BASIC SCIENCE: OBSTETRICS

Histone deacetylase inhibitors and a functional potent inhibitory effect on human uterine contractility

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OBJECTIVE: This study was undertaken to investigate the effects of 3 histone deacetylase inhibitors on human uterine contractility.

STUDY DESIGN: Biopsy specimens of human myometrium were obtained at elective cesarean section (n = 18). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous, and oxytocin-induced contractions, were subjected to cumulative additions of 3 histone deacetylase inhibitors: trichostatin A, suberic bishydroxamate (1 nmol/L-10 μmol/L) and valproic acid (100 nmol/L - 1 mmol/L). Control experiments were run simultaneously. Integrals of contractile activity were measured by using the PowerLab hardware unit and Chart v3.6 software. Data were analyzed by using 1-way analysis of variance, followed by post hoc analysis.

RESULTS: All 3 histone deacetylase inhibitor compounds exerted a potent and cumulative inhibitory effect on spontaneous (n = 18) and oxytocin-induced (n =18) contractility. The mean maximal inhibition values for the 3 compounds were as follows: trichostatin A, 46-54% ($P < .05$); valproic acid, 35-36% ($P < .05$); and suberic bishydroxamate, 53-65% ($P < .05$).

CONCLUSION: The histone deacetylase inhibitors trichostatin A, valproic acid, and suberic bishydroxamate exerted a potent inhibitory effect on human uterine contractions. This raises the possibility that this new class of compounds may have tocolytic potential, in addition to their current clinical indications. We speculate that this inhibitory effect may be linked, at least in part, to the ability of histone deacetylase inhibitors to induce the expression of genes involved in maintaining myometrial quiescence via epigenetic mechanisms but may also potentially involve nonepigenetic pathways.

Key words: histone deacetylase inhibitors, tocolytics, uterine smooth muscle

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Despite intensive research efforts, preterm labor and birth continue to be the single greatest cause of morbidity and mortality in obstetric practice in the developed world.[1] Furthermore, the incidence of preterm birth has steadily risen, with an increase of more than 18% since 1990, and today 1 in 8 infants in the United States is born preterm.[2] The cause of preterm labor is unclear, and although risk factors have been identified, these factors are not reliably predictive. Hence, the pursuit of novel effective tocolytics to prevent preterm birth is critically important in improving neonatal outcome.

The maintenance of myometrial quiescence is central to a pregnancy reaching term. Many factors are important in regulating myometrial quiescence; however, there is significant evidence indicating that levels of placentally derived human chorionic gonadotrophin (hCG) are important in maintaining early pregnancy. The levels of hCG have been shown to increase dramatically with pregnancy, reaching a peak at 8-10 weeks, and then declining thereafter.[3] Importantly, hCG has also been shown to exert a potent inhibitory effect on human uterine contractility in vitro, in the third trimester,[4] and this reduction in contractility may be mediated, at least in part, by activation of the BK_{Ca} channel.[5] The action of hCG is primarily mediated by coupling of chorionic gonadotrophin (CG)/lutenizing hormone (LH) receptors in the myometrium to the G protein, Gαs, and subsequent formation of cyclic adenosine monophosphate (cAMP), thus maintaining uterine quiesence.[6] The transition of the myometrium from a quiescent state to a contracting one is facilitated by the

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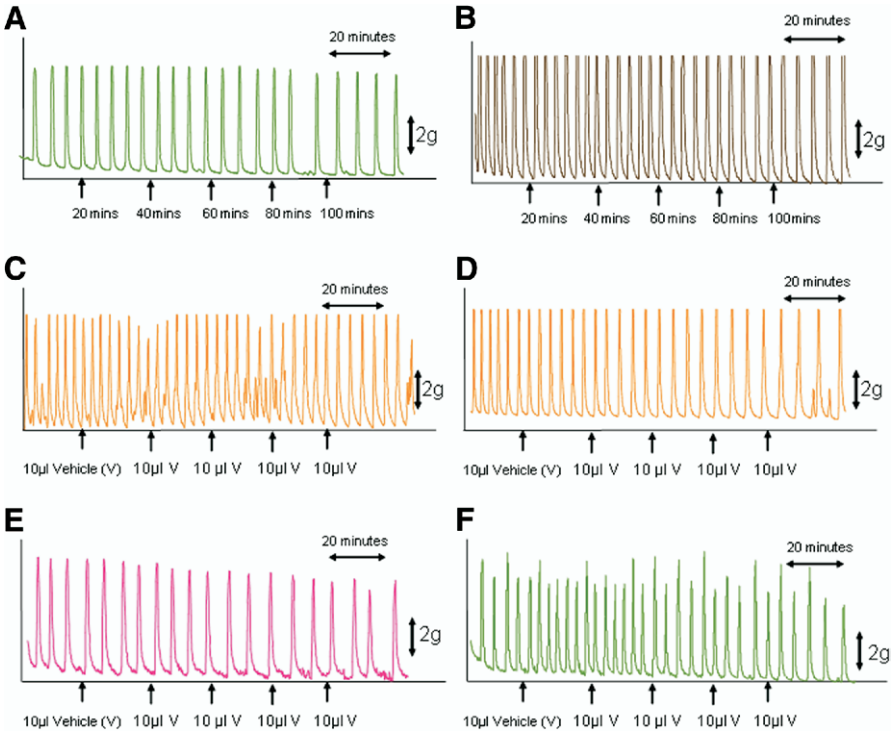
FIGURE 1
Representative recordings



