hERG K^+ channel activity controls human uterine contraction in labour and this fails in obesity

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Short title: hERG channel in failure to progress in human labour

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Abstract

Human ether-a-go-go-related gene (hERG) potassium channels determine cardiac action potential and contraction duration. Human uterine contractions are underpinned by an action potential that also possesses an initial spike followed by prolonged depolarization. Here we demonstrate for the first time the presence of hERG channel proteins (α-conducting and β-inhibitory subunits) and hERG currents in isolated patch-clamped human myometrial cells. hERG channel activity suppressed contraction amplitude and duration before labour, facilitating quiescence. During established labour β-inhibitory protein was markedly enhanced, resulting in reduced hERG activity that was associated with an increase in action potential and contraction duration. Thus, changes in hERG channels contribute to action potential mechanisms that produce the powerful contractions typical of labour. We also demonstrate that this system fails in women with elevated BMI, they have enhanced hERG activity, due to low β-inhibitory protein, which likely contributes to the poor labour outcomes observed in many obese women, necessitating cesarean delivery.

Key words (not in the title): Myometrium, cesarean delivery, KCNE2, failed labour

Introduction

Our poor understanding of the mechanisms regulating the onset and progress of human labour limits the ability to clinically control events when these mechanisms malfunction. Successful vaginal delivery demands strong contractions forcing the fetal head to dilate the cervix, separated by periods of relaxation permitting replenishment of placental blood flow[1,2]. A plateau phase is a particularly prominent feature of the action potential (AP) in human uterine smooth muscle (myometrium)[3,4] but the ionic conductances responsible for determining the amplitude, duration and rapid repolarization of the plateau to achieve relaxation between contractions, are unknown. The AP in cardiac muscle also has a prominent plateau component and the heart contracts forcefully during systole and then relaxes to permit refilling. The hERG1 potassium channel, I_{kr}, plays an important role in repolarization of the prominent plateau of the cardiac AP and hence in determining AP, contraction and diastolic durations [5]. We hypothesized that hERG might play a role in regulating human uterine contractions. Potassium passes through the hERG α-pore-forming subunit and this is negatively regulated by a β ancillary subunit[6,7]. The level of β subunit protein increases in late pregnancy in mouse uterus[8] but the situation in labour has not been addressed. hERG has been identified in a range of smooth muscle tissues and pharmacological manipulation of its activity impacts contractile function[8-15]. We obtained human myometrium following cesarean delivery at term not in labour (NIL, n=43) and during established (in) labour (IL, n=27). Obese women are more likely to experience failure to go into spontaneous labour and failure to progress through to vaginal delivery (18%) compared with normal weight controls (5%). This necessitates cesarean section, with the effects especially concentrated in the first stage of labour, when powerful contractions are required to dilate the cervix and move the fetus through the narrow pelvis[16-18]. Thus, body mass index (BMI) was determined at first antenatal visit.

Results

hERG, present in human uterus in late pregnancy, has a major influence on contractility. To test whether hERG regulated AP duration we first recorded membrane potential and contraction simultaneously in strips of myometrium from lean women (BMI < 30) at term prior to labour onset. We used dofetilide (1 μM) and E-4031 (1 μM) as they are selective for hERG blockade[19] and have been commonly used in smooth[8-15] and cardiac[5] muscle. Both blockers caused a striking prolongation of the AP plateau and contraction (Fig. 1a), from 0.9±0.2 min to 2.8±0.2 min (a 2.9 fold increase, n=10, $p<0.001$) (Fig. 1d). This occurred without a depolarizing effect on resting membrane potential (–57±1mV, Fig. 1a). The hERG activator ICA-195574 (5 μM)[20] reduced contraction duration to 54±4% (n=7) (Fig. 2).

Myometrium from the upper region (fundus) of the uterus was obtained from four women undergoing hysterectomy following cesarean delivery and dofetilide prolonged the AP and contraction duration in a manner indistinguishable from its effects in the lower segment (Fig 1b).

The concentration dependence of dofetilide was tested using 20 min applications per dose in tissues from 5 women. There was a concentration dependent increase in plateau duration (pD_2=7.70±0.11), a more depolarized level of the AP plateau (pD_2=7.43±0.23), and an after-hyperpolarization more negative than that observed basally (pD_2=6.51±0.65) (Fig 1a, c). Recovery from the after-hyperpolarization resulted in a prolongation of the time until the next AP from 6.8±1.4 min basally to 18.7±1.5 min in dofetilide ($p<0.0001$, n=5).

We acutely isolated myometrial cells from the same tissue samples and used whole cell patch clamp techniques to interrogate the cells for hERG channel activity. The hERG current had a maximum amplitude of 3.6±0.4 pA/pF (n=10) (Fig. 3a, c) and was blocked by dofetilide and E-4031 (Fig. 3b, c). Current was restored to about 70% upon washout of dofetilide for 20 min. The hERG current decayed with an exponential time-course whose time-constant displayed voltage-dependence in which the time-constant changed e-fold per 73mV (Fig. 3d). These data are consistent with a

mechanism by which, before labour, the depolarization during the AP activates hERG which shortens the duration of the plateau and its associated contraction, facilitating quiescence.

