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Authors: Wei-She Zhang, Kui-Lin Fei, Mei-Ting Wu, Xin-Hua Wu, and Qing-Hua Liang
Source: Biology of Reproduction, 86(5)
Published By: Society for the Study of Reproduction
URL: https://doi.org/10.1095/biolreprod.111.095984
Neuromedin B and Its Receptor Influence the Activity of Myometrial Primary Cells In Vitro Through Regulation of Il6 Expression via the Rela/p65 Pathway in Mice

Wei-She Zhang,1,2,3,4 Kui-Lin Fei,3 Mei-Ting Wu,3 Xin-Hua Wu,3 and Qing-Hua Liang2,4

1Department of Obstetrics and Gynecology, Xiangya Hospital, Central South University, Hunan, Changsha, China
2The Postdoctoral Mobile Station of the Institute of Combined Traditional Chinese and Western Medicine, Xiangya Hospital, Central South University, Hunan, Changsha, China

ABSTRACT

The neuromedin B receptor (Nmbr) is an important physiological regulator of spontaneous activities and stress responses through different cascades as well as its autocrine and paracrine effects. Previous studies have revealed that neuromedin B (Nmbr) and its receptor signal via the Rela (also known as p65)/Il6 pathway in a mouse model of pregnancy. This study investigated the mechanism of Nmbr signaling via the Rela/p65/Il6 pathway and regulation of the concentration of intracellular free calcium ([Ca2+]i) during the onset of labor in primary mouse myometrial cell cultures isolated from mice in term labor. Data demonstrated Nmbr agonist-mediated upregulation of the DNA binding activity of Rela/p65, Il6 expression, and [Ca2+]i in a concentration-dependent manner. Furthermore, a significant correlation was observed between DNA binding activity of Rela/p65 and Il6 expression. Moreover, this up-regulation was blocked by Nmbr and Rela/p65 knockdown, achieved by RNA interference (RNAi) technology. No significant differences were identified in the inhibition of Il6 expression as a result of Nmbr or Rela/p65 knockdown. However, significant differences were observed between the [Ca2+]i in Rela/p65-specific and that in the Nmbr-specific small interfering RNA (siRNA)-treated groups. These data demonstrated that the Nmbr/Nmbr interaction in pregnant myometrial primary cells in vitro predominantly influenced uterine activity through regulation of Il6 expression via the Rela/p65 pathway, although the effects of Nmbr on [Ca2+]i, involved several pathways that remain to be elucidated.

INTRODUCTION

Preterm birth is a leading cause of neonatal mortality and a major cause of pediatric morbidity and long-term disability. The global rate of preterm delivery is increasing and there is no effective means of prevention [1]. Current strategies to prolong pregnancy are based on inhibition of uterine myometrium contraction in preterm labor [2–4]. However, the mechanism by which spontaneous onset labor is initiated, both at term and preterm, is still unclear. Therefore, the effects of the tocolysis in preventing preterm labor do not satisfy clinical needs [5, 6]. Other strategies have been designed to maintain a state of uterine quiescence and pregnancy, preventing the uterine smooth muscle from initiating contractions and entering preterm labor [7, 8]. The use of cDNA microarray technology is critical for the identification of novel targets related to the contraction of uterine smooth muscles, in order to provide a greater understanding of the mechanisms underlying preterm birth. It has been reported that the neuromedin B receptor (Nmbr) gene is one of the group of G protein-coupled receptors (GPCR), which are typical drug targets that are differentially expressed during parturition, while oxytocin receptor genes and other tocolytic targets do not exhibit such changes in expression [9, 10]. Furthermore, the expression of Nmbr peaks at term and occurs before parturition in humans and mice, and the Nmbr agonist neuromedin B (NMB) shortens the gestational age of mice with an associated increase in DNA binding activity of Rela/p65 and Il6 mRNA expression [11]. These results suggest that the mechanism underlying the onset of labor involves changes in the expression of the Nmbr, Rela/p65, and Il6 genes in a mouse model of pregnancy. Other studies have shown that NMB is a member of the family of bombesin-like peptides that, like oxytocin, are predominantly secreted by the hypothalamus and pituitary gland [12]. NMB binds with high affinity to the NMBR to mediate the biological effects of smooth muscle contraction, spontaneous activity, and stress responses [13, 14]. These studies implicate Nmbr as a novel tocolytic drug target. Furthermore, the biological effects of the Nmbr/Nmbr interaction are mediated through the Gpcr pathway, resulting in phospholipase C/protein kinase C (PKC)-mediated myosin light chain phosphorylation and increased intracellular free calcium ([Ca2+]i) [12–14], which induce uterine smooth muscle cell contraction. Related research has demonstrated that PKC activates Rela/p65 [15–17], and there is now compelling evidence demonstrating that Rela/p65 is an important upstream regulator of labor-associated processes [18]. Indeed, Rela/p65 and its associated inflammatory reactions are widely accepted to be a key feature of labor onset [19–21]. Downstream effectors in the Rela/p65 pathway, including Il6 and interleukin 1b (Il1b), have been shown to promote cervical ripening, uterine contraction, and acceleration of human parturition [22–24]. Therefore, it is hypothesized that Nmbr/Nmbr regulates uterine smooth muscle contraction in parturition through regulation of the expression of and [Ca2+]i, via the P65 pathway.

