Effect of mexiletine on long QT syndrome model[1]

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ABSTRACT

AIM: To make a LQT_3 model (one form of the long QT syndromes) and to investigate the effect of mexiletine on LQT_3. **METHODS:** Sea anemone toxin (ATX II) was used to produce the LQT_3 model. The Effect of mexiletine on LQT_3 was performed on single Na channel, action potential, and electrocardiography in guinea pigs. **RESULTS:** With the binding of ATX II to the Na^+ channels, the probability of being in the open state and the open time constant of single Na^+ channel with long opening mode increased significantly. Action potential duration APD_{50}, APD_{90}, and the maximal upstroke velocity of phase 0 were increased by 25.8 %, 26.1 %, and 12 %, respectively. The QT interval and QTc, a rectified QT interval, increased by 12.8 % and 16.9 %. On the contrary, after application of mexiletine, the open probability of single Na^+ channel was reduced greatly. In the presence of ATX II (40 nmol/L), mexiletine (1, 5, 15, 45, 70 μmol/L) shortened the APD_{50} by 0.5 %, 6.7 %, 14.4 %, 19.4 %, and 18.8 %, respectively, and decreased the APD_{90} and V_{max} accordingly. In the experiments with ECG, mexiletine reversed the ATX II-produced prolongation effects on QTc in a dose-dependent manner. **CONCLUSION:** Mexiletine may be an effective drug in the treatment of LQT_3.

INTRODUCTION

Long QT syndromes is one of genetic diseases. Recent studies identified three types of long QT syndromes caused by mutations of ion channel genes located on chromosomes 3 (SCN5A), 7 (HERG) and 11 (KvLQT1)[1-3]. Mutations in SCN5A (LQT_3) resulted in the incomplete inactivation of sodium channel[4]. With the high affinity to cardiac sodium channel, sea anemone toxin (ATX II) is a useful tool to study Na^+ channel gating process[5]. Therefore, ATX II can be used to make models of LQT_3 carried on in three levels of single channel recording, action potential recording, and electrocardiography, respectively[6]. This paper is concentrated on the mechanisms by which mexiletine, a kind of anti-arrhythmia medicine, acts on the models of LQT_3 in three experimental levels in order to make sure if it is effective in preventing and curing the long QT syndromes.

MATERIALS AND METHODS

Isolation of ventricular myocytes The heart of guinea pig weighing 250-300 g was taken out and perfused consecutively with calcium-free Tyrode solution and collagenase-containing solution in a constant hydrostatic pressure of 76 cm H_2O for about 10 min (Langendorff perfusion). Then the heart was removed to Ca-free solution for 2 h for slowly-digesting the connective tissue by the collegenase left in the heart. Ventricles were cut out to pieces and shaked in K-B solu-

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tion to obtain the calcium-tolerant cells [7].

Single channel recording The isolated ventricular cells which sedimented to the bottom of bath chamber were perfused with oxygen-bubbled Tyrode solution, and the single channel current was recorded with cell-attached configuration of patch-clamp. The pipette was filled with solution containing in mmol/L: NaCl 180, KCl 1.3, $MgCl_2$ 0.5, $CaCl_2$ 1.5, Glucose 5, Hepes 5, $CoCl_2$ 3, TEA 10, 4-AP 10 and CsCl 10. Because the K and Ca channel were blocked by $CoCl_2$, TEA, 4-AP, and CsCl in the pipette solution, the only channel which can allow inward current pass through is the Na channel[8]. ATX II 0.2 μmol/L (Calbiochen, USA) or ATX II (0.2 μmol/L) and mexiletine (0.7 mmol/L, sigma, USA) were added to the pipette solution to observe their effects on Na channels. Oscilloscope DSS6521 (Kikusui, Japan) and Axopatch-1-C amplifier were used to monitor and record the voltage and channel currents. The data were stored and processed by Pentium II computer.

Intracellular recording of action potentials The right ventricular papillary muscle of guinea pig was isolated and pinned to the bottom of a chamber. The preparation was perfused and stimulated with extracellular electrode at a rate of 1 Hz and impaled with glass microelectrode which contained KCl solution of 3 mol/L and had a tip resistance of 10-20 MΩ. A VDF-3 amplifier and an oscilloscope were employed to measure the action potentials. Concentration of ATX II in Tyrode solution was 40 nmol/L while needed. When the preparation was treated with mexiletine, the Tyrode solution contained both ATX II (40 nmol/L) and mexiletine with different concentrations (1, 5, 15, 45, 70 μmol/L).

