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In Vitro *Electrophysiologic Effects of Morphine in Rabbit Ventricular Myocytes*

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Background: **Morphine is widely used in patients undergoing surgical operations and is also reported to mediate cardioprotection of preconditioning. The current study determined effects of morphine at therapeutic to pharmacologic concentrations on cardiac action potential, L-type Ca^{2+} current ($I_{Ca.L}$), delayed rectifier K^+ current (I_K), and inward rectifier K^+ current (I_{K1}) in isolated rabbit ventricular myocytes.**

Methods: **Ventricular myocytes were enzymatically isolated from rabbit hearts. Action potential and membrane currents were recorded in current and voltage clamp modes.**

Results: **Morphine at concentrations from 0.01 to 1 μM significantly prolonged cardiac action potential, and at 0.1 and 1 μM slightly but significantly hyperpolarized the resting membrane potential. In addition, morphine at 0.1 μM significantly augmented $I_{Ca.L}$ (at +10 mV) from 5.9 ± 1.9 to 7.3 ± 1.7 pA/pF (by 23%; $P < 0.05$ *vs.* control) and increased I_{K1} (at −60 mV) from 2.8 ± 1.0 to 3.5 ± 0.9 pA/pF (by 27%; $P < 0.05$ *vs.* control). Five μM naltrindole (a selective δ-opioid receptor antagonist) or 5 μM norbinaltorphimine (a selective κ-opioid receptor antagonist) prevented the increase in $I_{Ca.L}$ induced by morphine, but 5 μM CTOP (a selective μ-opioid receptor antagonist) did not. The three types of opioid antagonists did not affect the augmentation of I_{K1} by morphine. Morphine had no effect on I_K.**

Conclusions: **These results indicate that morphine prolongs action potential duration by increasing $I_{Ca.L}$, an effect mediated by δ- and κ-opioid receptors. It also hyperpolarizes cardiac resting membrane potential by increasing I_{K1}, which is not mediated by opioid receptors.**

MORPHINE is still widely used in postoperative and preoperative care[1,2] and sedation in critically ill patients.[3] The drug is also effective in relieving the chest pain associated with acute myocardial infarction.[4–6] Morphine has been shown to mediate cardioprotection of preconditioning,[7,8] and the effect of morphine results from direct action at the level of the cardiac myocytes *via* δ- and κ-opioid receptors.[9] In addition, morphine showed antiarrhythmic activity in ischemia-reperfusion–induced arrhythmias.[10] Therefore, morphine is used in prognostic myocardial damage in cardiac surgery.[11]

Electrophysiologic effects of morphine on cardiac myocytes are not well understood. In multicellular preparations, morphine at higher therapeutic concentrations decreases the maximal upstroke velocity of action potential depolarization and increases the action potential duration (APD).[12,13] At the cellular level, the compound inhibits Na^+ current in rat and human cardiac myocytes and has no effect on L-type Ca^{2+} current ($I_{Ca.L}$), transient outward K^+ current, and inwardly rectifying K^+ current (I_{K1}).[14] A recent study in ischemia-reperfused guinea pig myocardium showed that morphine at clinically relevant concentrations decreases ischemia-induced conduction blocks and reperfusion-induced ventricular arrhythmias, which is believed to be mainly due to partial reverse of ischemia-induced membrane depolarization and decrease in action potential amplitude and the maximal upstroke velocity of action potential depolarization.[15] However, the ionic mechanisms involved in the effects of morphine remain to be clarified. The current study was therefore designed to determine the effects of morphine at clinically relevant concentrations on the action potential and ionic currents in isolated rabbit ventricular myocytes using a whole cell patch clamp technique.

Materials and Methods

The New Zealand white rabbits used in the current study were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*,[16] and the experimental protocol was approved by the Ethics Committee on the Use of Animals in Teaching and Research, The University of Hong Kong (Pokfulam, Hong Kong SAR, China).