Reduced hERG activity is involved in the transition into normal labour. We then asked whether changes in hERG function might contribute to the stronger, more co-ordinated contractions at the time of labour in lean women. In IL tissues dofetilide increased AP duration from 2.8±0.1 to 3.6±0.1 min ($p=0.005$, $n=7$) (Fig. 1d), an increase of only 1.3 fold, and the maximum hERG current in single cells was reduced to 1.3±0.4 pA/pF (Fig. 3c).

hERG (KCNH2) α pore-forming subunit protein was detected in human myometrium using Western blotting (Fig. 4a). The plasma membrane-inserted glycosylated 155 KDa form (Fig. 4ci) and the endoplasmic reticulum-stored poorly-glycosylated 135 KDa form (Fig. 4cii) occurred and these bands were not observed in the presence of antibody blocking peptide (Fig. 4a). hERG α-subunit protein levels were unchanged IL ($n=10$) compared with NIL ($n=10$, Fig. 4Ci, ii). Similarly, hERG α subunit mRNA did not differ IL versus NIL (Fig. 4d).

Expression of β auxiliary inhibitory subunit (KCNE2) protein was significantly enhanced IL versus NIL ($p=0.0001$, Fig. 4b, ciii). The β auxiliary subunit reduces current flow through the α subunit of the hERG channel[6,7] and its increase in the IL samples explains the suppression of hERG current and consequent prolongation of the AP plateau and contraction in labour. These data demonstrate that hERG contributes to suppression of uterine contractile strength before labour and this effect is reduced in labour in lean women, facilitating the strong contractions required for vaginal delivery.

hERG effectiveness is increased as BMI increases. Women with elevated BMI are more likely to have longer pregnancies, necessitating induction of labour[16-18]. Here we asked whether inappropriate hERG function in late pregnancy could contribute to this effect of obesity. We used the dofetilide-induced increase in AP plateau duration as a measure of hERG activity and plotted

this against BMI. In tissues from women at term but NIL there was a strong positive correlation between dofetilide-induced increase in AP plateau duration and BMI. BMI explained 89% of the variance in plateau duration evoked by dofetilide (r^2=0.89, p<0.0001, Fig. 5a). Importantly, and consistent with this, hERG current density in isolated cells increased as BMI increased (r^2=0.59, p=0.001, Fig. 5b).

This functional effect of elevated BMI was supported by a marked increase in hERG α subunit protein (r^2=0.62, p<0.0001, Fig. 6a) and a decrease in inhibitory KCNE2 protein expression (r^2=0.33, p=0.004, Fig. 6b) with increasing BMI. The reduction in β subunit and increase in hERG expression with increasing BMI is consistent with greater hERG activity which would shorten AP plateau and contraction duration, thus diminishing the prospects for strong uterine contraction development in women with elevated BMI.

hERG activity persists at the time of labour in myometrium from obese women. In labour, women with a higher BMI more often fail to progress through to vaginal delivery, necessitating cesarean section[16,17]. The dramatic effects of BMI on hERG activity and on AP and contraction duration before labour prompted the critical question; does this suppressive effect of hERG persist in labour and account for the failure to progress in labour in obesity? In our cohort of lean women, BMI <30, the reason for cesarean delivery IL was fetal distress (indicated by cardiotocography, CTG) in 11/16 women, with failure to progress (FTP) in only 5 of the 16 (Fig. 6d). In contrast, for women with BMI >30, FTP occurred in 10/11 cases (Fig. 6d).

Dofetilide had only a modest effect on AP and contraction duration in myometrial strips from lean women IL (Fig. 6e), but was more effective in strips from obese women (Fig. 6f). The increase in AP plateau duration by dofetilide in IL tissues increased progressively as BMI increased (r^2=0.82, p<0.0001, Fig. 6c). While the increase in α subunit of hERG with BMI was similar in IL (r^2=0.76, p<=0.0001) and NIL tissues (r^2=0.62, p<0.0001, Fig. 6a), levels of β subunit protein declined as BMI increased to a greater extent in IL samples (r^2=0.57, p=0.001, Fig. 6b). Thus, for

larger BMI there is little difference in levels of β-subunit protein between IL and NIL. Since the β subunit suppresses hERG current, its increase in lean labour removes the hERG termination of the action potential and contraction permitting longer and likely more effective contractions, ensuring vaginal delivery. The strong decrease in β subunit protein levels in women with elevated BMI effectively means that the myometrium does not progress into labour and this provides a mechanistic explanation for the failure to progress in labour in many women with increasing BMI.

