Extracellular Hypotonicity Induces Disturbance of Sodium Currents in rat Ventricular Myocytes

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Summary

Hypotonic solution alters ion channel activity, but little attention has been paid to voltage-dependent sodium channels. The aim of this study was to investigate the effects of hypotonic solution on transient sodium currents (I_{NaT}) and persistent sodium currents (I_{NaP}). We also explored whether the intracellular signal transduction systems participated in the hypotonic modifications of sodium currents. I_{NaT} and I_{NaP} were recorded by means of whole-cell patch-clamp technique in isolated rat ventricular myocytes. The results revealed that hypotonic solution reduced I_{NaT} and simultaneously augmented I_{NaP} with an occurrence of interconversion between I_{NaT} and I_{NaP}. Hypotonic solution shifted steady-state inactivation to a more negative potential, prolonged the time of recovery from inactivation, and enhanced intermediate inactivation (I_{IM}). Rethenium red (RR, inhibitor of TRPV4), Bisindolylmaleimide VI (BIM, inhibitor of PKC), Kn-93 (inhibitor of Ca/CaMKII) and BAPTA (Ca^{2+}-chelator) inhibited the effects of hypotonic solution on I_{NaT} and I_{NaP}. Therefore we conclude that hypotonic solution inhibits I_{NaT}, enhances I_{NaP} and I_{IM} with the effects being reversible. TRPV4 and intracellular Ca^{2+}, PKC and Ca/CaMKII participate in the hypotonic modifications on sodium currents.

Key words

hypotonic solution • ventricular myocytes • sodium channels • whole-cell recording • signal transduction systems

Introduction

Extracellular hypotonic solution alters the electrical activity of excitable cells, which is a frequent pathological condition in many cases as ischemia, hypoxia, intoxication, infection, water and electrolytes metabolic disorder. Hypotonic cell swelling can cause arrhythmia and other heart disorders (Kawata *et al*. 1974, Tranum-Jensen *et al*. 1981, Jennings *et al*. 1986, Janse and Wit 1989, Vandenberg *et al*. 1996, Diaz *et al*. 2003, Kocic 2005). Many studies have reported that hypotonic cell swelling modulates several ion channels, ion exchangers or ion transporters (Vandenberg *et al*. 1996, Mongin and Orlov 2001, Kocic *et al*. 2001). For instance, Sorota S. found that hypotonic solution provoked a kind of outward rectifying chloride current ($I_{cl\ swell}$) in canine atrial myocytes which participated in regulatory volume decrease (RVD) (Sorota 1992, Hiraoka *et al*. 1998). While many studies focus on Cl, K and Na-K ATPase, little attention has been paid to hypotonicity-induced effects on voltage-dependent sodium channels (Sorota 1992, Vandenberg *et al*. 1996, Wang *et al*. 2005). Being responsible for the rapid upstroke of action potential, voltage-dependent sodium channels play important roles in the activities of myocardial excitability and conduction (Goldin 2002). The disorder of sodium currents contributes to ischemia, arrhythmia, cardiac failure and other acquired cardiac dysfunctions (Ju *et al*. 1996, Antzelevitch and Belardinelli 2006, Saint 2006, Song *et al*. 2006, Wang *et al*. 2007, Luo *et al*. 2007). Consequently, the study of hypotonicity-induced effects on the voltage-dependent sodium channels in ventricular myocytes is significant in understanding the mechanisms of cardiac dysfunctions.

Methods

Solutions and drugs

The calcium-free Tyrode solution contained (in mmol/L): NaCl 135, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.33, HEPES 10, Glucose 10 (pH 7.4). The KB solution contained (in mmol/L): KOH 70, KCl 40, $MgCl_2$ 3, KH_2PO_4 20, EGTA 0.5, L-glutamic acid 50, taurine 20, HEPES 10, Glucose 10 (pH 7.4).

For whole-cell current recording, the intracellular pipette solution contained (in mmol/L): CsCl 120, $CaCl_2$ 1, $MgCl_2$ 5, Na_2ATP 5, TEA 10, EGTA 11, HEPES 10 (pH 7.3).

In this research, transient sodium currents (I_{NaT}) and persistent sodium currents (I_{NaP}) were recorded simultaneously to reveal the correlation between them. So we made the [Na^+] as 65 mmol/L in the extracellular bath solution which contained (in mmol/L): NaCl 65, CsCl 23, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, Glucose 10, Mannitol 96, (300 mOsm/kg or 300M isotonic solution, pH 7.4). For the 280M and 260M hypotonic solution, mannitol was reduced to 78 mmol/L and 60 mmol/L respectively. For control isotonic solution, the Mannitol was replaced by D-sorbitol. Osmolarity of all test solutions was checked by freezing point osmometer immediately before experiments.

Collagenase type I was obtained from Gibco (GIBCO TM, Invitrogen Co., Paisley, UK). CsCl and HEPES were purchased from Amresco Co. (USA). L-glutamic acid, taurine, Mannitol and D-sorbitol were obtained from Wuhan Zhongnan Chemical reagent Co. (Wuhan, China). All other chemicals were purchased from Sigma (Saint Louis, Mo, USA).

Isolation of myocytes

Single cardiac ventricular myocytes were enzymatically isolated from healthy adult rats (250 - 300 g, of either sex, Grade II). In brief, rats were anesthetized with 20% urethane (1000 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 adding Ca^{2+} (Ca^{2+}-free) for 5 minutes; (2) Ca^{2+}-free Tyrode solution containing 0.2 mg/ml collagenase type I and 0.5 mg/ml BSA for 30 minutes; and (3) 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 Hubei Provincial Guide for the Care and Use of Laboratory Animals.

