Reproductive System & Sexual Disorders

Deák et al., Reprod Sys Sexual Disorders 2013, 2:1
http://dx.doi.org/10.4172/2161-038X.1000117

Research Article Open Access

Uterus-Relaxing Effects of Nociceptin and Nocistatin: Studies on Preterm and Term-Pregnant Human Myometrium *In vitro*

Deák BH[1], Klukovits A[1], Kormányos Z[2], Tekes K[3], Ducza E[1] and Gáspár R[1]*

[1]Department of Pharmacodynamics and Biopharmacy, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary
[2]Department of Obstetrics and Gynecology, Albert Szent-Györgyi Clinical Center, Faculty of General Medicine, University of Szeged, Hungary
[3]Department of Pharmacodynamics, Semmelweis University, H-1089 Budapest, Nagyvárad tér 4, Hungary

Abstract

The endogenous neuropeptides nociceptin/orphanin FQ and nocistatin translated from the prepronociceptin gene exert a relaxant effect on the rat uterus. Previous studies have reported their role in pain transmission in the central nervous system both in rodents and in humans, but to date only limited information is available on their effects in the periphery, and there are no data on their presence in the human uterus.

The expression of prepronociceptin mRNA in the human uterus was confirmed by an RT-PCR technique. *In vitro* contractility studies of the action of nociceptin and nocistatin on human uterine tissues were performed in an isolated organ system. Human myometrial strips from cesarean sections at full-term pregnancy and at preterm labor were stimulated with oxytocin, and the relaxant effects of nociceptin and nocistatin were studied.

The level of prepronociceptin mRNA was significantly decreased in full-term pregnant uterus samples as compared with preterm pregnancy samples. Nociceptin and nocistatin significantly and dose-dependently inhibited the oxytocin-evoked contractions in the human uterus. In the presence of nocistatin, the uterus-relaxant effect of nociceptin was enhanced. In contrast, nociceptin did not alter the uterus-relaxant effect of nocistatin.

We conclude that locally generated nociceptin and nocistatin both exert a relaxant effect on the human uterus, and nocistatin can potentiate the relaxant effect of nociceptin, though for this to occur nocistatin administration must precede the administration of nociceptin.

Keywords: Uterine contractility; Nociceptin; Nocistatin; Human uterus; Calcitonin gene-related peptide

Abbreviations: CGRP: Calcitonin Gene-related Peptide; NOP: Nociceptin Receptor; N/OFQ: Nociceptin/orphanin FQ; NST: Nocistatin; PNOC: Prepronociceptin; OT: Oxytocin; SP: Substance P

Introduction

Preterm birth complicates 5-9% of pregnancies in Europe and in many other developed countries; while the rate in the USA is 12-13% [1]. The statistics indicate that preterm birth is the leading cause of neonatal morbidity and mortality; and appropriate tocolytic therapy is therefore one of the greatest challenges in obstetrical practice. A number of agents have been used clinically as tocolytics; but their efficacy is questioned. The approach to new therapeutic targets brings us to the investigation of endogenous mediators which play a role in the modulation of uterine contractility.

After identification of the endogenous ligand nociceptin/orphanin FQ (N/OFQ) for the orphan opioid receptor (NOP); another heptadecapeptide; nocistatin (NST); was isolated from the same precursor protein Prepronociceptin (PNOC) from bovine brains by Okuda-Ashitaka et al. [2] in 1998. NST was thought to be a functional antagonist of N/OFQ on the basis of their effects in the central nervous system [3-8]. Although N/OFQ has no affinity to opiate receptors; interaction was reported between the N/OFQ and dynorphin in neuropathic pain [9]. Other paper suggests that the classical opioid and the novel nociceptin system regulate nociception in parallel [10].

