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Effects of U50,488H on transient outward and ultra-rapid delayed rectifier K^+ currents in young human atrial myocytes

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Abstract

The effects of *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate salt (U50,488H), a selective κ-opioid receptor agonist, on transient outward K^+ current (I_{to1}) and ultra-rapid delayed rectifier K^+ current (I_{Kur}) in young human atrial myocytes were evaluated with a whole-cell patch-clamp technique. At +10 mV, U50,488H decreased I_{to1} in a concentration-dependent manner (IC_{50} = 12.4 ± 3.5 μM), while at +50 mV, U50,488H produced biphasic effects on I_{to1}—increasing and decreasing the current at 1–3 and 10–30 μM, respectively. U50,488H at 10 μM shifted the midpoint ($V_{0.5}$) of I_{to1} activation in a depolarizing direction by ~5 mV, accelerated the inactivation, and slowed the recovery from inactivation of I_{to1}. In addition, U50,488H inhibited I_{Kur} in a concentration-dependent manner (IC_{50} = 3.3 ± 0.6 μM). The effects of U50,488H on the two types of K^+ currents were not antagonized by either 5 μM norbinaltorphimine or 300 nM naloxone. These results indicate that U50,488H affects both I_{to1} and I_{Kur} in young human atrial myocytes in an opioid receptor-independent manner.

Keywords: U50,488H; Atrium, human; Ion channel; Opioid; K^+ current, transient outward; K^+ current, ultra-rapid delayed rectifier

1. Introduction

Trans-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate salt (U50,488H), is a selective κ-opioid receptor agonist (Von-voigtlander et al., 1983). However, it has been shown to act on cardiac ion channels via mechanisms independent of opioid receptor. U50,488H inhibits Na^+ and K^+ currents in rat ventricular myocytes (Pugsley et al., 1994), and Ca^{2+} current in guinea pig ventricular myocytes (Utz et al., 1995). In mouse hippocampal CA3 pyramidal neurons, U50,488H directly inhibits A-type, transient outward K^+ current (I_A) and rapidly activating, delayed rectifier K^+ current (I_K) (Zou et al., 2001). In terms of kinetics, these two types of K^+ currents are similar to transient outward K^+ current (I_{to1}) and ultra-rapid delayed rectifier K^+ current (I_{Kur}) (Nattel et al., 1999; Rudy, 1988), which are important outward K^+ currents responsible for the repolarization in human atrium (Shibata et al., 1989; Van Wagoner, 2000; Wang et al., 1993). It is likely that U50,488H also inhibits I_{to1} and I_{Kur} in human atrium via mechanisms independent of opioid receptor. Therefore, in the present study we firstly determined the effects of U50,488H on these two types of K^+ currents in human atrial myocytes using the whole-cell patch-clamp technique. Secondly, we determined whether the effects of U50,488H were opioid receptor-mediated. Our results showed that U50,488H produced biphasic effects on I_{to1} and inhibited I_{Kur} via an opioid receptor-independent manner.

2. Materials and methods

2.1. Patient characteristics

Specimens of human right atrial appendage were obtained from 19 pediatric patients (10 females and 9 males) undergoing cardiac surgery for repair of atrial or ventricular septal defects. None of the patients had atrial arrhythmias. All specimens were grossly normal at the time

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of excision. The patients were recruited from the pediatric cardiac clinic. Their age ranged from 1 to 15 years of age (6.5 ± 3.2 years). The procedure for obtaining the tissue was approved by the Ethics Committee of the University of Hong Kong, and also a written consent was obtained from parents of the subjects.

2.2. Myocyte preparation

After the tissue was transferred to the laboratory, atrial myocytes were dissociated with a modified procedure as described previously (Du et al., 2003). Briefly, the tissue was minced with a sharp blade and then placed in a 15-ml tube containing 10 ml of the Ca^{2+}-free solution (36 °C), agitated by continuous bubbling with 100% O_2. After 15 min (5 min for three times in fresh solution), the tissue chunks were re-incubated in a solution containing 150–200 U/ml collagenase (CLS II, Worthington Biochemical, Freehold, NJ), 1.2 U/ml protease (type XXIV, Sigma, St. Louis, MO) and 1 mg/ml bovine serum albumin for 50 min. The supernatant was then removed and discarded. The chunks were re-incubated in a fresh solution with 150–200 U/ml collagenase. Microscopic examination of the medium was performed every 5–10 min to examine the number and quality of the isolated cells. When the yield appeared to be maximal, the digested tissue was suspended in high-K^+ storage solution and gently pipetted. The isolated myocytes were kept in the medium for at least 1 h before use.

