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Investigation of the effects of nociceptin and nocistatin on pregnant uterine contractions in vitro

Summary of Ph.D. Thesis

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Introduction

Preterm birth is a significant public health concern, as it is associated with high risk of infant mortality, various morbidities in both the neonatal period and later in life. As its underlying causes and molecular pathways have not been fully elucidated, there is a need for investigations of endogenous factors that might control uterine activity, with the perspective of improving tocolytic therapy.

The peptide nociceptin/orphanin FQ (N/OFQ) was isolated for the first time in 1995. The nociceptin receptor (NOP) is similar in sequence to opioid receptors. Moreover, N/OFQ has a primary structure similar to that of opioid peptides. N/OFQ may act as a transmitter in the brain by modulating nociceptive and locomotor behaviour. N/OFQ could modulate the outcome of some inflammatory diseases, allows the stimulation of food intake and it is an anxiolytic peptide that plays a role in adapting to stress. N/OFQ counteracts the rewarding properties of abused drugs and produce impairment of spatial learning. N/OFQ is present in the plasma of humans, in several pathological conditions such as female fibromyalgia syndrome and postpartum depression; it is a paracrine mediator of the FSH effects in the regulation of spermatogenesis and also regulates the LH surge and ovarian function. N/OFQ level changes may play a role in the function of the female and male reproductive tract. The NOP receptor is widely expressed in the central nervous system, in rat intestine and vas deferens as well as porcine gastrointestinal tract and kidney, in several guinea-pig ganglia and in the retina and the heart of the rat.

Nocistatin (NST) was isolated from bovine brains and showed antinociceptive activity. It has been demonstrated that NST binds to a binding site that is distinct from the NOP receptor. The putative receptor of NST is probably a G protein–coupled one. Given the myorelaxant actions of opioids, these neuropeptides may participate in the control of myometrial contractility.

Aims

1. The main aim of the study was to investigate the roles and mechanisms of N/OFQ and NST in the uterine contractility in term-pregnant rat (day 22 of pregnancy) and in human myometrium obtained from full-term pregnancy and from preterm birth.

2. We set out to investigate the effect of N/OFQ and NST on the uterine contractility in an isolated organ bath system *in vitro*, by using term-pregnant rat uterine samples and myometrial samples obtained from caesarean sections of term-pregnant women, and from preterm delivery. Our further aim was to test the expression of N/OFQ and NST

in the rat myometrium with radioimmunoassay and to detect the presence of their common precursor prepronociceptin (*PNOC*) mRNA with real-time PCR technique.

3. The following aim was to find out the signalling pathways of N/OFQ and NST in the uterus with the means of [^{35}S]GTPγS binding assay (for G-protein activation), enzyme immunoassay (cAMP accumulation) and in vitro contractility studies. We set out to investigate the possible role of the Ca^{2+}-dependent (outward rectifying) K^+ channels (BK_{Ca}) and the release of the sensory neuropeptide CGRP in the mechanism of action of N/OFQ and NST, as well as the influence of a poor Ca^{2+} environment in the uterus-relaxant effect of NST in the presence of naloxone (NX).

Materials and methods

Animal and human tissue samples

Sexually mature female Sprague-Dawley rats were mated in the early morning hours. Copulation was confirmed by the presence of a copulation plug or spermatozoa in the vagina. The day of copulation was considered to be the first day of pregnancy. Biopsy specimens of human myometrial tissue were obtained at cesarean section in the third trimester of pregnancy in two groups: at full-term birth (37-41 weeks of gestation) and at preterm birth (33-36 weeks).

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

On selected days of late pregnancy (days 18, 20 and 22), rats were killed by CO_2 inhalation, the uteri were excised and trimmed of fat and the feto-placental units were removed. The rat and human tissue samples were frozen immediately in liquid nitrogen, and then stored at -70 °C until analysis. The total RNA was isolated with the TRIsure Kit. RT-PCR was performed by using the ABI StepOne Real-Time cycler. 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).

Radioimmunoassay (RIA) for N/OFQ and NST in the rat uterus

The uterine levels of N/OFQ were measured in nonpregnant and 22-day pregnant rats and the levels of NST were evaluated in nonpregnant and 15-, 18-, 20- and 22-day pregnant rats. The extraction of N/OFQ and NST was carried out by a validated method. The tissue extracts were subjected to RIA for N/OFQ using a commercially available ^{125}I-N/OFQ RIA Kit with a minimum sensitivity of 1 pg/ml and for NST using a commercially available ^{125}I-Nocistatin

RIA kit with minimum sensitivity of 10 pg/ml. Data were evaluated with Isodata 20/20 software and with RIA-Mat 280. Significance was calculated by Student *t*-test.