The epithelial cells of the human endometrium express the Corticotropin-Releasing Hormone (CRH) and opioid peptide precursors' genes (i.e. proopiomelanocortin; proenkephalin; and prodynorphin) and their end-products. Additionally; given the myorelaxant actions of opioids; these endometrial neuropeptides may participate in the control of myometrial contractility [11]. Opioid agonists produce a concentration-dependent inhibition or excitation of spontaneous rhythmic or tonic contractions induced by potassium chloride (KCl) (50 mM) in the isolated rat uterus [12]. Thus; the opioid system plays a role in the regulation of uterine contractility.

As reported earlier by our group [13,14]; N/OFQ and NST; the translation products of the PNOC gene; inhibit uterine contractions in the pregnant rat. Despite a large body of literature on the role of opioid peptides in pain modulation in humans; limited information is available on their actions in the human uterus. The mechanisms of action of N/OFQ and NST remain largely unexplored [15].

In this work; we set out to detect the expression of PNOC mRNA in the human uterus; by means of an RT-PCR technique. We planned to study the *in vitro* effects of NST alone and also in combination with N/OFQ; and also to investigate the effects of N/OFQ alone and in combination with NST on the isolated human uterus.

Materials and Methods

Human specimens

Biopsy specimens of human myometrial tissue were obtained at cesarean section (performed in the Department of Obstetrics and

***Corresponding author:** Róbert Gáspár, Department of Pharmacodynamics and Biopharmacy, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary, Tel/Fax: (36) 62-545567; E-mail: gaspar@pharm.u-szeged.hu

Received February 18, 2013; **Accepted** March 14, 2013; **Published** March 18, 2013

Citation: Deák BH, Klukovits A, Kormányos Z, Tekes K, Ducza E, et al. (2013) Uterus-Relaxing Effects of Nociceptin and Nocistatin: Studies on Preterm and Term-Pregnant Human Myometrium *In vitro*. Reprod Sys Sexual Disorders 2: 117. doi:10.4172/2161-038X.1000117

Gynecology; University of Szeged) in the third trimester of pregnancy in two groups: at full-term birth (37-41 weeks of gestation; n=10) and at preterm birth (33-36 weeks; n=9). At full-term; cesarean delivery was indicated by a previous cesarean delivery; breech presentation; a suspected cephalopelvic disproportion or myopia. The parity varied from 0 to 3; and the mean maternal age was 28.4 years (21-35 years). None of the women received a tocolytic agent; and there were no signs of labor.

Preterm delivery occurred in mothers with twin pregnancies; or labor was indicated by an ongoing infection; leukocytosis; toxemia; fetal distress or growth restriction. In the preterm group; the parity varied from 0 to 3; and the mean maternal age was 28.2 years (18-38 years). Three of the 9 patients received tocolytic therapy (magnesium sulfate) to arrest preterm uterine contractions; which proved to be ineffective. All the operations were performed under spinal anesthesia. The Ethical Committee of Albert Szent-Györgyi Clinical Center; University of Szeged; approved the clinical protocol for the use of human tissue; the pregnant women signed an informed consent form; uninfluenced (registration number: 114/2009).

RT-PCR (Real-time reverse transcription polymerase chain reaction) studies

The human uterus tissue samples were frozen in liquid nitrogen; and then stored at -80°C until analysis. The frozen samples were ground with a Micro-Dismembrator S homogenizer (Sartorius; Germany); and the total RNA was isolated with the TRIsure Kit according to the manufacturer's instructions. RNA purity was controlled via the optical density at 260/280 nm with a BioSpec Nano instrument (Shimadzu; Japan); all samples exhibited an absorbance ratio in the range 1.6-2.0. RNA quality and integrity were assessed by agarose gel electrophoresis. One microgram of each sample of total RNA was used for reverse transcription and amplification (TaqMan RNA-to-C_T 1-Step Kit and the Sensi FAST Probe Hi-Rox One-Step Kit).

The following primers were used: assay ID Hs00173823_m1 for PNOC and Hs 01060665_g1 for *ß-actin* as endogenous control. RT-PCR was performed by using the ABI StepOne Real-Time cycler. The fluorescence intensities of the probes were plotted against PCR cycle numbers. The amplification cycle displaying the first significant fluorescence signal increase was defined as the threshold cycle (C_T).