This study evaluated the role and mechanism of the Nmbr/Nmbr interaction in regulation of the DNA binding activity of Rela/p65, Il6 expression, and [Ca2+]i, in primary murine myometrium cells isolated at the onset of labor. Furthermore, regulation of Il6 expression and [Ca2+]i was investigated by...
small interfering RNA (siRNA)-mediated knockdown of Nmbr and Rela/p65.

MATERIALS AND METHODS

Primary Culture of Myometrial Cells and Identification

All animals received humane care in compliance with the university’s guidelines. Experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Central South University. Inbred BALB/c pregnant mice were killed after parturition of the first neonate. Uterine horns and cervix were immediately excised, and fetalplacental units were removed. Myometrial tissue was rapidly isolated from connective tissue and adherent endometrium by scraping, under the guidance of histology in pre-experiment, and cut into fragments (approximately 1 mm³). Myometrial tissue was digested using 375 U/ml collagenase type II (Sigma) containing 25 U/ml phosphate saline at 37°C in 95% humified atmosphere containing 5% CO2 with agitation. Dissociated myometrial cells were collected by centrifugation (500 × g, 5 min), and resuspended in sterile, phenol red-free Dulbecco modified Eagle medium (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% fetal calf serum (FCS), 25 mM HEPES, 100 U/ml penicillin-streptomycin. Medium was replaced by fresh DMEM supplemented with 20% FCS 1 day later and by 10% FCS on the following day. Cells were used at confluence 3 days after plating. Myometrial cells were plated on 6-well silicone elastomer plates of culture plates in coated with type 1 collagen (Flexcell International Corp., McKeesport, PA) at a density of 3 × 10⁵ cells per well. The purity of the uterine smooth muscle cells (SMCs), and the expression of NMBR in the primary cultured cells was confirmed as described below.

Experimental Protocols

Myometrial cells were cultured for 72 h in 10% FCS/DMEM before being randomly divided into experimental groups and incubated for 24 h in 10% FCS/DMEM. The control groups were divided into untreated cells (control) randomly divided into experimental groups and incubated for 24 h. Experimental Protocols