Current recordings

Currents were recorded from isolated myocytes with similar size using a Multiclamp 700B amplifier (Axon Instruments, Inc. USA) by means of whole-cell configuration of the patch-clamp technique. The patch electrodes had resistances of 1.0 - 1.5 MΩ when filled with the pipette solution. Capacitance and series resistances were adjusted to obtain minimal contribution of the capacitive transients. An 90% compensation of the series resistance was usually achieved without ringing. Residual linear leak and capacitance were subtracted by using a P/4 leak-subtract protocol. 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 I_{NaT} and I_{NaP} recording, a single pulse protocol was used from a holding potential (HP) of -120 mV to -40 mV. The duration of the depolarizing pulse was 300 ms. For the I-V relationship and steady-state activation protocol, currents were elicited by 300 ms depolarizing pulses applied at 0.5 Hz from a holding potential of -120 mV, in 5 mV increments between -80 and +40 mV. The steady-state inactivation protocol was from a HP of

-120 mV; 50 ms conditioning prepulses were applied in 5 mV increments between -140 and -20 mV followed by a 30 ms test pulse to -30 mV at 0.5 Hz. For the inactivation recovery protocol, a double-pulse mode was used. The prepulse and the test pulse were all elicited from a HP of -120 mV to -40 mV, and the intervals of the two pulses were between 2 ms to 100 ms in 2 ms increments at 0.5 Hz. For the intermediate inactivation (I_{IM}) protocol, a prepulse was elicited from a HP of -140 mV to a depolarizing potential of -20 mV followed by a 20 ms test pulse with the same depolarizing potential. The depolarizing duration of prepulse was between 12 ms and 34242 ms in fifteen different increments at 0.2 Hz. The intervals of the prepulse and the test pulse were 20 ms. These measurements were made after a minimal time of 5 minutes after rupture of the patch to minimize the contribution of time-dependent shifts of steady-state gating parameter measurements.

Data analysis

Whole-cell recordings were analyzed using Clampfit 9.0. Sodium currents were defined by subtraction of the currents remaining in 20 μmol /L TTX. The amplitude of I_{NaP} was measured after 200 ms of the depolarizaton 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 curve, Y stands for the relative conductance. The chord conductance was calculated using the ratio of the currents to their electromotive for potentials in individual current-voltage relationships. Then these conductances were normalized to their individual maximal values. For steady-state inactivation curve, Y represents the relative current ($I_{NaT}/I_{NaT.Max}$). Data from the recovery

from inactivation were fitted to the exponential function: I/Imax = 1 – exp (-t/τ), where τ is the recovery time constant. Data from I_{IM} were fitted to the exponential function: y = b exp (-kx) + a, where *b* is the 'weight', *k* is the rate constant , and *a* is the Y-offset constant.

All data were expressed as mean ± 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 with control. $P < 0.05$ was considered significant. Figures were plotted by Origin (V7.0, OriginLab Co., MA, USA).

Results

The effects of hypotonic solution on I_{NaT} and I_{NaP}

We found that the membrane capacitance and series resistances did not evidently change when the cardiac myocytes were perfused by hypotonic solution. So we neglected the adjustment of them when the bath solutions were changed. To investigate whether mannitol affected sodium currents, we replaced mannitol by D-sorbitol in the isotonic extracellular solution and found there were no significant difference between the effects of them on the amplitudes of I_{NaT} and I_{NaP} (Data not shown).

In the present research, hypotonic solution suppressed I_{NaT} and increased I_{NaP} at the same time (Fig. 1, 2). When the isolated ventricular myocytes were perfused by 280M hypotonic solution, the current density of I_{NaT} was decreased from -249.2 ± 14.8 pA/pF to -162.2 ± 15.9 pA/pF and I_{NaP} was increased from -0.22 ± 0.07 pA/pF to -0.53 ± 0.07 pA/pF (n = 10, both $P < 0.01$ vs. 300M), the inhibition ratio of I_{NaT} being 34.9 ± 1.8%. I_{NaT} was reverted to -214.8 ± 30.6 pA/pF and I_{NaP} to -0.29 ± 0.07 pA/pF after being reperfused by 300M isotonic solution (Fig. 1). We found similar results when the myocytes were perfused by 260M hypotonic

solution. 260M hypotonic solution decreased I_{NaT} from -253.4 ± 23.2 pA/pF to -175.6 ± 35.3 pA/pF and increased I_{NaP} from -0.23 ± 0.08 pA/pF to -0.58 ± 0.07 pA/pF (n = 10, both $P <$ 0.01 vs. 300M), the inhibition ratio of I_{NaT} being 30.6 ± 6.2% (Fig. 1). We used 280M hypotonic solution to study I-V relationship, steady-state activation, steady-state inactivation, recovery from inactivation and intermediate inactivation.

Hypotonicity (280M) only decreased the amplitude of I-V curve and did not change its shape. The threshold potential was -70 mV and the corresponding potential of the maximum current was -40 mV (Fig. 3C).

Hypotonicity (280M) had no significant effect on the steady-state activation (n = 10, $P >$ 0.05 vs. 300M, Table 2, Fig. 3D), but shifted the steady-state inactivation to a more negative membrane potential (n =10, $P < 0.05$ vs. 300M Table 1, Fig. 4C). It revealed that hypotonicity had no significant effect on the activation process, but had significant effect on the inactivation process. Hypotonicity delayed the inactivation process and increased the time constant (τ value, n = 10, $P < 0.01$ vs. 300M) for recovery from inactivation significantly, indicating that recovery was slowed (Table 1, Fig. 4D).

We also studied the I_{IM} of sodium channels and found hypotonicity significantly enhanced the fraction of channels undergoing I_{IM}. The 'weight' (b) and rate constant (k) were increased and the amplitude of a was decreased significantly (n = 6, Table 2, Fig. 6).

The effects of TRPV4 and intracellular signal transduction system

We investigated whether TRPV4 and intracellular signal transduction system participated in the hypotonicity-adjusting process on sodium currents. H-89 dihydrochloride hydrate (H-89, selective inhibitor of PKA), KT5823 (selective inhibitor of PKG), Bisindolylmaleimide VI

(BIM, selective inhibitor of PKC), Kn-93 (selective inhibitor of Ca/CaMKII) and BAPTA (Ca^{2+}-chelator, used with Ca^{2+}-free and EGTA-free pipette solution) were filled into recording pipette solution respectively (for I_{IM} research, the Kn-93 was added to extracellular perfusing solution). Ruthenium red (RR, inhibitor of TRPV4) was used to incubate the single myocytes for 3 minutes before recording. A single pulse from -120 mV to -40 mV was used to record I_{NaT} and I_{NaP}.