Radioligand-binding experiments

Radioligand-binding experiments were carried out on nonpregnant or 22-day-pregnant rat uterus membrane preparations. Protein concentration was determined by the method of Bradford. The radioactivity of the dried filters was detected in UltimaGold^{TM} F scintillation cocktail with a Packard Tricarb 2300TR liquid scintillation counter. Specific binding was determined by subtracting the non-specific binding value from the total binding value. The experiments were individually analyzed and the maximum numbers of binding sites (B_{max}) and the equilibrium dissociation constants (K_d) were calculated.

[^{35}S]GTPγS-binding assay

The effect of N/OFQ was investigated together with 10^{-6} M NX. The G_i-protein-activating effect of N/OFQ and NX was also measured in the presence of 500 ng pertussis toxin (PTX)/ml. The radioactivity of the dried filters was detected in UltimaGold^{TM} F scintillation cocktail with a Packard Tricarb 2300TR liquid scintillation counter.

Measurement of uterine cAMP accumulation

Uterine tissue samples were incubated in de Jongh solution at 37 °C. cAMP accumulation was determined in the presence of the non-specific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 10^{-3} M; 10 min), OFQ alone (10^{-8} M; 10 min) or in combination with NX, NST alone (10^{-8} M; 10 min) or in combination with N/OFQ or NX, and the adenylyl cyclase activator forskolin (10^{-5} M; 10 min). Uterine cAMP accumulation was measured with a commercial competitive cAMP enzyme immunoassay (EIA) kit.

In vitro contractility studies

Animal studies: On day 22 of pregnancy, the rats were killed by CO_2 inhalation, and the uteri were removed and prepared for the in vitro contractility assay. Briefly, the isolated uterine horns were placed immediately in an organ bath perfused with carbogen and trimmed of fat, and the foeto-placental units were removed. The temperature was maintained at 37 °C. In the isolated uterine rings, rhythmic contractions were elicited with 25 mM KCl. The effects of N/OFQ and NST on the uterine rings were measured in the concentration range of 10^{-12} – 10^{-6} M in a non-cumulative manner. In another set of experiments, uterine contractions were

elicited with 10^{-8} M oxytocin and the contraction-inhibiting effects of N/OFQ were tested in a non-cumulative manner. These experiments were carried out in the presence of the non-selective K^+ channel blocker tetraethylammonium (TEA; 10^{-3} M) and the BK_{Ca} channel-selective blocker paxilline (PAX; $5x10^{-6}$ M).

The joint effect of NST and NX was studied in a modified de Jongh buffer, containing half the Ca^{2+} concentration (0.5 mM $CaCl_2$) of the standard de Jongh buffer. In order to investigate the participation of the outward rectifying K^+ channels in mediating the effects of NST, tests were performed in the presence of the BK_{Ca} channel-selective blocker PAX ($5x10^{-6}$ M), against spontaneous uterine contractions.

The possible involvement of the sensory neuropeptide CGRP in the actions of NST was also tested on uterine tissue. In this set of experiments, capsaicin (1 μM; 10 min) was used to deplete CGRP from the uterine sensory nerve endings. After thorough washing out, the tissues were incubated with CGRP (0.1 μM; 20 min) and washed again, and the effects of NST were tested as above.

Human studies: Each tissue sample 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 tested in parallel in an organ bath (Krebs–Henseleit solution at 37 °C).

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. 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 and recorded and analyzed with the SPEL Advanced ISOSYS Data Acquisition System. The areas under the curves of 4- or 6-min periods were evaluated; the effects of NST, N/OFQ and NX were expressed as percentages of KCl/oxytocin-induced or spontaneous contractions The maximum contraction-inhibitory values were calculated with the Prism 4.0 computer program.

Results

Animal studies

N/OFQ and NST tissue levels

In the 22-day pregnant rats, uterine N/OFQ concentration was significantly higher ($P<0.05$) than in the non-pregnant rats. The myometrial NST levels increased significantly as term was approached.