Discussion

Here we provide compelling evidence that hERG plays a significant role in the relative quiescence of the uterus before labour, essential for development of the fetus to maturity. This is achieved by the ability of hERG to shorten the plateau phase of the AP, providing insufficient time for the development of a large contraction in human myometrium. The resulting contraction is short and weak. The transition of the myometrium into a labouring phenotype is accompanied by changes in hERG activity, whereby hERG-associated suppression of the uterine AP is markedly reduced. In fact, when we blocked hERG in not in labour tissues, the AP duration was transformed into a basal labour-like duration (see Fig. 1d). This labour-associated reduction in hERG effectiveness occurs despite maintained levels of hERG α pore-forming subunit protein and mRNA. Rather, there is a marked increase in β auxiliary subunit expression, which suppresses hERG current[6].

Blockade of hERG with dofetilide or E4031 results in depolarization in rat stomach[12], bovine epididymal[11], and opossum esophageal[9] smooth muscles. This likely accounts for the increase in contraction frequency in most smooth muscle tissues studied[8,10,14]. In contrast, human myometrium was hyperpolarized between APs in the presence of dofetilide, which was accompanied by a decrease in contraction frequency. This hyperpolarization is likely a consequence of the prolonged nature of the human uterine AP plateau in the presence of hERG blockers, rather than as a direct result of hERG blockade. This interpretation arises from our previous finding that application of prostaglandin $F_{2\alpha}$ to human myometrial strips results in prolongation of the AP plateau and an after-hyperpolarization between APs, reminiscent of dofetilide application here. We established that the prostaglandin effect is due to an increase in Na/K ATPase activity as a result of AP plateau lengthening[21]. Thus, understandable differences in ion channel type and density occur in human uterine versus other smooth muscles[2].

Many smooth muscle tissues display plateau-like electrical activity which dictates the amplitude and duration of contraction[22-25], and this includes the circular muscle layer of the mouse myometrium[24]. While the level of the plateau in many cases is close to that observed in human

myometrium, −25 to −30mV, a striking feature of the AP plateau in human myometrium is the rapidity of repolarization. Block of hERG in guinea-pig gall bladder increases the duration of a proportion of pacemaker depolarisations permitting an increase in the number of superimposed spike APs[14]. In human jejunum E4013 increases the amplitude of all pacemaker depolarisations, again increasing the number of spike APs[10]. Taken together, the use of hERG blockers in the present study provides insights into the unique nature of the plateau-AP in human uterus, demonstrates an important role for hERG potassium channels, and provides an impetus for further study.

The voltage dependence of the rate of deactivation of hERG current in smooth muscle cells is much weaker than that of hERG in cardiomyocytes[13,15,26]. Furthermore, in mouse myometrium the voltage dependence appears weaker in late pregnancy compared with non-pregnant tissue[8]. Our results show a weak voltage dependence for myometrium of women at term, consistent with the earlier studies in late pregnant mouse myometrium[8].

Activation of protein kinase A (PKA) increases hERG protein phosphorylation, which facilitates hERG incorporation into the plasma membrane[27]. PKA may also influence activity of the inhibitory β subunit[28]. Within the myometrium, cAMP activation of PKA pathways is involved in maintaining relaxation during pregnancy, and components of PKA signalling are down regulated in human labour[29] permitting strong contractions. It remains to be determined if regulation of hERG activity in the transition into human labour involves the cAMP/PKA signalling system.

Obesity is an increasing scourge within the population in general and consequently is increasing in the pregnant population. Obesity is largely responsible for the recent increasing need for cesarean delivery, which increases maternal and neonatal morbidity and can predispose to problems for future pregnancies[30,31]. The hERG β subunit is upregulated by estrogen[32], and the estrogen profile can be dysfunctional in high BMI pregnancies[33]. Obesity is associated with increased circulating levels of cholesterol and leptin[34]. Cholesterol levels are higher in caveoli[35], the location of hERG α and β subunit insertions[36]. Direct interaction may occur between membrane

196 lipids and amino acids in membrane-traversing domains of ion channel proteins[37], and hERG
197 channel kinetics are sensitive to the cholesterol content of the plasma membrane[36]. Disruption of
198 membrane cholesterol suppresses human and rat uterine contractions[38]. Leptin is also increased in
199 obesity and in pregnancy[39], and leptin[40] and the adipokine apelin[41] also suppress human uterine
200 contractions. The role of these factors in regulating hERG α and β subunits in human myometrium
201 warrants investigation.

202 Here we demonstrate for the first time the presence of hERG protein and β ancillary subunit
203 protein in human myometrium in late pregnancy and labour. Our results demonstrate the dynamic
204 contribution of hERG channels, and the β subunit in particular, to uterine smooth muscle function
205 in the progression into labour. Importantly, we show that hERG is present and influences
206 contractility, not only in the lower segment but also within the main fundus region of the uterus.
207 Effectively, blockade of hERG by dofetilide transforms the pre labour myometrium into the
208 labouring phenotype (Fig. 1d). Significantly, our results draw together a strong link between obesity
209 and hERG function in human myometrium. Thus, our data present compelling evidence implicating
210 hERG channels in the established clinical problem of the rising incidence of cesarean delivery in
211 obesity and provides a focus for further investigation. We provide a solid mechanism towards
212 understanding the poor labour outcomes in obese women.