Ventricular Myocytes Preparation

Ventricular myocytes were isolated from the hearts of rabbits with a procedure previously described.[17] Briefly, New Zealand white rabbits of either sex (1.5–2.5 kg) were anesthetized with pentobarbital (30 mg/kg, intravenous injection), and their hearts were quickly removed and placed in oxygenated Tyrode solution. Hearts were mounted on a Langendorff system and perfused for approximately 5 min with normal Tyrode solution and for an additional 10–12 min with Ca^{2+}-free Tyrode solution. Then, perfusion solution was switched to the Ca^{2+}-free Tyrode solution containing 0.5 mg/ml collagenase (CLS II; Worthington Biochemical, Freehold, NJ) and 1 mg/ml bovine serum albumin. The myocytes isolated from the softened heart were stored in high-K^+

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storage solution. Myocytes were placed in the recording chamber (0.3 ml) mounted on the stage of an inverted microscope and superfused at approximately 2 ml/min with external solution. Only quiescent, rod-shaped cells showing clear striations were selected for experiments. The experiments were performed at 36°C for recording action potentials and delayed rectifier K^+ current (I_K, *i.e.*, I_{Kr} and I_{Ks}) or room temperature (21°–22°C) for recording $I_{Ca.L}$ and I_{K1}.

Solutions and Drugs

The high-K^+ storage solution contained 10 mM KCl, 10 mM KH_2PO_4, 20 mM glucose, 120 mM K-glutamate, 10 mM taurine, 0.5 mM EGTA, 10 mM HEPES, and 1.8 mM $MgSO_4$ (pH adjusted to 7.2 with KOH). The Tyrode solution contained 140 mM NaCl, 5.4 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.33 mM NaH_2PO_4, 10 mM glucose, and 10 mM HEPES (pH adjusted to 7.4 with NaOH). When Tyrode solution was used to record K^+ currents, 200 μM Cd^{2+} and 3 mM 4-aminopyridine were added to respectively block $I_{Ca.L}$ and transient outward K^+ current (I_{to}). The pipette solution for K^+ current recordings contained 20 mM KCl, 110 mM potassium aspartate, 1.0 mM $MgCl_2$, 10 mM HEPES, 5 mM EGTA, 0.1 mM GTP, 5 mM Mg_2ATP, and 5 mM sodium phosphocreatine (pH adjusted to 7.2 with KOH). An *N*-methyl-D-glucamine Tyrode solution, containing 140 mM *N*-methyl-*d*-glucamine, 5.4 mM CsCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.33 mM KH_2PO_4, 10 mM glucose, and mM 10 HEPES, was used when $I_{Ca.L}$ was measured (pH adjusted to 7.4 with CsOH). The pipette solution for $I_{Ca.L}$ recording contained 20 mM CsCl, 110 mM cesium aspartate, 1.0 mM $MgCl_2$, 10 mM HEPES, 5 mM EGTA, 0.1 mM GTP, 5 mM Mg_2ATP, and 5 mM sodium phosphocreatine (pH adjusted to 7.2 with CsOH). Morphine (sulfate salt) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and dissolved in distilled water. All other chemicals were obtained from Sigma-Aldrich.

Based on the pharmacokinetics of morphine in humans, *i.e.*, a volume of distribution of 3.2 l/kg and 35% protein binding, the equilibrate plasma concentration is approximately 0.06 μM after intravenous injection of 0.2 mg/kg morphine.[18] We therefore used clinically relevant concentrations (0.01–1 μM) of morphine. To study the interaction of morphine with opioid receptors, effects of morphine on $I_{Ca.L}$ and I_{K1} were determined in the presence of 5 μM naltrindole (NTD; a selective δ-opioid receptor antagonist), 5 μM nor-binaltorphimine (nor-BNI; a selective κ-opioid receptor antagonist), or 5 μM CTOP (a selective μ-opioid receptor antagonist).

