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Interactions of the narcotic *l*-α-acetylmethadol with human cardiac K^+ channels

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Abstract

l-α-acetylmethadol is a long-acting narcotic analgesic that is used in the treatment of opiate addiction. However, the drug has been associated with cases of QT interval prolongation and ventricular arrhythmia. To understand the mechanism underlying these clinical findings, we examined the effects of *l*-α-acetylmethadol on the cloned human cardiac K^+ channels HERG (human ether-a-go-go-related gene), KvLQT1/minK and Kv4.3. Using patch clamp electrophysiology, we found that *l*-α-acetylmethadol inhibited HERG channel currents in a voltage-dependent manner displaying an IC_{50} value of 3 μM. The major active metabolite of *l*-α-acetylmethadol, noracetylmethadol, inhibited HERG with an estimated IC_{50} values of 12 μM. *l*-α-acetylmethadol had little or no effect on Kv4.3 or KvLQT1/minK K^+ channel currents at concentration up to 10 μM. We conclude that the proarrhythmic effects of *l*-α-acetylmethadol are due to specific blockade of the HERG cardiac K^+ channel and that its active metabolite noracetylmethadol may provide a safer alternative in the treatment of opiate addiction.
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Keywords: HERG channel; K^+ channel; *l*-α-acetylmethadol; QT prolongation; Torsades de pointes

1. Introduction

Drug-induced (or acquired) long-QT syndrome is a potentially dangerous side effect caused by the administration of certain prescription medications. This syndrome is characterized by a delay in cardiac repolarization, leading to a lengthening of the QT interval on the electrocardiogram (ECG), and is believed to contribute to the generation of the life-threatening ventricular arrhythmia, torsades de pointes (Ben-David and Zipes, 1993). Block of repolarizing K^+ channel currents in the human heart is the most common mechanism underlying acquired long-QT syndrome. The three principal voltage-dependent K^+ channels in the human ventricular myocardium are HERG (human ether-a-go-go-related gene), KvLQT1/minK and Kv4.3. The HERG channel underlies the rapid component of the cardiac delayed rectifier current, I_{Kr} (Sanguinetti et al., 1995). KvLQT1/minK underlies the slow component of the cardiac delayed rectifier current, I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996), while Kv4.3 underlies the transient outward current, I_{to} (Dixon et al., 1996). Specific block of HERG/I_{Kr} appears to be the main mechanism whereby drugs act to produce acquired long-QT syndrome and associated ventricular arrhythmias (Brown and Rampe, 2000), although in some instances, other channels such as KvLQT1/minK may be the primary target (Kang et al., 2001).

l-α-acetylmethadol (Orlaam®, Roxane Laboratories, Columbus, OH) is a long-acting methadone derivative used in the treatment of heroin addiction (Eissenberg et al., 1997). However, postmarketing experience has revealed several cases of torsades de pointes arrhythmia associated with use of the drug (Deamer et al., 2001). These reports have prompted the United States Food and Drug Administration (FDA) to issue a "black box" labeling for the drug warning of QT prolongation and severe arrhythmia (Schwetz, 2001). To further understand the molecular mechanism(s) underlying these clinical findings, we examined the effects of *l*-α-acetylmethadol on the cloned human cardiac K^+ channels HERG, KvLQT1/minK and Kv4.3. In addition, we also examined the major active metabolite of *l*-α-acetylmethadol, noracetylmethadol, on HERG.

