10, glucose-free (pH 7.4). Patch electrodes were pulled with a two-stage puller (pp-830, Narishige Scientific Instrument Co., Tokyo, Japan). For whole-cell recordings, resistances were in the range of 1.5–3 M′Ω when filled with pipette solution containing (mmol/liter) CsCl 120, $CaCl_2$ 1.0, $MgCl_2$ 5, Na_2ATP 5, tetraethylammonium chloride (TEACl) 10, ethyleneglycoltetraacetic acid (EGTA) 11 and HEPES 10 (pH 7.4). The external solution was Tyrode's solution with $CdCl_2$ (200 μmol/liter). For single-channel recordings, the shanks of pipettes with resistance of 6–10 M′Ω were coated with Sylgard (Sigma Co., St. Louis, MO, USA) and the tips were heat-polished. The pipettes were filled with solution of the following composition (mmol/liter): NaCl 180, KCl 1.3, $CaCl_2$ 1.5, $MgCl_2$ 0.5, Na_2ATP 5, $CoCl_2$ 3.0, TEACl 10, 4AP 10, CsCl 10, HEPES 5 and glucose 5 (pH 7.4). All experiments were performed at room temperature (21 ± 2°C). Currents were conducted through an Ag/AgCl electrode and obtained with a patch-clamp amplifier (EPC-9; Heka, Lambrecht, Germany), filtered at 2 KHz, digitized at 10 KHz and stored on a computer hard disk for further analysis.