Methods

Studies were approved by the Royal Women's Hospital Research Ethics Committee, and the Hunter and New England Area Research Committee, adhering to guidelines of the Declaration of Helsinki. Participants gave informed written consent for collection of myometrial samples prior to surgery and tissue collection. BMI was obtained at first hospital visit. Women undergoing term (37–40 weeks gestation) elective cesarean delivery with no signs of labor formed the NIL cohort, while women undergoing term emergency cesarean section following the spontaneous establishment of labor formed the IL cohort. Infection, hypertension, diabetes were exclusion criteria. Clinical indications for elective NIL cesarean delivery were previous cesarean section, $3^{rd}/4^{th}$ degree tear, breech, while clinical indications for emergency IL cesarean section were fetal distress or failure to progress in labor. Following delivery of the placenta, all women were administered 5 units of oxytocin (syntocinon) directly into an intravenous line as part of standard care for the prevention of post-partum hemorrhage. Myometrial biopsies were excised 3–5 min after administration of oxytocin, thus all samples were briefly exposed to oxytocin. Myometrial samples (5×5×10 mm) from the lower uterine segment of term singleton pregnancies were collected. A portion of the sample was immediately frozen in liquid nitrogen for subsequent protein analysis, while the remaining tissue was immediately taken to the lab and electrophysiology and contraction studies were commenced within 1 h.

Strip electrophysiology and contraction. Membrane potential was recorded from smooth muscle cells in strips of tissue (3×1×0.5mm), using glass intracellular microelectrodes filled with 1M KCl (resistances ~100MΩ) to impale a single smooth muscle cell within the strip, and tension was recorded simultaneous (force transducer, AE801, SensoNor, Horton, Norway) as previously described[21]. The microelectrode was connected to an Axoclamp 2B amplifier (Axon Instruments, CA), low-pass filtered at 3KHz and digitized at 1KHz (Digidata 1440A, Molecular Devices, CA). Strips were set at 0.2 mN basal tension and continuously superfused at 3 ml/min and 36°C with physiological salt solution (PSS) containing (mM); NaCl 120, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1,

$MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11, gassed with 95% O_2, 5% CO_2, pH 7.4. Concentration response curves to dofetilide were fitted to a sigmoid curve, using the least-squares method and pD_2 (-log EC_{50}) was calculated (GraphPad Prism).

Patch clamp. Single smooth muscle cells were isolated (2 mg/mL type I collagenase, 2mg/ml trypsin inhibitor, 1mg/ml fatty acid-free albumin at 37°C for 50min) and studied within 6h. The cells were voltage clamped using whole cell or nystatin-perforated patches (Axopatch 200 and Digidata 1322A, pClamp 9) following. Cells were superfused with PSS (mM); NaCl 137, $NaHCO_3$ 4, NaH_2PO_4 0.3, KCl 5.4, KH_2PO_4 0.44, $MgCl_2$ 0.5, $MgSO_4$ 4, glucose 5.6, HEPES 10, $CaCl_2$ 1.3, at pH 7.4 and 22°C. Patch electrode solution contained (mM): KCl 130, $MgCl_2$ 1.2, ATP 3, EGTA 5, HEPES 10. To enhance K^+ currents, the PSS contained (mM): KCl 140, $MgCl_2$ 1, HEPES 10, glucose 10, $CaCl_2$ 0.1 immediately before testing. Cells were held at 0mV and stepped in 10mV increments to −120mV[8,9].

Western blotting. Protein analysis was performed as previously outlined[42]. Rabbit anti-K_v11.1 (hERG) (APC-109, 1:2000 dilution, Alomone Laboratories, Israel) and rabbit anti-KCNE2 (APC-054, 1:200 dilution, Alomone Labs) were applied in the presence or absence of blocking peptide (2μg/1μg antibody). Protein was expressed relative to α smooth muscle actin and analysed using t-tests with unequal variance. mRNA abundance was expressed relative to 18S rRNA and analyzed using the ΔΔCt method.

Reagents: Stock solutions of blockers and activators of hERG channels were prepared in DMSO at x1000 concentration or dH_2O as appropriate. The hERG blockers dofetilide and E-4031, activators PD-118057, NS1643 and ICA-195574, DMSO and all solution reagents were purchased from Sigma-Aldrich (St Louis, USA). DMSO 1:1,000 dilution was tested for the appropriate time exposure in every tissue. There was no detectable effect of DMSO on activity in whole tissues or isolated single smooth muscle cells.

Statistical analysis. Data were analysed using GraphPad Prism and GraphPad InStat (GraphPad Software Inc. SanDiego, CA, USA). For all data sets, equality of standard deviations and Gaussian

distribution, using the Kolmogorov/Smirnov method, were tested. Data are expressed as mean and standard error of the mean (SEM). Throughout, *n* represents the number of women studied and $p < 0.05$ was accepted as statistically significant. Experimenters were blinded to the clinical status of the women (NIL, IL, BMI values) and the status was provided by Dr P Sheehan at a stage in the analysis when it was required. Effects of DMSO diluent (0.01%) were tested in every tissue and were negative. For electrophysiology and contractility studies, correlation between dofetilide-induced plateau lengthening and BMI was determined using least products regression. Repeated measures ANOVA was used to compare between NIL versus IL. Two-way ANOVA was used to test hERG currents and dofetilide effects versus BMI, with Bonferonni *post-hoc* testing. Unpaired or paired Student t-tests were used for testing differences.