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2. Materials and methods

Chinese hamster ovary cells (American Type Culture Collection, Manassas, VA) were stably transfected with the cDNAs encoding the HERG, Kv4.3 or KvLQT1/minK human cardiac K^+ channels (Rampe et al., 1997; Kang et al., 2001). Cells used for electrophysiological recordings were seeded onto plastic coverslips 16–24 h prior to use. HERG, Kv4.3 and KvLQT1/minK currents were recorded using the whole-cell method of the patch clamp technique. Electrode (2–3 MΩ resistance) were fashioned from TW 150 F glass capillary tubes (World Precision Instruments, Sarasota, FL) and filled with an internal solution containing 120 mM potassium aspartate, 20 mM KCl, 4 mM Na_2ATP, 5 mM HEPES, 1 mM $MgCl_2$, pH 7.2, with KOH. For KvLQT1/minK current recordings, the internal solution was further supplemented with 14 mM sodium phosphocreatine, 0.3 mM sodium GTP and 50 U/ml creatine phosphokinase. The external recording solution contained 130 mM NaCl, 5 mM KCl, 2.8 mM sodium acetate, 1.0 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 1.0 mM $CaCl_2$, pH 7.4, with NaOH. Currents were recorded at room temperature using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and analyzed using the pCLAMP suite of software (Axon Instruments). *l*-α-acetylmethadol and noracetylmethadol were obtained from Cerilliant (Round Rock, TX). All other chemicals were obtained from Sigma (St. Louis, MO).

3. Results

Fig. 1 shows the effects of *l*-α-acetylmethadol on HERG channel currents. In these experiments, a 2-s depolarization to +20 mV from a holding potential of −80 mV was followed by repolarization of the cell to −40 mV to produce large, slowly deactivating tail currents characteristic of HERG. The effects of *l*-α-acetylmethadol are shown in Fig. 1A. *l*-α-acetylmethadol had no detectable effects on the shape of the current waveform. However, *l*-α-acetylmethadol inhibited HERG channel currents in a dose-dependent manner over the concentration range of 0.3–10 μM. The IC_{50} value for *l*-α-acetylmethadol block of peak HERG tail currents was 3.0 μM (1.8–5.3 μM, 95% C.L., Fig. 1C). The major active metabolite of *l*-α-acetylmethadol, noracetylmethadol, was also tested on HERG channel currents over the same concentration range. Noracetylmethadol was less potent at inhibiting HERG currents producing a maximum block of only 46 ± 4% at 10 μM resulting in an estimated IC_{50} value of 12 μM (Fig. 1B and C).

Fig. 2 shows the effects of *l*-α-acetylmethadol on HERG currents measured over a wide range of test potentials. In these experiments, cells were held at −80 mV and currents were elicited by 2-s depolarizations to potentials ranging from −40 to +30 mV. The membrane potential was then

