Influence of Opioid Agonists on Cardiac Human Ether-a-go-go-related Gene K⁺ Currents

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ABSTRACT

We have evaluated the ability of various opioid agonists, including methadone, l-α-acetylmethadol (LAAM), fentanyl, meperidine, codeine, morphine, and buprenorphine, to block the cardiac human ether-a-go-go-related gene (HERG) K⁺ current (IHERG) in human cells stably transfected with the HERG potassium channel gene. Our results show that LAAM, methadone, fentanyl, meperidine, and buprenorphine were effective inhibitors of IHERG, with IC₅₀ values in the 1 to 10 μM range. The other drugs tested were far less potent with respect to IHERG inhibition. Compared with the reported maximal plasma concentration (Cmax) after administration of therapeutic doses of these drugs, the ratio of IC₅₀/Cmax was highest for codeine and morphine (>455 and >400, respectively), thereby indicating that these drugs have the widest margin of safety (of the compounds tested) with respect to blockade of IHERG. In contrast, the lowest ratios of IC₅₀/Cmax were observed for LAAM and methadone (2.2 and 2.7, respectively). Further investigation showed that methadone block of IHERG was rapid, with steady-state inhibition achieved within 1 s when applied at its IC₅₀ concentration (10 μM) for IHERG block. Results from “envelope of tails” tests suggest that the majority of blockade occurred when the channels were in the open and/or inactivated states, although ~10% of the available HERG K⁺ channels were apparently blocked in a closed state. Similar results were obtained for LAAM. These results demonstrate that LAAM and methadone can block IHERG in transfected cells at clinically relevant concentrations, thereby providing a plausible mechanism for the adverse cardiac effects observed in some patients receiving LAAM or methadone.

Torsades de pointes is a potentially fatal form of ventricular arrhythmia that typically occurs under conditions where cardiac repolarization is delayed (as indicated by prolonged QT intervals from electrocardiographic recordings) (Goodman and Peter, 1995; Viskin, 1999). These conditions can be precipitated by drugs that block the cardiac potassium channels responsible for mediating ventricular repolarization. Remarkably, many different types of drugs, including some antiarrhythmics, antihistamines, antibiotics, gastrointestinal prokinetics, and antipsychotics (Faber et al., 1994; De Ponti et al., 2001), have been shown to cause QT prolongation, primarily through interference with the rapid component of the delayed rectifier potassium current, IKr (Antzelevitch et al., 1996; January et al., 2000; Tamargo, 2000; Tseng, 2001). The human ether-a-go-go-related gene (HERG) gene encodes for the major channel protein that underlies IKr, and a recently developed cell line that was stably transfected with the HERG gene (Zhou et al., 1998) has proven useful for evaluating drugs suspected of causing delays in cardiac repolarization (Mohammad et al., 1997; Ferreira et al., 2001). In April 2001, the United States Food and Drug Administration issued a new warning about adverse cardiac events (Deamer et al., 2001) associated with the use of l-α-acetylmethadol hydrochloride (LAAM), a µ-opioid agonist licensed for the treatment of narcotic addiction (Prendergast et al., 1995). This warning was prompted by 10 cases of serious cardiac arrhythmias reported to the Food and Drug Administration through their MedWatch surveillance program.

ABBREVIATIONS: IKr, delayed rectifier potassium current; HERG, human ether-a-go-go-related gene; LAAM, l-α-acetylmethadol; IHERG, cardiac human ether-a-go-go-related gene K⁺ current; HEK, human embryonic kidney; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
similar warning was issued by the European Agency for the Evaluation of Medicinal Products in March 2001. Their report indicated that of the 10 cases of serious cardiac arrhythmias reported for patients receiving LAAM, five of them were cases of cardiac arrest associated with ventricular arrhythmias. Methadone, another μ-opioid agonist with a chemical structure closely related to LAAM, has also come under recent suspicion of having arrhythmogenic properties because several case reports of cardiac abnormalities such as lengthening of the QT interval and ventricular tachycardia arrhythmias have been reported in patients receiving i.v. methadone via an International Registry for Drug-Induced Arrhythmias (QTRDRUGS.org). Because many drugs that have been associated with QT prolongation and the development of ventricular arrhythmias act by blocking the cardiac HERG potassium channel (Antzelevitch et al., 1996; Cavero et al., 2000; January et al., 2000; Tamargo, 2000; Tseng, 2001), we initiated the present investigation to evaluate the ability of LAAM, methadone, and six other opioids to influence the cardiac HERG K⁺ current, IHERG.