Radioligand-binding studies

The presence of NOP receptors in the uterus was detected by radioligand-binding experiments. In the uteri of nonpregnant females, the maximum binding capacity (B_{max}) of the NOP receptors was 87.3 ± 5.2 fmol protein/mg membrane, with a dissociation constant (K_d) of 2.19 ± 0.14 × 10^{-8} M. In the membrane fractions of the 22-day-pregnant uteri, the corresponding B_{max} and K_d values were 99.6 ± 2.31 fmol protein/mg membrane and 1.95 ± 0.09 × 10^{-8} M, respectively. No significant difference (P>0.05) was found between the B_{max} or K_d values in the nonpregnant versus the 22-day pregnant rat uteri.

[^{35}S]GTPγS-Binding Assay

The N/OFQ-stimulated G protein activation was additionally tested on the membrane fractions of the 22-day-pregnant rat uteri (**Fig. 1**). N/OFQ stimulated the [^{35}S]GTPγS binding through the NOP receptors. In the presence of NX, the N/OFQ-stimulated G protein activation was decreased. In the presence of the G_i protein inhibitor PTX and NX, however, the maximum G protein activation decreased further, with the activation declining to below the basal level.

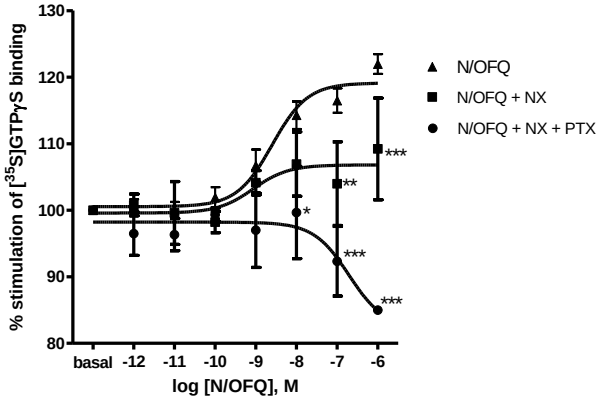

Fig. 1. Effect of nociceptin (N/OFQ) on G protein activation in the pregnant rat myometrium *in vitro*. n=6; *P<0.05, **P<0.01, ***P<0.001.

***In vitro* contractility studies**

Investigation of the uterus-relaxant effects of N/OFQ, NST, and NX

The KCl-evoked rhythmic contractions in the pregnant rat uterus were inhibited in a concentration-dependent manner by N/OFQ. In the presence of the opioid antagonist NX (10^{-8} M), the maximum contraction-inhibiting effect of N/OFQ was increased (**Figure 2A**). NST alone decreased the KCl-induced contractions concentration-dependently. Co-administration of N/OFQ (10^{-8} M) with NST, however, significantly increased the maximum contraction-inhibitory effect of NST; P<0.05 (**Fig. 2B**). The maximum inhibitory effect of NST was

decreased by NX (10^{-8} M); P<0.001. NX also decreased the maximum contraction-inhibitory effect of the combination NST+N/OFQ; P<0.05 (**Fig. 2B**).

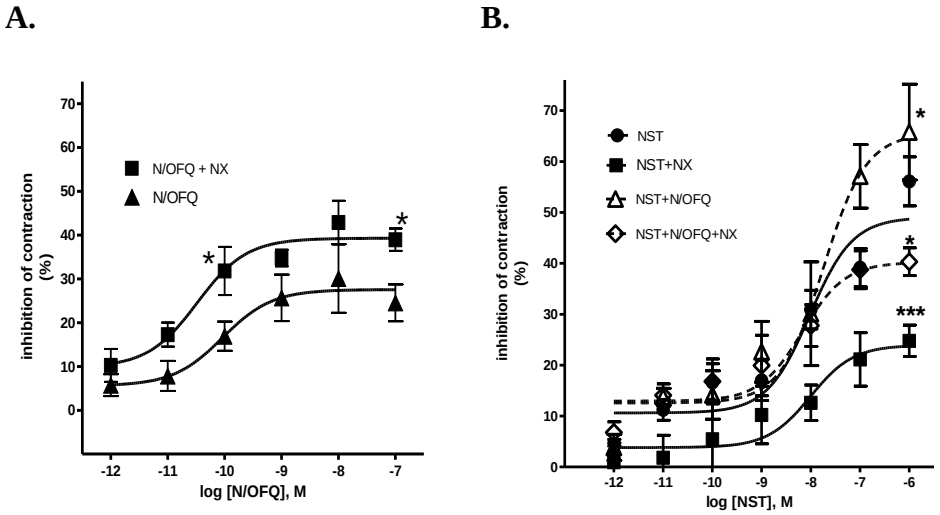

Fig. 2. Inhibitory effects of nociceptin (N/OFQ), nocistatin (NST) and naloxone (NX) on pregnant rat uterine contractions *in vitro*. n=8; *P<0.05, ***P<0.001.

