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Oxytocin does not directly alter cardiac repolarization in rabbit or human cardiac myocytes

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Abstract

Oxytocin, a nine amino acid peptide, is highly conserved in placental mammals, including humans. Oxytocin has a physiological role in parturition and parenteral administration of the synthetic peptide is used to induce labor and control postpartum hemorrhage. Endogenous levels of oxytocin before labor are ~20 pg/mL, but pharmacological administration of the peptide can achieve levels of 110 pg/mL (0.1 nmol/L) following intravenous administration. Cardiac arrhythmia and premature ventricular contractions have been associated with oxytocin administration in addition to QTc interval prolongation. In the conscious rabbit model, intravenous oxytocin produced QT and QTc prolongation. The mechanism of oxytocin-induced QTc prolongation is uncertain but could be the result of indirect changes in autonomic nervous tone, or a direct effect on the duration of cardiomyocyte repolarization. The purpose of this study was to examine the ability of oxytocin to alter cardiac repolarization directly. Two conventional models were used: QTc interval evaluation in the isolated rabbit heart (IRH) and assessment of action potential duration (APD) in human ventricular myocytes (HVM). Oxytocin did not prolong QTc intervals in IRH or APD in HVM when tested at suprapharmacological concentrations, for example, up to 1 μmol/L. The results indicate that oxytocin has very low risk for eliciting QTc and APD prolongation directly, and infer that the QTc changes observed in vivo may be attributed to an indirect mechanism.

Abbreviations

APD, action potential duration; CF, coronary flow; CPP, coronary perfusion pressure; HVM, human ventricular myocytes; IRH, isolated rabbit heart; LV, left ventricle; LVP, left ventricular pressure; LVDP, LV diastolic pressure; LVSP, LV systolic pressure; NO, nitric oxide; TdP, torsades de pointes.

Introduction

Oxytocin is a peptide of nine amino acids which is highly conserved in placental mammals, including human. It was the first polypeptide hormone to be sequenced and synthesized (du Vigneaud et al. 1954). Oxytocin has both peripheral and central actions as a hormone, and its physiological effects are mediated by specific, high-affinity oxytocin receptors. The oxytocin receptor is a G-protein-coupled receptor which belongs to the rhodopsin-type (class I) group of G-protein-coupled receptors (Gimpl and Fahrenholz 2001).

Injected oxytocin analogs (Oxytocin Label retrieved from http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/077453s000lbl.pdf) are used for labor induction and to support labor in cases of difficult parturition. In addition, they have been used as the principal agent to increase uterine tone in acute postpartum hemorrhage. Oxytocin is relatively safe when used at recommended doses, and side effects are uncommon. However, in its

label, cardiac arrhythmia in patients was listed as one of the adverse reactions of oxytocin injection. For example, it has been reported that intravenous oxytocin prolonged QTc intervals (Guillon et al. 2010) and triggered ventricular arrhythmia during cesarean section under spinal anesthesia (Liou et al. 1998; Thomas and Cooper 2002). A large and transient QTc interval prolongation has also been observed after oxytocin administration during a first-trimester-induced abortion curettage under general anesthetics (Charbit et al. 2004). In all the human studies referenced above, anesthetics were present, which could complicate the interpretation of QTc effects, because anesthetics can alter ventricular repolarization through a direct effect upon myocardial electrophysiology (Spevak et al. 2012) or by an interaction with oxytocin. To understand if oxytocin could prolong QT and QTc intervals independent of anesthetics, Uzun et al. (2007) evaluated the effect of oxytocin on QT and QTc intervals in both conscious male and female rabbits. The results revealed that QT and QTc intervals were prolonged in male and female conscious rabbits by administration of oxytocin. That study demonstrated that oxytocin prolonged ventricular repolarization independent of anesthesia and suggested that rabbit heart is a good model for translating the QTc findings of oxytocin in human.

To assess the effect of oxytocin on cardiac repolarization directly, this peptide was evaluated for its ability to prolong QT intervals in the isolated rabbit heart (IRH), and on action potential duration (APD) in human ventricular myocytes (HVM). The IRH model has been used for cardiac safety assessment to evaluate the propensity of small molecule drugs to cause QT interval prolongation and arrhythmia (Lawrence et al. 2006), and it has demonstrated sensitivity to detect QT prolongation induced by BeKm-1, a specific peptide inhibitor of the hERG channel (Qu et al. 2011). Therefore, this isolated whole heart model can detect QTc prolongation caused by small or large molecules inhibitors that directly interfere with cardiac repolarization. In addition, APD recorded in ventricular myocytes is another sensitive model for predicting effects on the QT interval in vivo (Hayashi et al. 2005).

Materials and Methods

IRH using Langendorff perfusion

Pathogen-free rabbits (New Zealand White; 2.5–3.5 kg; female) were maintained in accord with the Guide for the Care and Use of Laboratory Animals (8th Edition; NRC 2011) at an AAALAC-accredited facility. All experiments were conducted in compliance with the Amgen Institutional Animal Care and Use Committee and USDA regulations. Animals had daily access to pelleted feed (#2031; Harlan Teklad, Madison, WI) and water (reverse osmosis-purified) via automatic watering system. Animals were maintained on a 12:12 h light:dark cycle in temperature-controlled rooms and had access to enrichment which included enrichment devices of either a dumbbell, jiggle ball, or hanging whisk in their cages. Enrichment devices were rotated every 2 weeks. Rabbits were also provided loose hay and a hay cube once a week.