Materials and Methods

Cell Culture. Stably transfected HEK 293 cells expressing high levels of the HERG K⁺ channel were obtained from Dr. Craig January (University of Wisconsin, Madison, WI) and maintained as described previously (Zhou et al., 1998).

Solutions and Drugs. Cells were superfused with HEPES-buffered Tyrode's solution containing 137 mM NaCl, 5.4 mM KCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂, 10 mM glucose, and 10 mM HEPES (pH was adjusted to 7.4 with NaOH). Pipette internal solution contained 130 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 5 mM MgATP, and 10 mM HEPES (pH was adjusted to 7.2 with KOH). Solution exchange near the cell in the bath was estimated to be complete in 30 s. All experiments were performed at room temperature (22 ± 1°C). Race-methadone was obtained from Eli Lilly & Co. (Indianapolis, IN). Codeine phosphate and morphine sulfate powder were obtained from Mallinckrodt (St. Louis, MO). Meperidine HCl powder was obtained from Winthrop Labs (New York, NY). Fentanyl citrate i.v. ampules (50 μg/ml) were obtained from Elkins-Sinn (Cherry Hill, NJ). LAAM and EDDP powder were obtained from the National Institute on Drug Abuse (Baltimore, MD). For each compound tested, a concentrated stock solution (10 or 20 μM) was prepared by dissolving the concentrated stock solution (10 or 20 mM) was prepared by dissolving the powder in deionized Milli-Q water. Small aliquots of the concentrated stock were immediately frozen and stored at −80°C. Aliquots were thawed immediately before use and diluted to the desired final concentration in Tyrode's solution.

Voltage-Clamp Recordings. HEK cells expressing the HERG gene were seeded onto collagen-coated glass coverslips 24 to 48 h before analysis. For electrophysiological recording, individual coverslips were transferred to a ΔTC3 (0.5-mm thickness) culture dish (Biotechs, Inc., Butler, PA) installed on the table of an inverted phase contrast microscope. Membrane currents were measured by the whole-cell patch-clamp method (Hamill et al., 1981) using an Axopatch 200B amplifier (Axon Instruments, Union City, CA) as described previously (Liu et al., 1998a,b). Micropipettes were pulled from borosilicate glass capillaries (MTW150F-3; WPI, Sarasota, FL) on a programmable horizontal puller (S-87; Sutter Instruments, San Rafael, CA). The suction pipettes had inner tip diameters of about 1 to 1.5 μm. When filled with internal solutions, they had resistances of 2 to 4 MΩ. Liquid junction potential was not corrected. Series access resistance was 3 to 5 MΩ, and 80% of its compensation resulted in a voltage error of less than 1 mV when current was equal to 1 nA. Data were filtered at 1 kHz with a four-pole low-pass Bessel filter and sampled at 2 kHz. All experiments were performed using pCLAMP 8.01 software (Axon Instruments).

Voltage-clamp protocols to study HERG currents activation were performed as described previously (Mohammad et al., 1997). Voltage steps were applied in increments of 10 mV from −60 to +50 mV for 2 to 8 s from a holding potential of −80 mV and repolarization to −50 mV for 6 s. Interval between pulses was 20 s. Steady-state and peak tail currents were evaluated for I-V plots in control conditions and in the presence of the drug. To study the concentration dependence of opioid action, tail currents were measured at −50 mV in the absence or presence of different opioid concentrations. Voltage was stepped from a holding level of −80 mV to +20 mV for 2 s followed by repolarization to −50 mV for 6 s. The cells were exposed to a given concentration of drug for 30 to 40 s before evaluating the next highest concentration.

Data Analysis. All data were analyzed with pCLAMP8.01 and Origin 6.1 software (Microcal Software, Northampton, MA). Data are presented as mean ± S.E.M. Two-tailed Student's t test or one-way analysis of variance tests were used to compare means, with p < 0.05 required to reject the null hypothesis.

