Slowing Na\(^+\) Channel Inactivation Prolongs QT Interval and Aggravates Adrenaline-Induced Arrhythmias

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ABSTRACT—We investigated the effects of prolonged repolarization induced by slowed inactivation of Na\(^+\)/G2b channel on adrenaline-induced arrhythmias in halothane anesthetized, closed-chest dogs. We used sea anemone toxins (ATX-II and Anthopleurin-A) to prolong ventricular repolarization and examined their effects on adrenaline arrhythmias. Sea anemone toxins prolonged the QTc- and JTc-intervals ($P<0.01$), but did not affect the PQ interval, QRS duration, heart rate and mean blood pressure. Although sea anemone toxins did not induce any arrhythmias by themselves, under the treatment with these toxins, arrhythmias were induced by non-arrhythmia-inducing doses of adrenaline in four dogs out of seven and the control arrhythmias induced by adrenaline were aggravated. These results indicate that, similar to the inhibition of K\(^+\)/G2b channels by class III drugs, which we have already reported, slowing Na\(^+\) channel inactivation with QTc prolongation also aggravates adrenaline-induced arrhythmias.

Keywords: Adrenergic, ECG, long QT syndrome, Na\(^+\) channel, Ventricular arrhythmia

Prolongation of cardiac repolarization is often associated with induction and aggravation of ventricular arrhythmias including torsades de pointes and ventricular fibrillation (1, 2). Long-QT syndrome (LQTS) is a hereditary disease associated with life-threatening ventricular tachycardia, which may lead to sudden death. Experimentally QT-prolonging agents are used to mimic some types of congenital LQTS. Class III antiarrhythmic drugs, inhibitors of K\(^+\) channels, are used as models of the LQT2 (3 – 5) in which K\(^+\) efflux through the rapid component of the delayed rectifier current (I\(_{\text{Kr}}\)) is impaired. On the other hand, sea anemone toxins, such as anthopleurin-A (AP-A) and ATX-II, are used as models of the LQT3 (4 – 6), in which inactivation of the Na\(^+\) channel is slowed or incomplete.

We have shown that class III drugs, MS-551, KCB-328 and azimilide, prolong QTc and have proarrhythmic effects on the adrenaline-induced arrhythmias (7, 8). We used an adrenaline-induced arrhythmia model to mimic a clinical situation of arrhythmia and sudden death in LQTS associated with sympathetic overactivity. We have also shown that class I drugs such as disopyramide suppress adrenaline-induced arrhythmias even though this agent prolonged the QTc interval. We have suggested that inhibition of Ca\(^2+\) current by disopyramide might have counteracted the arrhythmogenic effects of K\(^+\) current inhibition (9).

It has been suggested that sea anemone toxins prolong action potential duration in vitro (10 – 12) or monophasic action potentials in vivo (13). In addition, isoproterenol decreases the action potential duration prolonged by anthopleurin (4), suggesting that $\beta$-adrenergic stimulation may not be arrhythmogenic when cardiac repolarization is prolonged by the slowed Na\(^+\) channel inactivation. However, it is not clear whether suppressing the Na\(^+\) channel inactivation has proarrhythmic effects on adrenaline-induced arrhythmias in vivo, such as inhibition of K\(^+\) channels.

Therefore, in this study, we evaluated the effects of sea anemone toxins on adrenaline-induced arrhythmias.

MATERIALS AND METHODS

Experimental preparation

These animal experiments were approved by the Yamanashi Medical University Animal Experimentation Committee, and animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University.

Adult Beagle dogs of either sex, weighing 9.0 – 11.5 kg, were anesthetized initially with thiopental sodium. After tracheal intubation, 2.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator (20 ml/kg, 15 strokes/min; Model SN-480-4; Shinano, Tokyo). Both vagal nerves were cut at the mid-cervical...
region. The lead II electrocardiogram (ECG) and atrial electrogram from the catheter tip electrodes in the right atrium were continuously monitored. The QT interval was measured from the onset of the QRS complex to the end of the T-wave. The corrected QT interval (QTc) was calculated using Bazett’s formula, \( QTc = QT/\sqrt{RR} \). The corrected JT interval (JTc) was calculated by the formula, \( JTc = JT/\sqrt{RR} \), in which the JT interval was calculated by subtracting the QRS duration from the QT interval. A femoral artery catheter was inserted for blood pressure monitoring. The ECG, atrial electrogram and blood pressure were recorded with a polygraph system (Nihon Kohden, Tokyo). The femoral vein was also cannulated for administering toxins and adrenaline.