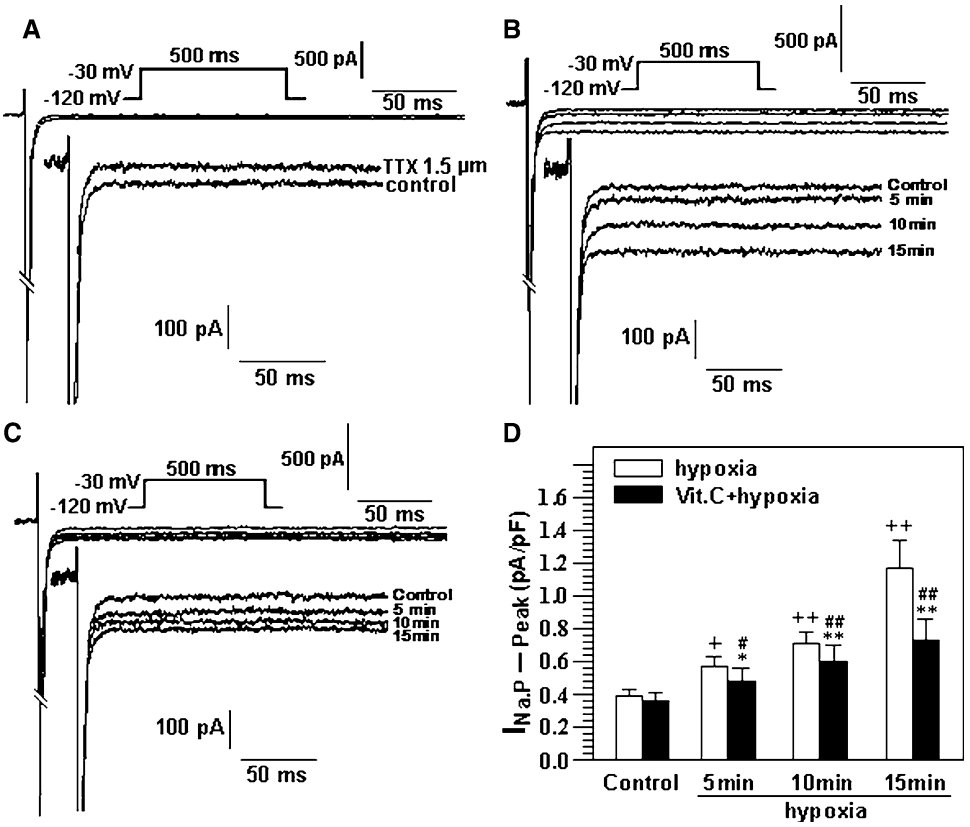

Fig. 1. Effects of hypoxia and vitamin C + hypoxia on I_{NaP} recorded by whole-cell patch clamp. (**A**) Recorded I_{NaT} and I_{NaP} during normoxia. I_{NaP} was blocked completely by TTX (1.5 μM), and this proved the recorded current was I_{NaP} ($n = 6$, cells from six guinea pigs). (**B**) Current traces show the recordings at hypoxia 15 min. (**C**) Current traces show the recordings at vitamin C + hypoxia 15 min. (**D**) Mean current density of I_{NaP} in both groups increased significantly during hypoxia; however, values in the hypoxia group increased more acutely ($n = 9$, $P < 0.05$ *vs.* vitamin C + hypoxia group). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* hypoxia (at simultaneity); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* vitamin C + hypoxia control; $^{+}P < 0.05$, $^{++}P < 0.01$ *vs.* hypoxia control.

Reagents

Collagenase type I and CsCl were obtained from GIBCO Invitrogen (Paisley, UK). Vitamin C, protease E, 4AP, TEACl, Na_2ATP and egtazic acid were purchased from Sigma (St. Louis, MO). BSA, HEPES and taurine were obtained from Roche (Basel, Switzerland). Tetrodotoxin (TTX) was purchased from Hebei Fisheries Research Institute (Qinhuangdao, P.R. China). All other chemicals were purchased from Sigma.

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, while the leak current was not subtracted during experiments. Single-channel recordings were analyzed using TAC+TACFit (X4.0.9; Bruxton, Seattle, WA). Capacitance transients and leakage currents were nullified by off-line subtracting fits of average blunt traces. The channel activity after a 200-ms depolarization pulse was calculated as persistent sodium channel. Open probability was calculated from the total open times of 50 sweeps divided by the total sweep duration. Histograms of channel open time distribution were fitted to single exponentials using TACFit. All histograms of mean current density were plotted by Origin (5.0; Microcal Software, Northampton, MA, USA). Statistical significance between two groups was evaluated by Student's *t*-test for paired data. The significance of differences between multiple groups was evaluated by one-way analysis of variance. All values were expressed as mean ± standard deviation (SD), and the number of cells in each group was given. $P < 0.05$ was considered statistically significant.

Results

Whole-Cell Currents

Under the whole-cell recording model, currents were elicited by the depolarization pulse with 500-ms duration, which depolarized from a holding potential of −120 mV to −30 mV. Both I_{NaP} and I_{NaT} were recorded for 30 min. I_{NaP} changed very little during the whole depolarization and persisted. After adding TTX (1.5 μmol/liter) into the bath solution, I_{NaT} did not change obviously, while I_{NaP} was blocked completely ($n = 6$, Fig. 1A). The recorded currents were proved to be I_{NaP}.

Eighteen cells were separated into two groups equally, i.e., hypoxia and vitamin C + hypoxia (each group containing nine cells). In the hypoxia group, no medical interventions were administered. However, in the vitamin C + hypoxia group, vitamin C (1 mmol/liter) was added to the perfusates during normoxia (before hypoxia treatment) as pretreatment; 15 min later, we simulated hypoxia and currents were recorded alternately using two pulse protocols under the whole-cell recording model. The first was the pulse protocol described above and the second was depolarizing pulses with a duration of 16 ms applied at 0.5 Hz from a holding potential of −120 mV in 10-mV steps between −110 and + 50 mV. Therefore,