Results

The identities and structures of the opioid compounds tested in this study are shown in Fig. 1. To determine whether these compounds could influence IHERG, HEK cells stably transfected with the HERG gene were evaluated in the absence and presence of increasing concentrations of each opioid compound using the whole-cell patch-clamp technique. An example of IHERG recorded in the absence and presence of increasing concentrations of LAAM is shown in Fig. 2. This result clearly shows that LAAM can block IHERG in a dose-dependent manner. At relatively low concentrations (<100 nM), little or no block was observed. When the concentration of LAAM was increased to 300 nM, however, significant decreases in IHERG were found (24 ± 3% decrease, n = 12, p < 0.001). IHERG continued to show a decline in the presence of greater LAAM concentrations, with a 60 ± 3% decrease in IHERG occurring in the presence of 3 μM LAAM (n = 12, p < 0.001). Nearly complete blockade (96 ± 3% decrease in IHERG, n = 5, p < 0.001) was achieved in the presence of 10 μM LAAM (Fig. 2B). After removal of the drug (washout), up to ~75% of IHERG returned over a period of 5 min. These results demonstrate that LAAM significantly blocks IHERG in stably transfected HEK cells at concentrations ≥300 nM, with ~96% inhibition occurring in the presence of 10 μM LAAM.

Similar experiments were repeated for all of the opioid compounds tested in this study and the results are shown graphically in Fig. 3. These data show that of the compounds tested, LAAM and fentanyl were clearly the most potent, whereas codeine and morphine were the least potent with respect to blockade of IHERG. The data presented in Fig. 3 were used to derive IC50 values for each compound tested, and these numbers are listed in Table 1. For comparative purposes, the maximal plasma concentrations (Cmax) reported after therapeutic dosing for each compound are also listed, and as an estimate of the therapeutic index for each compound, the ratio of IC50/Cmax was calculated (Table 1). These data indicate that LAAM and methadone had by far the smallest IC50/Cmax values (2.2 and 2.7, respectively), thereby indicating that of the compounds tested, LAAM and methadone may have the greatest potential for causing IHERG block in patients. Interestingly, EDDP had relatively little influence on IHERG (IC50 > 50 μM), despite having a chemical structure similar to that of methadone. Morphine
Fig. 1. Drug structures of the opioid agonists tested in this study.
To investigate possible mechanisms underlying opioid inhibition of \( I_{\text{HERG}} \), a modified pulse protocol was used to record \( I_{\text{HERG}} \) before (control) and after application of 10 \( \mu \)M methadone, as shown in Fig. 4A. Twenty-five measurements were performed before the addition of methadone with little or no change in \( I_{\text{HERG}} \) (Fig. 4B). Methadone was then added to the bath during which time no pulses were delivered, and 1 min later, another 25 recordings were performed using the same protocol. Upon the very first activation pulse in the presence of methadone, blockade of \( I_{\text{HERG}} \) was evident (Fig. 4A). In fact, steady-state inhibition was essentially achieved because no further block (or recovery) was seen in \( I_{\text{HERG}} \) during the next 24 activation pulses (Fig. 4B). These results demonstrate that steady-state inhibition of \( I_{\text{HERG}} \) by methadone was achieved rapidly with no indication of use dependence.

Although steady-state inhibition of \( I_{\text{HERG}} \) was achieved after the first pulse following a 1-min pulse-free period in the presence of methadone (Fig. 4), this result does not necessarily mean that HERG channels were blocked in a closed state. In fact, because we measured tail currents that were generated during the return step to \(-100\) mV, the channels could have been blocked in an open, inactivated, and/or a closed state. To examine which of these states may be affected by methadone, we performed an envelope of tails test (Sanguinetti and Jurkiewicz, 1990; Salata et al., 1996). This test delivers pulses of increasing duration that progressively increases the number of channels in the open and/or inactivated states. As shown in Fig. 5B, there was a progressive decrease in the ratio of \( I_{\text{HERG}} \) tails in methadone versus control during the initial pulse period with stabilization being achieved between 750 and 1000 ms after the first pulse, thereby indicating that \( I_{\text{HERG}} \) was blocked by methadone primarily when the HERG channel was in an open or inactivated state. These results demonstrate that the onset of \( I_{\text{HERG}} \) block by methadone was fast and progressed to steady-state levels (40–50% decrease in \( I_{\text{HERG}} \)) within 1 s of recording in the presence of 10 \( \mu \)M methadone using the indicated protocol.