In vitro contractility studies

Uterus preparation: Each tissue sample (10×10×30 mm) was obtained from the upper edge of a lower-segment transverse incision; after delivery of the child; but before oxytocin was given to the mother. Tissues were stored in Krebs–Henseleit solution (containing in mM: 118 NaCl; 5 KCl; 2 $CaCl_2$; 0.5 $MgSO_4$; 25 $NaHCO_3$; 1 $KHPO_4$; 10 glucose; pH 7.4) at 4°C; until investigation; but within 12 hours of collection.

Longitudinal myometrial strips (~3×5×10 mm) were mounted vertically in an organ bath containing 10 ml Krebs–Henseleit solution. The organ bath was maintained at 37°C; and carbogen (95% O_2+5% CO_2) was bubbled through it. After mounting; the initial tension was set at 3.0 g and the rings were equilibrated for 90 min; with a solution change every 15 min.

***In vitro* studies in human uterus tissues from full-term births and from preterm births:** In the isolated uterine rings; rhythmic contractions were elicited with 10^{-8} M oxytocin. The effects of N/OFQ and NST on the uterine contractions were tested in the concentration range 10^{-12}–10^{-6} M; in a non-cumulative manner. After each concentration of N/OFQ and NST; the rings were washed 3 times; allowed to recover for 5 min; and then contracted again with oxytocin. The uterus-relaxant effect of NST was also investigated in the presence of N/OFQ. Additionally; the uterus-relaxant effect of N/OFQ was investigated in the presence of NST.

The tension of the myometrial rings was measured with a strain gauge transducer (SG-02; Experimetria Ltd; Budapest; Hungary); and recorded and analyzed with the SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd; Budapest; Hungary). The areas under the curves of 6-min periods were evaluated; the effects of NST and N/OFQ were expressed as percentages of the oxytocin-induced contractions. The maximum relaxations were calculated with the Prism 4.0 computer program (GraphPad Inc.; San Diego; CA; USA). Statistical analyses were carried out with the ANOVA Newman-Keul test.

Materials

N/OFQ and NST were purchased from PolyPeptide Laboratories France SAS; Strasbourg; France. Oxytocin was purchased from Richter Gedeon Ltd.; Budapest; Hungary. The TRIsure Kit and the Sensi FAST Probe Hi-Rox One-Step Kit were from Bioline Ltd.; Budapest; Hungary. TaqMan RNA-to-C_T 1-Step Kit and ß-actin primer were obtained from Life Technologies; Budapest; Hungary.

Results

Measurement of PNOC mRNA in the human uterus

The myometrial PNOC mRNA levels were significantly higher in preterm uterine samples (RQ=35.40 ± 3.31) as compared with samples from full-term pregnancy (RQ=0.81 ± 0.12); p<0.001 (Figure 1).

In vitro contractility studies in the full-term pregnant human myometrium

N/OFQ alone decreased the uterine contractility concentration-dependently. NST (10^{-8} M) increased the maximum uterus-relaxant

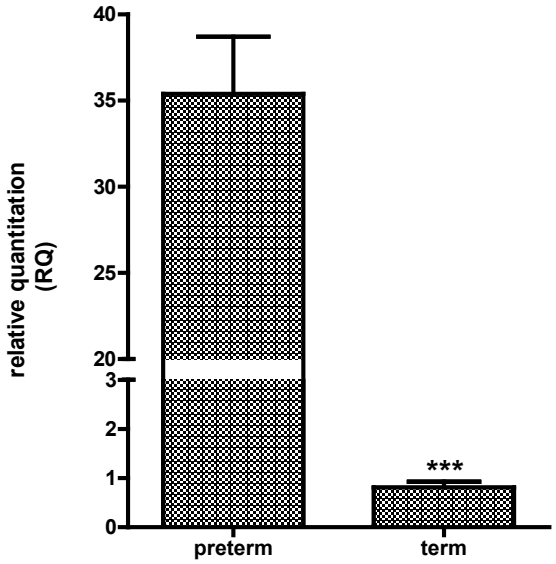

Figure 1: Levels of expression of *Pnoc* mRNA in human uterus samples obtained from preterm birth and full-term birth. By term pregnancy, the *Pnoc* mRNA level was decreased; there was a significant decline in the level of *Pnoc* in full-term uterine samples as compared with preterm samples; ***p<0.001.