A small aliquot of the solution containing the isolated cells was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope. Myocytes were allowed to adhere to the bottom of the dish for 5–10 min and were then superfused at 2–3 ml/min with Tyrode's solution. The experiments were performed at room temperature (21–22 °C). Only quiescent and rod-shaped cells showing clear striations were selected for experiments.

2.3. Solutions and drugs

The standard Tyrode's solution contained (in mM): NaCl, 140; KCl, 5.4; $MgCl_2$, 1; $CaCl_2$, 1.8; NaH_2PO_4, 0.33; HEPES, 5; and glucose, 10 (pH adjusted to 7.4 with NaOH). For atrial tissue chunk wash, Ca^{2+} was omitted. High-K^+ storage solution contained (in mM): KCl, 10; KH_2PO_4, 10; glucose, 20; K-glutamate, 120; taurine, 10; EGTA, 0.5; HEPES, 10; and $MgSO_4$, 1.8 (pH adjusted to 7.2 with KOH). The pipette solution contained (in mM): KCl, 20; K-aspartate, 110; $MgCl_2$, 1; HEPES, 10; EGTA, 5; GTP, 0.1; Na_2-phosphocreatine, 5; and Mg_2ATP, 5 (pH adjusted to 7.2 with KOH). For I_{to1} and I_{Kur} determination, 200 μM $BaCl_2$ and 200 μM $CdCl_2$ were added to the bath solution to block I_{K1} and I_{Ca}. Atropine (1.0 μM) was used to minimize possible $I_{K,Ach}$ contamination during the current recording. In some experiments, sodium current was inhibited with the use of a holding potential of −50 mV and equimolar *N*-methyl-D-glucamine replacement of Na^+ in the bath solution. The K^+ channel blocker 4-aminopyridine was prepared as a 5 M stock solution in distilled water, with pH adjusted to 7.4 with HCl.

U50,488H and naloxone (a nonselective opioid receptor antagonist) were purchased from Sigma. Nor-binaltorphimine, a selective κ-opioid receptor antagonist, was purchased from Tocris Cookson. All other chemicals were obtained from Sigma. All chemicals were dissolved in distilled water. The concentrations of naloxone and nor-binaltorphimine used in this study were based on previous studies (Grudt and Williams, 1993; Sheng et al., 1997; Zhang and Wong, 1998).

2.4. Data acquisition and analysis

The whole-cell patch-clamp technique was used. Borosilicate glass electrodes (1.2-mm OD) were pulled with a Brown-Flaming puller (model P-97, Sutter Instrument, Novato, CA, USA) and had a tip resistance of 2–3 MΩ when filled with pipette solution. The tip potentials were compensated before the pipette touched the cell. After a gigaseal (>10 GΩ) was obtained, the cell membrane was ruptured by gentle suction to establish the whole cell configuration. Data were acquired with the use of EPC-9 (Heka, Lambrecht, Germany). Command pulses were generated by a 12-bit digital-to-analog converter controlled by Pulse software (Heka Instruments). The series resistance was electrically compensated to minimize the capacitive surge on the current recording. Current recordings were low-pass filtered at 5 kHz and stored on the hard disk of an IBM-compatible computer.

Values were presented as mean ± S.E.M. Nonlinear curve fitting was performed using PulseFit (Heka) and Sigmaplot (SPSS, Chicago, IL). Paired and/or unpaired Student's *t*-tests were used to evaluate the statistical significance of differences between two groups, while one-way analysis of variance was used for multiple groups. A difference at $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of U50,488H on I_{to1}

Fig. 1 illustrates I_{to1} traces recorded from a representative cell in the absence and presence of U50,488H. The current was elicited by 300-ms voltage steps to between −40 and +60 mV from a holding potential of −50 mV. U50,488H at 1 μM increased slightly its amplitude (Fig. 1B). On the other hand, at a higher concentration (30 μM), it decreased its amplitude substantially and accelerated its decay (Fig. 1C). The effect was partially reversed after 10 min of drug washout (Fig. 1D).