The heart preparation and perfusion procedure has been described previously (Qu et al. 2011). Briefly, rabbits were anesthetized with pentobarbital (50 mg/kg) by ear vein injection, hearts removed rapidly, cannulated via aorta, and perfused (retrogradely) according to the Langendorff technique. The perfusion media was a modified Krebs–Henseleit solution composed of (mmol/L): 120 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 11.1 Glucose, 2 Na-pyruvate, 1.8 $CaCl_2$, and bubbled with O_2/CO_2 (95%/5%). Two lead ECGs were recorded with flexible unipolar electrodes (Harvard Apparatus, Holliston, MA) placed on the heart, one over the epicardium of ventricles and the other over the epicardium of the left atria. For measuring left ventricular pressure (LVP), a metal cannula with a latex balloon on the tip was inserted into the left ventricle (LV). The balloon was expanded with water to achieve a LV diastolic pressure (LVDP) of ~5–10 mmHg. Once the end diastolic pressure was stabilized at the baseline, it was not adjusted during the course of the experiment. A pressure transducer was connected to the balloon for measuring LVDP and LV systolic pressure (LVSP). Coronary perfusion pressure (CPP) was measured with a pressure transducer connected to the aortic block. Coronary flow (CF) was measured with an inline transonic flow probe (Harvard Apparatus, Holliston, MA) inserted in the aortic block. The heart was stabilized for at least 45 min in Krebs–Henseliet solution before baseline measurements. Following baseline measurement, the hearts were perfused with 4–5 concentrations of testing compounds with each concentration applied for 20 min. The hearts were maintained at ~37°C.

ECG measurements were continuously monitored with the Notocord HEM (Croissy sur Seine, France) v3.5 data capture system and EMKA IOX software (EMKA Technologies, Paris, France). Digital markers were used to indicate drug application periods. ECG was analyzed with ECG-Auto v2.4 (EMKA Technologies) automatically and 1 min consecutive ECG waveforms were analyzed for HR, PR, QRS, and QT intervals. Values from each individual heart were pooled to determine an average for each variable at individual concentrations. Average percent change in each variable between baseline and each concentration

was also determined. Fridericia equation (QTcF = QT/RR1/3) was used to correct QT intervals for heart rate change (Qu et al. 2011).

AP recording in isolated HVM

This study was conducted at Zenas Technologies (New Orleans, LA). Myocytes were obtained from human LV epicardium of two male Class IV (New York Heart Association) heart failure patients (63 and 54 years) undergoing cardiac transplant. Tissues were obtained from the explanted failing heart and quickly immersed in a cardioplegia solution consisting of (in mmol/L): 50 KH_2PO_4, 8 $MgSO_4$, 10 $NaHCO_3$, 5 adenosine, 25 taurine, 140 glucose, and 100 mannitol, titrated to a pH of 7.4, and bubbled with 100% O_2 at 0–4°C. Specimens were minced into 0.5–1 mm cubes and transferred to a 50 mL conical tube containing an ultra-low calcium wash solution containing (in mmol/L): 137 NaCl, 5 KH_2PO_4, 1 $MgSO_4$, 10 taurine, 10 glucose, 5 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 0.1 ethylene glycol tetraacetic acid; pH = 7.4 (22–24°C). The tissue was then gently agitated by continuous bubbling with 100% O_2 for 5 min. The tissue was next incubated in ~5 mL of solution containing (in mmol/L): 137 NaCl, 5 KH_2PO_4, 1 $MgSO_4$, 10 taurine, 10 glucose, 5 HEPES, supplemented with 0.1% bovine albumin, ~2.2 mg/mL collagenase type V, and ~1.0 mg/mL protease (Sigma Chemical), pH = 7.4 (37°C), and bubbled continuously with 100% O_2. The supernatant was removed after 40 min and discarded. The precipitates were then incubated in a solution of the same ionic composition but supplemented with only collagenase and 100 μmol/L $CaCl_2$. Microscopic examination of the medium was performed every 10–20 min to determine the number and quality of the isolated cells. When the yield appears to be maximal, the cell suspension was centrifuged for 2 min, and the resulting pellet was resuspended in a modified Kraftbruhe solution containing (in mmol/L): 25 KCl, 10 KH_2PO_4, 25 taurine, 0.5 EGTA, 22 glucose, 55 glutamic acid, and 0.1% bovine albumin, pH = 7.3 (22–24°C). Cells were used within 24 h after isolation.

Action potentials were recorded using the intracellular microelectrode technique. The microelectrode had a resistance of 25–40 MΩ when filled with 3 mol/L KCl. Cells were perfused (perfusing rate ~2 mL/min) with a bath solution containing (mmol/L): NaCl 137, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and HEPES 10 (pH = 7.4 with NaOH). Experiments were performed at 36.0 ± 0.5°C, and myocytes were stimulated with a frequency of 0.5 Hz. Control APs were recorded 2 min after the first AP was generated. Cells were then perfused with the bath solution containing a certain concentration of a test article. The AP was then recorded 5 min after initiation of a test article superfusion. Cells were only exposed to 1 concentration of test article.

Electrophysiological recording of hERG, hNav1.5, and L-type Ca^{2+} current

Whole-cell hERG or hNav1.5 currents were recorded using standard procedures in PatchXpress 7000A (Molecular Devices, Sunnyvale, CA). Experiments were conducted at room temperature (20–22°C) and currents were elicited by a series of voltage pulses given at 0.1 Hz.