Data Acquisition and Analysis

The whole cell patch clamp technique was used[17,19] to record membrane current. Borosilicate glass electrodes (1.2 mm OD) were pulled with a Brown-Flaming puller (model P-97; Sutter Instrument Co., Novato, CA) 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 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 Electronik, Lambrecht, Germany). Command pulses were generated by a 12-bit digital-to-analog converter controlled by Pulse software (Heka Electronik). The recording of ionic currents were low-pass filtered at 5 kHz and stored on the hard disk of a computer. The series resistance (Rs) was electrically compensated to minimize the capacitive surge on the current recording and voltage drop across the clamped membrane and was kept at a constant value during the current recording. Membrane capacitance was 116.5 ± 12.8 pF (n = 45). To account for differences in cell size, all mean data are expressed as current density (*i.e.*, normalized to cell membrane capacitance). For recording action potential, a perforated patch was applied using a procedure of back-filling amphotericin-B (160 μg/ml; Sigma-Aldrich) in the K^+ pipette solution.[20] Action potentials were recorded in current clamp mode. Recorded membrane potentials were corrected by 10 mV for the liquid junction potential between the pipette and external solutions.

Data Analysis and Statistics

Nonlinear curve fitting was performed using Sigmaplot (SPSS, Chicago, IL). Concentration–response effects of morphine were fit to the Hill equation: $E = E_{max}/[1 + (EC_{50}/C)^b]$, where E is the effect at concentration C, E_{max} is the maximal effect, EC_{50} is the concentration for half-maximal effect, and *b* is the Hill coefficient. Curves for steady state activation (*d*) and inactivation (*f*) of $I_{Ca.L}$ were fit to Boltzmann relations for activation and inactivation as follows: d (or f) $= 1/\{1 + \exp[(V_{0.5} - V)/K]\}$, where V is the membrane potential, $V_{0.5}$ is a midpoint of potential for activation or inactivation, and K is a slope factor.

Comparison of two means was performed using the paired and unpaired Student *t* tests, and comparison of several means was performed using analysis of variance, then with a Dunnett *post hoc* test for the comparison with control. Statistical differences were considered significant if the *P* value was less than 0.05. Results are presented as mean ± SD.

Results

Effects of Morphine on Action Potentials in Isolated Rabbit Ventricular Myocytes

Figure 1 shows representative action potentials recorded at 1 Hz in current clamp mode from a rabbit ventricular myocyte in the presence of 0.01, 0.1, and 1 μM morphine and after washout of the drug. The APD

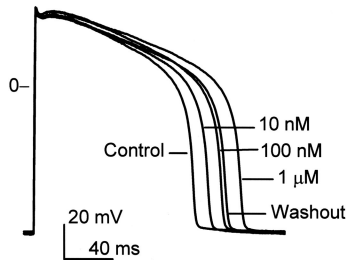

Fig. 1. Effect of morphine on action potentials. Action potentials were recorded from a representative rabbit ventricular myocyte, showing that action potential duration is prolonged and resting membrane potential is slightly hyperpolarized by the application of morphine at 0.01, 0.1, and 1 μM (6 min for each concentration). The effect was only partially reversed upon drug washout (10 min).

was substantially increased, and the resting membrane potential was slightly hyperpolarized by application of morphine. The effects were partially reversed by washout of the drug for 10 min. The effects on action potential variables in each experiment exposed to three concentrations of morphine are summarized in table 1.

Effect of Morphine on $I_{Ca.L}$

Because $I_{Ca.L}$ is mainly responsible for the plateau of action potentials of the cardiac muscle, we determined the effect of morphine on the current. Figure 2A displays $I_{Ca.L}$ tracings recorded from a representative cell with 300-ms voltage steps to between −40 and +60 mV from −50 mV as shown in the inset. Morphine at 0.1 μM substantially increased $I_{Ca.L}$ after administration for 6 min. The effect was partially reversed upon washout for 10 min. Figure 2B shows current–voltage relations of mean values of $I_{Ca.L}$ density (n = 12) in the absence and presence of 0.1 μM morphine. The density of $I_{Ca.L}$ at +10 mV corresponding to peak current was significantly increased by 23 ± 8% (from 5.9 ± 1.9 to 7.3 ± 1.7 pA/pF; $P < 0.05$).