siRNA Preparation and Selection

Knockdown of Nmbr (GenBank accession number NM-008703) and Rela gene (GenBank accession number NM-000045, also known as p65) was achieved by the transfection of sequence-specific siRNA. The dominant Nmbr and Rela/p65 sequences and negative control sequences were constructed by GenePharma Company (Shanghai, China). Blast analysis was performed to confirm target gene specificity of the designed siRNA duplexes. Transfections were carried out at a final concentration of 100 nM, using Lipofectamine2000 (Invitrogen), according to the manufacturer’s instructions. Medium was changed 24 h after transfection, and analyses were performed after a further 24 h. Transfection efficiencies were validated by visualization of coexpressed enhanced green fluorescent protein under fluorescence microscopy. The effects on cell growth were measured by methyl thiazolyl tetrazolium (MTT) assay according to the reference methods [25]. The knockdown efficiency of three siRNA and protein levels using real-time PCR and immunocytochemistry (ICC), data not shown. The most effective siRNA were confirmed as Nmbr -987 siRNA and Rela/p65-1477 siRNA (Table 1).

Rela/p65 DNA Binding Activity Assay

Nuclear proteins were extracted from myometrial cells, and relative Rela/p65 DNA binding activity was quantified and calculated using the NoShift transcription factor assay kit (Merk Corporation) according to the manufacturer’s instructions.

RNA Extraction and Real-Time PCR

Real-time PCR analyses of Nmbr, Rela/p65, and Il6 mRNA expression were performed as previously described [25]; primer sequences are shown in Table 2.

Table 2. Primers used for Nmbr, Rela/p65, and Il6.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Direction</th>
<th>Sequence</th>
<th>Size (bp)</th>
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<tbody>
<tr>
<td>Nmbr</td>
<td>Sense</td>
<td>5’-CATCCGCATCTTCCTCAAAATC-3’</td>
<td>331</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’-CCAGAAGGTACATGCTGTCGAC-3’</td>
<td>225</td>
</tr>
<tr>
<td>Rela/p65</td>
<td>Sense</td>
<td>5’-CTACTGTCGTCGTCGAATTAA-3’</td>
<td>499</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’-ATGGTTGCGGATGGGACCTG-3’</td>
<td>250</td>
</tr>
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Table 2. The most effective siRNA are indicated in boldface. The number is the location of the mRNA sequence on the target gene which is blocked via siRNA interference.

Table 2. Briefly, RNA was extracted from SMCs using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. RNA samples were first treated with DNase I (Qiagen) according to the manufacturer’s instructions to remove possible DNA contamination. mRNA was reverse transcribed to cDNA with ReverTra Ace (TOYOBO). Quantitative analysis of gene expression was performed with the ABI Prism 7500 sequence detection system (PE Applied Biosystems) using SYBR Green real-time PCR Master Mix Plus (TOYOBO). Values for target genes were related to their controls using the 2^-ΔΔCT calculation method [26]. Absolute gene transcription was normalized to the reaction with an end volume of 25 μl. Primers used for the analysis of gene transcription are described in Table 2. The PCR amplification conditions were as follows: 95°C for 5 min, 94°C for 20 sec, 55°C for 20 sec, 72°C for 20 sec, 72°C for 5 min, and 55°C for 10 sec for 30–35 cycles. Total RNA (1 μg) was then reverse transcribed using the Supercript III reverse transcription kit (Invitrogen) according to the manufacturer’s instructions.

Detection of Protein Expression

Protein expression of α-smooth muscle actin (α-SMA) was analyzed by direct immunofluorescence and ICC using fluorescein isothiocyanate (FITC)-labeled α-SMA antibody (Sigma-Aldrich) and the primary antibody α-SMA (1:200 dilution; Santa Cruz Biotechnology). Interleukin 6 (IL6) protein in culture supernatants was detected by ELISA using mouse IL6 Immunoassay Kits (Biosource) according to the manufacturer’s instructions.

Measurement of Intracellular Free Calcium Concentration

Cells were seeded on culture slides and washed with serum-free RPMI 1640 medium twice before loading with 1% fluo-3 AM (code sc-206212; Santa Cruz Biotechnology) fluorescent indicator dye at 37°C for 45 min in the absence or presence of CaCl2, 2 mM (pH 7.4). Slides were washed three times with distilled water to remove extracellular fluo-3 AM. Calcium concentration [Ca2+] was determined using laser confocal scanning microscopy (model TCS-SP5; Leica) according to the manufacturer’s instructions.