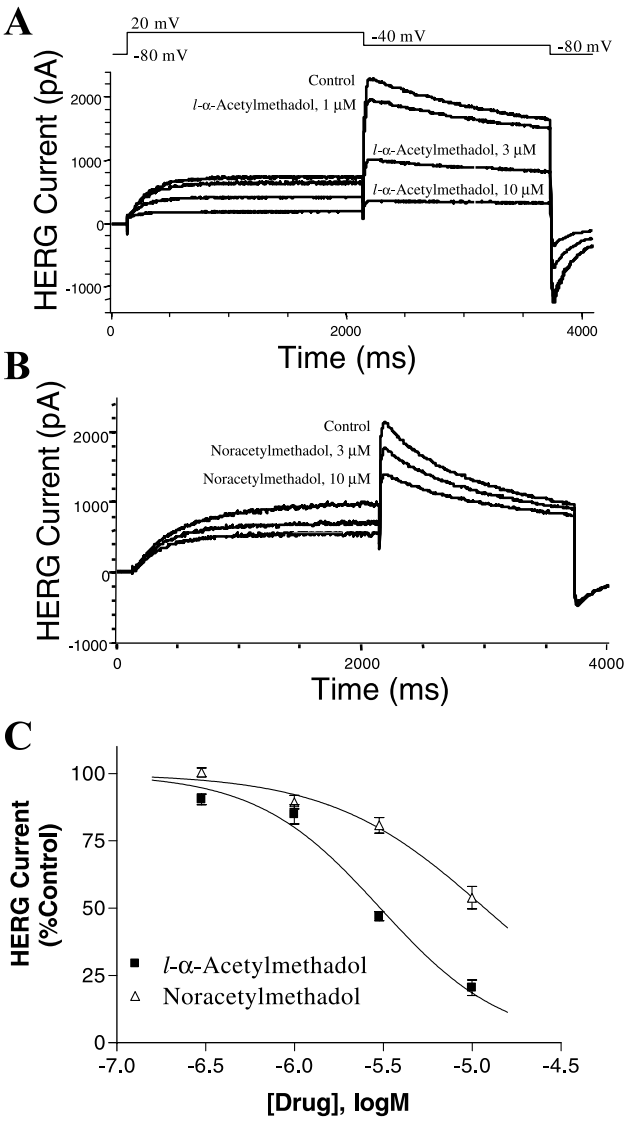

Fig. 1. Effects of *l*-α-acetylmethadol and noracetylmethadol on HERG. (A) Whole-cell HERG K^+ channel currents were elicited by 2-s test depolarizations to +20 mV from a holding potential of −80 mV at 40-s intervals. The cells were then returned to −40 mV and peak tail current amplitudes were recorded. The effects of 1, 3 and 10 μM *l*-α-acetylmethadol are shown. (B) Effects of noracetylmethadol on HERG channel currents. Currents were elicited as described in (A). The effects of 3 and 10 μM noracetylmethadol are shown. (C) Dose–response relationship for L-α-acetylmethadol and noracetylmethadol inhibition of HERG. The IC_{50} value for *l*-α-acetylmethadol was 3 μM. Error bars denote S.E.M. (n = 4–6).

returned to −100 mV and peak tail currents were recorded. Currents in the absence and presence of 3 μM *l*-α-acetylmethadol are shown in Fig. 2A and B, respectively. Following depolarization to +20 mV, tail currents decayed with time constants of 54 ± 3 and 69 ± 4 ms in the absence and presence of drug, respectively (n = 5). *l*-α-acetylmethadol inhibited the amplitude of these inward tail currents in a voltage-dependent manner. The current–voltage (I–V) relationships averaged from five cells are shown in Fig. 2C. When inhibition of HERG current is plotted as a function of