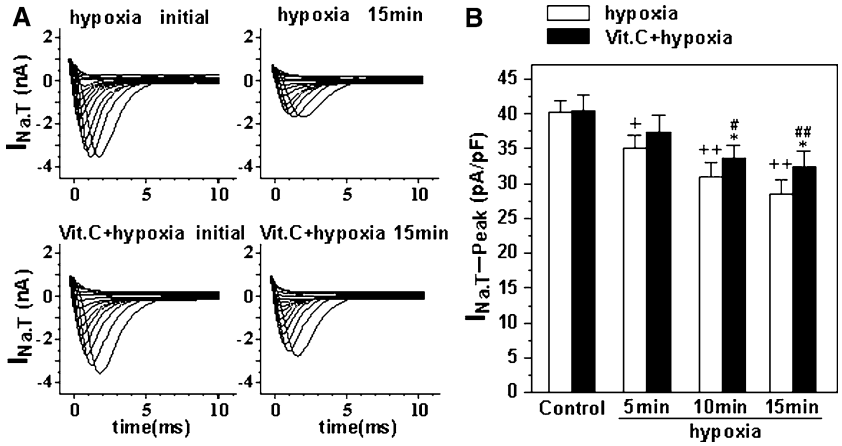

Fig. 2. Effects of hypoxia and vitamin C + hypoxia on I_{NaT} recorded by whole-cell patch clamp. (**A**) Samples of I_{NaT} recorded during hypoxia. Currents were elicited by 16-ms depolarizing pulses, applied at 0.5 Hz from a holding potential of −120 mV, in 10-mV steps between −110 and +50 mV. (**B**) Current density of I_{NaT} decreased gradually during hypoxia in both groups from 0 to 15 min; however, it decreased slightly when vitamin C was pretreated before hypoxia (each group $n = 9$). $^{*}P < 0.05$ *vs.* hypoxia (at simultaneity); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* vitamin C + hypoxia control; $^{+}P < 0.05$, $^{++}P < 0.01$ *vs.* hypoxia control.

two kinds of Na^+ currents (I_{NaT} and I_{NaP}) were recorded. The recordings showed that vitamin C had no significant influence on myocardial I_{NaT} and I_{NaP} during normoxia. However, under hypoxic conditions (100% N_2, glucose-free), we observed that current intensity of I_{NaT} in both groups decreased gradually (Fig. 2A) and mean current density of I_{NaT} in each group decreased from the control values of 40.2142 ± 1.7735 pA/pF (hypoxia group) and 41.6038 ± 2.9762 pA/pF (vitamin C+ hypoxia group) to 27.1663 ± 1.8441 pA/pF and 34.6341 ± 1.9651 pA/pF ($n = 9$, $P < 0.05$ *vs.* control; Fig. 2B, D), respectively, at 15 min. However, mean current density of I_{NaT} in the vitamin C + hypoxia group decreased slightly compared with the hypoxia group ($n = 9$, $P < 0.05$ *vs.* hypoxia group, still significant *vs.* control; Fig. 2B, D). While I_{NaT} decreased, I_{NaP} increased gradually as hypoxia persisted. Current density of I_{NaP} in each group increased from the control values of 0.3987 ± 0.0474 pA/pF (hypoxia group) and 0.3843 ± 0.0636 pA/pF (vitamin C + hypoxia group) to 1.1854 ± 01994 and 0.6734 ± 0.1057 pA/pF ($n = 9$, $P < 0.05$ *vs.* control; Fig. 1B–D), respectively, at 15 min. In Figure 1D, it can be seen clearly that the mean current density of I_{NaP} in the vitamin C + hypoxia group increased slightly compared with the hypoxia group ($n = 9$, $P < 0.05$ *vs.* hypoxia group, still significant *vs.* control). To eliminate the influence of I_{NaT}, all amplitudes of I_{NaP} were tested at 200 ms in depolarization testing pulse.

Cell-Attached, Single-Channel Currents

To further confirm the above findings in the whole-cell recording model, a cell-attached configuration of the patch-clamp technique was carried out. Currents were evoked by a 700-ms voltage pulse to −50 mV from a holding potential of −120 mV. Dividing 12 cells into the hypoxia and the vitamin C + hypoxia groups equally, we recorded the activity of I_{NaP}. Recordings indicated that the mean open probability and the mean open time of I_{NaP} on the cell patch increased significantly in both groups at hypoxia 15 min (Fig. 3A–C). However, the increased current values in the hypoxia group were still marked compared with the vitamin C + hypoxia group ($n = 6$, $P < 0.05$; Fig. 3B, C). The results of single-channel recordings coincided with whole-cell recordings very well. In addition, Figure 4 gives an example of corresponding all-time histograms and mean open-time histograms from another cell-attached patch. These indicated that vitamin C can definitely attenuate the influence of hypoxia on myocardial Na^+ currents.