During hyopotonicity, RR (10 μmol/L) diminished the inhibition ratio of I_{NaT} to 15.8 ± 6.1% (n = 6, $P < 0.01$ vs. control, 34.9 ± 1.8%) and inhibited I_{NaP} to -0.24 ± 0.05 pA/pF (n = 6, $P < 0.01$ vs. control, -0.53 ± 0.07 pA/pF). Kn-93 (10 μmol/L) diminished the inhibition ratio of I_{NaT} to 20.8 ± 3.4% (n = 8, $P < 0.01$ vs. control, 34.9 ± 1.8%) and inhibited I_{NaP} to -0.24 ± 0.04 pA/pF (n = 8, $P < 0.01$ vs. control, -0.53 ± 0.07 pA/pF). BIM (1.5 μmol/L) diminished the inhibition ratio of I_{NaT} to 21.8 ± 3.0% (n = 7, $P < 0.01$ vs. control, 34.9 ± 1.8%), and inhibited I_{NaP} to -0.28 ± 0.05 pA/pF (n = 7, $P < 0.01$ vs. control, -0.53 ± 0.07 pA/pF). BAPTA (1 mmol/L) diminished the inhibition ratio of I_{NaT} to 16.2 ± 4.0% (n = 6, $P < 0.01$ vs. control, 34.9 ± 1.8%), and inhibited I_{NaP} to -0.25 ± 0.05 pA/pF (n = 6, $P < 0.01$ vs. control, -0.53 ± 0.07 pA/pF) (Fig. 1). These results revealed that TRPV4 and Ca^{2+}, Ca/CaMKII and PKC participated in the adjusting process. We further investigated the effects of BIM and Kn-93 on I-V relationship, steady-state activation, steady-state inactivation, recovery from inactivation. Both of the drugs inhibited the effects of hypotonocity on them (Table 1, 2, Fig. 2, 5). Furthermore, Kn-93 also inhibited the effects of hypotonicity on I_{IM} (Fig. 6). Additional experiments using Kn-92 showed that it, unlike Kn-93, failed to inhibit the effects of hypotonicity on sodium currents (Data not shown).

H-89 (1 μmol/L) and KT5823 (2 μmol/L) had no significant effects on I_{NaT} and I_{NaP} during hypotonicity, suggesting PKA and PKG did not participate in the adjusting process (Fig. 1).

Discussion

TRPV4, a Ca^{2+}-permeable cation channel in the transient receptor potential (TRP) channels family, has been cloned from the rat, mouse, human and chicken. The TRPV4 channel is widely expressed in mammaliam tissues, including heart, sensory neurons, etc (Guler *et al*. 2002, Inoue *et al*. 2006, Plant and Strotmann 2007). Activation of TRPV4 channel by hypotonic solution increases intracellular Ca^{2+} concentration (Strotmann *et al*. 2003, essandri-Haber *et al*. 2003, Inoue *et al*. 2006), which can activate Ca/CaMKII and PKC. So TRPV4 may play an important role in Ca^{2+} signaling during hypotonic stimulation (Chen *et al*. 2008a, Chen *et al*. 2008b). In the present study, RR, BAPTA, KN-93 and BIM diminished the hypotonic effects on the ventricular myocytes, revealing the possible role of TRPV4 during hypotonic stimulation. According to previous studies, many intracellular signals are involved in the hypotonic swelling process (Mongin and Orlov 2001, Stutzin and Hoffmann 2006, Hoffmann and Pedersen 2006). Our experiments revealed that Ca^{2+}, PKC and Ca/CaMKII participated in the hypotonic regulating process on sodium channels. Nevertheless, the inhibitors of the first three did not completely eliminate the hypotonic effects on I_{NaT}, suggesting that there were other mechanisms involved.

Our study showed that hypotonicity decreased I_{NaT} and increased I_{NaP} simultaneonsly. The two currents were reverted after being reperfused with 300M isotonic solution. The changes of I_{NaT} were accompanied by the opposite changes of I_{NaP}. That is to say, when I_{NaT} was decreased, I_{NaP} was increased and vice versa. This demonstrated the existence of an

interconversion between I_{NaT} and I_{NaP} during hypotonicity (Fig. 1C,D Fig. 2A). The interconversion was reversed by RR, BIM, Kn-93 and BAPTA, indicating TRPV4 and intracellular Ca^{2+}, PKC and Ca/CaMKII systems participated in the above-mentioned hypotonic modifications.

The previous studies in our group have demonstrated the interconversion between I_{NaT} and I_{NaP} during redox reaction (Wang *et al*. 2007, Luo *et al*. 2007). Based on recent studies, we presume that I_{NaT} and I_{NaP} may be two different gating modes of sodium channels and can exhibit interconversion modulated by some factors. For instance, it is probable that part of the sodium channels are switched to I_{NaP}-charged gating mode resulting in more I_{NaP} and less I_{NaT}. The phenomenon may be induced by the phosphorylation of some structural domains of sodium channels (Qu *et al*. 1996, Wang *et al*. 2007, Luo *et al*. 2007). Of course, the real mechanisms of the hypotonicity-induced interconversion between I_{NaT} and I_{NaP} needs be interpreted by more penetrating studies.

In this study, hypotonicity shifted steady-state inactivation to a more negative potential and prolonged the recovery time from inactivation. These results indicate that the availability of sodium channels is reduced during depolarizing. At the same potential, hypotonicity transforms more sodium channels into inactivation state and prolongs the refractory period of cardiocytes for reactivation. The downward shift of I_{IM} curve reveals the augmentation of inactivation, further suggesting that when I_{NaP} increases while I_{NaT} decreases simultaneously. Since sodium currents play important roles in ventricular muscle electric activity, we can safely conclude that hypotonicity may inhibit the excitability and excitation conductability of ventricular myocytes.

Solute flux pathways activated by hypotonic swelling permits intracellular osmotic materials efflux and restricts extracellular osmotic materials inflow, tending to restore cell volume to its original size (Vandenberg *et al*. 1996). Na^+ inflow may be, without exception, restricted and I_{NaT} suppressed. Our experimental results are consistent to this deduction.