HEK 293 cells stable-transfected with hERG cDNA were purchased from Millipore (Billerica, MA). The hERG current was recorded by automatic whole-cell voltage-clamp using the following solutions (in mmol/L): extracellular, NaCl, 135; KCl, 5; $CaCl_2$, 1.8; HEPES, 10; glucose, 5; pH = 7.40 adjusted with NaOH; intracellular, KF, 60; KCl, 70; EGTA, 10; K2ATP, 5; $MgCl_2$, 1; HEPES, 10, pH = 7.20 with KOH. A standardized protocol was used to elicit ionic current through the hERG potassium channel. Cells were held at −80 mV. Membrane potential was first depolarized to −50 mV for 0.5 sec. Since hERG channel is not activated at –50 mV, this is used for baseline subtraction. A depolarization to +30 mV for 2 sec was used to activate hERG channel followed by a repolarization to −50 mV for 2 sec to elicit a tail current.

HEK 293 cells stable-transfected with hNav1.5 cDNA were purchased from Millipore (Billerica, MA). The hNav1.5 current was recorded with extracellular solution (mmol/L): NaCl, 70; *N*-methyl-D-glucamine 67; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; KCl, 4; HEPES, 10; glucose, 10; pH = 7.40 adjusted with HCl, and intracellular solution containing the following (in mmol/L): CsF, 130; NaCl, 10, EGTA, 10; $MgCl_2$, 2; HEPES, 10, pH = 7.20 with CsOH. A standardized step protocol was used to elicit ionic current through the hNav1.5 sodium channel. Cells were held at −80 mV. Onset and steady state block of hNav1.5 sodium current was measured using a pulse pattern with fixed amplitudes (conditioning prepulse: −120 mV for 50 msec; depolarizing test step to –30 mV for 20 msec).

L-type Ca channel was recorded using HVM conducted at Zenas Technologies LLC (New Orleans, LA). Whole-cell variant of the patch clamp method was used. Current was elicited by a pulse to 0 mV (200 msec) given at 0.1 Hz from a holding potential of −40 mV. The external solution consisted of (mmol/L): 1.8 $CaCl_2$, 137 NaCl, 20 CsCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, 10 dextrose, pH = 7.4 with NaOH. The internal solution had a composition of (mmol/L): 120 CsCl, 20 TEA-Cl, 5 NaCl, 1 $CaCl_2$, 10 EGTA, 5 Na2 ATP, 10 HEPES, adjusted to pH 7.2 with CsOH. Experiments were performed at 37 ± 1°C, and each concentration of testing agents was tested on at least 3 cells.

Data analysis and statistics

All numeric values derived in these studies are presented as mean ± SE, and group comparisons were conducted using statistical software (GraphPad Prism 4.01, GraphPad Software Inc.). One way repeated measures ANOVA (with Dunnett's multiple comparison test) was used for comparison and $P < 0.05$ was the significance level.

Reagents

Moxifloxacin (CAS #354812-41-2) was purchased from ChemPacific (Baltimore, MD) and dissolved in DMSO to make stock solutions. Human oxytocin (CAS #: 50-56-6) was purchased from Sigma-Aldrich (St. Louis, MO), and was dissolved in distilled H_2O to make stock solutions.

Results

Oxytocin: no effect on QT and QTc intervals in IRH

Oxytocin was administered cumulatively in a rising dose perfusion (0.003–1 μmol/L) to determine its ability to cause QTc prolongation in this model. Moxifloxacin (10–300 μmol/L) was also evaluated and served as a positive control. Figure 1 shows sample ECG traces from hearts perfused with moxifloxacin (left panel) or oxytocin (right panel). It is apparent that QT interval prolongation was observed following moxifloxacin, but not oxytocin treatment.

To characterize the effect of the peptide on cardiac conduction and repolarization, ECG parameters (PR, QRS, and QT intervals) were analyzed during each application (20 min) of oxytocin. The average percent change in each parameter between baseline and each test concentration is shown in a minute-by-minute plot (Fig. 2). Over a broad exposure range, oxytocin did not alter QT and QTc intervals in this model, however, at 0.1 μmol/L, the PR and QRS intervals were prolonged significantly.

The absolute values for HR (bpm) and ECG intervals (msec) before and after treatment with oxytocin are in Table 1. Consistent with the minute-by-minute plot in Figure 2, no QT or QTc prolongation was observed at any of the test concentrations up to 1 μmol/L oxytocin. The PR and QRS intervals were prolonged significantly at 0.1 and 1 μmol/L.

Oxytocin: no effect on APD in HVM

To examine the effects of oxytocin on AP parameters, including APD90, APD30, and Vmax, a study was designed to record APs in isolated HVM.