<table>
<thead>
<tr>
<th>Primer</th>
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<td>331</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’-UUGACGAUGUGACUGAATT-3’</td>
<td>225</td>
</tr>
<tr>
<td>Rela/p65</td>
<td>Sense</td>
<td>5’-GAUCUCCUGUGACCAATT-3’</td>
<td>499</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’-UUGAGAGGGACCCAUUACATTT-3’</td>
<td>250</td>
</tr>
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</table>
**Statistical Analysis**

All data are presented as means ± SEM. Comparison of NMB-treated group and control group data was analyzed using one-way ANOVA. Comparison of NMB-treated group and siRNA+ NMB-treated group data was analyzed using two-way ANOVA. Pearson and linear analyses were performed for analysis of the relationship between genes and proteins. A P value of <0.05 was considered significant. Statistical analyses were performed using SPSS version 17.0 software for Windows (Microsoft).

**RESULTS**

**Primary Cell Culture and Verification**

Cultured primary SMCs within passage 4 were long and fusiform in shape. Cell cloning was performed after 48-h culture, and fusion was apparent in some clones 1 week later (Fig. 1A). Immunofluorescence and ICC results showed that the majority of cultured SMCs were long and fusiform or polygonal in shape. Intracytoplasmic green filamentous structures were observed, correlating with actin protein expression (Fig. 1, B1 and B2), thus confirming the identity of SMCs. ICC results confirmed that NMBR expression (Fig. 1C) in the cell membrane of the majority of cultured smooth muscle cell was positive. Thus, these results provide direct evidence that the cultured smooth muscle cell within passage 4 from the mice term myometrium demonstrate abundant expression of α-SMA and NMBR protein.

**NMB-Induced DNA Binding Activity of Rela/p65 Blocked by Nmbr-Specific siRNA Pretreated Myometrial Cells**

Significant differences were observed among the binding activity of Rela/p65 DNA in pregnant SMCs treated with $10^{-8}$ M NMB, $10^{-6}$ M NMB, and $10^{-4}$ M NMB ($P < 0.05$). Higher binding activity was also detected in these three groups than in the control group ($P < 0.05$), but not in the $10^{-10}$ M NMB-treated group ($P > 0.05$). The binding activities of Rela/p65 DNA in Nmbr siRNA+ NMB ($10^{-10}$ M $~ 10^{-8}$ M) treated groups were lower than that of the control group and in the same concentrations of NMB before siRNA pretreatment, respectively ($P < 0.05$). No significant differences were identified among the four groups pretreated by siRNA ($P > 0.05$) (Fig. 2). Thus, these findings demonstrate that $10^{-8}$ M $~ 10^{-4}$ M NMB can up-regulate the DNA binding activity of Rela/p65, and this role is blocked by Nmbr-specific siRNA in pregnant SMCs.

**NMB-Induced Il6 Expression Reduced by Nmbr- or Rela/p65-Specific siRNA Pretreated Myometrial Cells**

Significant differences was observed among the levels of Il6 mRNA and protein in pregnant SMCs treated with $10^{-8}$ M NMB, $10^{-6}$ M NMB, and $10^{-4}$ M NMB ($P < 0.05$). Higher levels were also detected in these three groups than in the control group ($P < 0.05$), but not in the $10^{-10}$ M NMB-treated group ($P > 0.05$). The levels of Il6 mRNA and protein in Nmbr siRNA+ NMB ($10^{-10}$ M $~ 10^{-8}$ M) groups were lower than that of the control group and that in the same concentration of NMB before siRNA pretreatment, respectively ($P < 0.05$). No significant differences were identified among the four groups pretreated by siRNA ($P > 0.05$) (Fig. 3A). The levels of Il6 mRNA and proteins in Rela/p65 siRNA+ NMB ($10^{-10}$ M $~ 10^{-8}$ M) treated groups were also lower than that in the control group and that in same concentration of NMB before siRNA pretreatment, respectively ($P < 0.05$). No significant differences were identified among the four siRNA groups ($P > 0.05$) (Fig. 3B), and no remarkable differences were detected between the effects of siRNA on Nmbr and Rela/p65 on the levels of Il6 mRNA and protein ($P > 0.05$). Taken together, these findings provide strong evidence that NMB can induce the expression of Il6, and this up-regulating role is interrupted equally via Nmbr- or Rela/p65-specific siRNA in pregnant SMCs.