I_{to1} was analyzed by measuring the current from the peak current to the steady-state level at the end of voltage

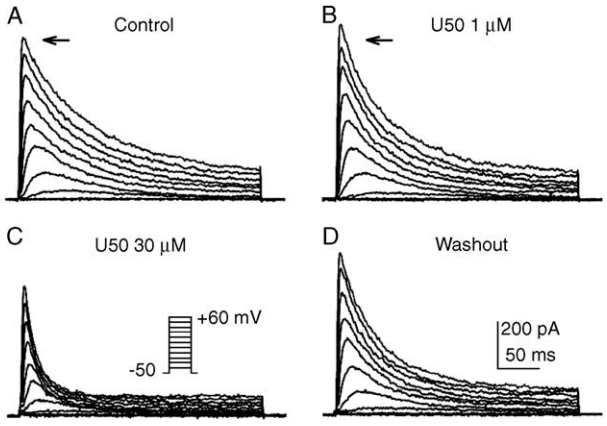

Fig. 1. Effect of U50,488H (U50) on I_{to1}. Recordings from a representative cell are shown under control conditions (A), in the presence of 1 μM (B) and 30 μM (C) U50, and washout of the drug for 10 min (D). I_{to1} was elicited by 300-ms voltage steps to between −40 and +60 mV from −50 mV as shown in the inset of panel C.

steps and normalized by individual cell membrane capacitance. Fig. 2A displays current–voltage relationship of I_{to1} density in seven cells studied under control conditions, in the presence of 1, 3, 10, 30 and 50 μM U50,488H, and after drug washout. Fig. 2B displays percent changes of I_{to1} by U50,488H at test potentials from 0 to +60 mV, showing significant voltage-dependent effect of U50,488H on I_{to1}. U50,488H decreased I_{to1} at all test voltages in a concentration-dependent manner with the exception of a small enhancing effect by 1 and 3 μM U50,488H at +50 and +60 mV.

Due to the voltage dependence of U50,488H on I_{to1}, we analyzed the concentration–response relationship at +10 and +50 mV, respectively. Fig. 2C shows the concentration–response relationship of U50,488H effect on I_{to1} at +10 mV. The dose-dependent curve was fitted by Hill equation in individual cell. The half-maximal inhibitory concentration (IC_{50}) was 12.4 ± 3.5 μM (n = 6), and Hill coefficient was 0.96 ± 0.35. Fig. 2D displays the concentration–response relationship of U50,488H effect on I_{to1} at +50 mV (n = 6). U50,488H at 1 and 3 μM increased I_{to1} by 8.3 ± 2.6% (P < 0.05) and 6.4 ± 3.2% (P < 0.05), respectively. In contrast, at 10, 30 and 50 μM the compound decreased the current by 8.1 ± 3.4% (P < 0.05), 20.2 ± 3.8% (P < 0.01) and 31.4 ± 2.9% (P < 0.01), respectively.

3.2. Effects of U50,488H on voltage- and time-dependent kinetics of I_{to1}

To study voltage-dependent activation of I_{to1}, the activation variables of I_{to1} were determined from I_{to1} current–voltage relationship in Fig. 2A with the procedure as described previously (Li et al., 1998). Calculated values were normalized to the maximal value in each cell. The inactivation variable of I_{to1} was determined by normalizing the current at different prepulses by the current without prepulse under Na^+-free conditions with the voltage protocol shown in the inset in Fig. 3A. Data from individual cells were fit to Boltzmann distributions to obtain the half activation or inactivation voltage ($V_{0.5}$) and the slope factor (k). Mean data for activation and inactivation before and after exposure to 10 μM U50,488H are shown in Fig. 3A. With 10 μM U50,488H, a concentration close to the IC_{50} of I_{to1}, mean values of $V_{0.5}$ for activation were shifted in depolarizing direction (from 15.5 ± 2.4 to 20.0 ± 2.2 mV; n = 6, P < 0.01), and the k was not changed (10.5 ± 0.8 for control and 10.1 ± 0.8 for U50,488H; P>0.05). However, $V_{0.5}$ for inactivation of I_{to1} was not significantly shifted by 10 μM U50,488H (−23.5 ± 2.2 for control and −25.7 ± 3.1 mV for U50,488H; n = 6, P>0.05). The k for inactivation was 4.9 ± 0.7 and 5.0 ± 0.7 in control and U50,488H, respectively (P>0.05).