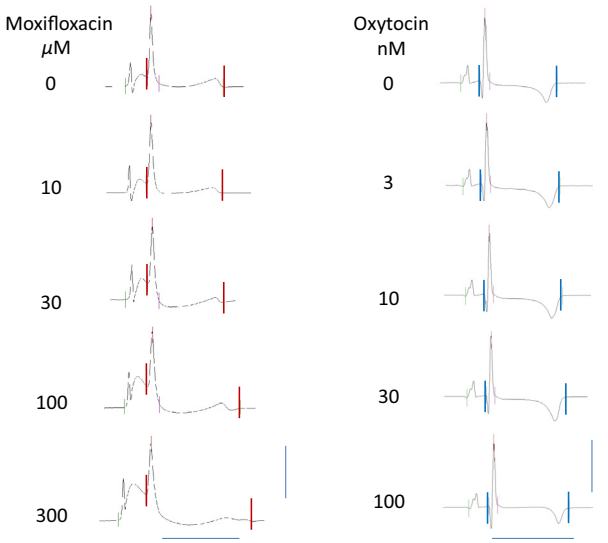

Figure 1. Example ECG traces from IRH showing moxifloxacin prolonged QT intervals, while oxytocin did not prolong QT intervals in IRH. For moxifloxacin, ECG traces were shown in control (0) and after 20 min with 10, 30, 100, and 300 μmol/L as labeled. For oxytocin, ECG traces were shown in control (0) and after 20 min with 3, 10, 30, and 100 nmol/L as labeled. Each waveform is marked with beginning of P, Q, R, end of S and T waves. Calibration: 200 msec, 2 mV.

Action potential traces before and after 1 μmol/L oxytocin exhibited no change in AP morphology or duration (Fig. 3A). From a total of three cells recorded at 0.03–0.3 μmol/L and a total of six cells recorded at 1 μmol/L, the APD90 following oxytocin treatment was not prolonged compared to baseline values (Fig. 3B). Oxytocin had no discernible effect on Vmax and repolarization endpoints following treatment up to 1 μmol/L (Table 2).

Application of moxifloxacin did cause significant prolongation of the APD recorded in HVM (Fig. 3C and D). Figure 3C depicts an AP trace following 300 μmol/L moxifloxacin, which demonstrates that this agent causes a predominant delay of phase 4, that is, the late repolarization. Moxifloxacin prolonged APD90 in a dose-dependent manner (10–300 μmol/L), with significant prolongation observed at ≥30 μmol/L (Fig. 3D).

Oxytocin does not affect hERG, hNav1.5, or L-type Ca^{2+} channel function

To further understand if oxytocin could have inhibitory effects on human cardiac ion channels directly, hERG potassium channel and hNav1.5 channel functional assays were performed using an automatic patch clamp system. In addition, L-type Ca^{2+} channels were recorded in HVM using a whole-cell variant of the patch clamp method. Figure 4 presents the average percent inhibition at each

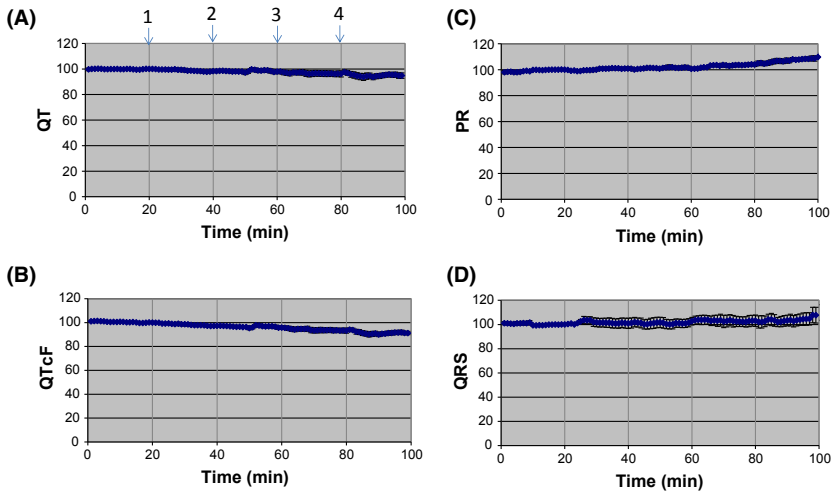

Figure 2. Concentration- and time-dependent plots of oxytocin on ECG parameters in IRH. QT (A), QTcF (B), PR (C), and QRS (D) were derived from 1 min consecutive ECG waveforms. Average percent change in each variable between baseline and each concentration was determined and presented from $n = 6$ hearts. Baseline values are represented in the first 20 min, each concentration is perfused for 20 min (1 grid line). Data are 1 min mean ± SE. Numbers in (A) indicated the beginning of oxytocin application at 1, 3 nmol/L; 2, 10 nmol/L; 3, 30 nmol/L; 4, 100 nmol/L. No prolongation of QT or QTc was observed.

Table 1. ECG parameters recorded under control and oxytocin treatments in IRH.

	HR (bpm)		PR (msec)		QRS (msec)		QT (msec)		QTcF (msec)		
Oxytocin (μmol/L)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*
0	128	4	57	2	27	1	212	7	272	8	6
0.003	126	4	57	2	28	1	207	8	264	8	6
0.01	121	3	57	2	28	1	206	9	260	10	6
0.03	121	3	59	2	28	2	200	9	253	11	6
0.1	114*	3	62*	2	29*	2	207	10	257	11	6
1	109*	4	68*	2	29*	2	213	10	260	11	6

*Statistically significant ($P < 0.05$).

Table 2. ECG parameters recorded under vehicle control.