Investigation of the role of BK_{Ca} channels in mediating the effects of N/OFQ and NST

Because the central actions of N/OFQ are mediated via the activation of the BK_{Ca} channels and hyperpolarization of the neurons, the effects of N/OFQ on the uterine samples were also tested in the presence of the nonselective K^+ channel inhibitor TEA (10^{-3} M) and the BK_{Ca} channel-selective PAX ($5x10^{-6}$ M) (**Fig. 3A**). The uterus-relaxing effect of N/OFQ on oxytocin-evoked rhythmic contractions was significantly attenuated by TEA and PAX. The effect of NST on the spontaneous contractions of the term-pregnant rat uterus were also tested in the presence of the selective BK_{Ca} channel inhibitor PAX ($5x10^{-6}$ M) (**Fig. 3B**). In the presence of PAX, the maximum contraction-inhibitory effect of NST was decreased significantly.

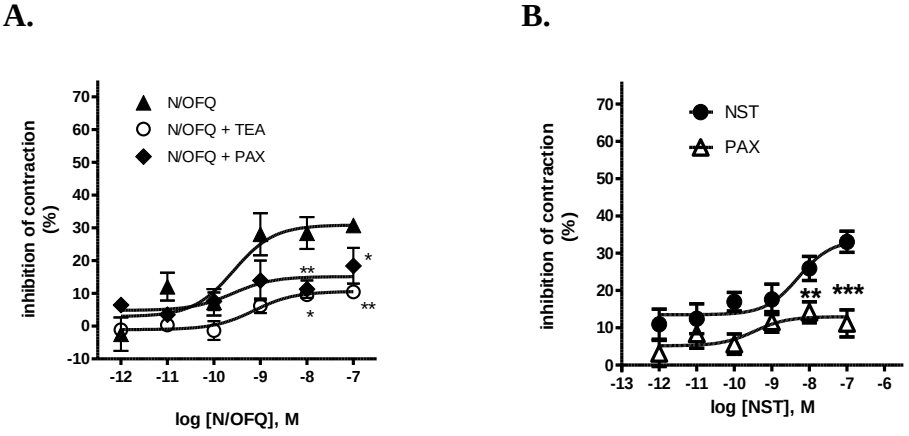

Fig. 3. Effects of potassium channel inhibitors on the pregnant contraction-inhibitory effects of nociceptin (N/OFQ) and nocistatin (NST) *in vitro*. A) The contractions were elicited with 10^{-8} M oxytocin in 22-day-pregnant uterine rings from the rat. B) The spontaneous contractions were recorded in 22-day-pregnant uterine rings from the rat. n= 8; *P<0.05, **P<0.01, ***P<0.001.

Investigation of the contraction-inhibitory effects of NST and NX in a hypocalcemic environment

In the hypocalcemic environment, the concentration—response curves of NST alone and of NST in combination with NX were both shifted to the left as compared with the curves in standard de Jongh solution. The logEC_{50} values of NST alone and of NST with NX were significantly lower in the hypocalcemic environment than in standard de Jongh solution ($P<0.05$). At the same time, NX did not decrease the inhibitory effect of NST in the hypocalcemic environment as it did in the standard buffer. The maximum contraction-inhibitory effect of NST alone did not differ from that of NST in combination with NX in the hypocalcemic buffer (**Fig. 4**).

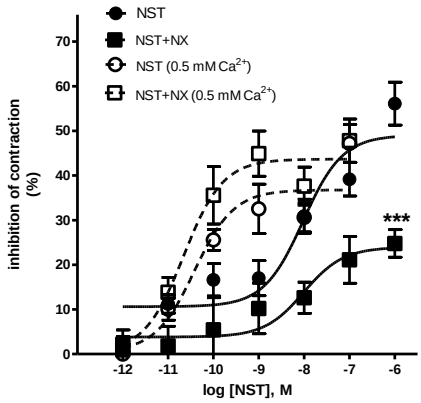

Fig. 4. Effects of nocistatin (NST) and naloxone (NX) on pregnant uterine contractions in a hypocalcemic environment *in vitro*. n= 6; P<0.001.