Figure 2C illustrates the concentration–response relation for the augmentation of $I_{Ca.L}$ by morphine. The mean data were fit to the Hill equation, and the EC_{50} (at

Table 1. Effects of Morphine on Action Potential Variables in Rabbit Ventricular Myocytes

	RMP, mV	APA, mV	APD_{50}, ms	APD_{90}, ms
Control	−81.4 ± 1.3	122.5 ± 7.6	120.8 ± 29.7	130.8 ± 30.3
Morphine				
10 nM	−82.1 ± 1.0	123.1 ± 11	131.5 ± 48.5*	142.7 ± 54.1*
100 nM	−82.6 ± 1.0*	122.1 ± 9.6	139.0 ± 48.2*	148.4 ± 51.8*
1 μM	−83.0 ± 1.0*	122.3 ± 10.2	142.9 ± 42.2*	154.7 ± 44.8*
Washout	−82.5 ± 1.0*	122.6 ± 8.3	135.5 ± 51.8*	146.2 ± 54.8*

Action potentials recorded at 1 Hz in current clamp mode at 36°C. Data are expressed as mean ± SD. n = 12.

* $P < 0.05$ *vs.* control.

APA = action potential amplitude; APD_{50} and APD_{90} = action potential duration at 50 and 90% of repolarization, respectively; RMP = resting membrane potential.

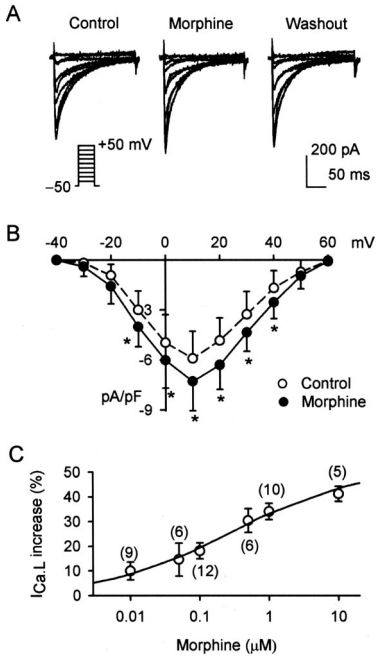

Fig. 2. Effect of morphine on L-type Ca^{2+} current ($I_{Ca.L}$). (*A*) $I_{Ca.L}$ traces recorded in a representative myocyte using 300-ms steps to between −40 and +60 mV from −50 mV as shown in the *inset* during control, after the application of 0.1 μM morphine for 6 min, and after washout of the drug for 10 min. $I_{Ca.L}$ was increased by the application of 0.1 μM morphine and was partially reversed by drug washout. (*B*) Current–voltage relations of $I_{Ca.L}$ density (n = 12) in the absence (control; *open circles*) and presence of 0.1 μM morphine (*filled circles*). Data are expressed as mean ± SD. * $P < 0.05$ *versus* control. (*C*) Concentration–response relation of morphine effect on $I_{Ca.L}$. *Numbers within parentheses* are number of experiments.

+10 mV) was 0.47 μM with a Hill coefficient of 0.31, and the E_{max} was 47%.

Effects of morphine on steady state voltage dependence of $I_{Ca.L}$ activation and inactivation were evaluated in rabbit ventricular cells. Voltage-dependent activation of $I_{Ca.L}$ was determined, as described previously,[21] from the current–voltage relation of $I_{Ca.L}$ in figure 2B on the basis of the formulation: $d_t = I_t/[g_x(V_t - V_r)]$, where d_t is the activation variable and I_t is $I_{Ca.L}$ at a test potential V_t, g_x is the maximum conductance, and V_r is the reversal potential. Calculated values were normalized to the maximum value in each cell to obtain the activation variable *d*. Figure 3A illustrates the voltage protocol and representative recordings used to assess $I_{Ca.L}$ inactivation. The inactivation variable *f*) was determined as $I_{Ca.L}$ at a given prepulse potential divided by the maximum $I_{Ca.L}$ in the absence of a prepulse. Figure 3B shows the results obtained from analysis of voltage-dependent activation and inactivation before and after 0.1 μM morphine treatment. Mean data are shown by the symbols, and the curves shown are best-fit Boltzmann distributions. The midpoint of voltage ($V_{0.5}$) and slope factor for activation averaged −5.7 ± 1.2 mV and 6.4 ± 1.1 for control, and those for morphine (0.1 μM) were −6.7 ± 1.3 mV and 6.1 ± 1.0 (n = 9), respectively. The $V_{0.5}$ and slope factor