Production of adrenaline-induced arrhythmias

After surgical preparation, 30–45 min was allowed for stabilization, and then adrenaline diluted in 20 ml saline was intravenously infused for 50 s, according to the method of Hashimoto and coworkers (14, 15). The starting dose of adrenaline was 0.5 \( \mu g/kg \). If 0.5 \( \mu g/kg \) adrenaline did not produce arrhythmia, the dose of adrenaline was increased. The maximum adrenaline dose in the control period was the dose that produced severe ventricular tachycardia or occasionally fatal ventricular fibrillation. If 0.5 \( \mu g/kg \) adrenaline produced arrhythmia, a lower dose of 0.25 \( \mu g/kg \) adrenaline was infused. Between the challenges of the adrenaline infusion, a recovery period was allowed, during which the hemodynamic parameters, e.g., heart rate and blood pressure, became stable. We defined the arrhythmia-inducing dose of adrenaline (referred to as the inducible dose) as the lowest dose that produced ventricular arrhythmias, including premature ventricular complexes, bigeminy or ventricular tachycardia, which was defined as more than 3 consecutive premature ventricular complexes; and the non-arrhythmia-inducing dose of adrenaline (referred to as the non-inducible dose) was defined as the highest dose that did not induce any arrhythmias.

As we previously reported (7), inducible- and non-inducible doses of adrenaline were not changed by saline infusion, indicating the reproducibility of the arrhythmia.

Sea anemone toxins (5 \( \mu g/kg \) ATX-II, \( n = 4 \) or 20 \( \mu g/kg \) AP-A, \( n = 3 \)) were administered as an intravenous bolus. The doses of toxins were selected to prolong the QTc-interval by 10%. To obtain the stable prolongation of QTc-interval took less than 12 min after which adrenaline was infused at a non-inducible dose.

Drugs

AP-A and ATX-II were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Calbiochem-Novabiochem Co. (La Jolla, CA, USA), respectively. These toxins were dissolved in saline. Thiopental sodium (Tanabe Seiyaku, Tokyo), halothane (Takeda Chemical Industries, Osaka) and adrenaline hydrochloride (Daiichi Pharmaceutical Co., Ltd., Tokyo) were purchased.

Evaluation of proarrhythmic effects

The arrhythmic ratio was calculated by dividing the number of premature ventricular complexes by the total heart rate, i.e., the number of premature ventricular complexes plus the number of conducted beats, and the ventricular beats were judged by the different shape of the ventricular complex from the normal QRS complex. If the arrhythmic ratio and the number of the ranks expressing the severity of adrenaline-induced arrhythmias after the toxin administration were significantly higher than those of the control period, the toxin was judged to have proarrhythmic effects.

Statistics

The hemodynamic and electrocardiographic parameters, and the arrhythmic ratio were expressed as means \( \pm S.E.M. \) (\( n = 7 \)). Differences between values were evaluated by paired Student’s t-test (Figs. 2 and 3) and, where appropriate, repeated measured analysis of variance (ANOVA) were performed (Fig. 1). In the latter case, when a statistical difference was detected, Dunnett’s multiple comparison test was used to determine the difference between the 0 time value and the other values in hemodynamic and electrocardiographic parameters. The severity of arrhythmias was compared by the Wilcoxon signed-ranks test (Fig. 4). Differences were regarded as significant if the P values were less than 0.05.

RESULTS

As shown in Fig. 1, sea anemone toxin (5 \( \mu g/kg \) ATX-II, \( n = 4 \) or 20 \( \mu g/kg \) AP-A, \( n = 3 \)) did not change the PQ interval, QRS duration, the heart rate and mean blood pressure. On the other hand, QTc- and JTc-intervals were increased gradually, and a significant increase was observed after 6 min of the administration. Twelve min after administration, QTc and JTc increased from 397 ± 9 to 441 ± 15 ms/\( \sqrt{RR} \) (by 11%) and from 303 ± 11 to 354 ± 15 ms/\( \sqrt{RR} \) (by 17%), respectively. These toxins did not induce any arrhythmias by themselves in all dogs (\( n = 7 \)). Even after the challenge of adrenaline infusion, the QTc- and JTc-interval prolongations were sustained without significant changes in other ECG parameters and mean blood pressure (Fig. 1).

Figure 2 shows effects of adrenaline on the QTc- and JTc-intervals prolonged by sea anemone toxins. In Fig. 2, values before each adrenaline infusion were taken as 100%. Adrenaline decreased these intervals and there was a significant difference at 0.75 \( \mu g/kg \) adrenaline. However, even
in the presence of 0.75 μg/kg adrenaline, QTc- and JTc-intervals were 426 ± 18 and 337 ± 20 ms/s, respectively, which were longer than the pre-toxins values (QTc: 397 ± 9 ms/s, JTc: 303 ± 11 ms/s).