Discussion

As a water-soluble compound, vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body (Carr & Frei, 1999). Many biochemical, clinical and epidemiological studies have indicated that vitamin C may be of benefit in chronic diseases such as cardiovascular disease, cancer and cataract, probably through antioxidant mechanisms (Carr & Frei, 1999). Vitamin C has several important functions in the body for the synthesis of amino acids and collagen, wound healing and metabolism of iron, lipids, cholesterol, etc. In particular, ascorbic acid may be the front line of defense against free radicals created by metabolism (Duell, 1996; Kitts, 1997).

ROS play a central role in myocardial ischemic injury and are a target for therapeutic intervention. They can attack the lipids of the cell membrane, membrane and cytoplasmic proteins and both nuclear and mitochondrial DNA, resulting in serious or mortal cellular injury (Cochrane, 1991). Myocardial ischemic injury is mediated in part by ROS produced during the ischemic process (Lefer & Granger, 2000; Dhalla et al., 2000), which can affect a variety of molecules of particular relevance to the

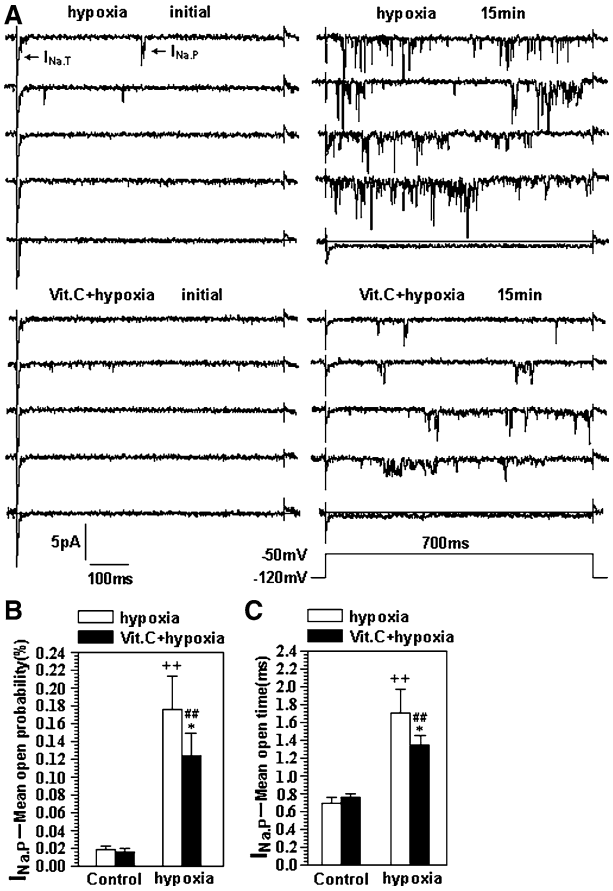

Fig. 3. Activity of I_{NaP} in both groups recorded with the cell-attached configuration of single-channel patch clamp. (**A**) Individual current traces were evoked by a voltage step to –50 mV from a holding potential of –120 mV with 700-ms duration. *Upper four traces* were original current record, and the *fifth trace* in each panel shows the ensemble average of 50 sweeps. The fifth current trace in the hypoxia group at 15 min moves away from the basic zero line; however, in the vitamin C + hypoxia group, it got closer to the zero line. This indicated that I_{NaP} on the cell patch in the vitamin C + hypoxia group was minor compared with the hypoxia group. (**B, C**) Mean open probability and mean open time of I_{NaP} in the hypoxia and vitamin C + hypoxia groups at hypoxia 15 min. $^{*}P < 0.05$ *vs.* hypoxia, $^{\#\#}P < 0.01$ *vs.*vitamin C + hypoxia control, $^{++}P < 0.01$ *vs.* hypoxia control.