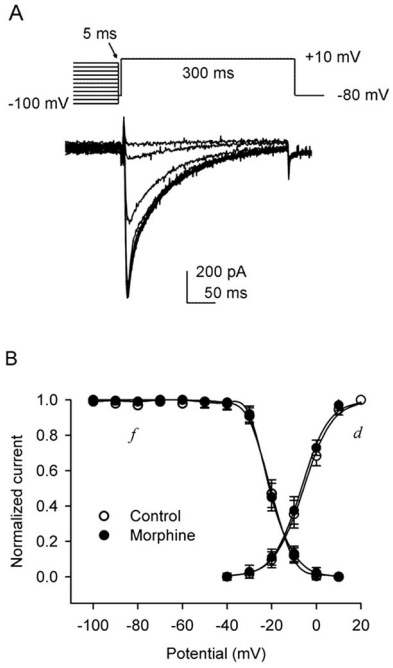

Fig. 3. Absence of effect of morphine (0.1 μM) on voltage-dependent activation and inactivation of L-type Ca^{2+} current ($I_{Ca.L}$). (*A*) Representative current recordings used to determine voltage dependence of $I_{Ca.L}$ inactivation. Cell was conditioned with 500-ms prepulses from holding potential of −80 mV to between −100 and 0 mV and back to −80 mV for 5 ms, then subjected to 300-ms test pulse to +10 mV. (*B*) Mean values of voltage-dependent activation and inactivation relations for $I_{Ca.L}$. No significant differences of $V_{0.5}$ and slope factor were observed for activation and inactivation of $I_{Ca.L}$ before (control; *open circles*) and after application of 0.1 μM morphine (*filled circles*).

for inactivation averaged −20.1 ± 1.1 mV and −4.6 ± 0.9 for control and −20.4 ± 0.8 mV and −4.5 ± 0.7 for morphine (0.1 μM) treatment (n = 10), respectively. The steady state activation and inactivation of $I_{Ca.L}$ were not affected by morphine. In addition, recovery of $I_{Ca.L}$ from inactivation, determined with the procedure as previously described,[21] was not influenced by 0.1 μM morphine, either (n = 7).

Figure 4 shows the effects of 0.1 μM morphine on $I_{Ca.L}$ at +10 mV in the absence and presence of opioid receptor antagonists. These three types of opioid receptor antagonists themselves had no effect on $I_{Ca.L}$. NTD or nor-BNI, but not CTOP, abolished the action of morphine on the current, suggesting that the enhancement of $I_{Ca.L}$ by morphine is mediated by both δ- and κ-opioid receptors.

In addition, the prolongation of cardiac APD by morphine was prevented by NTD or nor-BNI, but not by CTOP. In cells with pretreatment of 5 μM NTD and 5 μM nor-BNI, APD_{50} (at 1 Hz) was 124.2 ± 31.6 and 121.7 ± 36.5 ms, respectively, and 122.9 ± 39.1 and 125.7 ± 35.3 ms after application of 0.1 nM morphine (n = 6; not significant). Nevertheless, in cells pretreated with 5 μM CTOP, APD_{50} was 126.8 ± 34.9 and 142.3 ± 41.3 ms in the absence and presence of 0.1 μM morphine, respectively (n = 8; $P < 0.05$). These results indicate that the

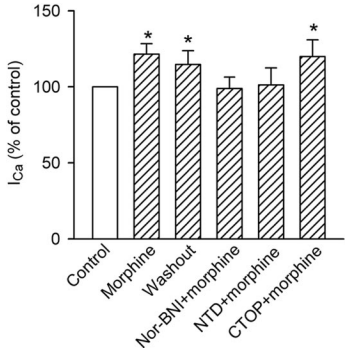

Fig. 4. Opioid receptor antagonists and morphine effects on L-type Ca^{2+} current ($I_{Ca.L}$). $I_{Ca.L}$ was increased by the administration of 0.1 μM morphine, and the increase was partially reversed upon drug washout for 10 min. Pretreatment (5 min) with nor-binaltorphimine (nor-BNI, 5 μM, n = 7) and naltrindole (NTD, 5 μM, n = 8), but not CTOP (5 μM, n = 6), prevented morphine-induced increase of $I_{Ca.L}$. Data are expressed as mean ± SD. * $P < 0.05$ *versus* control.

prolongation of cardiac APD by morphine is related to the increase of $I_{Ca.L}$ and mediated by δ- and κ-opioid receptors.