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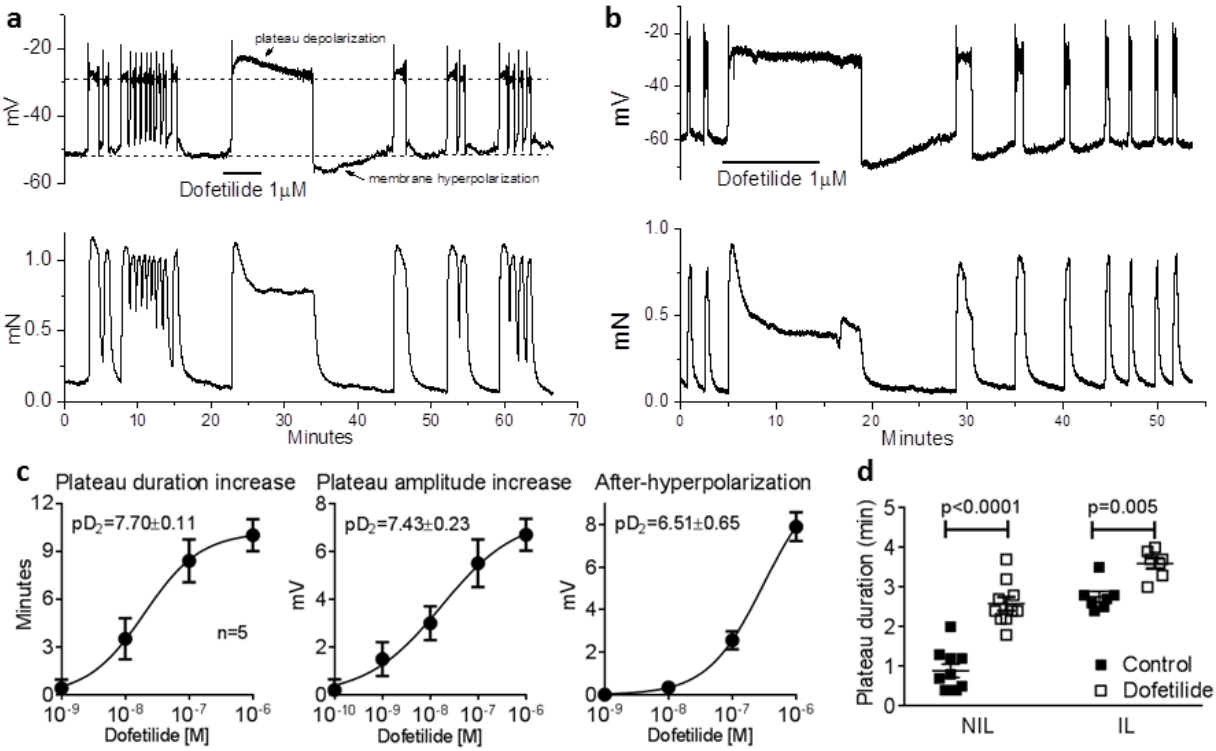
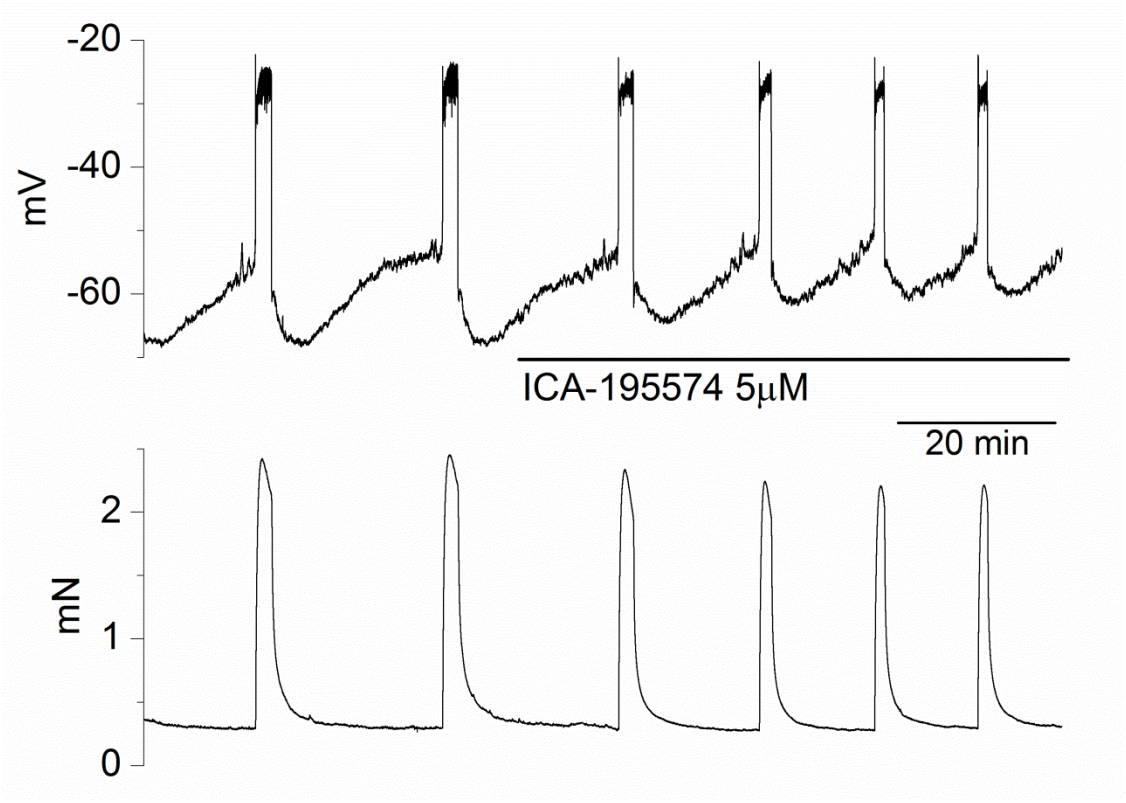
Disclosure of Interests: Roger Smith has patents held at the University of Newcastle related to the use of hERG modulators in pregnancy. The other authors have nothing to disclose.