Recording of ECG The guinea pig was anesthetized by injection of urethane (20 %, 5 mL/kg) into the abdomen. ECG of standard limb lead II was sampled by the Power Lab System (AD Instrument, Australia). ATX II (1.5 μg/kg) was injected from the saphenous vein to make the LQT_3 model. Mexiletine (5, 15, 45, 70 μg/kg) was introduced into the body of the guinea pig in the same way as ATX II. The rectified QT interval, QTc, was used to correct the effects of heart rate on QT interval produced by the drugs used above. The formula is as follows:

$QTc=QT/RR^{1/2}$ (RR: the interval between two R waves).

Data analysis Data were expressed as mean±SD. Differences between groups were evaluated with a two-tailed student *t* test.

RESULTS

Effects of ATX II and mexiletine on single sodium channel In the configuration of cell-attached patch, the holding potential was kept at -120 mV. If the membrane was depolarized to -50 mV ($E_{command}$), four modes of late Na channel currents (brief, drizzle, long-open and burst modes) were observed[8-9]. When ATX II was contained in the pipette solution, the long open mode occurred more often (Fig 1B). The probability of being in the open state of long open mode of Na channel was increased from (0.054±0.016) % to (9.14±0.25) % (n=6). And the open time constant prolonged from (1.08±0.09) ms to (18.2±2.4) ms (P<0.01, n=6).

When both mexiletine and ATX II were contained in the electrode, open probability and open time constant of single Na channel had no significant difference compared with the controls (Fig 1C).

Effects of ATX II and mexiletine on action potentials The right ventricular papillary muscle of guinea pig was consecutively perfused by the Tyrode solution, ATX II-contained solution, and ATX II-mexiletine-contained solution. The preparation was stimulated by suprathreshold electric pulse at a rate of 1 Hz and fast response action potentials were recorded. The results showed that ATX II (40 nmol/L) prolonged the action potential duration (APD_{50} and APD_{90}) from 143 ms and 176 ms to 180 ms and 222 ms, respectively. The maximum upstroke velocity of phase 0 changed from 236 to 264 V/s by ATX II.

When the perfusion solution contained both mexiletine (1, 5, 15, 45, 70 μmol/L) and ATX II, the APD and V_{max} decreased according to different concentration of mexiletine (Tab 1, Fig 2A). Fig 2B was concentration-response curve of mexiletine. The changes of excitability of the preparation could also be observed, for the stimulus threshold reduced from 0.3 mA to 0.2 mA or less after the effect of ATX II, but it would increase to 0.5 mA as mexiletine added into the perfusing solution.

Effects of ATX II and mexiletine on ECG of guinea pig ECG of guinea pig was recorded by standard limb lead II. One of 6 experiments was shown in Fig 3. ATX II was injected from the saphenous vein, 2 min after the injection, QT interval of ECG began to increase, and within 8 min the changes became stable. Ten minutes later mexiletine was injected, it needed 2-4 min to show its effect (Tab 2). The results indicated