Besides, the suppression of I_{NaT} may results from a different mechanism. I_{NaP} is enhanced by some factors and drugs such as ischemia, hypoxia, redox reaction, H_2O_2, NO, GSSG and Veratridine (Ju *et al*. 1996, Hammarstrom and Gage 1999, Ma *et al*. 2005, Saint 2006, Wang *et al*. 2007, Luo *et al*. 2007). Some studies have revealed that intracellular Na^+ concentration ($[Na^+]_i$) increases during myocardial ischemia due to persistent Na^+ inflow (I_{NaP}) (Williams et al. 2007). The increase of intracellular $[Na^+]$ may facilitate hypotonic swelling during the initial stage of myocardial ischemia (Qu *et al*. 1996). The increase of intracellular $[Na^+]$ decreases the $[Na^+]$ gradient difference between both sides of the cell membrane. Consequently, the I_{NaT} would also be suppressed.

But, why I_{NaP} increased under this condition? It is very likely that the adjusting mechanisms of intracellular signal transduction are activated during hypotonicity. Literatures have demonstrated that intracellular PKC and Ca^{2+}/CaMKII systems are related to I_{NaP} (Qu *et al*. 1996, Murray *et al*. 1997, Kim *et al*. 2004, Wagner *et al*. 2006). PKC activates the phosphorylation of serine and threonine located in the inactivation gate of sodium channels protein which conduces to inactivation loss and increases I_{NaP} (Qu *et al*. 1996, Murray *et al*. 1997). CaM bonding with some motif at the C termination of sodium channels probably adjusts I_{NaP} via Ca^{2+}/CaMKII (Kim *et al*. 2004, Wagner *et al*. 2006). The present study revealed that BIM, Kn-93 and BAPTA weakened the I_{NaP} induced by hypotonicity. These

results not only indicate that PKC and Ca/CaMKII participate in the hypotonicity-adjusting process on I_{NaP}, but also verify that intracellular Ca^{2+} is correlated to I_{NaP}. The enhanced I_{NaP} prolongs action potential duration, resulting in LQT_3 symptom and other arrhythmia (Noble and Noble 2006). I_{NaP} increases intracellular [Na^+] and provokes reverse Na^+/Ca^{2+} exchange which induces intracellular Ca^{2+} overload (Williams *et al*. 2007). I_{NaP} and Ca^{2+} overload are important agents causing myocardial ischemia diseases and are related to arrhythmia (Noble and Noble 2006). Consequently, the blockage of I_{NaP} would contribute to alleviation of ischemia damage. All of these experimental results may provide new perspectives for treating myocardial ischemia diseases.

In conclusion, we maintain that hypotonicity decreases I_{NaT} while increases I_{NaP} simultaneously with an interconversion between them, these effects being reversible. TRPV4 and intracellular Ca^{2+}, PKC and Ca/CaMKII participate in the hypotonic modifications on sodium currents in rat ventricullar myocytes.

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Table 1. Effects of hypotonic solution on steady-state activation , inactivation and recovery from inactivation.

Treatments	Steady-state activation			Steady-state inactivation			Inactivation recovery	
	V1/2	k	n	V1/2	k	n	τ	n
300M	-53.8 ± 2.2	3.29 ± 0.25	10	-78.7 ± 2.2	-8.55 ± 0.33	10	4.40 ± 0.25	10
280M	-55.2 ± 1.9*	3.68 ± 0.30*	10	-84.3 ± 2.5†	-7.70 ± 0.41†	10	8.02 ± 0.23‡	10
300M r	-54.4 ± 1.4	3.57 ± 0.29	10	-81.9 ± 3.2	-9.05 ± 0.45	10	5.94 ± 0.24	10
300M+Kn-93 p	-51.6 ± 2.0	3.03 ± 0.21	8	-77.8 ± 2.0	-8.88 ± 0.25	8	4.42 ± 0.31	8
280M+Kn-93 p	-52.5 ± 2.0§	3.20 ± 0.25§	8	-79.9 ± 1.8§	-8.29 ± 0.18§	8	5.89 ± 0.22‖	8
300M+BIM p	-51.9 ± 2.0	3.20 ± 0.23	7	-78.0 ± 2.2	-8.75 ± 0.30	7	4.45 ± 0.40	7
280M+BIM p	-52.3 ± 2.3¶	3.35 ± 0.27¶	7	-79.2 ± 2.4¶	-8.18 ± 0.23¶	7	5.90 ± 0.36‖	7

* $P > 0.05$ vs. 300M, † $P < 0.05$ vs. 300M, ‡ $P < 0.01$ vs. 300M, §$P > 0.05$ vs. 300M+Kn-93, ‖ $P < 0.01$ vs. 280M, ¶ $P > 0.05$ vs. 300M+BIM

p = drug was added into pipette solution, r = reperfuse.

Table 2. Effects of hypotonic solution on intermediate inactivation (I_{IM})

Treatments	Intermediate inactivation (I_{IM})			
	k ($\times 10^{-4} ms^{-1}$)	b	a	n
300M	0.68 ± 0.07	0.19 ± 0.04	0.78 ± 0.05	6
280M	1.12 ± 0.08*	0.31 ± 0.05*	0.63 ± 0.04*	6
280M+Kn-93 b’	0.70 ± 0.08†	0.24 ± 0.04†	0.71 ± 0.04†	6

* $P < 0.05$ vs. 300M, † $P < 0.05$ vs. 280M

b’ = Kn-93 was added to bath solution.

Figure legends

Fig. 1. Effects of hypotonic solution on transient sodium currents (I_{NaT}) and persistent sodium currents (I_{NaP}). H-89 dihydrochloride hydrate (H-89), KT5823, Bisindolylmaleimide VI (BIM), Kn-93, BAPTA and Rethenium red (RR) were filled into recording pipette solution respectively. The corresponding bars of these drugs represented hypotonic values. A:

Amplitudes of I_{NaT} and I_{NaP}. * $P < 0.01$ vs. 300M, ** $P > 0.05$ vs. 280M, ** $P < 0.01$ vs. 280M. B: Percentage inhibition of I_{NaT} and percentage augment of I_{NaP}. * $P > 0.05$ vs. 280M, ** $P < 0.01$ vs. 280M. C: Tendency of I_{NaT} along time. D: Tendency of I_{NaP} along time. For panels C and D, the ventricular myocytes were perfused with isotonic solutions in the first 3 minutes, with hypotonic solutions in the subsequent 3 minutes, and reperfused with isotonic solutions in the last several minutes without or with Kn-93 and BAPTA-filled pipette solution.