effect of N/OFQ significantly ($p<0.001$; Figure 2A; Table 1). There was no significant difference between the EC_{50} values. NST alone exerted a uterus-relaxant effect. However; co-administration of N/OFQ (10^{-8} M) with NST did not alter the uterus-relaxant effect of NST ($p>0.05$; Figure 2B). There was no significant difference between the EC_{50} values.

In vitro contractility studies in the pregnant human myometrium from preterm births

N/OFQ alone decreased the uterine contractility concentration-dependently. NST (10^{-8} M) increased the maximum uterus-relaxant effect of N/OFQ significantly ($p<0.001$; Figure 2C). There was no significant difference between the EC_{50} values (data not shown). NST alone demonstrated a uterus-relaxant effect; which was not altered by N/OFQ ($p>0.05$; Figure 2D). There was no significant difference between the EC_{50} values.

Discussion

It is accepted that; whilst hormones such as oxytocin; vasopressin and prostaglandin F2alpha induce myometrial contractions; essentially via an elevation of the intracellular calcium level; other ligands; such as beta-adrenoceptor agonists and Calcitonin Gene-Related Peptide (CGRP); promote uterine quiescence via their ability to increase

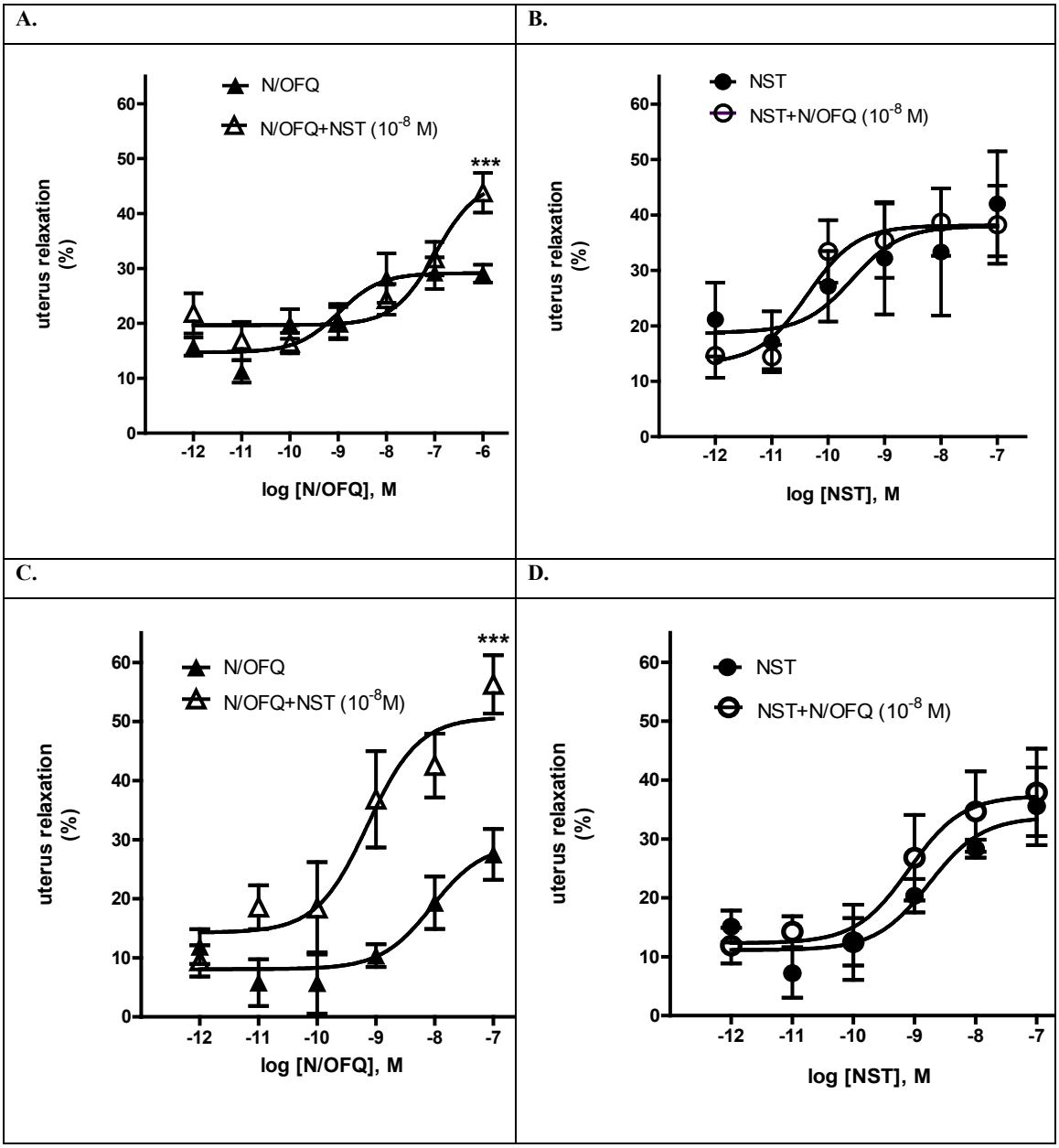

Figure 2: Uterus-relaxant effects of nociceptin (N/OFQ) and nocistatin (NST) on the term- and preterm-pregnant human myometrium *in vitro*. The contractions were elicited with 10^{-8} M oxytocin in uterine rings from full-term (A, B) and preterm (C, D) human pregnancies. A) The concentration-dependent relaxing effect of