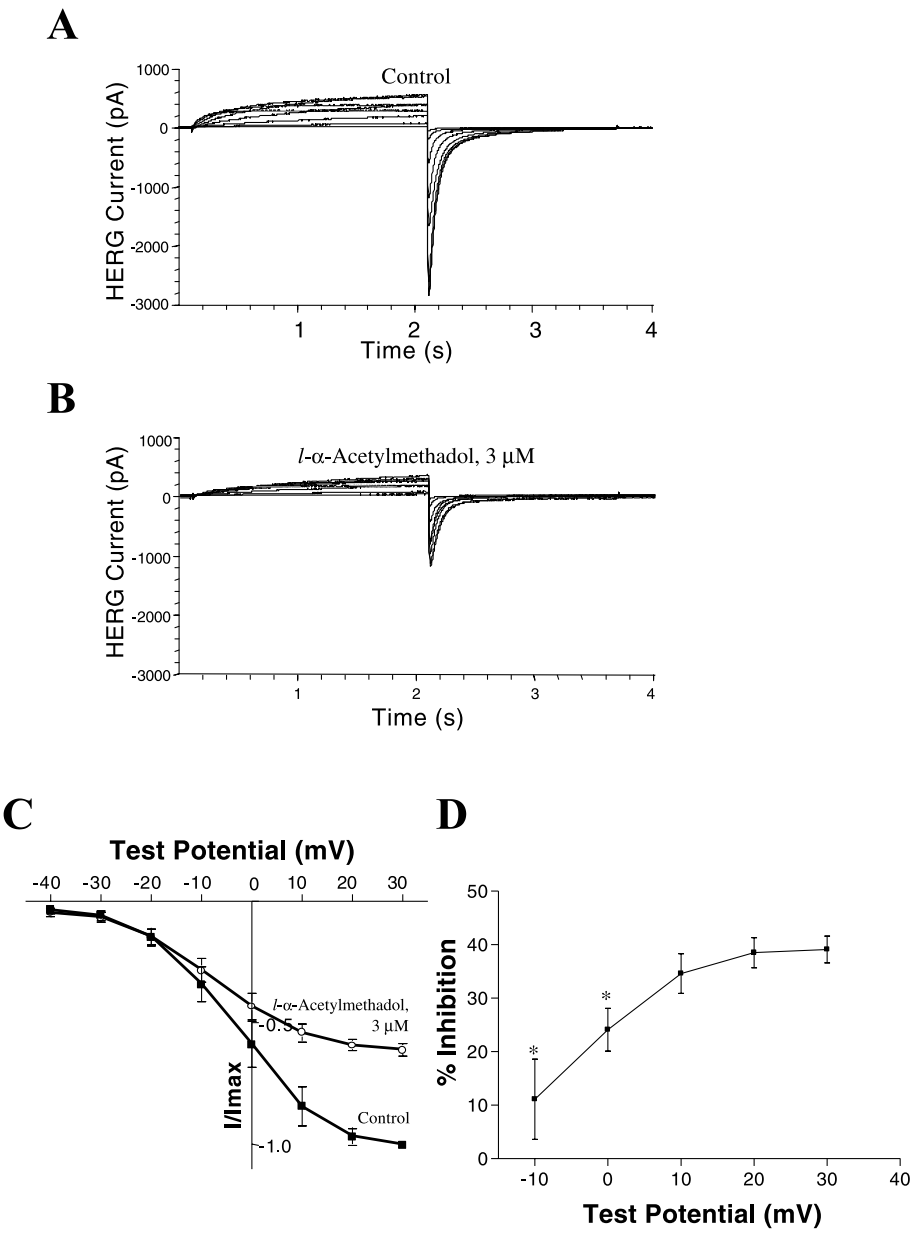

Fig. 2. Effects of membrane potential on *l*-α-acetylmethadol block of HERG. Cells were held at − 80 mV and depolarized for 2-s to potentials ranging from − 40 to + 30 mV in 10-mV increments. The cells were then returned to − 100 mV to generate inward tail currents. Traces in the absence and presence of 3 μM *l*-α-acetylmethadol are shown in (A) and (B), respectively. (C) Peak tail currents amplitudes at − 100 mV were normalized to those obtained after the + 30 mV pulse in the absence of drug. The normalized tail currents are plotted as a function of test potential. The data in the absence of drug (filled squares) and after the addition of 3 μM *l*-α-acetylmethadol (open circles) are shown. Error bars denote S.E.M. ($n = 5$). (D) Inhibition of peak tail current amplitude is plotted as a function of test potential. Asterisks indicate statistical significance compared with the inhibition obtained after the + 30 mV test pulse ($P < 0.05$, analysis of variance). Error bars denote S.E.M. ($n = 5$).

test potential, a statistically significant ($P < 0.05$, analysis of variance) positive correlation between voltage and drug effect is observed with inhibition ranging from 11 ± 8% following the − 10 mV pulse to 39 ± 3% following the + 30 mV pulse (Fig. 2D).

We also examined the effects of *l*-α-acetylmethadol on two other human cardiac K^+ channels KvLQT1/minK and Kv4.3. KvLQT1/minK currents were elicited by 2-s depolarizing pulses to + 20 mV from a holding potential of − 80 mV. *l*-α-acetylmethadol at concentrations of 1, 3 and 10 μM showed no significant reduction of KvLQT1/minK currents in five cells tested. Kv4.3 currents were elicited by 300 ms test pulses to + 20 mV from a holding potential of − 80 mV. *l*-α-acetylmethadol inhibited Kv4.3 channel currents by 11 ± 2, 17 ± 3 and 30 ± 4% at concentrations of 1, 3 and 10 μM, respectively ($n = 5$).