**Influence of Nmbr- or Rela/p65-Specific siRNA Pretreatment on NMB-Induced [Ca$^{2+}$i] in Pregnant Myometrial Cells**

A remarkable difference was observed in the levels of average or maximum [Ca$^{2+}$i] in pregnant SMCs among cultures treated with $10^{-8}$ M $~ 10^{-4}$ M NMB jointly with extracellular calcium ($P < 0.01$). The differences were also shown between the joint group with the NMB or extracellular
calcium-alone treatment group or 10 \textsuperscript{-10} M joint group (P < 0.01, respectively), but no differences were found between NMB- or CaCl\textsubscript{2}-alone treated groups (P > 0.05) (Fig. 4A). These findings demonstrate the role of NMB in inducing the increase of [Ca\textsuperscript{2+}]\textsubscript{i} in pregnant SMCs relies on the presence of extracellular calcium.

The levels of [Ca\textsuperscript{2+}]\textsubscript{i} in Nmbr siRNA+ NMB (10 \textsuperscript{-10} M \sim 10 \textsuperscript{-4} M) treated groups were lower than that of the control group and that in same concentration of NMB before siRNA pretreatment groups, respectively (P < 0.05). No significant differences were identified among the four groups by siRNA pretreatment (P > 0.05). The [Ca\textsuperscript{2+}]\textsubscript{i} in Rela/p65 siRNA+ NMB (10 \textsuperscript{-10} M \sim 10 \textsuperscript{-4} M) groups were lower than that in the control group and that in the same concentrations of NMB groups before siRNA pretreatment, respectively (P < 0.05). No significant differences were identified among the four groups pretreated by siRNA (P > 0.05) (Fig. 4, B and C). However, significant differences were observed in [Ca\textsuperscript{2+}]\textsubscript{i} between the Rela/p65-specific and Nmbr-specific siRNA-treated groups (P < 0.05) (Fig. 4, B and C). Therefore, these findings showed that NMB (10 \textsuperscript{-8} M \sim 10 \textsuperscript{-4} M) jointly with extracellular calcium can dramatically increase the levels of [Ca\textsuperscript{2+}]\textsubscript{i}, and this role is impaired by Nmbr- or Rela/p65-specific siRNA in pregnant smooth muscle cells, but this regulating role is unbalanced between Nmbr and Rela/p65.

**Correlations Between the NMB-Induced DNA Binding Activity of Rela/p65, Il6 mRNA Levels, and [Ca\textsuperscript{2+}]\textsubscript{i} in Smooth Muscle Cells Pretreated with Nmbr-Specific siRNA**

Positive correlations were identified between the DNA binding activity of Rela/p65 and Il6 mRNA levels (r = 0.952; P < 0.01) and between [Ca\textsuperscript{2+}]\textsubscript{i} and the DNA binding activity of Rela/p65 and levels of Il6 mRNA (r = 0.278; P < 0.05; and r = 0.293; P < 0.05, respectively) induced by a different concentration gradient of NMB (10 \textsuperscript{-10} M \sim 10 \textsuperscript{-7} M) with joint extracellular calcium. However, this regulating relationship between levels of Rela/p65 and Il6 is not observed after Nmbr-specific siRNA treatment was performed. Thus, these findings not only reveal the parallel relationship in the regulation of Rela/p65 and Il6 induced by NMB but also provide evidence that the induction of Il6 by NMB up-regulated in the cultured SMCs within passage 4 from the pregnant myometrium requires the Rela/p65 signaling pathway and extracellular calcium.