Time-dependent recovery of I_{to1} from inactivation was analyzed under Na^+-free conditions using a 300-ms paired-pulse protocol shown in the inset in Fig. 3B. Fig. 3B shows mean values of the time course of I_{to1} recovery in seven cells. I_{to1} was completely recovered within 210 ms under

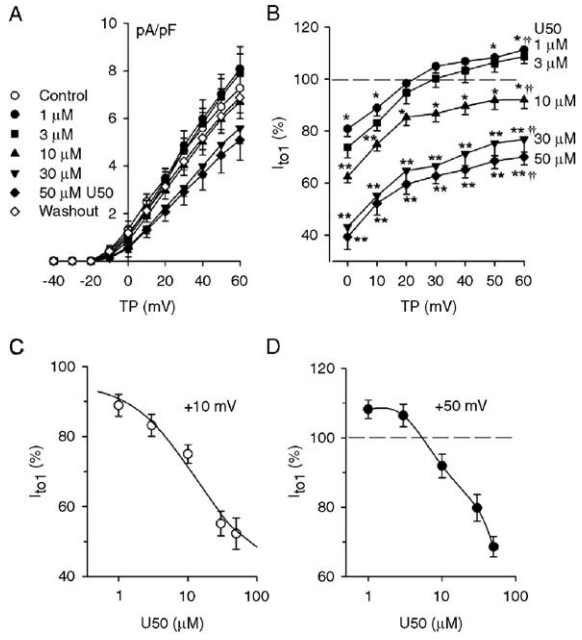

Fig. 2. Effects of U50,488H (U50) on voltage-dependent I_{to1}. (A) Current–voltage (I–V) relationship of I_{to1} under control conditions, in the presence of 1, 3, 10, 30 and 50 μM U50 and the drug washout (n = 7). (B) Percent changes of I_{to1} by U50 with various concentrations (n = 7). I_{to1} is expressed as percentage of the control current. *P < 0.05, **P < 0.01 vs. control. $^{\dagger\dagger}P$ < 0.01 for voltage dependence of drug effect. (C) Concentration–response relation for U50 effect on I_{to1} at +10 mV. Symbols are mean experimental data (relative to percentage of control, n = 6). Solid line is best-fit equation of the form $E = E_{max}/[1+(C/IC_{50})^b]$, where E is the effect at concentration C, E_{max} is the maximum effect, IC_{50} is the concentration for half maximal action, and b is Hill coefficient. The IC_{50} was 12.4 ± 3.5 μM, and Hill coefficient was 0.96 ± 0.35. (D) Concentration–response relation for U50 effect on I_{to1} recorded at +50 mV. Symbols are mean experimental data (relative to percentage of control, n = 6).

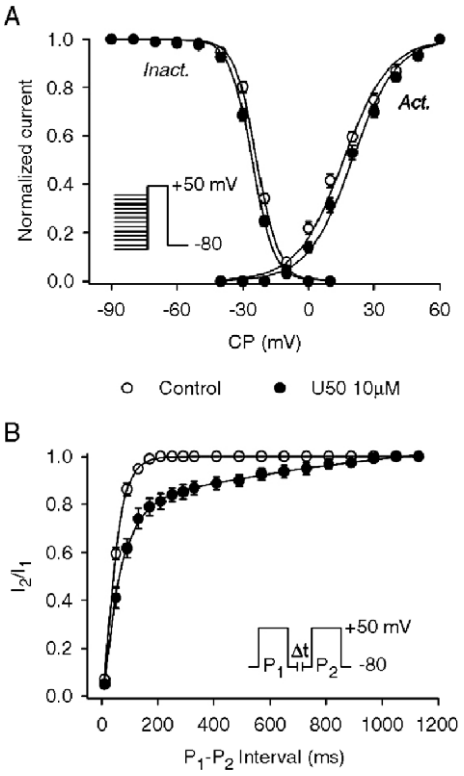

Fig. 3. U50,488H (U50) effects on voltage dependence and recovery of I_{to1}. (A) Mean data for voltage dependence of I_{to1} inactivation (*Inact.*) and activation (*Act.*) in the absence (open circles) and presence (filled circles) of 10 μM U50 ($n=6$). Inactivation was assessed with a 1-s conditioning pulse from voltages between −90 and +30 mV, followed by a 300-ms test pulse to +50 mV (inset). Activation was obtained by normalizing calculated values of different test potentials to the maximal value in each cell. The curves shown are fit to Boltzmann distribution function. (B) Time course of recovery from inactivation of I_{to1} in the absence (open circles) and presence (filled circles) of 10 μM U50. The recovery of I_{to1} was determined using 300-ms paired pulses by varying P_1–P_2 interval. Mean values for I_{to1} of the test pulse (P_2) normalized to current during a basic pulse (P_1) are shown as a function of the P_1 to P_2 interval in seven cells. Data are best fit to a mono-exponential function.

control conditions. However, the current was not recovered until 1050 ms after the application of 10 μM U50,488H. The recovery curves of I_{to1} were fitted by a mono-exponential function with time constants of 18.2 ± 3.9 ms under control conditions and 48.3 ± 2.2 ms in the presence of U50,488H ($P<0.01$). The results indicated that U50,488H significantly decreased the recovery of I_{to1} from inactivation.