Fig. 2. A: The interconversion between I_{NaT} and I_{NaP}. The peaks of I_{NaT} and the fractions of I_{NaP} from the same raw current traces were shown on left and right-lower sides on panel A. Hypotonic solution decreased I_{NaT} and increased I_{NaP} at the same time. Isotonic solution reperfusion (showed as gray traces) reversed the aforesaid effects. B: Effects of hypotonic solution on I_{NaP}. Kn-93 (10 μmol/L, pipette solution) and BIM (1.5 μmol/L, pipette solution) antagonized the hypotonic effects on I_{NaP}.

Fig. 3. Effects of hypotonic solution on I-V relationship and steady-state activation. A: Original traces of I_{NaT} selected from -80 mV to 0 mV with 5 mV steps. B: I-V traces from -65 mV to -40 mV selected from the original traces on panel A. C: I-V relationship curves of I_{NaT}. D: Steady-state activation curves. Hypotonic solution decreased I_{NaT} shown on panels A, B and C. Isotonic solution reperfusion reversed the inhibited effect partly. No significant effects of hypotonic solution on steady-state activation shown on panel D.

Fig. 4. Effects of hypotonic solution on steady-state inactivation and recovery from inactivation. A: Original I_{NaT} traces elicited by test pulses of recording protocol for steady-state inactivation. B: Original I_{NaT} elicited by prepulse and test pulses of recording protocol for recovery from inactivation. The I_{NaT} amplitudes and tendency were altered by

hypotonic solution. Isotonic reperfusion reversed it partly. C: Steady-state inactivation curves. Hypotonic solution shifed steady-state inactivation curve leftward significantly, and isotonic reperfusion reversed it partly shown on panels C. D: Curves of recovery from inactivation. Hsypotonic solution shifted it right-lower-ward significantly and prolonged recovery time.

Fig. 5. Kn-93 (10 μmol/L in pipette solution) antagonized the effects of hypotonic solution on I-V relationship, steady-state inactivation and recovery from inactivation. A: Original current traces. B: I-V curves of I_{NaT}. I_{NaT} amplitudes were decreased by hypotonic solution, but in lower percentage inhibition versus pure hypotonicity. C: Original I_{NaT} traces elicited by test pulses of recording protocol for steady-state inactivation. D: Steady-state inactivation curves. Steady-state inactivation curves were unaltered by hypotonic solution with Kn-93. E: Original I_{NaT} elicited by prepulse and test pulses of recording protocol for recovery from inactivation with 10 μmol/L Kn-93 in pipette solution. F: Curves of recovery from inactivation. Hypotonic solution shifted it right-lower-ward in smaller amplitude versus pure hypotonicity.

Fig. 6. Effects of hypotonic solution on intermediate inactivation (I_{IM}). A: Original current traces of I_{NaT} elicited by prepulse and test pulses of recording protocol for recording I_{IM}. B: I_{IM} curves. Hypotonic solution increased the amount of accumulating I_{IM} versus isotonic, and the effect was reversed by Kn-93 (10 μmol/L in perfused solution).

Figure 1.

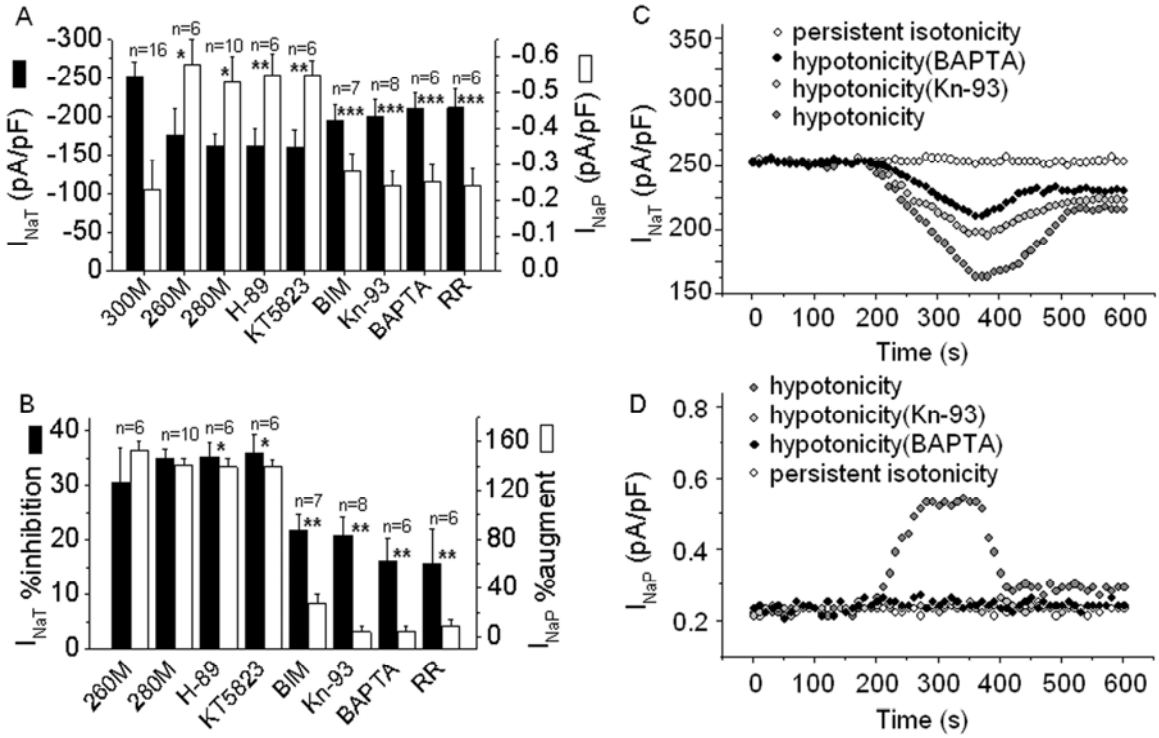


Figure 2.