**Correlations Between NMB-Induced [Ca\textsuperscript{2+}]\textsubscript{i}, and Il6 mRNA Levels and in SMCs Pretreated with Rela/p65-Specific siRNA**

No correlations were identified between NMB-induced [Ca\textsuperscript{2+}]\textsubscript{i}, and levels of Il6 mRNA in SMCs pretreated with Rela/p65-specific siRNA (P > 0.05). Thus, these results suggested that the up-regulation of [Ca\textsuperscript{2+}]\textsubscript{i} induced by NMB via inflow from extracellular calcium is not in line with the regulation of Il6 and can cross-talk with other pathways.

**Comparison of the Influences of siRNA on Nmbr and Rela/p65**

In NMB-Induced Il6 and [Ca\textsuperscript{2+}]\textsubscript{i}, Levels in SMCs

No significant differences were detected between the effects of siRNA on Nmbr and Rela/p65 on the levels of Il6 mRNA (P > 0.05) (Fig. 3A). However, significant differences were observed in [Ca\textsuperscript{2+}]\textsubscript{i}, between the Rela/p65-specific and Nmbr-specific siRNA-treated groups (P < 0.05) (Fig. 4B). Overall, these results strongly indicate that regulation of Il6 and [Ca\textsuperscript{2+}]\textsubscript{i}, induced by NMB do not occur in the single signal pathway and can cross-talk with other pathways.

**DISCUSSION**

Investigation of the molecular mechanisms involved in the Nmb/Nmbr interaction in myometrial contraction in vitro is currently limited by the lack of availability of a suitable model cell line. However, primary cell cultures have been shown to retain morphological and functional characteristics [27].
Previous studies in vivo have shown that the expression level of \( \text{Nmbr} \) reached a peak at term and decreased sharply after labor [11]. Thus, this study was conducted using primary cultures of myometrial primary cells isolated at the onset of labor immediately after parturition of the first neonate or in the interval between delivery of two pups. Standard immunohistochemical staining techniques were used to demonstrate \( \alpha_\text{-actin} \) and \( \text{Nmbr} \) expression, thus confirming the myogenic origin of cultured cells. Furthermore, morphological characteristics and expression of NMBR were shown to be retained for at least four generations. Therefore, this primary myometrial cell culture constitutes a suitable model of the onset of labor for the investigation of the role and mechanism of the \( \text{Nmbr}/\text{Nmbr} \) interaction in delivery, using RNA interference (RNAi) technology for the identification of genes involved in the regulation of this interaction.

The use of this model in this study revealed that \( \text{Rela/p65} \) and \( \text{Il6} \) levels induced by the \( \text{Nmb} \) agonist NMB were increased in a concentration-dependent manner jointly with extracellular calcium. This is in accordance with previous reports of \( \text{Nmb} \) agonist-mediated up-regulation of \( \text{Rela/p65} \) and \( \text{Il6} \) expression in a mouse model of pregnancy in addition to the down-regulatory role of \( \text{Nmbr} \) antagonists [28]. Increased DNA binding activity of \( \text{Rela/p65} \) is regarded as the key step in the onset of labor [18]. Moreover, the up-regulation of \( \text{Rela/p65} \) activity enhances gene transcription of proinflammatory cytokines including \( \text{Il6} \) and \( \text{Il8} \) [19–21], which are closely associated with the contraction of uterine smooth muscle and dilation of the cervix [22–24]. This study demonstrated that the regulatory role of the \( \text{Nmbr}/\text{Nmbr} \) interaction on \( \text{Rela/p65} \) and \( \text{Il6} \) expression is blocked by siRNA-mediated \( \text{Nmbr} \) knockdown. Furthermore, this effect on \( \text{Il6} \) expression was also blocked by \( \text{Rela/p65} \) silencing. It was noted that both \( \text{Nmb} \) and \( \text{Rela/p65} \) knockdown resulted in similar inhibition of \( \text{Il6} \) expression at the mRNA and protein level. Mouse and human \( \text{Nmbr} \) have been shown to be coupled to phospholipase C, resulting in a breakdown of phosphoinositides, mobilization of cellular calcium, and activation of protein kinase C [13–16]. Gastrin-releasing peptide (GRP) activation of its receptor (GRPR), another bombesin-like peptide and receptor, has also shown to result in PKC activation and to elicit increased \( [\text{Ca}^{2+}]\text{i} \), in cancer and neuroendocrine cells [16–17]. The activity of \( \text{Nmbr} \) in BALB/3T3 cells is governed by PKC activation [13], which is known to activate \( \text{Rela/p65} \)-mediated regulation of the expression of proinflammatory genes during the onset of labor [15]. These data suggest that the \( \text{Nmb}/\text{Nmbr} \) interaction regulates \( \text{Il6} \) expression via the \( \text{Rela/p65} \) pathway in primary cultured myometrial cells from pregnant mice.