Fig. 4 shows the effects of U50,488H on time-dependent inactivation of I_{to1}. Representative recordings (points) of I_{to1} upon depolarization to +50 mV from −50 mV were well fitted by a mono-exponential function (solid line) with the time constant shown in the absence and presence of U50,488H (Fig. 4A). The inactivation time constant (τ) was substantially reduced in the presence of 10 μM U50,488H. Fig. 4B displays mean values of time constants under control conditions and in the presence of 1, 3, 10, 30 and 50 μM U50,488H. U50,488H significantly accelerated I_{to1} inactivation in a concentration-dependent manner ($n=8$, $P<0.01$), indicating blockade of open channels (Dukes et al., 1990).

3.3. Effect of U50,488H on I_{Kur}

Fig. 5 illustrates the effect of U50,488H on I_{Kur}. Fig. 5A displays time-dependent effect of U50,488H on I_{Kur} in a representative cell. I_{Kur} was recorded with a 100-ms prepulse to +40 mV to inactivate I_{to1}, followed by a 150-ms test pulse to +60 mV from −50 mV (shown in the left inset of the panel), and its amplitude was measured from zero current to the steady-state level at the end of depolarization steps. The inhibitory effect of U50,488H on I_{Kur} occurred within a few seconds after exposure to the drug and reached a steady-state level in about 2 min, and partially recovered upon the drug washout. The original I_{Kur} traces at the specified time points are shown in the right inset of the panel. Fig. 5B shows I_{Kur} recordings before and after application of 10 μM U50,488H with the voltage protocol shown in the inset. Currents under control conditions showed rapid activation with a little inactivation typical of I_{Kur} (Fig. 5B,a). U50,488H at 10 μM caused substantial reductions in both step current elicited by depolarization and tail current at −20 mV (Fig. 5B,b). The effect was partially reversed after 10 min of drug washout (Fig. 5B,c).

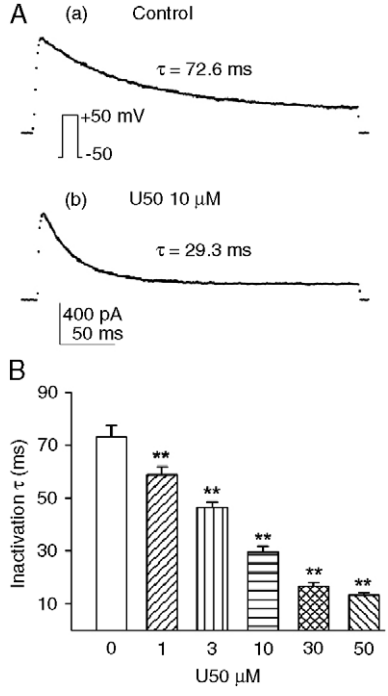

Fig. 4. Effects of U50,488H (U50) on I_{to1} inactivation. (A) Representative I_{to1} tracing from one cell upon depolarization to +50 mV from −50 mV under control conditions (a) and in the presence of 10 μM U50 (b). Raw data (points) before and after application of U50 were well fit to mono-exponential functions (solid lines) with time constants shown. (B) Time constants of I_{to1} inactivation in the absence and presence of 1, 3, 10, 30 and 50 μM U50 ($n=8$). **$P<0.01$ vs. control.

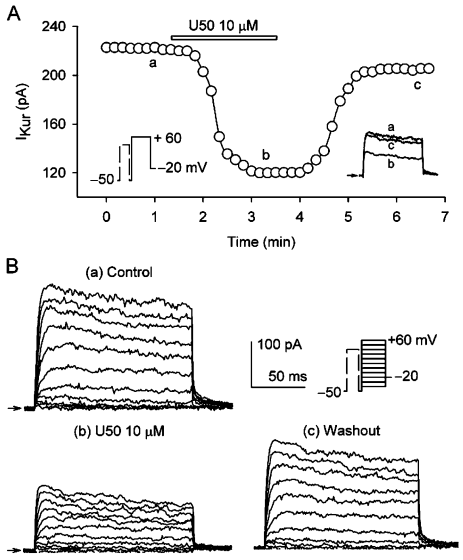

Fig. 5. Effect of U50,488H (U50) on I_{Kur}. (A) Time-dependent effect of 10 μM U50 on I_{Kur} elicited by the voltage protocol shown in the left inset in a representative cell. The original I_{Kur} tracings are shown in the right inset from control (a), steady-state level after U50 exposure (b), and drug washout (c) at corresponding time points. (B) Representative voltage-dependent I_{Kur} traces in one cell under control conditions (a) and then in the presence of 10 μM U50 (b), followed by drug washout (c). I_{Kur} was recorded with a 100-ms prepulse to +40 mV to inactivate I_{to1}, followed by 150-ms test pulses between −40 and +60 mV from −50 mV.