Effect of Morphine on I_K

Figure 5 displays the effect of morphine on I_K (I_{Kr} and I_{Ks}) in a typical experiment. Morphine at 0.1 μM had no effect on I_K. In a total of six ventricular myocytes, the tail current (measured at −30 mV from the peak tail to the completed deactivation level) of I_K was 2.2 ± 0.8 pA/pF in control and 2.2 ± 0.9 after application of 0.1 μM morphine (n = 6; not significant). No change in I_K tail

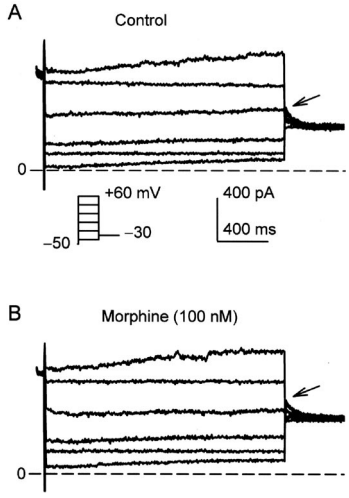

Fig. 5. Effect of morphine on delayed rectifier K^+ current (I_K). (*A*) I_K (including I_{Kr} and I_{Ks}) was recorded during control in a representative cell in the presence of 200 μM Cd^{2+} (to block L-type Ca^{2+} current) and 3 mM 4-aminopyridine (to block I_{to}). The current was elicited by 2-s voltage steps to between −40 and +60 mV (20-mV increment) from −50 mV, then to −30 mV (to record I_K current; *arrow*). (*B*) Current traces recorded after application of 100 nM morphine in the same cell as in *A*. No change in I_K (assessed by tail current) was observed with morphine administration. The *dashed lines* indicate the zero current.

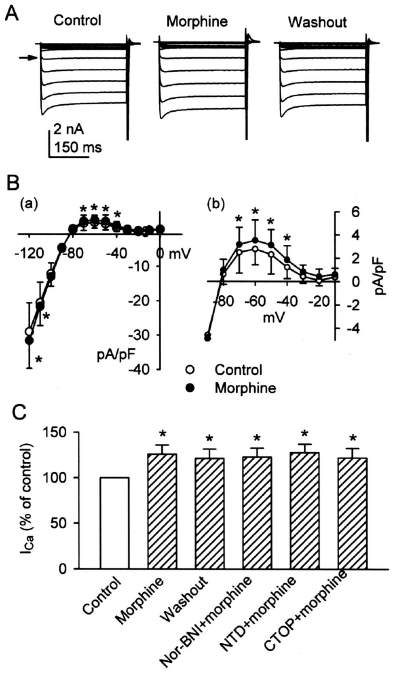

Fig. 6. Effect of morphine on inward rectifier K^+ current (I_{K1}). (*A*) I_{K1} traces recorded using 300-ms voltage steps to between −120 and 0 mV from a holding potential of −40 mV in a representative ventricular myocyte during control, after the application of 0.1 μM morphine (6 min), and after washout of the drug (10 min). (*B*) (*a*) Current–voltage relation of I_{K1} density determined with the same protocol as *A* in the absence (*open circles*) and presence of 0.1 μM morphine (*filled circles*). (*b*) Expanded plots for the outward components of I_{K1} from *B*(*a*) to show the difference before and after the application of 0.1 μM morphine. (*C*) Mean values of relative I_{K1} at −60 mV (n = 12) in the absence and presence of 0.1 μM morphine, and opioid receptor antagonists, *i.e.*, 5 μM nor-binaltorphimine (nor-BNI), 5 μM naltrindole (NTD), or 5 μM CTOP. The augmentation of I_{K1} by morphine was not prevented by the pretreatment (5 min) with any opioid receptor antagonist. Data are expressed as mean ± SD. * $P < 0.05$ *versus* control.

current was observed even when the concentration of morphine was increased to 1 μM (2.3 ± 0.7 pA/pF, n = 6; not significant).