Representative recordings of **A**, spontaneous myometrial contractions in a control strip; **B**, oxytocin-induced contractions in a controls strip; **C**, spontaneous contractions treated with vehicle for TSA only; **D**, oxytocin-induced myometrial contractions treated with vehicle for TSA; **E**, spontaneous contractions treated with vehicle for SBHA; and **F**, oxytocin-induced contractions treated with vehicle for SBHA.

Moynihan. Histone deacetylase inhibitors and a functional potent inhibitory effect on human uterine contractility. Am J Obstet Gynecol 2008.

downregulation of the myometrial hCG/LH receptors.[6] In this context Phillips et al[7] have recently described the epigenetic mechanism by which hCG/LH receptors are regulated in the human myometrium. Data from this study indicated that Sp1, Sp3, Sp4, and Sp-like proteins recruit HDACs 1 and 2 to the 2 GC boxes within the proximal promoter region of the hCG/LH receptor gene resulting in histone deacetylation and transcriptional repression. This silencing effect on expression of the gene was reversed by the HDAC inhibitor trichosatin A (TSA), which provoked transcriptional activation of the hCG/LH receptor gene via chromatin remodeling because of increased histone acetylation. Moreover, we now have data indicating that HDACs may also play an important part in regulating expression of both the PKA RIIα and Gαs genes in the human myometrium,[8] both of these genes are known to play significant roles in the maintenance of myometrial quiescence during pregnancy.[9,10] The pharmacologic properties of HDAC inhibitors have generated significant interest as anticancer agents and several of them, including TSA, valproic acid (VPA), sodium butyrate, and suberic bishydroxamate (SBHA) are currently undergoing evaluation in phase I/II clinical trials.[11]

Further evidence for HDACs/HDAC inhibitors having a role in regulating parturition is provided by Condon et al.[12] By using the mouse model, they observed that administration of TSA late in gestation delayed the initiation of parturition by 24-48 hours, suggesting a functional importance for the decline in histone acetylation in the onset of labor. Even though TSA (and VPA) have been observed to have teratogenetic effects during organogenesis in mammals, viable mice were born after TSA treatment with no apparent adverse effects on the mothers. Although this study indicated that TSA may affect myometrial activity in this animal model, no functional studies have yet been carried out on the effects of HDAC inhibitors on human uterine contractility. Consequently the aims of this current study were to investigate the effects of TSA, VPA, and SBHA on human uterine contractility in vitro, on spontaneous contractions, and those elicited by the agonist oxytocin.

MATERIALS AND METHODS

Tissue collection

Biopsy specimens of human myometrial tissue were obtained at elective cesarean section in the third trimester of pregnancy in the Department of Obstetrics and Gynaecology, University College Hospital, Galway, Ireland. The biopsies were excised from the upper lip of the lower uterine segment incision in the midline, ie, upper portion of lower uterine segment. Ethical committee approval for tissue collection was obtained from the Research Ethics Committee at University College Hospital Galway and recruitment was by written informed consent. Once collected, all tissue biopsy specimns were placed in Krebs-Henseleit physiologic salt solution (PSS) at pH 7.4 containing the following: 4.7 mmol/L potassium chloride, 118 mmol/L sodium chloride, 1.2 mmol/L magnesium sulphate, 1.2 mmol/L calcium chloride, 1.2 mmol/L potassium phosphate, 25 mmol/L sodium bicarbonate, and 11 mmol/L glucose (Sigma-Aldrich, Dublin, Ireland). Tissues were stored at 4°C and used within 12 hours of collection.

Tissue bath experiments

Longitudinal myometrial strips (measuring approximately 2 × 2 × 10 mm) were dissected free of uterine deciduas and serosa and mounted for isometric recording under 2 g of tension in organ baths as previously described.[13,14] The tissue baths contained 10 mL of Krebs-Henseleit PSS maintained at 37°C, pH 7.4, and were gassed continuously with