4. Discussion

The present study is the first to examine the effects of *l*-α-acetylmethadol on the cloned human cardiac K^+ channels thought to be responsible for repolarization of the human ventricular myocardium. We found that *l*-α-acetylmethadol inhibits HERG cardiac K^+ channels with an IC_{50} value of 3 μM. The threshold concentration for inhibition of HERG was approximately 300 nM where we saw small (10 ± 2%) but consistent inhibition in all cells tested. The inhibitory effects of *l*-α-acetylmethadol on HERG were voltage-dependent with greater block observed following more depolarized potentials. By contrast, at concentrations up to 10 μM, *l*-α-acetylmethadol displayed little inhibitory effects on the Kv4.3 cardiac K^+ channel and no detectable effects on KvLQT1/minK. These results suggest that *l*-α-acetylmethadol inhibits HERG via an interaction with an activated state of the channel and that this interaction is selective for HERG relative to other human cardiac K^+ channels.

l-α-acetylmethadol is an effective maintenance therapy for opiate dependence and is administered thrice-weekly at addiction clinics (Eissenberg et al., 1997). However, treatment with *l*-α-acetylmethadol has been associated with a number of cases of QT prolongation and ventricular arrhythmia, including torsade de pointes, resulting in a "black box" warning from the FDA (Deamer et al., 2001; Schwetz, 2001). Peak plasma levels of *l*-α-acetylmethadol can approximate 1–2 μM following therapeutic doses (Henderson et al., 1976; Finkle et al., 1982). We have recently shown that when peak total plasma levels of a drug approximate or exceed their HERG IC_{50} values, QT interval prolongation is observed (Kongsamut et al., 2002). The data presented here suggest that the proarrhythmic effects of *l*-α-acetylmethadol can be explained by a specific, clinically achievable block of the HERG cardiac K^+ channel by this drug. In addition, since *l*-α-acetylmethadol is mainly metabolized via the cytochrome *P*450 3A4 enzyme (Moody et al., 1997), concurrent administration of drugs that inhibit this enzyme (e.g. ketoconazole) could increase *l*-α-acetylmethadol plasma levels and enhance the likelihood of proarrhythmia. Finally, self-administration of other popular drugs of abuse, specifically cocaine, is a common occurrence in this patient population (Eissenberg et al., 1997; Deamer et al., 2001) and may result in additive inhibitory effects on HERG/I_{Kr} (O'Leary, 2001) furthering the possibility of serious arrhythmia.

l-α-acetylmethadol is metabolized into two major active species, noracetylmethadol and dinoracetylmethadol. Dinoracetylmethadol was not commercially available and therefore not tested in this study. We did, however, find that noracetylmethadol was several-fold less potent at inhibiting HERG channel currents relative to *l*-α-acetylmethadol. Following administration of *l*-α-acetylmethadol, peak plasma levels of noracetylmethadol approximate 1 μM (Henderson et al., 1976; Finkle et al., 1982). Noracetylmethadol has a long half-life (ca. 30 h or more) and is reported to be 10–15 times more potent as a narcotic compared to *l*-α-acetylmethadol (Kaiko and Inturrisi, 1975; Walsh et al., 1998). Based on its higher efficacy and lower HERG affinity, we propose that noracetylmethadol may represent an effective treatment of opioid dependence with a lower potential to produce ventricular arrhythmia compared to *l*-α-acetylmethadol.

In conclusion, this report is the first to detail the effects of the narcotic analgesic *l*-α-acetylmethadol on human cardiac K^+ channels. We find that *l*-α-acetylmethadol is a selective antagonist of HERG and that block occurs at concentrations achieved in the plasma following therapeutic doses. The risk of proarrhythmia from *l*-α-acetylmethadol is likely to be enhanced by high doses, inhibition of cytochrome *P*450 3A4 and additive pharmacodynamic interactions at HERG by co-administered drugs such as cocaine. Noracetylmethadol was a weaker inhibitor of HERG suggesting a greater cardiovascular safety window for this potent active metabolite. *l*-α-acetylmethadol can now be included in the list of compounds that produce QT prolongation and the attending proarrhythmia via specific inhibition of HERG.

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