The adrenaline dose-arrhythmic ratio curve was not significantly shifted by the sea anemone toxins, although there was a tendency to move left and upward (Fig. 3). Sea anemone toxins induced arrhythmias by non-inducible
doses of adrenaline in 4 out of 7 dogs; however, there were no significant differences between the arrhythmic ratio in the absence and the presence of sea anemone toxins (Fig. 4A, $P = 0.068$). On the contrary, arrhythmias produced by inducible doses of adrenaline were aggravated by the sea anemone toxins (Fig. 4B, $P = 0.018$).

**DISCUSSION**

It has been suggested that sea anemone toxins (AP-A and ATX-II) prolong the cardiac action potential by selectively inhibiting the inactivation of Na$^+$ channels (11, 16, 17). QTc- and JTc-intervals were prolonged by these toxins (Fig. 1), although PQ, QRS, heart rate and mean blood pressure were not changed, which is consistent with a previous observation in dogs (18). These prolongations of QTc- and JTc-intervals lasted until the completion of the experiments (Fig. 1), suggesting the long lasting effects of these toxins. In the absence of adrenaline, we did not observe any proarrhythmic effects of these toxins. This may be due to the fact that effects of sea anemone toxins on Na$^+$ currents are cycle length-dependent (13). The cycle length-dependent effects of these toxins may have also contributed to the less severe prolongation of the QTc interval (11%) than those obtained by class III antiarrhythmic drugs of 20–30% prolongation (7, 8) in our halothane-anesthetized dogs.

QTc- and JTc-intervals prolonged by sea anemone toxins were partially inhibited by adrenaline (Fig. 2). These results are consistent with an observation in vitro study (4). However, the effects were not dose-dependent, as shown in Fig. 2. Since multiple-channel effects of adrenaline may influence the duration of the repolarization in the heart, we could not clarify the possible mechanisms of variable

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**Fig. 2.** Effects of adrenaline on QTc- and JTc-intervals prolonged by sea anemone toxins. Values before each adrenaline infusion were taken as 100%. *$P<0.05$ by paired Student’s $t$-test.

**Fig. 3.** Effects of sea anemone toxins on the arrhythmic ratio in dogs. A: Representative trace, showing the adverse effect of AP-A. B: The arrhythmic ratios in the drug-treated groups was compared with those in the control group at corresponding adrenaline doses. Each data point represents the mean, and vertical lines show S.E.M.
effects of adrenaline on the QTc- and JTc-intervals.

The mechanism of adrenaline-induced arrhythmias is thought to be induction of abnormal automaticity and triggered activity due to the augmentation of cardiac Ca\textsuperscript{2+} channels opening (14, 19, 20), and halothane is known to interfere with the cell-to-cell coupling (14, 21). As we have previously reported, the adrenaline-induced arrhythmias are aggravated by blocking of K\textsuperscript{+} channels (7), but are suppressed by blocking Ca\textsuperscript{2+} channels, using class IV anti-arrhythmic drugs (22, 23) or class IA drugs, cibenzolin or disopyramide, even though the latter class IA drug prolongs QTc-interval (9).

After the injection of sea anemone toxins, the adrenaline dose-arrhythmic ratio curve moved to the left and upward (Fig. 3), and arrhythmias induced by the inducible doses of adrenaline were aggravated (Fig. 4). These proarrhythmic effects of these toxins are similar to those induced by class III drugs as we reported previously (7, 8). Therefore, prolonged repolarization of action potential, regardless of the ionic mechanisms, may be proarrhythmic when the adrenergic activity is increased. Thus the proarrhythmic effects of sea anemone toxins on this model may be due to induction of early afterdepolarization by \beta-adrenergic stimulation associated with prolonged action potential, and spatial dispersion of repolarization induced by these toxins may precipitate arrhythmias by reentry mechanisms. Recently, Schwartz et al. reported that in 65 LQT3 patients, 39% of cardiac events occurred during sleep or rest, 32% of cardiac events occurred during exercise or emotional stress and 50% of patients with \beta-blocker therapy had no recurrences of attacks (24). These observations are consistent with results obtained in this study. However, it has been suggested that none of the experimental findings reproduce the clinical features of arrhythmias in LQT patients (25), and the use of halothane and high doses of adrenaline in this study, may not accurately represent the sympathetic overactivity of LQT patients.

In summary, the inhibition of inactivation of Na\textsuperscript{+} channels resulted in prolonged QTc and JTc and aggravated the adrenaline-induced arrhythmias. These results suggest that lengthening cardiac repolarization, which is induced not only by the inhibition of K\textsuperscript{+} currents but also the inhibition of inactivation of Na\textsuperscript{+} currents, is proarrhythmic in the presence of adrenaline.

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