N/OFQ (▲) was significantly increased in the presence of NST (10^{-8} M; Δ); n=6. B) The concentration-dependent relaxant effect of NST (●) was not altered by N/OFQ (10^{-8} M; ○); n=4. C) The concentration-dependent relaxing effect of N/OFQ (▲) was significantly increased in the presence of NST (10^{-8} M; Δ); n=4. D) Co-administration of N/OFQ (10^{-8} M) with NST (○), however, did not significantly alter the uterus-relaxant effect of NST (●); n=5.

	E_{max} (% ± S.E.M.)			E_{max} (% ± S.E.M.)	
Term-pregnant uterus			**Preterm-pregnant uterus**		
N/OFQ	29.07 ± 1.61		N/OFQ	27.51 ± 4.31	
N/OFQ + NST	43.79 ± 3.61	***	N/OFQ + NST	56.29 ± 4.94	***
NST	42.01 ± 9.48		NST	35.55 ± 6.58	
NST + N/OFQ	38.24 ± 7.05	ns	NST + N/OFQ	37.92 ± 7.40	ns

p<0.001; significances are expressed relative to N/OFQ. ns: non-significant; significances are expressed relative to NST

Table 1: Maximum uterus-relaxant values of nociceptin (N/OFQ) alone and in the presence of Nocistatin (NST); and of NST alone and in the presence of N/OFQ on oxytocin-stimulated uterine contractions on the term- and preterm-pregnant human myometrium *in vitro*.

intracellular cyclic AMP levels [16]. As NOP is expressed in a high proportion of substance P (SP)/CGRP-positive neurons; and as a major subpopulation of N/OFQ neurons is located in juxtaposition to SP/CGRP-positive neurons; it is proposed that N/OFQ released locally in the dorsal root ganglia may (in a paracrine manner) modulate SP/CGRP-containing neurons expressing NOP. Thus; N/OFQ modulates both central and peripheral SP- or CGRP-mediated neurotransmission [17].