Even though methadone seems to primarily block HERG channels when they are in open or inactivated states, we cannot eliminate the possibility that some minor portion of the channels was also blocked in a closed state. To estimate the amount of HERG channel blocked in the closed state, the curve shown in Fig. 5B was extrapolated back to the zero time point using a single exponent curve-fit function (\( \tau \) of decay \(-300\) ms). This extrapolation indicates that the ratio of HERG tail currents in methadone versus control recordings was approximately 0.9 at the zero time point. This result suggests that \(-10\%\) of the available HERG channels were blocked in the closed state. As the voltage was switched to +10 mV, most of the HERG channels were in an activated (“open”) and/or inactivated rather than a closed state. Because most of the blockade of \( I_{\text{HERG}} \) by methadone occurred during depolarizations to +10 mV, it seems that methadone interfered with the HERG channels primarily when they were in an open and/or inactivated state. Consistent with this idea, we found that the \( \tau \) of decay was much faster when the level of depolarization was increased to +40 mV compared with that observed when the cells were depolarized to +10 mV (\( \tau \sim 30\) ms at +40 mV versus \(-300\) ms at +10 mV).

This finding further supports the hypothesis that methadone...
blocks the HERG channel primarily in the open and/or inactivated states rather than the closed state.

**Discussion**

Our results provide the first direct evidence showing that opioid agonists can block cardiac HERG K⁺ currents. The mechanism of I_{HERG} inhibition by opioids has not been fully explored, but the majority of the observed blockade of I_{HERG} seems to occur when the channels are in the open and/or inactivated states. This may be a common feature of HERG-blocking drugs because previous reports have suggested that

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC_{50} for HERG Block (μM)</th>
<th>Maximum Plasma Conc. (C_{max})</th>
<th>Reference (C_{max})</th>
<th>Ratio: IC_{50}/C_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAAM</td>
<td>2.2</td>
<td>1 μM</td>
<td>Henderson et al., 1977</td>
<td>2.2</td>
</tr>
<tr>
<td>Methadone</td>
<td>9.8</td>
<td>3.6 μM</td>
<td>de Vos et al., 1995</td>
<td>2.7</td>
</tr>
<tr>
<td>EDDP</td>
<td>&gt;50</td>
<td>1 μM</td>
<td>de Vos et al., 1995</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Meperidine</td>
<td>75</td>
<td>1.3 μM</td>
<td>Spigge et al., 1982</td>
<td>58</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1.8</td>
<td>30 nM</td>
<td>Ahonen et al., 2000</td>
<td>60</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>7.5 (μM)</td>
<td>36 nM</td>
<td>USP DI, 1999</td>
<td>208</td>
</tr>
<tr>
<td>Morphine</td>
<td>&gt;1</td>
<td>2.5 μM</td>
<td>Faura et al., 1996</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Codeine</td>
<td>&gt;300</td>
<td>0.66 μM</td>
<td>Band et al., 1994</td>
<td>&gt;455</td>
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* C_{max} values listed represent the upper limit of the range of values reported in the indicated references.
several HERG-blocking drugs, including dofetilide, quinidine, MK-499, terfenadine, and cisapride, bind to open and/or inactivated HERG channels (Fickler et al., 1998; Lees-Miller et al., 2000; Mitcheson et al., 2000). Furthermore, Lees-Miller et al. (2000) suggested that dofetilide may specifically interfere with the transition from the open to the inactivated state because a mutant form of HERG that fails to inactivate was relatively resistant to dofetilide. Amino acid residues in the S6 transmembrane segment of the HERG channel are thought to be important points of drug interaction, although MK-499 seems to also bind to residues in the pore region (Mitcheson et al., 2000). Further studies are required to determine the specific amino acid residues involved in opioid-HERG interactions.

Interestingly, the methadone metabolite EDDP was not a potent inhibitor of IHERG, despite having a chemical structure similar to methadone and LAAM. The biphenyl moieties present in LAAM, methadone, and EDDP could contribute to anti-HERG activity because some antihistamine drugs with similar biphenyl moieties also produce IKr block and QT prolongation (Wang et al., 1998). This idea is consistent with recent data from Ekins et al. (2002), who showed that the biphenyl rings present in terfenadine could represent two of the four hydrophobic moieties predicted by a computer model of an idealized HERG “pharmacophore” generated using published HERG data. The EDDP results suggest that although these biphenyl groups may contribute to the ability of drugs to block IHERG, other structural considerations must also be taken into account. In the case of EDDP, the additional cyclization and positive charge that are not present in either LAAM or methadone may have diminished its ability to block IHERG. Additional studies are necessary to delineate the structural features predictive of an ability to block IHERG. Clearly, however, the prototypical opioid agonists morphine and codeine were relatively poor blockers of IHERG, a finding that is consistent with the fact that the chemical structures for morphine and codeine are similar to each other and dissimilar to those for LAAM and methadone.