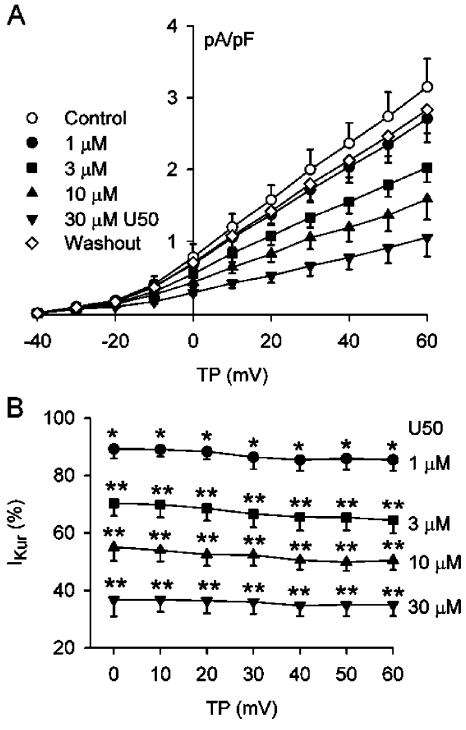

Fig. 6. Concentration-dependent effects of U50,488H (U50) on I_{Kur}. (A) Current–voltage (I–V) relationship of I_{Kur} under control conditions, in the presence of 1, 3, 10 and 30 μM U50 and the drug washout (n=7). (B) Percent changes of I_{Kur} by U50 at various concentrations. I_{Kur} is expressed as percentage of the control current. *P<0.05, **P<0.01 vs. control (n=7).

Fig. 6A shows current–voltage relationship of I_{Kur} density (normalized by individual cell membrane capacitance) in seven cells studied under control conditions, in the presence of 1, 3, 10 and 30 μM U50,488H, and after drug washout. U50,488H produced a concentration-dependent inhibition of I_{Kur}, and the effect was partially reversible upon washout. Fig. 6B shows percent changes of I_{Kur} by U50,488H with various concentrations at test potentials from 0 to +60 mV. Significant decrease of I_{Kur} began at an U50,488H concentration of 1 μM and a maximal reduction of 65.1 ± 4.0% (n=7) was reached at 30 μM. No significant voltage-dependent effect was observed at any concentration.

Because a time-independent nonselective cation current was also elicited by depolarization in human atrial cells (Crumb et al., 1995), we evaluated the effect of U50,488H on I_{Kur} in the presence of 5 mM 4-aminopyridine, shown to block I_{Kur} completely (Wang et al., 1993). Fig. 7A displays representative recordings in one cell under control conditions, in the presence of 30 μM U50,488H, and then 5 mM 4-aminopyridine. Fig. 7B shows 4-aminopyridine-sensitive current (a), obtained by digital subtraction of current in the presence of 4-aminopyridine from control, and U50,488H-sensitive current (b), obtained by digital subtraction of

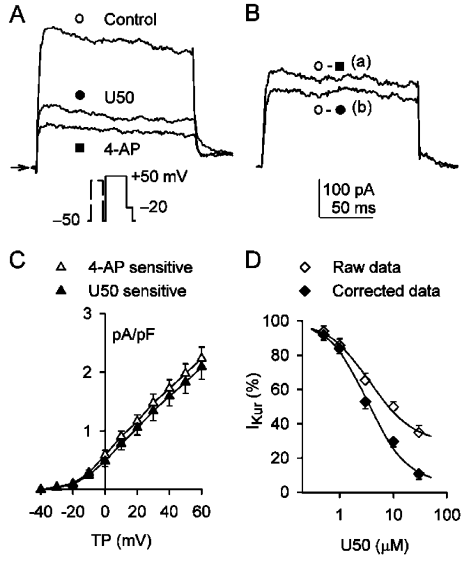

Fig. 7. Effects of U50,488H (U50) on I_{Kur} with and without 4-aminopyridine (4-AP) correction. (A) I_{Kur} recorded from one cell with the protocol as shown in the inset under control conditions and then after exposure to 30 μM U50 and 5 mM 4-aminopyridine. (B) Currents sensitive to 5 mM 4-aminopyridine (a) and to 30 μM U50 (b), obtained by digital subtraction of currents in the presence of drug from those in the absence of drug in panel A. (C) Current–voltage (I–V) relationship of 4-aminopyridine- and U50-sensitive currents obtained as illustrated in panels A and B (n=7). (D) Concentration–response relation of U50 inhibition of I_{Kur} at +50 mV (relative to percentage of control, n=6) without and with 4-AP correction. Solid lines are fit to Hill equation. The IC_{50} was 3.6 ± 0.6 and 3.3 ± 0.6 μM, Hill coefficient was 1.09 ± 0.13 and 1.20 ± 0.17, and the E_{max} was 65.1 ± 4.0 and 89.1 ± 3.4% before and after the correction, respectively.