Investigation of the role of CGRP in mediating the effects of NST

Since the exact site of action of NST is still unclear, we tested whether it might act by modulating neuropeptide release from capsaicin-sensitive sensory nerve endings in the pregnant rat uterus. Neuropeptide depletion from the capsaicin-sensitive primary afferents was induced with capsaicin (**Fig. 5**). The maximum contraction-inhibitory effect of NST was decreased significantly ($P<0.01$) after preincubation with capsaicin (1 μM). The solvent of capsaicin (control) did not change the effect of NST ($P>0.05$). When the neuropeptide depletion was followed by the addition of CGRP (0.1 μM), the maximum contraction-inhibitory effect of NST was significantly higher than after incubation with capsaicin ($P<0.01$). The addition of CGRP restored the inhibitory effect of NST as compared with the control ($P>0.05$).

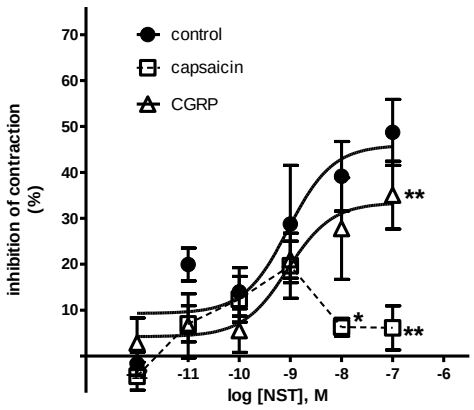

Fig. 5. The role of calcitonin gene-related peptide (CGRP) on the contraction-inhibitory effect of nocistatin (NST) *in vitro*. n= 6; *P<0.05, **P<0.01.

Measurement of *PNOC* mRNA in the rat uterus

The myometrial *PNOC* mRNA levels increased significantly as term was approached. The PCR study showed that the level of *PNOC* mRNA/*β-actin* mRNA was lowest on pregnancy day 18. The relative expression of *PNOC* mRNA on day 20 was not different from that on day 18, but it was increased significantly by day 22, the day of delivery; P<0.001 (**Fig. 6**).

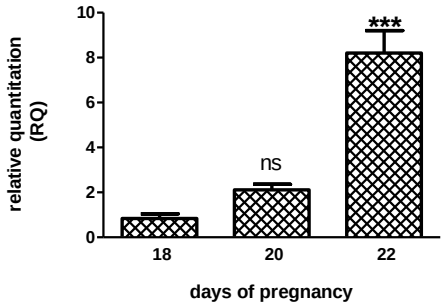

Fig. 6. Levels of expression of *PNOC* mRNA in the rat uterus on days 18, 20 and 22 of pregnancy. n=3; ***P<0.001, ns: non-significant. Significances are expressed relative to the previous column.

Measurement of uterine cAMP accumulation

The effect of N/OFQ on uterine cAMP accumulation was also measured (**Fig. 7A**). N/OFQ alone did not evoke a significant increase (P>0.05) in the uterine cAMP accumulation as compared with the non-treated control samples. Its combination with NX caused a significant elevation (P<0.05) in the uterine cAMP level. Moreover, if the uterine tissue samples were preincubated with the G_i protein inhibitor PTX (500 ng/ml), N/OFQ with NX elevated the uterine cAMP level far higher (P<0.01) than that without preincubation, which points to the involvement of G_s-proteins in the intracellular signalling pathways of N/OFQ and NX in the pregnant rat uterus.

We also investigated whether cAMP accumulation plays a role in the contraction-inhibitory effect of NST (**Fig. 7B**). NST evoked a significant increase ($P<0.001$) in the uterine cAMP level as compared with the basic activity. Co-administration of N/OFQ with NST caused a further elevation in the cAMP level ($P<0.01$). However, when NX was co-administered with NST, a significant decrease was detected in the cAMP level as compared with the effect of NST alone ($P<0.05$).