	HR (bpm)		PR (msec)		QRS (msec)		QT (msec)		QTcF (msec)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*
0.3% DMSO	126.4	11.1	59.3	3.7	27.2	1.9	221.5	11.2	282.0	6.0	4
0.3% DMSO	124.0	10.5	59.3	4.1	26.5	1.7	220.4	11.1	278.9	6.3	4
0.3% DMSO	123.8	10.2	58.9	3.8	26.8	1.6	220.8	11.1	279.2	6.4	4
0.3% DMSO	120.6	9.3	59.7	3.9	26.7	1.8	222.9	9.7	279.8	5.1	4
0.3% DMSO	119.5	9.3	58.9	3.9	26.4	1.6	223.0	8.4	279.1	3.7	4
0.3% DMSO	118.3	9.1	59.0	3.8	26.3	1.6	222.6	8.1	277.9	3.6	4

test concentration of oxytocin for all three ion channel assays. Compared to vehicle treatment, no significant inhibitory effect were detected, which indicated that oxytocin had no effect on hERG, hNav1.5, or L-type Ca^{2+} currents up to 1 μmol/L.

Effects of oxytocin on hemodynamic parameters in IRH

The effects of oxytocin on CF and LVP were assessed in IRH (Table 3). Oxytocin did not alter coronary function

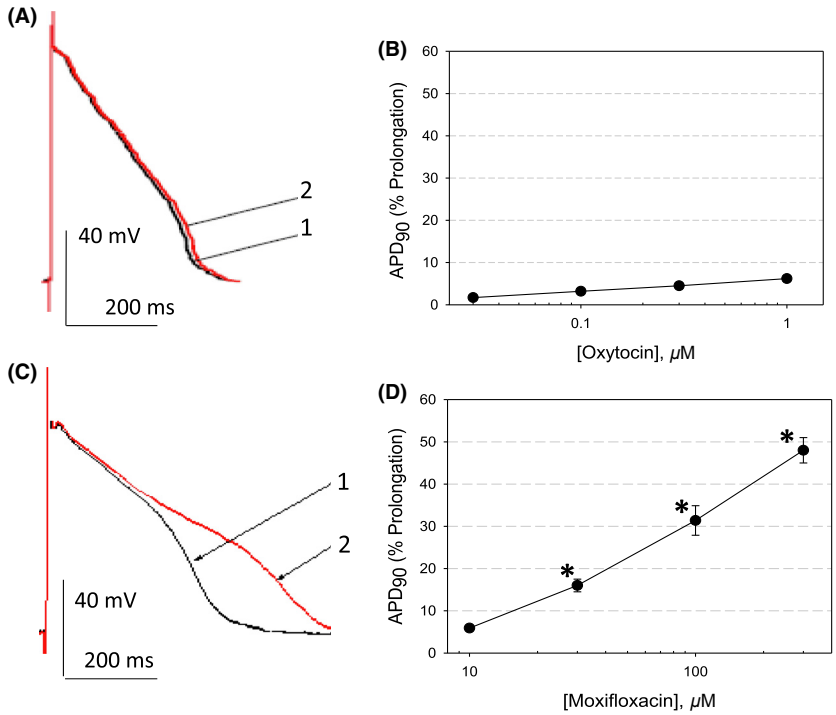

Figure 3. APD was not prolonged by oxytocin in HVM. (A) Example action potentials recorded in HVM under 1, control and 2, 1 μmol/L oxytocin. (B) Average concentration–response relationship of oxytocin on APD_{90} (n = 3–6 cells for each concentration). There was no significant prolongation observed at all concentrations. (C) Example action potentials recorded in HVM under 1, control and 2, 300 μmol/L moxifloxacin. (D) Average concentration-response relationship of moxifloxacin on APD_{90} (n = 3 cells for each concentration). Significant prolongation was observed at 30, 100, and 300 μmol/L, which was marked as * in (D).

or contractility at 0.003 μmol/L, but CF, LVSP, dP/dt_{max}, dP/dt_{min}, and LVDevP were all decreased significantly at higher concentrations of the peptide (Tables 4 and 5).

Discussion and Conclusions

To the best of our knowledge based on the literature, this was the first study to examine the pharmacological effect of oxytocin on ventricular repolarization in IRH and HVM. The findings clearly demonstrate that oxytocin does not prolong QT or QTc intervals in the IRH model, nor cause APD prolongation in HVM. In addition, oxytocin did not affect the amplitude of hERG, hNav1.5, or L-type Ca^{2+} currents evaluated by patch clamp technique. Together, these in vitro findings lead us to conclude that oxytocin does not prolong cardiac repolarization through a direct on-target or off-target electrophysiological action on rabbit or human cardiomyocytes. Thus, the prolongation of QT or QTc intervals observed in animals and human patients treated with oxytocin is likely due to an indirect mechanism, which remains unknown.

Oxytocin and ventricular repolarization

Prolongation of QT or QTc intervals has been recognized as a biomarker for proarrhythmia, especially torsades de pointes (TdP; Heist and Ruskin 2010; Pollard et al. 2010). The most common cause of acquired long QT syndrome is drug induced, with antiarrhythmics being the drug class most commonly implicated, due to their ability to delay cardiac repolarization therapeutically. However, a few noncardiac drugs have been pulled from the market due to their liability in producing QT prolongation. A common pharmacological feature of QT prolonging drugs is their ability to inhibit hERG potassium channels (Redfern et al. 2003; Gintant 2011).