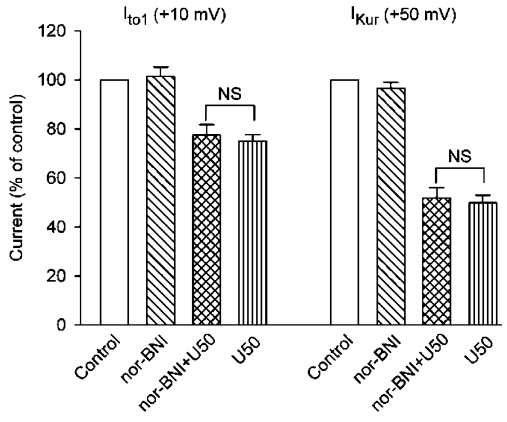

Fig. 8. Effects of U50,488H (U50) on I_{to1} and I_{Kur} with pretreatment of nor-binaltorphimine (nor-BNI). Nor-binaltorphimine at 5 μM was administered for 10 min before application of U50. U50 at 10 μM was then employed together with 5 μM nor-binaltorphimine for 5 min ($n=9$). Data are expressed as mean ± S.E.M. Nor-BNI at 5 μM had no effect on both I_{to1} and I_{Kur}, and did not antagonize U50-induced decrease of the two types of K^+ currents (P=NS vs. U50 alone).

current in the presence of U50,488H from control. Mean current–voltage relation curves of 5 mM 4-aminopyridine-sensitive and 30 μM U50,488H-sensitive currents are shown in Fig. 7C ($n=7$). Although slight difference was observed between the two currents at all test potentials, the difference was not statistically significant ($P>0.05$). Fig. 7D displays concentration-dependent inhibition of I_{Kur} by U50,488H without and with 4-aminopyridine correction. The data were fitted by Hill equation. The IC_{50} was 3.6 ± 0.6 and 3.3 ± 0.6 μM, Hill coefficient was 1.09 ± 0.13 and 1.20 ± 0.17 before and after the correction ($n=6$), respectively. However, the maximum effect (E_{max}) was greater with the 4-aminopyridine correction (89.1 ± 3.4%) than that without the 4-aminopyridine correction (65.1 ± 4.0%, $n=6$, $P<0.05$).

3.4. Effects of U50,488H on I_{to1} and I_{Kur} upon opioid receptor blockade

Nor-binaltorphimine and naloxone were used to study whether the effects of U50,488H on I_{to1} and I_{Kur} were mediated by opioid receptor. Fig. 8 shows the inhibitory effects of 10 μM U50,488H on I_{to1} at +10 mV and on I_{Kur} at +50 mV were the same in the presence and absence of 5 μM nor-binaltorphimine, which itself had no effect on either current. Similarly, 300 nM naloxone did not antagonize the actions of U50,488H either (data not shown). The result indicates that U50,488H-induced inhibitory effects on I_{to1} and I_{Kur} are not mediated by opioid receptor.

4. Discussion

The present study has provided the first evidence that the selective κ-opioid receptor agonist U50,488H significantly affects I_{to1} and I_{Kur} in young human atrial myocytes. At +10 mV, U50,488H inhibits I_{to1} in a concentration-dependent manner. At +50 mV, U50,488H produces biphasic effects on I_{to1}—increasing the current at 1–3 μM and decreasing the current at 10–50 μM. In addition and more importantly, U50,488H inhibits I_{Kur} in a concentration-dependent manner. The effects of U50,488H on the two types of K^+ currents are not mediated by opioid receptor.