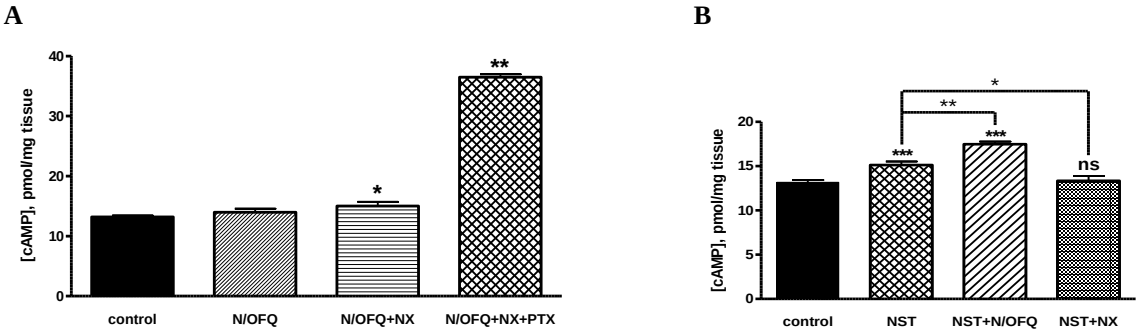

Fig. 7. Effects of nociceptin (N/OFQ) and nocistatin (NST) on intracellular cAMP levels in the pregnant rat myometrium. n=6; *P<0.05, **P<0.01, ***P<0.001.

Human myometrial studies

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 contraction-inhibitory effect of N/OFQ significantly ($P<0.001$; **Fig. 8A**). NST alone exerted a contraction-inhibitory effect. However, co-administration of N/OFQ (10^{-8} M) with NST did not alter the inhibitory effect of NST ($P>0.05$; **Fig. 8B**).

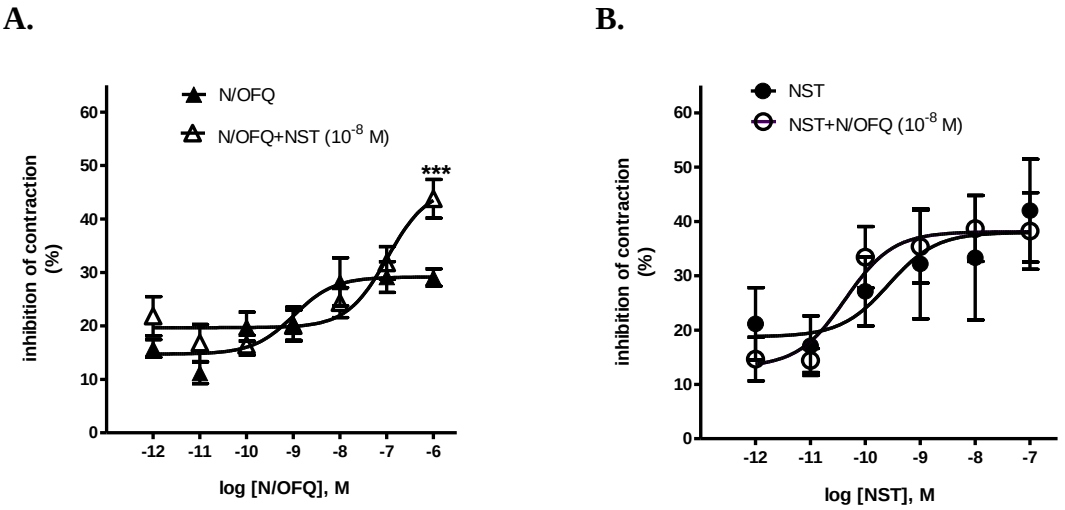

Fig. 8. Uterus-relaxant effects of nociceptin (N/OFQ) and nocistatin (NST) on the term-pregnant human myometrium *in vitro*. The contractions were elicited with 10^{-8} M oxytocin. n=4; ***P<0.001.

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 contraction-inhibitory effect of N/OFQ significantly (P<0.001; **Fig. 9A**). NST alone demonstrated a contraction-inhibitory effect, which was not altered by N/OFQ (P>0.05; **Fig. 9B**).