Oxytocin administration has been observed to prolong QTc intervals, and trigger ventricular arrhythmia, under clinical procedures in humans (Liou et al. 1998; Thomas and Cooper 2002; Charbit et al. 2004; Guillon et al. 2010). In addition, QT and QTc intervals were prolonged by oxytocin injection in conscious rabbits (Uzun et al. 2007). These findings have led to the perception that this peptide may carry a proarrhythmic risk, like some small molecule drugs that are associated with the occurrence of

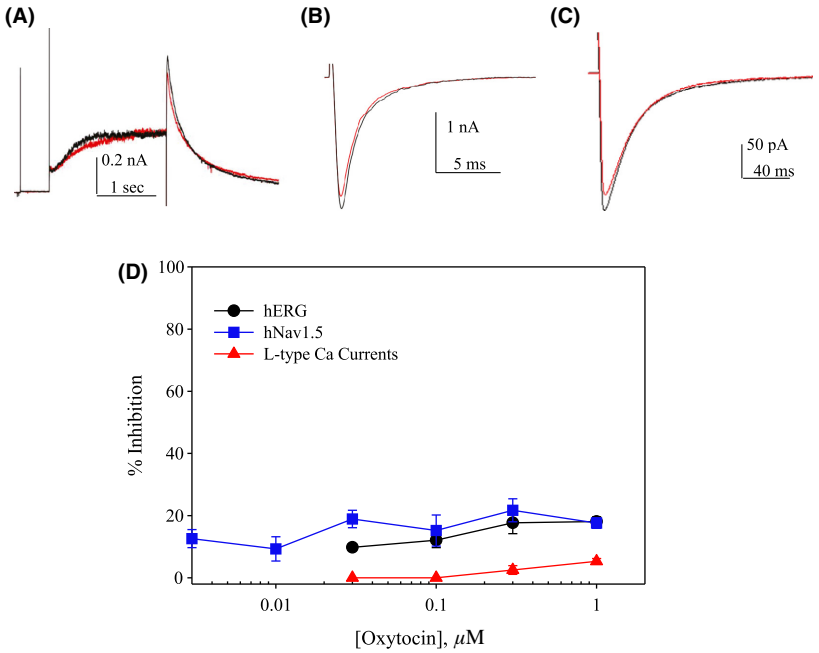

Figure 4. Lack of ion channel inhibition by oxytocin. 1 μmol/L oxytocin had minimal effects on hERG (A), Nav1.5 (B), and L-type Ca channel (C). Currents traces were shown in control (black) and 1 μmol/L oxytocin (red). (D) Concentration–response relationships of oxytocin in inhibiting hERG (black), hNav1.5 (blue), and L-type Ca currents (red). The averaged data were derived from three to six cells for each concentration. For all three channels, there was a lack of concentration-dependent inhibition.

TdP (see list of drugs at www.crediblemed.org). Charbit et al. (2012) had shown that oxytocin did not prolong APD in rabbit Purkinje fibers. However, no studies have been done to examine the effects of oxytocin on ventricular repolarization in an isolated heart and HVM. Furthermore, the pharmacological profile of oxytocin in ion channel assays has not been reported prior to the current study, so the mechanism of QTc prolongation remained uncertain. Radioligand-binding studies (Cicutti et al. 1999) confirmed the presence of a specific oxytocin-binding site in rat LV and in human atrium, thus it is plausible that the peptide may have an inherent effect on myocardial repolarization (Cicutti et al. 1999) mediated by oxytocin receptors that could underlie these QTc observations.

During oxytocin-induced labor, plasma levels of synthetic oxytocin increased linearly with dose, and achieved

Table 3. Percent changes of AP parameters in HVM under oxytocin treatments (n = 3–6 cells).

Condition	APD_{90}	APD_{30}	V_{max}	n
Vehicle	0.2 ± 0.6	0.2 ± 0.4	−1.2 ± 0.2	6
0.03 μmol/L	1.7 ± 0.3	1.1 ± 0.2	−1.5 ± 0.4	3
0.1 μmol/L	3.2 ± 0.6	2.5 ± 0.5	−0.2 ± 0.8	3
0.3 μmol/L	4.5 ± 0.7	3.7 ± 0.7	−0.2 ± 0.9	3
1 μmol/L	6.2 ± 0.7	5.0 ± 0.6	−1.6 ± 0.6	6

Table 4. Percent changes of hemodynamic parameters in IRH under oxytocin application.

	LVSP		LVDP		dP/dt_{max}		dP/dt_{min}		CPP		CF		LVDevP		
Oxytocin (nmol/L)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	N
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
3	−10	2	9	2	−11	2	−11	3	1	0	−8	3	−12	2	6
10	−17	3	22	5	−20	4	−21	3	3	1	−15	2	−22	4	6
30	−23	5	31	8	−25	5	−34	7	4	1	−17	6	−30	6	6
100	−28	7	35	11	−30	7	−39	9	4	2	−28	9	−36	9	6
1000	−42	6	46	15	−44	6	−57	6	4	2	−44	9	−52	7	6

Significant changes (P < 0.05) occurred at 10 nmol/L for all parameters other than CPP.

Table 5. Percent changes of hemodynamic parameters in IRH under vehicle application.