Effect of Morphine on I_{K1}

Figure 6A illustrates representative I_{K1} tracings elicited by 300-ms voltage steps to between −120 and 0 mV from −40 mV during control, after the application of 0.1 μM morphine, and after washout of the drug. I_{K1} was increased after the application of 0.1 μM morphine for approximately 5 min. The effect was reversed only partially after washout of the drug for 10 min. Figure 6B is the current–voltage relation of I_{K1} density (n = 12) in the absence and presence of 0.1 μM morphine. I_{K1} was significantly increased by morphine in both inward (−120 and −110 mV) and outward (−70 to −40 mV; $P < 0.05$) components. At −60 mV, I_{K1} was 2.8 ± 1.0 pA/pF during control. After the application of 0.01, 0.1, and 1 μM morphine, I_{K1} was increased to 3.1 ± 1.1, 3.5 ± 0.9, and 3.7 ± 1.0 pA/pF, respectively (*i.e.*, increased by 11 ± 7, 25 ± 9, and 32 ± 11%; $P < 0.05$ for 0.1 and 1 μM morphine).

Figure 6C summarizes the mean data of morphine effect on I_{K1} at −60 mV in the absence and presence of opioid receptor antagonists. Pretreatment with 5 μM NTD, 5 μM nor-BNI, or 5 μM CTOP did not affect the action of morphine on the current, indicating that the increase of I_{K1} by morphine is not mediated by opioid receptors.

Discussion

In the current study, we demonstrated that morphine at 0.01–0.1 μM prolonged APD and increased $I_{Ca.L}$ in isolated rabbit ventricular myocytes. The effects were prevented by blockade of δ- or κ-opioid receptors. At 0.1–1 μM, morphine also hyperpolarizes the resting membrane potential and enhances I_{K1}, but this effect was not mediated by opioid receptors. Based on the pharmacokinetics of morphine in humans, *i.e.*, a volume of distribution of 3.2 l/kg and 35% protein binding, the equilibrate plasma concentration is approximately 0.06 μM after intravenous injection of 0.2 mg/kg morphine.[18] Therefore, the concentrations at 0.01–0.1 μM used in the current study are within therapeutic concentrations.

It is well known that prolongation of action potential is dependent on increase of inward current (*e.g.*, $I_{Ca.L}$) or decrease of outward K currents (I_K or I_{K1}). A previous report demonstrated that morphine prolonged cardiac APD in cardiac tissue,[13] but the ionic mechanisms are not understood. In the current study, using a whole cell patch clamp technique, we provided evidence that morphine prolonged cardiac APD by increasing $I_{Ca.L}$, not by decreasing I_K or I_{K1}. The increase of $I_{Ca.L}$ by morphine had an EC_{50} of 0.5 μM with a Hill coefficient of 0.31. The small Hill coefficient suggested a wide effective concentration range of morphine.

The current observation on the increase of cardiac $I_{Ca.L}$ by morphine was supported by previous studies using $^{45}Ca^{2+}$ or spectrofluorometry, in which morphine was found to induce transmembrane Ca^{2+} influx in cardiac myocytes.[9,22] In contrast to the current finding, stimulation of opioid receptor with 0.01 μM leucine-enkephalin, a δ-opioid receptor agonist, was shown to reduce $I_{Ca.L}$ in rat ventricular myocytes.[23] This discrepancy may be due to the use of different opioid receptor agonists in the two studies: morphine in the current study and leucine-enkephalin in the previous study.[23] Actually, the opposing effects of opioids on voltage-dependent Ca^{2+} channels (I_{Ca}) were documented in nerve cells. Most of the studies indicated inhibitory effects of opioids on $I_{Ca.L}$,[24–26] but stimulation was also observed.[27]