401

402 **Figure 1: Significant influence of hERG on human uterine contractility.** a. Blockade of
403 hERG with dofetilide (1 μM) prolonged the plateau phase of the action potential (AP) (upper
404 trace) and contraction (lower trace) recorded simultaneously. b. Similar effects in a strip from
405 the fundus of the uterus. c. Dofetilide induced a concentration-dependent increase in AP
406 plateau duration, an increase in the level of plateau depolarization and an after-
407 hyperpolarization between APs (n=5). d. hERG block with dofetilide caused a greater
408 prolongation of the AP plateau before (NIL) versus during labour (IL), effectively turning a
409 NIL AP into an IL AP. Mean ± SEM.

410

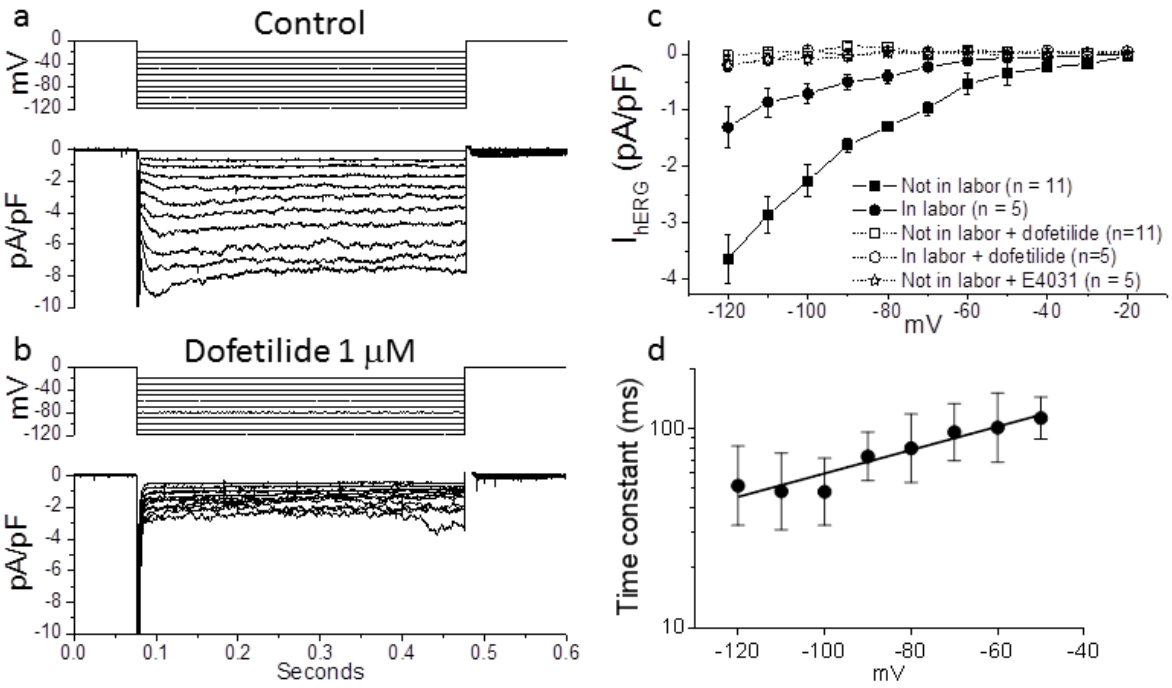


411

412 **Figure 2: Reduction in plateau duration by activation of hERG.** Action potential plateau

413 and contraction durations were reduced in the presence of the hERG activator ICA-195574.