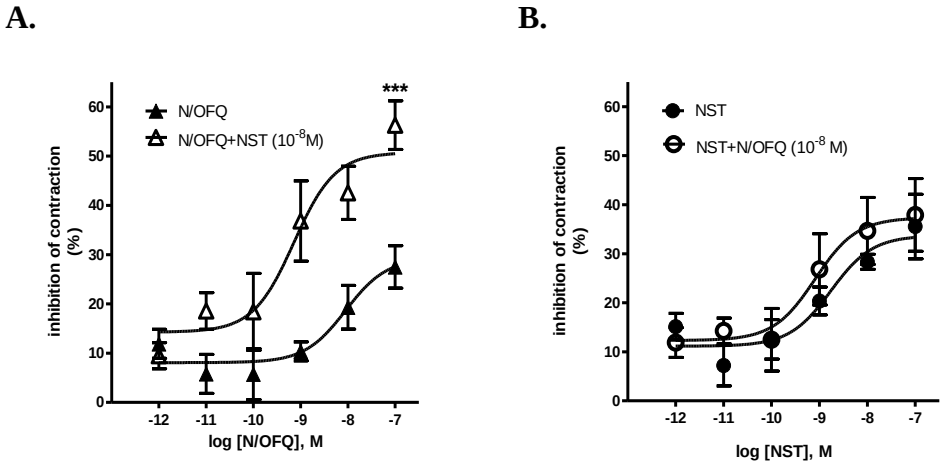

Fig. 9. Uterus-relaxant effects of nociceptin (N/OFQ) and nocistatin (NST) on the preterm-pregnant human myometrium *in vitro*. The contractions were elicited with 10^{-8} M oxytocin. n=6; ***P<0.001.

Measurement of *PNOC* mRNA in the human uterus

The myometrial *PNOC* mRNA levels were significantly higher in preterm uterine samples as compared with samples from full-term pregnancy; P<0.001 (**Fig. 10**).

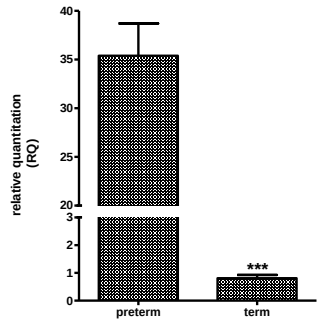

Fig. 10. Levels of expression of *PNOC* mRNA in human uterus samples obtained from preterm birth and full-term birth. n=3; ***P<0.001.

Discussion

We found that the common precursor for N/OFQ and NST, the *PNOC* mRNA, is expressed locally in the pregnant rat and human uterus, indicating that N/OFQ and NST are synthesized locally. The RIA results confirmed the presence of both N/OFQ and NST in the nonpregnant and the pregnant rat uterus. NST at term is more abundant than N/OFQ, so it seems that *PNOC* mRNA is translated mainly to NST. We also detected specific binding sites for NOP both in the nonpregnant and term-pregnant rat uterus.

N/OFQ was found to inhibit both KCl- and oxytocin-evoked rhythmic contractions. NX increased the uterus-relaxing effect of N/OFQ. N/OFQ was able to potentiate the inhibitory effect of NST. NST does not counteract the effects of N/OFQ on the myometrium contractility, as was presumed in previous studies relating to their actions in the central nervous system. NX inhibits the contraction-inhibitory effect of NST. NX induces an increase in inward Ca^{2+} currents, thus we used a hypocalcemic environment, where NX was unable to promote a Ca^{2+} influx, and hence it could not overcome the relaxation induced by NST. When N/OFQ was present, NX was able to decrease the common contraction-inhibitory effect of NST and N/OFQ.

We found a significant elevation of [^{35}S]GTPγS binding through the NOP receptors. Thus, G proteins at least in part, mediate the actions of N/OFQ on the uterus. The presence of NX decreased the maximum [^{35}S]GTPγS binding. NX may interfere with the G_i protein-activating potency of N/OFQ. In the presence of NX and PTX, we detected a dramatic fall in N/OFQ-induced G protein production.

Co-administration of NX with N/OFQ increased the uterine cAMP level. We presume that N/OFQ and NX compete for intracellular G_i protein activation. We detected elevation of cAMP levels in the presence of NST. In the presence of N/OFQ with NST, a further cAMP level elevation was found, which can be explained by the mutual cAMP-accumulating effects

of N/OFQ and NST-induced CGRP liberation. NX decreased the cAMP levels elevated by NST, which suggests that NX interferes with NST at the level of G-protein activation, too.

In the uterus, BK_{Ca} channels are abundant and play an important role in limiting depolarization, thereby relaxing the uterine smooth muscle. We found that blockade of the K^+ channels with TEA or PAX diminishes the uterus-relaxing effect of N/OFQ. PAX inhibited the contraction-inhibitory effect of NST. We suggest that the Ca^{2+}-dependent K^+ channels play a role in the intracellular signalling of N/OFQ and NST.

Opioid-like nociceptive peptides have been reported to release neurotransmitters such as CGRP or substance P (SP) from capsaicin-sensitive primary sensory neurons. CGRP has been reported to inhibit smooth muscle contractility in a variety of tissues, including the pregnant rat uterus. We investigated the effect of NST either on capsaicin-induced CGRP-depleted uterus samples or on CGRP-reloaded samples. Capsaicin blocked the contraction-inhibitory effect of NST, which was restored after the tissue samples were incubated with CGRP. We assume that CGRP is an important factor in the contraction-inhibitory effect of NST.