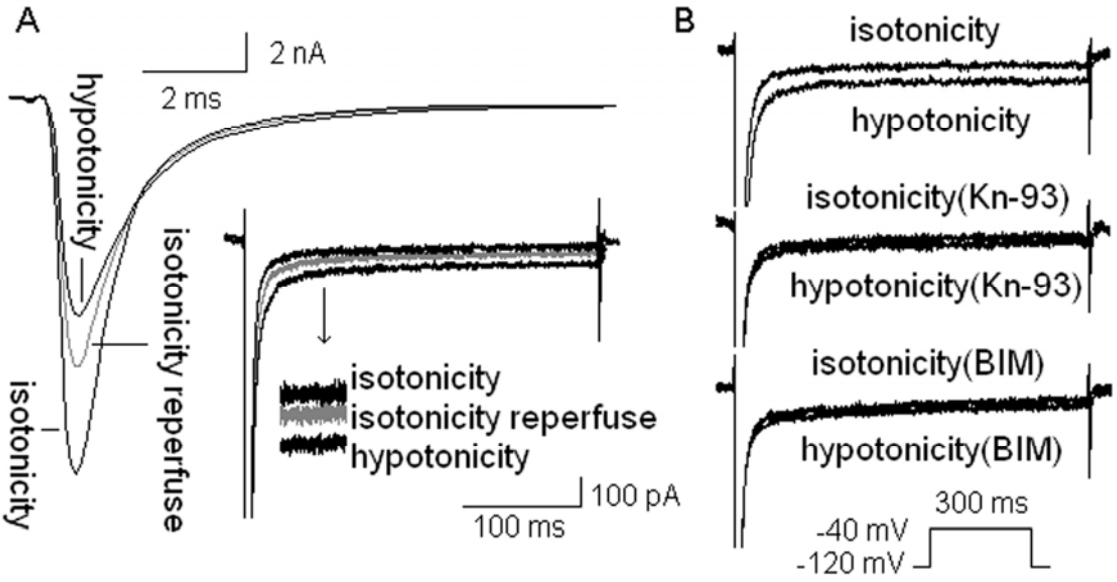


Figure 3.

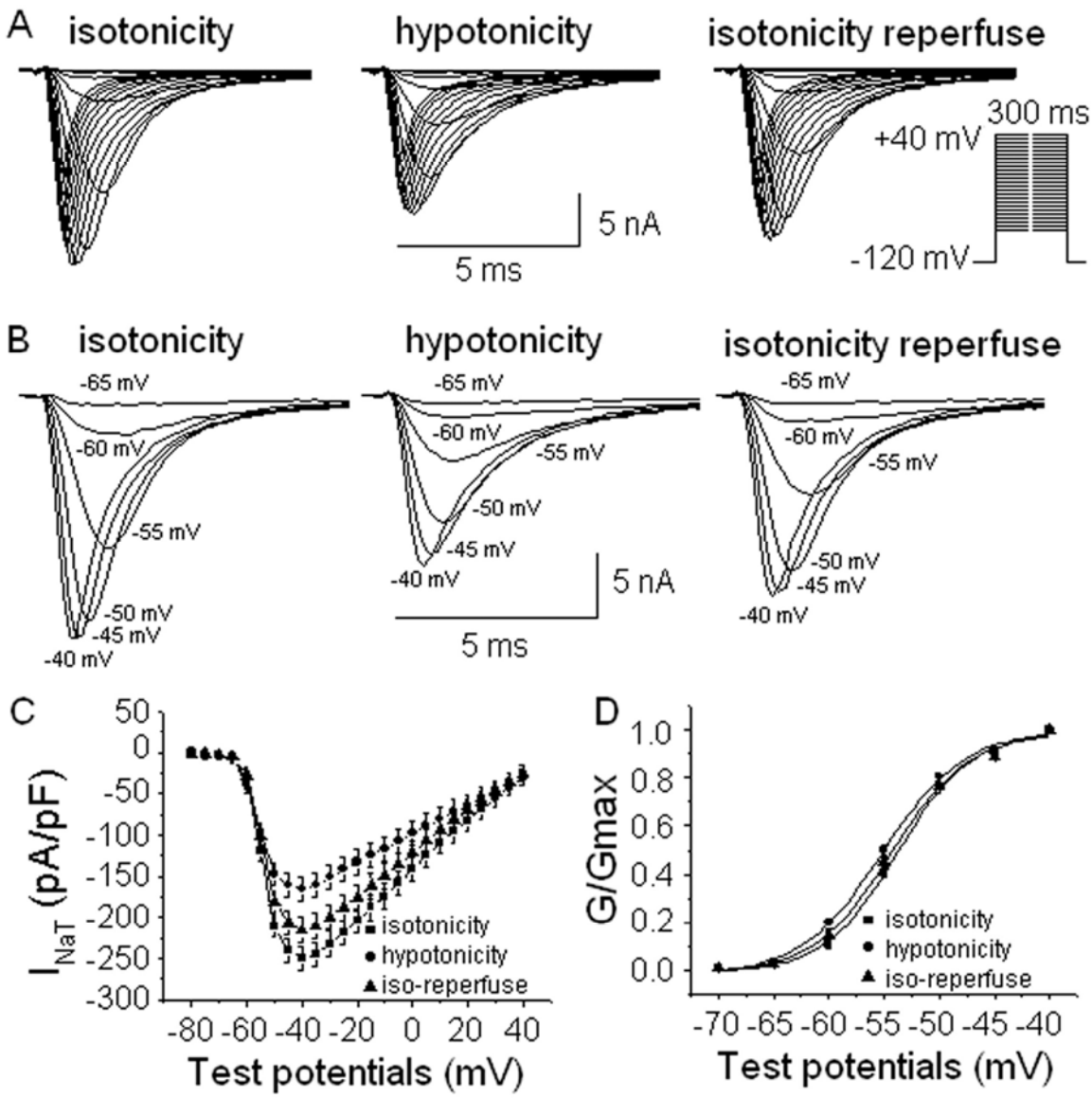

A
isotonicity
hypotonicity
isotonicity reperfuse
300 ms
+40 mV
-120 mV
5 nA
5 ms
B
isotonicity
hypotonicity
isotonicity reperfuse
-65 mV
-60 mV
-55 mV
-50 mV
-45 mV
-40 mV
5 nA
5 ms
C
I_{NaT} (pA/pF)
50
0
-50
-100
-150
-200
-250
-300
isotonicity
hypotonicity
iso-reperfuse
-80 -60 -40 -20 0 20 40
Test potentials (mV)
D
G/Gmax
1.0
0.8
0.6
0.4
0.2
0.0
isotonicity
hypotonicity
iso-reperfuse
-70 -65 -60 -55 -50 -45 -40
Test potentials (mV)

Figure 4.