414



415

416 **Figure 3: hERG currents in isolated human myometrial cells. a**. Following depolarization

417 to 0mV, in 140mM extracellular K^+ solution, progressive step repolarizations to −120mV

418 evoked currents **b**. that were blocked by dofetilide, indicating hERG. **c**. hERG current was

419 reduced to one third in labour and blocked by dofetilide 1 μM and E4031 1μM. **d**. The voltage

420 dependence of the time-constant of deactivation changed e-fold per 73mV (further analysis of

421 data in **a** not in labour). N = number of women tested, mean ± SEM.

422

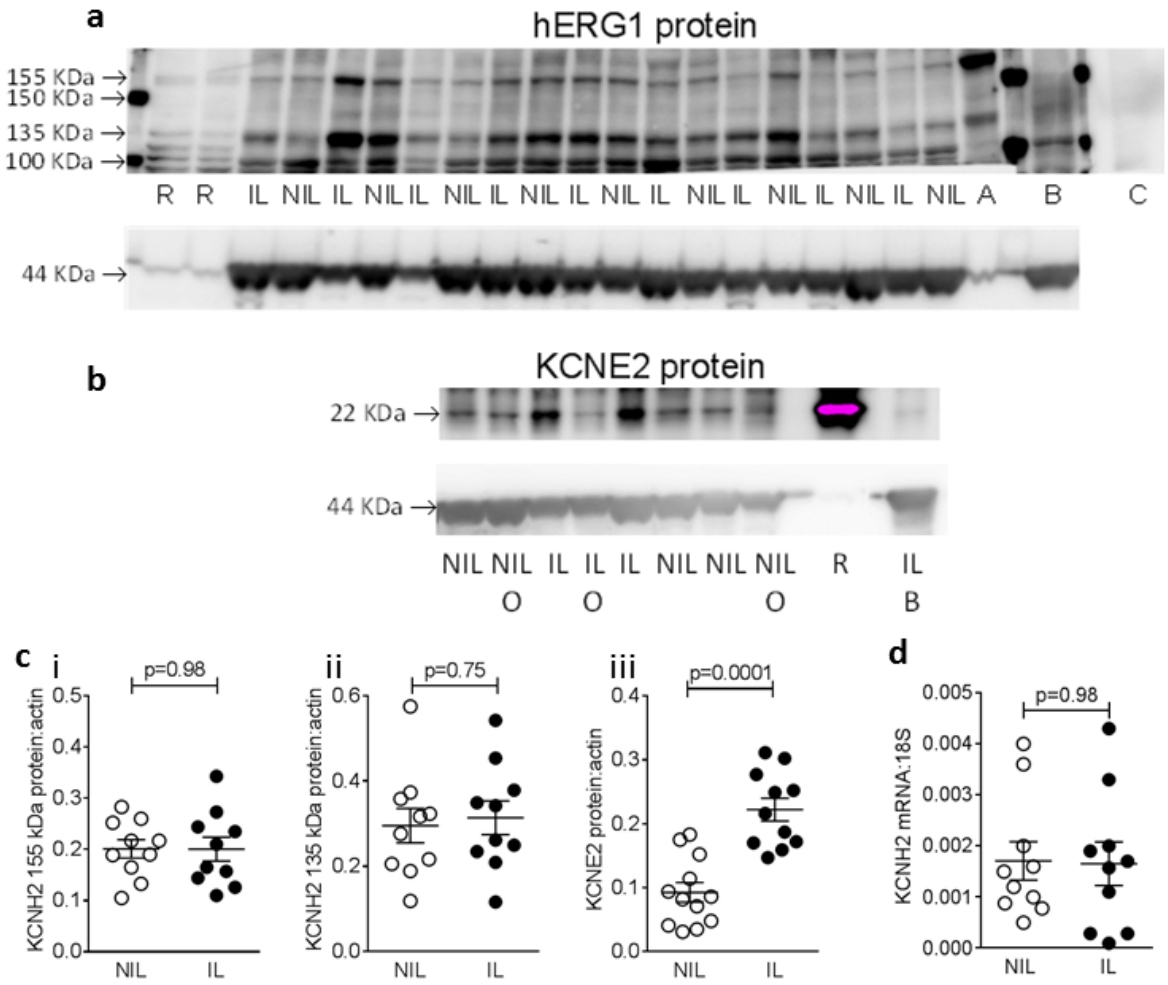


Figure 4: hERG α and β subunit protein levels in human myometrium. a. Plasma membrane-inserted glycosylated 155 KDa and poorly-glycosylated 135 KDa forms of hERG (upper trace) and α actin 44 KDa (lower trace, loading control) in myometrium from lean women. R, rat heart; IL, in labour and NIL, not in labour myometrium; A, human adipose tissue positive control; B blocking protein; C antibody only. **b**. KCNE2 β subunit 22 KDa (upper trace); O, obese, BMI >30. **c**. Levels of pore-forming hERG α-subunit (KCNH2) 155 KDa (i) and 135 KDa (ii) were not different before (NIL) versus during labour (IL). (iii) KCNE2 was significantly increased IL. **d**. hERG α-subunit mRNA was not different NIL versus IL. Mean ± SEM.