	LVSP		LVDP		dP/dt_{max}		dP/dt_{min}		CPP		CF		LVDevP		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*N*
0.3% DMSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
0.3% DMSO	1	2	−1	5	3	1	−1	2	2	2	2	1	1	2	4
0.3% DMSO	2	4	4	2	4	4	4	5	2	2	2	1	1	4	4
0.3% DMSO	2	3	7	1	2	4	5	3	2	3	0	1	1	3	4
0.3% DMSO	5	3	10	6	10	7	13	9	4	3	1	1	4	3	4
0.3% DMSO	6	2	10	4	11	8	14	9	4	3	0	1	6	3	4

a highest drug level of 110 ± 23 pg/mL following intravenous infusion of 10–16 mU/min (Fuchs et al. 1983). Based on the molecular weight of oxytocin (1007 g/mol), this concentration equates to a molar equivalent of 0.1 nmol/L (approximately). In the current electrophysiological studies, oxytocin was tested from 3 to 1000 nmol/L in the cardiac tissue assays, which represents a 30–10,000-fold range over the therapeutic plasma concentration (Fuchs et al. 1983). Over this broad range of concentrations, no significant prolongation of ventricular repolarization was observed in either the IRH or HMV models. The inability of oxytocin to alter cardiac repolarization strongly indicates that this peptide has no ability to alter cardiac repolarization due to target (oxytocin receptors) or off-target effects (ion channel blockade; see below) in isolated cardiac tissue. On the other hand, moxifloxacin, a drug known for its ability to cause hERG channel blockade and QTc prolongation in humans (Bloomfield et al. 2008), prolonged QTc intervals in IRH and lengthened APD in HVM, confirming the sensitivity of these two in vitro models to detect delayed ventricular repolarization.

It is generally accepted that biopharmaceuticals, including peptides like oxytocin, have low potential to inhibit cardiac ion channel function in a nonselective manner (Vargas et al. 2008, 2013). The potential for oxytocin to interfere with three major cardiac ion channels, for example, hERG, hNav1.5, and L-type Ca^{2+} channels, was assessed using standard ion channel recording methodology. As expected, oxytocin did not alter the function of cardiac ion channels when tested at suprapharmacological levels of the peptide, for example, 1000 nmol/L. Therefore, the inability of oxytocin to alter cardiac ion channel function is consistent with the inability of this peptide to alter repolarization in rabbit and human cardiomyocytes. Together, this pharmacological evidence indicates that this peptide may not alter cardiac repolarization like typical hERG channel blockers. The exact mechanism of QTc prolongation and/or TdP seen following oxytocin administration could be secondary to other cardiovascular effects of this peptide that modulate repolarization, or be related to coincident patient factors, for example, underlying cardiovascular disease or electrolyte imbalance, that made them susceptible to ventricular arrhythmia.

Aside from drugs, many conditions have been identified which may cause prolonged ventricular repolarization. These conditions include: (1) organic heart disease, such as congenital long QT syndrome, ischemic heart condition, and congestive heart failure; (2) electrolyte imbalance (Meenagh et al. 2004), such as hypokalemia, hypocalcemia, and hypomagnesemia; (3) hypoglycemia (Robinson et al. 2003); and (4) hypothermia (Amin et al. 2008; Hsieh et al. 2009). Furthermore, the interplay between the sympathetic and parasympathetic effects on the ventricular myocardium is another important factor that determines the duration of the ventricular AP and therefore the QT interval (Lee et al. 2003). It has been recognized early on that neural activity could predispose heart to ventricular fibrillation (Lown and Verrier 1976). Consistent with this knowledge, QT interval duration and ventricular refractory periods are strongly influenced by parasympathetic and sympathetic autonomic nervous system inputs (Kolman et al. 1976). The established relationship between autonomic tone and ventricular repolarization has been helpful in clarifying the mechanisms behind some conditions, such as hypoglycemia (Robinson et al. 2003). It could also shed light on the potential mechanism for oxytocin-induced QTc prolongation in vivo.

Effect of oxytocin on heart rate, CF, and LV contractility

Oxytocin has been reported to alter a number of cardiovascular endpoints, including BP, HR, and contractility in both human and animal models (reviewed by Gutkowska and Jankowski 2012). In addition, oxytocin inhibits inflammation and potentiates glucose uptake. Mechanisms of oxytocin actions in cardiovascular system could be possibly secondary to ANP release (Gutkowska et al. 1997) and oxytocin-induced nitric oxide (NO) pathway (Leitman et al. 1988). For example, in the human clinical

situation, tachycardia has been observed following oxytocin (Liou et al. 1998; Charbit et al. 2004), but bradycardia has been observed in conscious rabbits (Uzun et al. 2007) and anesthetized dog (Mukaddam-Daher et al. 2001). Mechanistic studies in the anesthetized dog model (Mukaddam-Daher et al. 2001) revealed that bradycardia could be induced by a localized perfusion of oxytocin through the sinus node artery, which was reversed with an oxytocin receptor antagonist. This finding indicates that oxytocin can modulate heart rate directly, presumably through the activation of oxytocin receptors at the level of the sinus node. Contractility measurements demonstrated that oxytocin was unable to alter contractile force in human atrial trabeculae in vitro (Rosaeg et al. 1998); however, in an isolated rat heart study oxytocin was shown to increase LVSP and contractility in a dose-dependent manner (Coulson et al. 1997). The effects of oxytocin on BP in in vivo studies have also been controversial. For example, in anesthetized dogs, hypotension (Nakano and Fisher 1963), hypertension (Desiderio and Hanson 1981), and no effect (Brooks et al. 1984) have been reported following oxytocin administration. In addition to neuronal involvement, the in vivo effects of oxytocin are also complicated by the presence of chlorobutanol in the oxytocin preparations (Rosaeg et al. 1998), which could depress cardiac contractility by itself. In summary, the reported effects of oxytocin on HR, BP, and contractility are conflicting.