The CGRP system might play a role in the maintenance of normal pregnancy; and a defect in this system might lead to complications [18]. During pregnancy; circulating CGRP levels become significantly raised in humans [19,20] and rats [21]. The increase in CGRP levels begins in the third month of gestation in women; continuing until parturition; with the peak value (300% increase) in the ninth month [19].

We have previously demonstrated the CGRP-liberating effect of NST; and also the cAMP-accumulating and potassium channel opening effects of N/OFQ [13,14]. These mechanisms may additionally explain the uterus-relaxant effects of N/OFQ and NST and their intracellular signaling in the human uterus. As potassium channel inhibitors block CGRP-induced uterus relaxation; we assume that potassium channel opening is another signaling mechanism to relax the uterine smooth muscle by N/OFQ and NST.

RT-PCR studies confirmed that PNOC; the precursor for both N/OFQ and NST; shows an elevated level in preterm pregnancies; which is in correlation with the uterine quiescence during pregnancy. As a consequence; much more NST and N/OFQ can be translated from PNOC. The PNOC level drops by the end of pregnancy; when uterine contractility becomes stronger.

The synergistic action of N/OFQ and NST is really surprising; because studies reported on their opposite effects in several biological functions [3-8,22,23]. However these studies were carried out in the Central Nervous System (CNS) and gave no information about the peripheral interaction of these two neuropeptides. Hence; the peripheral synergism of N/OFQ and NST is a novel finding and seems to be opposite as compared with their CNS effect.

We can also conclude that NST administration must precede the administration of N/OFQ with the aim of enhancing the common uterus-relaxant effect. We assume that the CGRP-liberating effect of NST results in a weaker relaxation as compared with the potassium channel opening effect of N/OFQ; repeated and increasing administrations of N/OFQ following NST can therefore further increase the uterus relaxation through the potassium channels. On the other hand; if N/OFQ is administered first; the CGRP liberating effect of NST cannot exceed the relaxation caused by a single dose of N/OFQ through potassium channel opening; therefore the relaxing effect of NST cannot be potentiated by N/OFQ significantly.

The more prominent potentiating effect of NST in N/OFQ-stimulated uterus relaxation in preterm birth as compared with term pregnancy correlates with the finding that PNOC is more abundant in preterm birth.

As a conclusion; both N/OFQ and NST; possibly generated locally; have relaxant effects on the human uterus. Moreover; if they are present together; N/OFQ demonstrates an increased relaxant effect when NST is administered first. Further studies are required to reveal the precise mechanism of action of this potentiation. This potentiating effect is more prominent in the preterm myometrium; revealing the importance of the PNOC-N/OFQ-NST axis in the maintenance of quiescence in the pregnant human uterus.