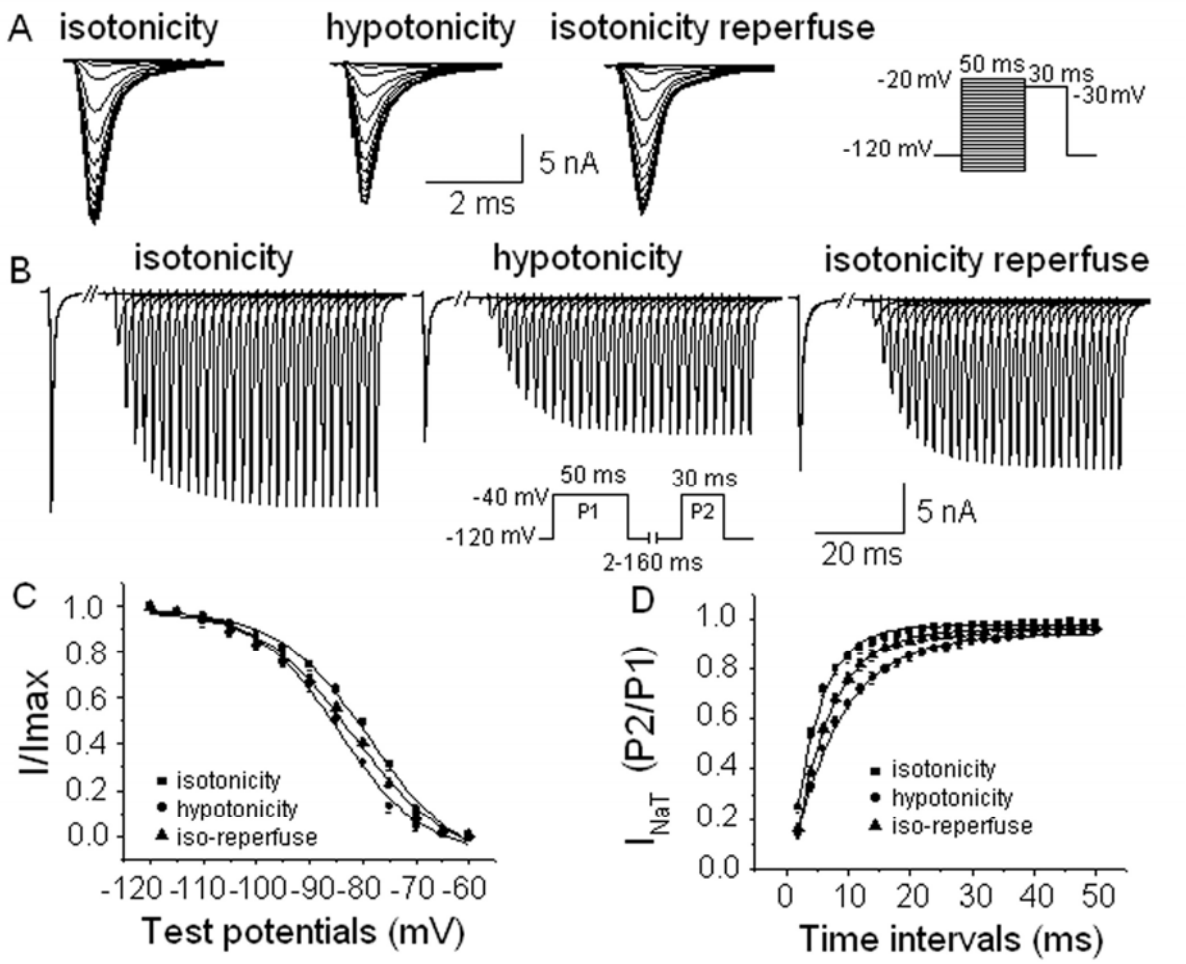

A
isotonicity
hypotonicity
isotonicity reperfuse
5 nA
2 ms
-20 mV
50 ms
30 ms
-30 mV
-120 mV
B
isotonicity
hypotonicity
isotonicity reperfuse
-40 mV
50 ms
P1
30 ms
P2
-120 mV
2-160 ms
5 nA
20 ms
C
I/Imax
1.0
0.8
0.6
0.4
0.2
0.0
isotonicity
hypotonicity
iso-reperfuse
-120 -110 -100 -90 -80 -70 -60
Test potentials (mV)
D
I_NaT (P2/P1)
1.0
0.8
0.6
0.4
0.2
0.0
isotonicity
hypotonicity
iso-reperfuse
0 10 20 30 40 50
Time intervals (ms)

Figure 5.