It was reported that morphine at 30 μM did not affect $I_{Ca.L}$ in rat ventricular myocytes.[14] The discrepancies

between these findings might be related to drug concentration, species, experimental procedures, opioid receptor subtypes involved, or other unknown factors. In the heart, L-type calcium channels play an important role in generating electrical activity and excitation–contraction coupling. It is well established that increases in $I_{Ca.L}$ produce positive inotropic effects and induce triggered arrhythmias, which is highly undesirable in patients with acute myocardial infarction. However, increases in $I_{Ca.L}$ by Bay Y5959, a Ca^{2+} promoter, have recently been shown to prevent the initiation of reentrant ventricular tachycardia in the epicardial border of healing infarcted canine heart,[28] indicating antiarrhythmic actions of an increase in $I_{Ca.L}$.

A previous study reported that activation of opioid receptors would hyperpolarize cell membrane in neurons.[29] In the current study, we showed that morphine at the therapeutic concentration of 0.1 μM increased I_{K1} in cardiac myocytes *via* an opioid receptor–independent pathway. There is evidence that a reduction in I_{K1} is associated with arrhythmias. Patients with Andersen syndrome, a rare inherited disease with a mutation in a major contributor of the I_{K1} channel, Kir2.1, which causes a reduction in I_{K1}, exhibit complex ventricular ectopy and polymorphic ventricular tachycardia.[30,31] Therefore, it is likely that an increase in I_{K1} hyperpolarizes the resting membrane potential, thus producing a membrane stabilizing effect.

It should be noted that the concentrations of morphine used in the previous study[29] were much higher than those used in clinical practice. Recently, it was found that morphine at low concentrations produced preconditioning in brain Purkinje cells.[32] Yvon *et al.* [15] reported that morphine at clinically relevant concentrations did not modify action potential variables in guinea pig ventricle in normoxic conditions but significantly attenuated the ischemia-induced depolarization and increase in amplitude of action potential and V_{max}, and therefore reduced the incidence of conduction block during ischemia and reperfusion-induced arrhythmias. Nevertheless, we observed that morphine at 0.1 and 1 μM hyperpolarized the resting membrane potential significantly and prolonged APD in single ventricular myocytes from rabbit hearts. Analyses of ionic currents showed that morphine increases both $I_{Ca.L}$ and I_{K1}. The increase in $I_{Ca.L}$ and I_{K1} would prolong and shorten the cardiac APD, respectively, thus resulting in a moderate prolongation of the APD. The effects of morphine on these two types of currents would be responsible for decreased ischemia-reperfusion–induced arrhythmias as observed by Yvon *et al.* [15] Together with its analgesic and cardioprotective action, morphine at the therapeutic concentrations may be very useful in the management of acute myocardial infarction.

The following limitations must be considered in the assessment of the relevance of our study. The species differences, *in vitro* study, healthy myocardium *versus* diseased myocardium, normal physiologic conditions *versus* abnormal conditions (*e.g.*, hypoxia, ischemia),[33] and interaction with other anesthetic agents[34] should all be taken into consideration. In addition, no clear explanation was available for the observed slow reversibility of morphine effect (by washout). Possible explanations for this phenomenon would be the tight binding of morphine with cell membrane protein and the participation of intracellular events. It has been found that several signal pathways (*e.g.*, protein kinase A, protein kinase C, protein kinase G) are involved in physiologic actions mediated by opioid receptors.[35–37] The slow reversibility is likely related to the fact that the effects from these pathways may require a longer time to disappear after washout of the receptor ligand.

It is interesting to note that blockade of either δ- or κ-opioid receptors abolished the effect of morphine. A similar phenomenon has been reported previously by us. The infarct-sparing effect of preconditioning of remifentanil, an ultrarapid-acting opioid receptor agonist, is abolished by blockade of κ- or δ-opioid receptors.[38] Further study is required to determine whether this is related to interaction of κ-opioid receptors with δ-opioid receptors.

In conclusion, the current study has demonstrated that morphine at a clinically relevant concentration of 0.1 μM increases $I_{Ca.L}$ and I_{K1} in isolated rabbit ventricular myocytes. Its action on $I_{Ca.L}$ is mediated by δ- and κ-opioid receptors, whereas its effect on I_{K1} is not mediated by opioid receptors.

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