U50,488H is known to exert cardiac effects via the κ-opioid receptor. For example, U50,488H at 2.5–40 μM reduces the contraction, electrically induced intracellular calcium transient and forskolin-stimulated cAMP accumulation in rat ventricular myocytes, which are antagonized by its selective antagonist, nor-binaltorphimine (Bian et al., 1998; Ventura et al., 1992). Pharmacological preconditioning with U50,488H (3–30 μM) also confers both immediate (Wang et al., 2001) and delayed (Wu et al., 1999) cardioprotection as does preconditioning with ischemia or metabolic inhibition and the protection is abolished with nor-binaltorphimine. However, Pugsley et al. (1994) reported that U50,488H inhibits the Na^+ current (IC_{50} = 15 μM) and sustained K^+ current (I_{sus}) in rat ventricular myocytes, and the effects are independent of opioid receptor. In addition, U50,488H has also been shown to inhibit Ca^{2+} current (IC_{50} = 20 μM) in guinea pig ventricular myocytes via an opioid receptor-independent manner (Utz et al., 1995). In the present study, we found that the effects of U50,488H on I_{to1} and I_{Kur} are not mediated via opioid receptor. So U50,488H could produce opioid receptor-dependent and/or -independent effects in the heart.

Direct effects of U50,488H on K^+ currents have previously been reported in rat ventricular myocytes (Pugsley et al., 1994). At high concentrations of 40–50 μM, U50,488H inhibits I_{sus}, and increases the rate of decay of I_{to1} without affecting the voltage-dependent activation and inactivation, and recovery from inactivation of I_{to1} (Pugsley et al., 1994). We found in the present study that U50,488H at 10–50 μM inhibited I_{to1}. At 1–3 μM, U50,488H produced biphasic effects on I_{to1}—inhibiting the current at 0 and +10 mV and stimulating the current at +50 and +60 mV. In addition, U50,488H shifted the activation curve of I_{to1} to depolarizing direction, accelerated the inactivation of I_{to1} and decreases the I_{to1} recovery from inactivation. Interestingly, U50,488H significantly also inhibited I_{Kur} in a concentration-dependent manner. It should be noted that the IC_{50} for U50,488H to inhibit I_{Kur} is 3.3 μM, which is much lower than those required to inhibit other ion channels. In ventricular myocytes, it was reported that 40–50 μM U50,488H was required for inhibition of I_{sus} (Pugsley et al., 1994), and the IC_{50}s for blocking Na^+ and Ca^{2+} currents were 15 and 20 μM U50,488H (Pugsley et al., 1994; Utz et al., 1995). The inhibitory effect of U50,488H on I_{Kur} at lower concentrations might make the drug a desirable therapeutic agent. Similarly, U50,488H directly inhibited I_A (IC_{50} = 20.1 μM) and I_K in neuronal cells (IC_{50} = 3.7 μM). The effects are believed to protect brain function against ischemic damage (Zou et al., 2001).

It is well known that I_{to1} and I_{Kur} are major repolarizing currents in human atrium (Shibata et al., 1989; Van Wagoner, 2000; Wang et al., 1993). Inhibition of the two currents, especially I_{Kur}, would prolong action potential duration and refractory period. It has been demonstrated that 50% reduction of I_{Kur} was found to prolong action potential duration by ~60% in human atrial myocytes (Wang et al., 1993). Therefore, decrease of I_{Kur} would be expected to inhibit atrial tachyarrhythmias including fibrillation induced by reentrant activity. In addition, I_{Kur} is found in atrium, but not in ventricle of human heart (Li et al., 1996), and therefore I_{Kur} is believed to be a potential target in the development of selective antiarrhythmic drugs (Nattel et al., 1999; Van Wagoner, 2000). In the present study we found that U50,488H at 1–3 μM inhibited I_{Kur} by 15–50%, suggesting that the drug may be useful in prophylaxis and/or treatment of atrial tachyarrhythmias. Moreover, U50,488H also inhibited I_{to1}, which would be a plus for prolongation of action potentials (Courtemanche et al., 1999). These effects and the cardioprotective actions of U50,488H (Wang et al., 2001; Wu et al., 1999) would make it a potential antiarrhythmic agent.

A drawback of the present study is that effects of U50,488H on rapid and slow components of delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) (Wang et al., 1994) were not evaluated. This was due to the fact that the cells obtained with the current isolation procedure was not favorable for recording I_{Kr} and I_{Ks}, and no I_{Kr} and/or I_{Ks} were detected even at 36 °C. Whether U50,488H would regulate I_{Kr} and I_{Ks} needs further study.

In conclusion, the present study has demonstrated for the first time that U50,488H at relatively low concentrations produces a marked inhibition of I_{Kur} in young human atrial myocytes in an opioid receptorindependent manner. It also exerts biphasic effects on I_{to1}. Further study is warranted to determine the therapeutic value of the compound as an antiarrhythmic drug against atrial tachyarrhythmias.

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