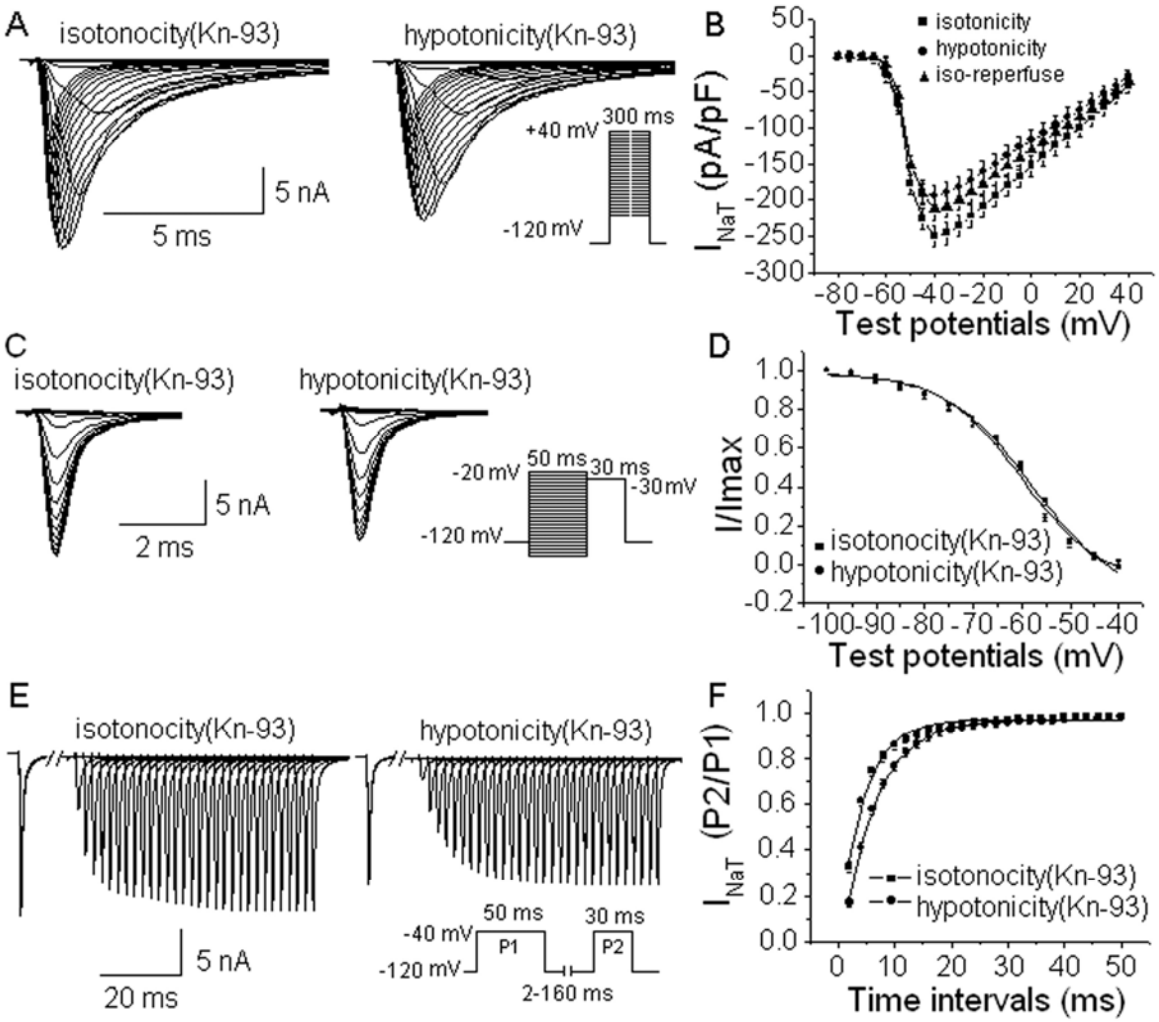
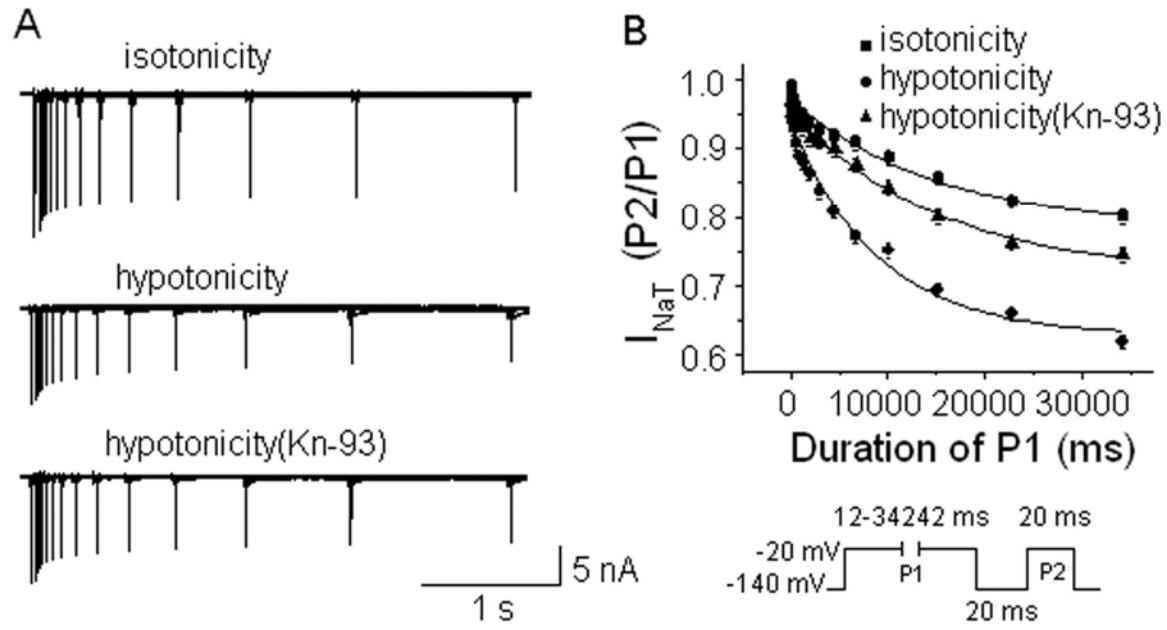

A
isotonocity(Kn-93)
hypotonicity(Kn-93)
5 nA
5 ms
+40 mV
300 ms
-120 mV
B
isotonicity
hypotonicity
iso-reperfuse
I_{NaT} (pA/pF)
Test potentials (mV)
C
isotonocity(Kn-93)
hypotonicity(Kn-93)
5 nA
2 ms
-20 mV
50 ms
30 ms
-30 mV
-120 mV
D
I/Imax
isotonocity(Kn-93)
hypotonicity(Kn-93)
Test potentials (mV)
E
isotonocity(Kn-93)
hypotonicity(Kn-93)
5 nA
20 ms
-40 mV
-120 mV
50 ms
P1
30 ms
P2
2-160 ms
F
I_{NaT} (P2/P1)
isotonocity(Kn-93)
hypotonicity(Kn-93)
Time intervals (ms)

Figure 6.


A
isotonicity
hypotonicity
hypotonicity(Kn-93)
5 nA
1 s
B
isotonicity
hypotonicity
hypotonicity(Kn-93)
1.0
0.9
0.8
0.7
0.6
I_NaT (P2/P1)
0
10000
20000
30000
Duration of P1 (ms)
12-34242 ms
20 ms
-20 mV
-140 mV
P1
P2
20 ms