In our study using the spontaneously beating IRH model, oxytocin was able to decrease heart rate significantly at high test concentrations. This finding is consistent with previous in vitro studies using isolated rat heart (Coulson et al. 1997) and dog atria (Mukaddam-Daher et al. 2001). Since oxytocin levels were not determined in the whole animal studies, it is difficult to compare the peptide concentrations achieved with the concentrations used in the IRH model. Based on the IRH findings, however, it is concluded that oxytocin has the ability to reduce heart rate, as has been observed in prior rabbit and dog studies, and that this effect is suspected to be target mediated, for example, oxytocin receptor activation. However, a limitation of the current study is that an oxytocin receptor antagonist was not employed, which would be needed to confirm that the bradycardia is target mediated.

In IRH, low concentrations of oxytocin (3 nmol/L) did not alter cardiac contractility which suggests that this peptide has minimal direct effects on cardiac contractility at therapeutic concentrations, but higher concentrations (>10 nmol/L) of the peptide decreased LV contractility. All indices of cardiac contractility (e.g., LVDevP and dP/dt max) were inhibited in a concentration-dependent manner from 10 to 1000 nmol/L. At the same concentrations, CF was also reduced. A decrease in CF under constant perfusion pressure suggests that oxytocin increases coronary resistance, which is consistent with previous reports that oxytocin produced constriction of human basilar artery (Abrams et al. 1985). The decrease in LV contractility could be due to the reduction in CF, because ischemic conditions would lead to LV dysfunction, or be related to the bradycardia observed. The IRH results indicated that oxytocin has minimal direct effects on HR, CF, and LV contractility at therapeutic concentrations. At supratherapeutic concentrations, oxytocin could constrict coronary vasculature and produce negative chronotropic and inotropic effects directly on the heart, which could be due to unspecific binding to other receptors. The structure of oxytocin is very similar to that of vasopressin (Gimpl and Fahrenholz 2001), oxytocin could bind and activate vasopressin receptor at a higher dose. However, these effects occur at high multiples of its therapeutic concentration (≥100-fold) that makes these effects irrelevant to clinical findings.

Limitations

There are potential limitations in this study, (1) HVM used in this study were isolated from diseased hearts that may have different pharmacological responses compared to normal hearts. It is well known that electrical remodeling occurs in heart failure and heart failure alone increases arrhythmic risk. While changes in ion channel amplitude do occur as a result of remodeling in the failing heart, the ion channels are still present as evidenced by the clinical effects of class III antiarrhythmics in patients with heart failure. The use of myocytes from HF patients has not only a limitation but also an advantage. The limitation is that drug response in normal tissue would remain unknown; it would be ideal to evaluate drug response in normal and HF myocytes, but access to HF hearts is easier relative to the acquisition of heart tissue from healthy human donors. The advantage is that electrical remodeling of HF heart may make the human HF myocytes more sensitive for the detection of proarrhythmia, as seen with other sensitized models. (2) Different in vitro models were used in this study including IRH, HVM, and HEK cells, as opposed to the ideal situation of conducting all studies in human cardiomyocytes. Heterologous expression systems were used for Nav1.5 and hERG in HEK-293 cells because of direct access to high-throughput assays for these two channels (but not CaV1.2); (ii) hERG currents are more robust in HEK cells and no pharmacological methods are needed to separate hERG currents from other cardiac K currents; (iii) HEK-293 cells are much smaller than HVM and spatial clamp is less a problem when Na currents are recorded. Given

that the pharmacological sensitivity of the transfected cells has been established, the hERG and NaV1.5 findings can be interpreted with high confidence, and would expected to be similar to findings in native HVM. Another potential limitation is that rabbit and human cardiac myocytes may have different oxytocin receptors and signaling pathways. Human oxytocin was used in the IRH model, so it is uncertain whether potency differences (e.g., effects observed at high exposures) are influenced by species differences in oxytocin receptor activation and function. (3) The metabolic stability of oxytocin in the various assay systems remains unknown. Oxytocin is cleaved by aminopeptidase and postproline endopeptidase in both cytosol and microsomal fractions (Mitchell and Wong 1995), but it is presumed that clearance of oxytocin was negligible in the various in vitro test systems used in the study. (4) The potential downregulation of oxytocin receptor in the assay systems is also uncertain. Like most other GPCRs, oxytocin receptors could be desensitized following persistent agonism due to receptor internalization (Evans et al. 1997). When HEK 293 cells expressing the human oxytocin receptor were treated for 18 h with high concentrations of oxytocin (1 μmol/L), 50% of the initial binding capacity remained at the cell surface (Jasper et al. 1995). In the current study, maximal oxytocin exposure lasted for 100 min, so the potential effect of oxytocin receptor internalization would be expected to be minimal. (5) Finally, an oxytocin receptor antagonist was not used to assess target effects of the peptide, therefore it is uncertain whether the HR and contractility effects observed in the IRH are target mediated specifically.

Conclusion

The collective data suggest that high pharmacological concentrations of oxytocin, up to a 10,000-fold over the effective therapeutic concentration in humans, has very low risk for causing QTc and APD prolongation directly. Therefore, the QTc interval prolongation observed in vivo is attributed to an indirect mechanism.

Author Contributions

Y. Qu: designing study, analyzing data, and writing manuscript. M. Fang, B. Gao, S. Amagasu, and W. J. Crumb: collecting and analyzing experimental data. H. M. Vargas: designing study, reviewing, and editing manuscript.

Disclosures

Authors are employed by companies that do not sell one or more of the drugs or devices mentioned in the article. The authors state no conflict of interest.

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