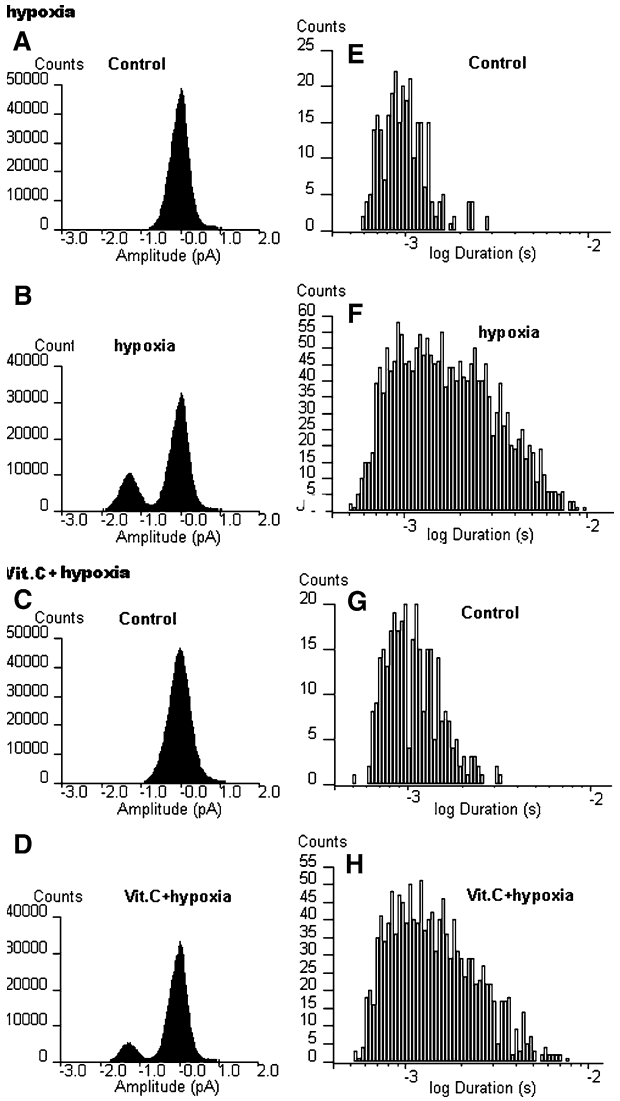

Fig. 4. Effects of hypoxia and vitamin C + hypoxia on mean open probability and mean open time of persistent Na^{+} channels on single cell patch. Each figure came from 50 individual current traces in a cell-attached patch. (**A-D**) All-point histograms. (**E-H**) Mean open time histograms. (**B**, **F**) Recordings at hypoxia 15 min. (**D**, **H**) Recordings at vitamin C + hypoxia 15 min. (**E-H**) Fitted mean open times were 0.6971, 1.773, 0.7966 and 1.342 ms, respectively.

myocardium and the pathogenesis of heart failure (Daly & Sole 1990). ROS generated from metabolic processes are normally quenched by antioxidants that are part of physiological cellular defense mechanisms. In disease conditions such as sudden hypoxia, however, overproduction of ROS and consumption of antioxidants result in oxidative cellular damage (Halliwell, 1999). The hydrophilic antioxidant vitamin C, which is widely distributed in heart tissues, can act in the plasma to scavenge free radicals and reactive oxygen, nitrogen and chlorine species (Ferrari et al., 1991; Carr, 1999; Guaiquil et al., 2001). In addition, ascorbic acid has the ability to regenerate the activity of lipid-soluble antioxidants such as α–tocopherol and β-carotene by interacting with biological membranes at the aqueous-lipid interphase (Stadtman, 1991). These data suggest that vitamin C may have a dual antioxidant function in biological systems, i.e., to inactivate the damaging radicals in the plasma and to preserve the activity of lipophilic antioxidants (Bandyopadhyay et al., 2004). Vitamin C may thus be a useful cardioprotectant to protect the heart from ROS-induced oxidative damage in ischemic heart disease. However, the protective electrophysiological mechanisms of vitamin C on cardiac myocytes against hypoxia remain unclear, so we studied the effects of vitamin C on I_{NaT} and I_{NaP} during hypoxia by patch-clamp techniques.