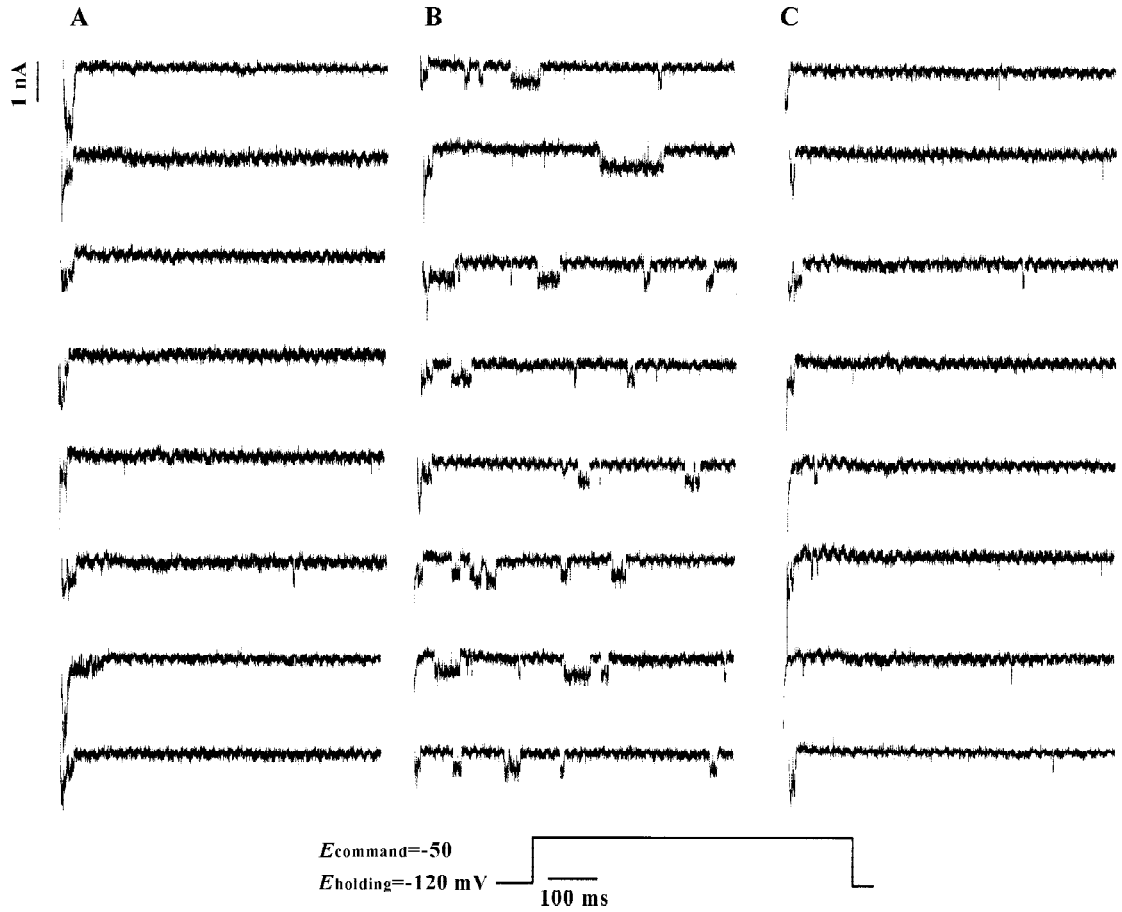

Fig 1. Effect of AXT II and mexiletine plus AXT II on single sodium channel. A) Control. B) ATX II (0.2 mmol/L). C) Mexiletine (0.7 mmol/L) plus ATX II (0.2 mmol/L).

Tab 1. Effect of ATX II (40 nmol/L) and mexiletine (MEX) at different concentrations on action potentials of guinea pig. *n*=5. Mean±SD. $^{c}P<0.01$ *vs* control. $^{e}P<0.05$, $^{f}P<0.01$ *vs* ATX II. $^{h}P<0.05$, $^{i}P<0.01$ *vs* ATX II+MEX 5 mmol/L. $^{k}P<0.05$ *vs* ATX II+MEX 15 mmol/L.

	APD_{50}/ms[1)]	APD_{90}/ms	V_{max}/V·s^{-1}	APA/mV
Control	143±22	176±26	236±16	122±5
ATX II	180±24^{c}	222±27^{c}	264±16^{c}	126±4
ATX II+MEX 1 μmol/L	179±20 (0.5%)	220±29	263±18	125±5
ATX II+MEX 5 μmol/L	168±20^{e} (6.7%)	202±17^{e}	258±14	123±3
ATX II+MEX 15 μmol/L	154±21^{fi} (14.4 %)	186±16^{fi}	250±14^{fi}	124±3
ATX II+MEX 45 μmol/L	145±23^{fki} (19.4 %)	176±27^{fh}	246±10^{eh}	122±3
ATX II+MEX 70 μmol/L	146±25^{fki} (18.8 %)	178±29^{f}	236±22^{fi}	122±2

1) The percentage represents the changes *vs* ATX II group.

that ATX II prolonged the QT interval by 12.8 % and mexiletine (5, 15, 45, 70 μmol/L) reduced it by 3.0 %, 5.3 %, 9.8 %, 9.0 %, respectively. For QTc, it was increased by ATX II from 248 ms (control) to 290 ms, and then decreased by mexiletine in a concentration-dependent manner (Tab 2). It was also observed that the effect of mexiletine could last more than 60 min, but the heart rate would slow down as time passed.

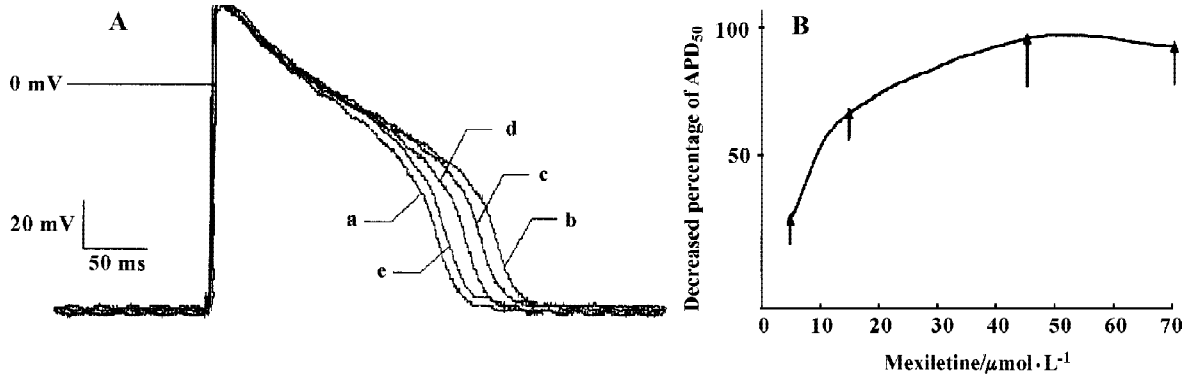

Fig 2. Effect of AXT II and mexiletine on action potential of guinea pig and the dose-effect curve of mexiletine. A: action potential of guinea pig. a) Control. b) ATX II (40 nmol/L). c) ATX II+mexiletine 5 mmol/L. d) ATX II+mexiletine 15 mmol/L. e) ATX II+mexiletine 45 mmol/L. B: The dose-effective curve of mexiletine.