In this study, \( [\text{Ca}^{2+}]\text{i} \), was induced by NMB with extracellular calcium jointly pretreated in a concentration-dependent manner that correlated with \( \text{Rela/p65} \) activity and \( \text{Il6} \) expression. However, this role can be reduced in the absence of extracellular calcium, and differences in the levels of \( [\text{Ca}^{2+}]\text{i} \), were not identified among the different concentration gradients of NMB groups. Moreover, the up-regulation of \( [\text{Ca}^{2+}]\text{i} \), by NMB was remarkably diminished by \( \text{Rela/p65} \)-specific and \( \text{Nmbr} \)-specific siRNA treatment. The significant differences were also noticed in levels of \( [\text{Ca}^{2+}]\text{i} \), in \( \text{Rela/p65} \)-specific and \( \text{Nmbr} \)-specific siRNA-treated groups. A higher inhibition level of \( [\text{Ca}^{2+}]\text{i} \), was detected in response to \( \text{Rela/p65} \)-specific knockdown than to \( \text{Nmbr} \)-specific knockdown. These results suggest that the NMB elicit increased \( [\text{Ca}^{2+}]\text{i} \), depending on the joint presence of extracellular calcium via PKC, and it may involve other pathways in \( \text{Nmbr} \)-mediated effects on myometrial cell activity. Related studies have shown that \( \text{Nmbr} \) activation stimulates phospholipase A2 and phospholipase D via both PKC-dependent and independent mechanisms [29]. Phospholipase A2 activity is a crucial enzyme in the regulation of the production and release of prostaglandin, which induces myometrium contraction and cervix ripening [29, 30]. Moreover, \( \text{Nmbr} \) stimulation also results in activation of tyrosine kinases and tyrosine phosphorylation of p125FAK by a phospholipase C-independent mechanism which requires p21 and the integrity of the actin cytoskeleton [31]. \( \text{Nmbr} \) activation also stimulates tyrosine phosphorylation of paxillin and MAP kinase activation [32]. Moreover, native and transfected rat \( \text{Nmbr} \) exhibit similar binding and signaling characteristics in BALB/3T3 cells [13]. These results have clearly demonstrated that \( \text{Nmbr} \) is involved in parturition via a number of signaling pathways in addition to its function in the regulation of the nervous system [33–37].

In conclusion, this study of the molecular mechanisms of parturition has demonstrated that the \( \text{Nmb}/\text{Nmbr} \) interaction influences the activity of myometrial primary cells in vitro predominantly through regulation of \( \text{Il6} \) expression via the \( \text{Rela/p65} \) pathway. Furthermore, data also indicate that the effects of \( \text{Nmbr} \) on the \( [\text{Ca}^{2+}]\text{i} \), involve the joint presence of extracellular calcium, but those effects involving transmitted pathways remain to be elucidated.

REFERENCES