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R (2008) Epidemiology and causes of preterm birth. Lancet 371: 75-84.
2. Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, et al. (1998) Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature 392: 286-289.
3. Zeilhofer HU, Selbach UM, Guhring H, Erb K, Ahmadi S (2000) Selective suppression of inhibitory synaptic transmission by nocistatin in the rat spinal cord dorsal horn. J Neurosci 20: 4922-4929.
4. Minami T, Nishihara I, Uda R, Ito S, Hyodo M, et al. (1994) Characterization of EP-receptor subtypes involved in allodynia and hyperalgesia induced by intrathecal administration of prostaglandin E2 to mice. Br J Pharmacol 112: 735-740.
5. Minami T, Uda R, Horiguchi S, Ito S, Hyodo M, et al. (1994) Allodynia evoked by intrathecal administration of prostaglandin E2 to conscious mice. Pain 57: 217-223.
6. Taiwo YO, Levine JD (1988) Prostaglandins inhibit endogenous pain control mechanisms by blocking transmission at spinal noradrenergic synapses. J Neurosci 8: 1346-1349.
7. Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, et al. (1998) Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature 392: 286-289.
8. Hiramatsu M, Inoue K (1999) Effects of nocistatin on nociceptin-induced impairment of learning and memory in mice. Eur J Pharmacol 367: 151-155.
9. Mika J, Obara I, Przewlocka B (2011) The role of nociceptin and dynorphin in chronic pain: implications of neuro-glial interaction. Neuropeptides 45: 247-261.
10. Miller-Pérez C, Sánchez-Islas E, Pellicer F, Rodríguez-Manzo G, Cruz SL, et al. (2008) Role of nociceptin/orphanin FQ and the pseudopeptide [Phe1Psi(CH2NH)Gly2]-nociceptin(1-13)-NH2 and their interaction with classic opioids in the modulation of thermonociception in the land snail Helix aspersa. Eur J Pharmacol 581: 77-85.
11. Gravanis A, Stournaras C, Margioris AN (1999) Paracrinology of endometrial neuropeptides: corticotropin-releasing hormone and opioids. Semin Reprod Endocrinol 17: 29-38.
12. Ohia SE, Laniyonu AA (1989) Naloxone-insensitive inhibitory and excitatory effects of opioid agonists in the rat isolated uterus. J Pharm Pharmacol 41: 168-172.
13. Klukovits A, Tekes K, Gündüz Cinar O, Benyhe S, Borsodi A, et al. (2010) Nociceptin inhibits uterine contractions in term-pregnant rats by signaling through multiple pathways. Biol Reprod 83: 36-41.
14. Deák BH, Klukovits A, Tekes K, Ducza E, Falkay G, et al. (2013) Nocistatin inhibits pregnant rat uterine contractions in vitro: roles of calcitonin gene-related peptide and calcium-dependent potassium channel. Eur J Pharm.

15. Chen Y, Sommer C (2007) Activation of the nociceptin opioid system in rat sensory neurons produces antinociceptive effects in inflammatory pain: involvement of inflammatory mediators. J Neurosci Res 85: 1478-1488.

16. Price SA, Bernal AL (2001) Uterine quiescence: the role of cyclic AMP. Exp Physiol 86: 265-272.

17. Mika J, Li Y, Weihe E, Schafer MK (2003) Relationship of pronociceptin/orphanin FQ and the nociceptin receptor ORL1 with substance P and calcitonin gene-related peptide expression in dorsal root ganglion of the rat. Neurosci Lett 348: 190-194.

18. Yallampalli C, Chauhan M, Thota CS, Kondapaka S, Wimalawansa SJ (2002) Calcitonin gene-related peptide in pregnancy and its emerging receptor heterogeneity. Trends Endocrinol Metab 13: 263-269.

19. Stevenson JC, Macdonald DW, Warren RC, Booker MW, Whitehead MI (1986) Increased concentration of circulating calcitonin gene related peptide during normal human pregnancy. Br Med J (Clin Res Ed) 293: 1329-1330.

20. Saggese G, Bertelloni S, Baroncelli GI, Buggiani B, Biver P (1989) Variations of circulating calcitonin gene-related peptide levels during pregnancy and perinatal period. Neuroendocrinol Lett 103–108.

21. Gangula PR, Wimalawansa SJ, Yallampalli C (2000) Pregnancy and sex steroid hormones enhance circulating calcitonin gene-related peptide concentrations in rats. Hum Reprod 15: 949-953.

22. Kazi JA (2012) Nocistatin and nociceptin modulate c-Fos expression in the mice thalamus. Neurol Sci 33: 1233-1237.

23. Kazi JA, Liu EH, Lee TL, Tachibana S (2007) Nocistatin attenuated the nociceptin induced c-Fos expression in the mouse hippocampus. Neuropeptides 41: 227-231.

Citation: Deák BH, Klukovits A, Kormányos Z, Tekes K, Ducza E, et al. (2013) Uterus-Relaxing Effects of Nociceptin and Nocistatin: Studies on Preterm and Term-Pregnant Human Myometrium *In vitro*. Reprod Sys Sexual Disorders 2: 117. doi:10.4172/2161-038X.1000117