In the human tissues 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. 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.

Conclusions

These results provide evidence that both N/OFQ and NST generated locally in the uterus have contraction-inhibitory effect in the pregnant rat and human uterus, and when they are administered together, they can potentiate each others effect, this mechanism being mediated mainly by BK_{Ca} channels and consequent hyperpolarization. NST additionally acts by release of the sensory neuropeptide CGRP. NX shows an additive relaxant effect with those of N/OFQ; on the other hand it overcomes the relaxation caused by NST by activating inward rectifying Ca^{2+} channels and by decreasing the cAMP-accumulating effect of NST. We have provided evidence that the NOP receptors are coupled to multiple G proteins. We assume that, N/OFQ or NOP-related (preferably non-peptide) agonists as well as NST derivatives might be considered as prospective candidates for tocolytic therapy. The findings of this *in vitro* study need to be evaluated under *in vivo* conditions, and further experiments on human tissue are necessary in order to allow conclusions on the relevance of the present findings as concerns human disease.

Publications related to the Ph.D. thesis

1. Klukovits A, Tekes K, Gündüz Cinar O, Benyhe S, Borsodi A, **Deák BH**, Hajagos-Tóth J, Verli J, Falkay G, Gáspár R. Nociceptin inhibits uterine contractions in term-pregnant rats by signaling through multiple pathways. Biol Reprod 2010; 83:36-41. **IF: 3.87**

2. **Deák BH**, Klukovits A, Tekes K, Ducza E, Falkay G, Gáspár R. Nocistatin inhibits pregnant rat uterine contractions in vitro: Roles of calcitonin gene-related peptide and calcium-dependent potassium channel. Eur J Pharmacol 2013; 714: 96-104. **IF: 2.592**

3. **Deák BH**, Klukovits A, Kormányos Z, Tekes K, Ducza E, Gáspár R. Uterus-Relaxing Effects of Nociceptin and Nocistatin: Studies on Preterm and Term-Pregnant Human Myometrium In vitro. Reprod Sys Sexual Disorders 2013; 2:117.

Abstracts related to the Ph.D. thesis

1. **Deák Beáta**, Klukovits Anna, Kormányos Zsolt, Falkay György, Gáspár Róbert. A nociceptin és nocisztatin hatása a terhes humán és patkány uterusz kontraktilitására. A Magyar Élettani Társaság (MÉT) és a Magyar Kísérletes és Klinikai Farmakológiai Társaság (MFT) II. Közös tudományos konferenciája; Szeged, 2010. június 16-18.

2. **Beáta H Deák**, Anna Klukovits, Eszter Ducza, Zsolt Kormányos, Attila Pál, George Falkay, Róbert Gáspár. The signalling pathways of nociceptin and nocistatin in the pregnant human and rat uterine contractility. Pharmaceutical Sciences for the Future of Medicines Conference; Prague, Czech Republic, 13-17 June, 2011.

3. **Beáta H Deák**, Anna Klukovits, Eszter Ducza, Zsolt Kormányos, Attila Pál, George Falkay, Róbert Gáspár. The signalling pathways of nociceptin and nocistatin in the pregnant human and rat uterine contractility. Molekulától a gyógyszerig; Szeged, 2012. május 24-25.

NYILATKOZAT

Alulírott, Dr. Klukovits Anna egyetemi adjunktus nyilatkozom, hogy a „**Nociceptin inhibits uterine contractions in term-pregnant rats by signaling through multiple pathways**" című (szerzők: Klukovits A, Tekes K, Gündüz Cinar O, Benyhe S, Borsodi A, Deák BH, Hajagos-Tóth J, Verli J, Falkay G, Gáspár R; megjelenés helye és ideje: Biol Reprod. 2010 Jul;83(1):36-41.) első szerzős közleményemet Deák Beáta PhD hallgató a doktori fokozatának megszerzéséhez szabadon felhasználhatja.

Deák Beáta a közlemény kiemelkedő fontosságú szerzője, aki a kísérletes munkában aktívan részt vett; az izolált szervi vizsgálatokat és a cAMP méréseket irányítás mellett önállóan végezte. Emellett közreműködött az SZBK-ban végzett receptor vizsgálatok kivitelezésében.

Szeged, 2012. február 23.

Dr. Klukovits Anna

SZTE Gyógyszerhatástani és Biofarmáciai Intézet