I_{NaT}, an important cation current in ventricular myocytes, evokes phase 0 depolarization of the action potential and influences the excitability and conduction of excitation in the myocardium. Accumulated extracellular K^+ and inhibited I_{NaT} in ventricular myocytes during hypoxia may be the important ion basis for the decline of excitability and conductibility, which often cause hypoxia-induced arrhythmia in ischemic heart disease (Whalley et al., 1994). Our present results show that the current intensity and mean current density of I_{NaT} decreased significantly during hypoxia time-dependently (Figs. 2 and 3), and this proves that I_{NaT} was inhibited by hypoxia prominently. However, vitamin C attenuates the impact of hypoxia on I_{NaT} of cadiocytes effectively (Fig. 2).

Persistent sodium current (also called *late sodium current*) is the current of sodium channel activity clamped in several hundred milliseconds after voltage depolarization and plays an important role in maintaining the action potential plateau (Kiyosue & Arita, 1989). Hypoxia has been reported to increase open probability and open time of I_{NaP} significantly (Hammarstrom & Gage, 1998, 1999; Ju et al., 1996; Bielefeldt et al., 1999), and our previous results showed that H_2O_2 and NO generated from hypoxia increased I_{NaP} in cardiac myocytes prominently. However, we previously found that another water-soluble antioxidant, GSH, could reverse the I_{NaP} increase by H_2O_2 effectively (Ma et al., 2005), so we presume that GSH has the same protective effect on Na^+ current against hypoxia. In this experiment, I_{NaP} was enhanced significantly during hypoxia but vitamin C inhibited it significantly. Moreover, we incidentally noted a novel feature: as I_{NaT} decreased gradually during hypoxia from 0 to 15 min, I_{NaP} increased (Figs. 1D and 2B). Actually, this is not difficult to understand. According to what we have presented above, hypoxia brings about a decrease of I_{NaT} and an increase of I_{NaP}. When both are recorded at the same time during hypoxia, their variation should be opposite in direction.

Ion regulation and the maintenance of ion gradients across the cell membrane are important for cell homeostasis. However, free redicals such as NO generated during hypoxia can directly affect sodium channels by nitrosylation of cysteine residues (Li et al., 1998). In cardiac tissue, ionic imbalance and current disorder can be precursors to the genesis of arrhythmias (Levi et al., 1997), which may degenerate to fibrillation and sudden cardiac death. The negative impact of hypoxia on I_{NaT} and I_{NaP} in ventricular myocytes often induces symptoms of ischemic heart disease such as ischemic arrhythmia. Myocardial ischemia/hypoxia impairs I_{NaT} and decreases the amplitude and velocity of phase 0 depolarization by inhibiting the transient Na^+ channel directly, which will result in conduction-blockade arrhythmia (Whalley et al., 1994). Hypoxia-enhanced I_{NaP} may cause ischemic arrhythmia in two ways. Firstly, as I_{NaP} distributes mostly at the medium of ventricular myocardium (called *M cells*) and increases significantly during hypoxia, the increased I_{NaP} prolongs the action potential plateau in M cells significantly; but the plateau of endothelial and extima cells in ventricular myocardium changes slightly compared with that in M cells (Sakmann et al., 2000; Zygmunt et al., 2001). Therefore, the repolarization dispersion of the ventricular wall is enlarged and reentry arrhythmia is induced more easily (Antzelevitch, 2000). Secondly, the increased I_{NaP} raises the concentration of intracellular Na^+ and consequently causes overload of intracellular Ca^{2+} through the Na^+-Ca^{2+} exchanger, finally inducing EAD (early after depolarization) (Sugiyama & Hashimoto, 1999; Zabel & Lichtlen, 1998). However, our results indicate that vitamin C can attenuate the negative effects of hypoxia on I_{NaT} and I_{NaP} in ventricular myocytes effectively, which suggests that it may prevent or reduce the occurrence of ischemic arrhythmia.

Conclusion

In this experiment, we found that vitamin C inhibits the negative impact of hypoxia on I_{NaT} and I_{NaP} during hypoxia significantly; and these results indicate that as an important *in vivo* antioxidant, vitamin C can attenuate hypoxia-induced electrophysiological injury on ventricular myocardium prominently and protect the heart from ischemia/hypoxia damage effectively. This is supposed to be one of the cardioprotective mechanisms of vitamin C in ischemic heart diseases, which may have never been discovered before.

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