Of the opioid agonists tested in this study, LAAM and methadone were two of the most potent inhibitors of IHERG, with IC50 values of approximately 0.2 and 0.1 μM, respectively. Fentanyl and buprenorphine also had IC50 values for blockade of IHERG in this concentration range, but because the Cmax values for these two drugs are much lower than those reported for LAAM and methadone, the therapeutic index (IC50/Cmax) for fentanyl and buprenorphine was substantially better than that observed for LAAM or methadone with respect to IHERG. This does not necessarily mean that LAAM and methadone are more likely to cause arrhythmias because other factors such as the degree of protein binding in plasma, subject variability, route of administration, and dosage equivalence could have significant influence on the ability of these opioids to block HERG currents in vivo. One study suggested, for example, that up to 89% of plasma methadone is protein bound (Inturrisi et al., 1987), thereby possibly reducing the in vivo amount of methadone available to inhibit IHERG to 11% (free fraction) and raising the therapeutic index for methadone approximately 10-fold. Drug metabolites may also play a role, because the metabolites for some opioid compounds can attain plasma concentrations that are >60-fold higher than those achieved by the parent drug (Faura et al., 1996). In addition, one has to consider drug formulations. For example, the cases of QTc lengthening and ventricular tachycardia reported to QTdrugs.org pertained only to patients receiving i.v. methadone. The parenteral preparation of methadone contains 5% chlorbutanol as a preservative, and this compound could contribute to cardiovascular toxicity (Hermesmeyer and Aprigliano, 1976; Bowler et al., 1986). These caveats notwithstanding, our results demonstrate that LAAM and methadone could effectively block IHERG at concentrations that may be clinically relevant.

As with any study of this type, however, one always has to be careful when trying to relate in vitro data to the clinical setting. Blockade of IHERG can certainly lead to cardiac repolarization deficits and has been associated with the development of life-threatening ventricular arrhythmias such as torsades de pointes (Antzelevitch et al., 1996; January et al., 2000; Tamargo, 2000; Tseng, 2001), but such influence could be masked by other drug effects that have yet to be identified (Yang et al., 2001). Certainly, methadone has been used clinically for many years (Food and Drug Administration-approved use since 1947) with relatively few reports of adverse reactions, almost none of which were recognized to be related to the development of cardiac arrhythmias (Joseph et al., 2000). Nevertheless, sudden death is a long-accepted event during methadone therapy that is typically thought to be related to complications arising from long-term narcotic abuse, but it may actually be torsades de pointes in some cases. Moreover, in the past several years, high-dose i.v. administration of methadone has been gaining popularity for the alleviation of chronic pain (Ayonrinde and Bridge, 2000; Ripamonti and Dickerson, 2001; Bruera and Sweeney, 2002). Methadone is generally used to treat cancer pain when other opioids are not effective and, therefore, its substitution with another opioid drug may be problematic (Manfredi et al., 1997; Santiago-Palma et al., 2001).

Although buprenorphine is not recommended for the treatment of cancer pain due to its partial antagonist activity, our data suggest that buprenorphine may represent a safer alternative (with respect to IHERG) to methadone or LAAM for the treatment of narcotic addiction. In France, where buprenorphine has been widely used in the treatment of narcotic addiction for several years, a recent study found that between 1994 and 1998, the proportion of patients whose death was classified as “treatment-related” was 3 times greater for patients during methadone treatment compared with those that received buprenorphine treatment (Auricomer et al., 2001). As the authors of this report remark, there are several sources of possible inaccuracies in these data, including biases in the determination of the cause of death. Our experimental data, however, substantiate the hypothesis that buprenorphine may be a safer drug than methadone for the treatment of narcotic addiction. Additional studies will be required to validate this hypothesis and to determine the mechanisms of methadone and LAAM action in the heart.

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