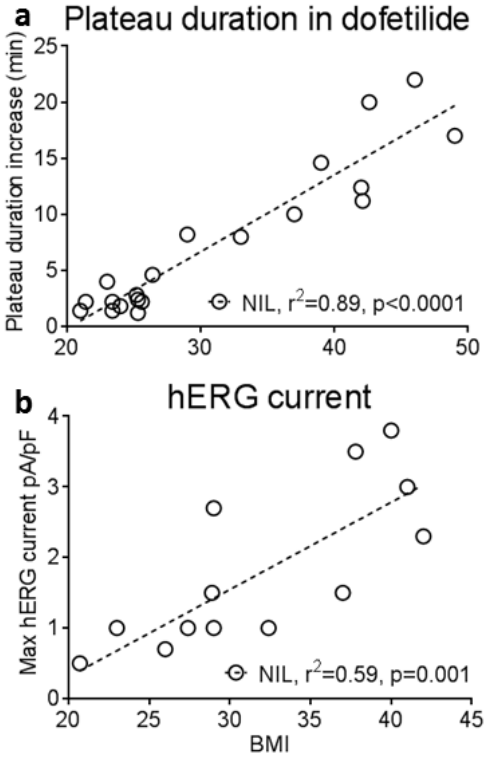
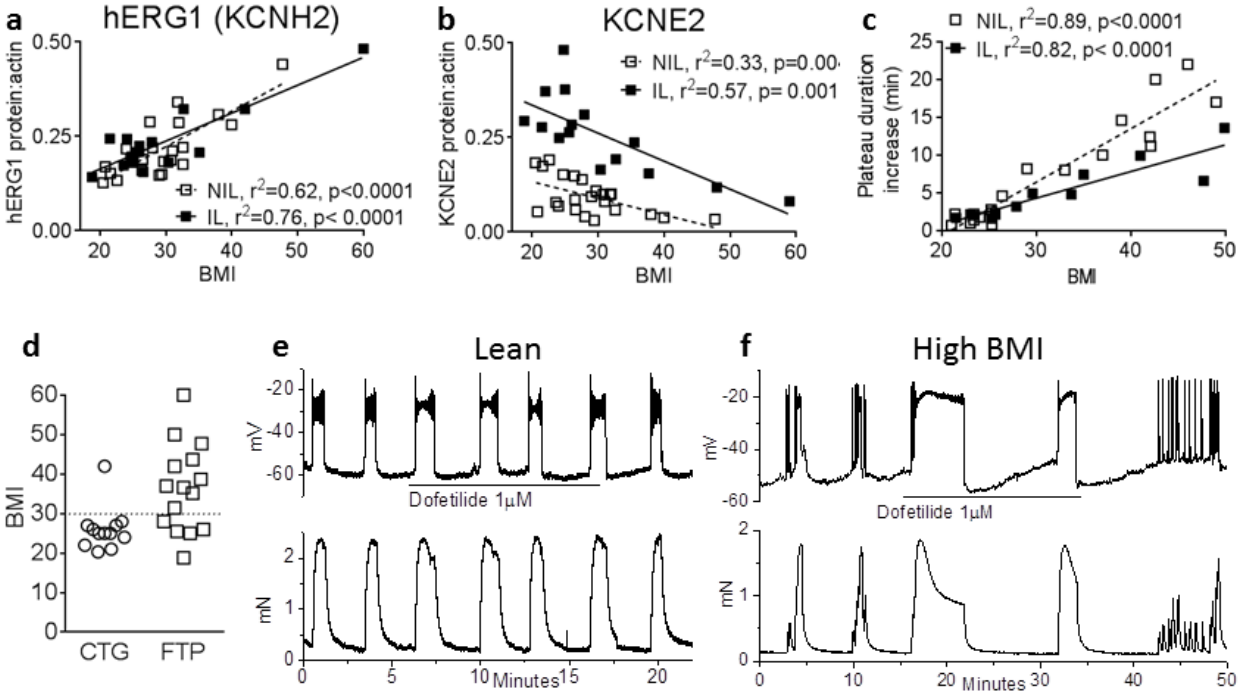


Figure 5: BMI positively associated with increased hERG function in human myometrium. a. The increase in plateau duration by dofetilide at term not in labour (NIL) was tightly correlated with increasing BMI. **b**. hERG current was similarly increased with increasing BMI.



439

440 **Figure 6: hERG activity in myometrium persists in labour in obese women. a**. hERG 155 KDa
441 protein increased with BMI in a similar manner before (NIL) and during labour (IL). **b**. While
442 KCNE2 protein was increased IL in lean tissue, this increase failed to occur as BMI increased. **c**.
443 The ability of dofetilide to increase AP plateau duration increased, indicating greater hERG and
444 suppression of the AP and contraction with increasing BMI. **d**. All but one woman with BMI >30
445 failed to progress (FTP) in labour, while fetal distress (measured by cardiotocography, CTG)
446 necessitated cesarean delivery in 11/16 lean. **e**. Dofetilide was less effective in tissue from lean
447 women IL compared with **f**. tissue from obese women.