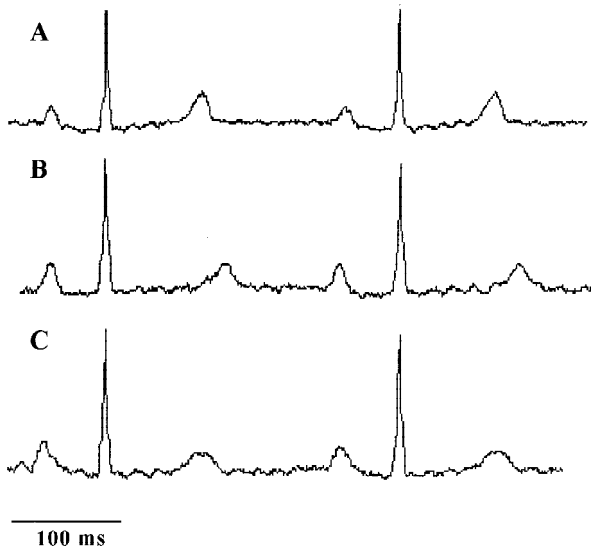

Fig 3. Effect of AXT II and mexiletine on ECG of guinea pig. A) Control. B) Injected with ATX II (1.5 mg/kg). C) Injected with mexiletine (15 mg/kg).

Tab 2. Effect of ATX II (1.5 mg/kg) and mexiletine (MEX) at different doses on QT interval of ECG. *n*=5. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{e}P<0.05$, $^{f}P<0.01$ *vs* ATX II. $^{h}P<0.05$, $^{i}P<0.01$ *vs* ATX II+MEX 5 mg/kg. $^{k}P<0.05$ *vs* ATX II+MEX 15 mg/kg.

	QT/ms	QTc/ms
Control	117±20	248±30
ATXII	132±19^{c}	290±50^{b}
ATXII+MEX 5 μg/kg	128±17^{e}	276±47^{f}
ATXII+MEX 15 μg/kg	125±16^{fh}	269±48^{fh}
ATXII+MEX 45 μg/kg	119±18^{fik}	260±42^{eh}
ATXII+MEX 70 μg/kg	120±18^{fki}	262±45^{eki}

DISCUSSION

In the previous research, late sodium channel was divided into four modes[9]. They are brief opening, drizzle opening, long opening, and burst mode. The opening of late sodium channel contributes to the formation of action potential plateau. In this study, we used ATX II, a high affinity toxin to cardiac sodium channel, to change its kinetics. It caused an incompleted inactivation of Na channel. The channel opening lasted much longer than normal. In this way, the long opening mode became the dominant one among the four modes. Its open time constant increased significantly. Meanwhile, open probability increased 170 times of the control.

The changes of Na channel modes should be responsible for prolongations of APD_{50} and APD_{90}, for the increased inward current going through the long open mode would last the plateau phase and action potential duration. As QT interval of ECG is supposed to correlate with action potential plateau, that is why the changes of Na channel mode caused by ATX II could make the QT and QTc of ECG prolonged in the ATX II-injected guinea pigs[10]. Our results are similar to those of other authors[11,12] who applied ATX II to produce LQTs models.

Mexiletine is one of I_b anti-arrhythmic medicine. As K and Ca channels are blocked by 4-AP, TEA, $CoCl_2$, and CsCl in the pipette solution, the target of mexiletine is then on sodium channels. As shown in Fig 1C, mexiletine inhibits inward sodium current when contained in electrode. The open time constant and open probability of long opening mode were reverted to con-

trols by mexiletine (Fig 1).

In the presence of ATX II, mexiletine (1, 5, 15, 45, 70 μmol/L) shortened APD_{50}, APD_{90}, and V_{max} in a dose-dependent manner. At the concentration of 45 μmol/L , mexiletine showed its maximum effect on those parameters. But mexiletine had no statistical significant effect on APA in our experiment.

Studying from ECG of guinea pig, we found that mexiletine shortened the QT interval dramatically after the animal was treated with ATX II. Not only QT but also QTc, were significantly reduced by mexiletine. The dose of mexiletine should be used very carefully. In our experiments, we observed that the mexiletine inhibited the excitability of the preparation and reduced heart rate of the animals.

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