JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2020, 71, 2, 257-264
www.jpp.krakow.pl | DOI: 10.26402/jpp.2020.2.10

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THE POTENTIAL IMPLICATION OF NEUROKININ B IN THE MODULATION OF PROLACTIN SECRETION AT THE PITUITARY LEVEL IN CYCLIC GILTS

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Based on the previous studies, neurokinin B (NKB) participation in the modulation of prolactin secretion at the pituitary level can be assumed, but information concerning this topic is largely insufficient. Therefore, in the present study, we aimed: 1) to evaluate changes in the expression of NKB precursor (*Tac3*) and its receptor (*Tacr3*) genes as well as the content of NKB and TACR3 proteins in the porcine anterior pituitary throughout the estrous cycle (days 2 – 3, 9 – 10, 12 – 13, 15 – 16, 19 – 20); 2) to determine *in vitro* the influence of NKB on the expression of *Prl*, *D2r* and *Trhr* genes in the anterior pituitary cells (incubated for 4 h) as well as on prolactin secretion by these cells (incubated for 4 and 24 h) during chosen days of the estrous cycle (9 – 10, 15 – 16, 19 – 20). The experiments have shown alterations in the expression of *Tacr3* mRNA and TACR3 protein content, but not in *Tac3* mRNA and NKB protein. The treatment with NKB stimulated the expression of *Prl* (days 15 – 16), *D2r* (days 9 – 10) and *Trhr* (days 19 – 20) genes, but its potential to modulate prolactin secretion was observed only following 24-h incubation, specifically inhibition by NKB alone and stimulation by NKB with dopamine on days 19 – 10 of the cycle. These results indicate some implications of NKB in the modulation of prolactin secretion at the pituitary level in cyclic pigs, however further experiments are required to better clarify its role in this process.

Key words: *neurokinin B, prolactin, estrous cycle, tachykinin receptor, anterior pituitary, dopamine, thyrotropin-releasing hormone*

INTRODUCTION

Neurokinin B (NKB) is a decapeptide that belongs to the tachykinin peptide family, along with neurokinin A (NKA), substance P (SP), neuropeptide K (NPK) and neuropeptide γ (NPγ). NKB is a product of preprotachykinin-B gene (*Tac3*) expression and preferentially exerts its action on target cells through subtype 3 of tachykinin receptors (TACR3) (1). At the hypothalamic level, NKB - besides kisspeptin and dynorphin - is an important component of the neural KNDy system, located in the arcuate nucleus, which controls an activity of gonadotropin-releasing hormone (GnRH) neurons (2-4). In addition to robust expression of NKB and its receptor in hypothalamus (5, 6), several studies documented the presence of their mRNAs and/or proteins in peripheral reproductive tissues, such as: ovary (7, 8), uterus (9) and oviduct (10, 11). The expression of NKB and/or TACR3 has been demonstrated in the anterior pituitary gland of ewes (12), rats (13), pigs (14) and fish (15). Importantly, TACR3 receptors have been also found on PRL-secreting GH3 cells (16). These data imply a potential participation of NKB in the modulation of secretory activity of the anterior pituitary gland.

Prolactin is one of the anterior pituitary hormones, produced by the lactotroph cells, which is involved in the regulation of numerous functions in different species, including those connected with reproductive processes. In females, the ovarian physiology is primarily controlled by pituitary hormones, gonadotropins and prolactin. Depending on stage of the porcine estrous cycle, prolactin acts as a luteotropic or luteolytic factor and modulates secretion of estrogens (17). In mature gilts, prolactin concentration in plasma undergoes specific alterations throughout the estrous cycle. Namely: (i) during the luteal phase it is maintained at comparatively low level, (ii) at the turn of luteal and follicular phases a few peaks correlated with $PGF_{2\alpha}$ surges are observed and (iii) in the periovulatory period a significant rise in prolactin concentration appears (18, 19). Due to pleiotropic nature and wide range of prolactin functions, its secretion by the anterior pituitary is submitted to more complex regulation than other pituitary hormones (20). Basal prolactin secretion by the lactotroph cells is relatively high, therefore it predominantly remains under inhibitory influence of hypothalamic dopamine (21). The stimulatory effect on prolactin release from lactotrophs is exerted by thyrotropin-releasing hormone (TRH) and at lower extent by oxytocin, vasoactive intestinal peptide (VIP) and estrogens (22, 23). In addition, several other factors - *e.g.* opioid peptides, kisspeptins and tachykinins - have been considered as modulators of prolactin secretion (24-26), however the mechanism of their action in different species still remains to be elucidated.

For many years, SP has been most extensively studied member of tachykinin family in relation to its potential role in the regulation of prolactin secretion (27). Recently, the changing expression of NKA precursor (*Ppt-a*) and its receptor (*Tacr2*)

mRNAs as well as NKA and TACR2 proteins in the porcine pituitary tissue throughout the estrous cycle has been found. Moreover, the *in vitro* effects of NKA on the expression of prolactin (*Prl*), dopamine receptor (*D2r*) and TRH receptor (*Trhr*) in the porcine pituitary cells and prolactin secretion by these cells on different days of the cycle have been described (28). Up to now, there are only few studies supporting NKB participation in the modulation of prolactin secretion. Henriksen *et al.* (13) demonstrated an increase in prolactin secretion by the anterior pituitary cells of female rats in the presence of NKB. Studies utilizing GH3 cell line revealed a stimulatory effect of NKB on the promoter of prolactin gene only in the presence of TRH (16). Furthermore, experiments performed on the grass carp model have also proved that NKB could upregulate *Prl* mRNA expression and prolactin secretion from pituitary cells (15).

Based on the quoted studies, it can be hypothesized that NKB is implicated in the modulation of prolactin secretion at the pituitary level in female pigs. It should be added that notably differentiated profile of prolactin secretion in cyclic pigs - as stated earlier - makes this animal model appropriate for our study. Therefore, we have undertaken an attempt: 1) to evaluate the expression of *Tac3* and *Tacr3* genes as well as NKB and TACR3 proteins in the porcine anterior pituitary on various days of the estrous cycle, representing luteal and follicular phases (Experiment I) and 2) to determine whether NKB can affect the expression of *Prl*, *D2r* and *Trhr* genes in the porcine pituitary cells as well prolactin secretion by these cells on selected days of the estrous cycle, reflecting specific changes in prolactin secretion (Experiment II).

MATERIAL AND METHODS

Animals and pituitary collection

The experiments were performed in accordance with the principles and procedures of the Animal Ethics Committee of the University of Warmia and Mazury in Olsztyn, Poland (82/2012/DTN).

In the first part of the study (Experiment I), anterior pituitaries were harvested from mature cross-bred (Large White × Polish Landrace, 90 – 110 kg) gilts on days 2 – 3, 9 – 10, 12 – 13, 15 – 16 and 19 – 20 of the estrous cycle (n = 5 per each studied group). The animals were selected from different litters and housed in the same breeding area with unlimited access to food and water. Symptoms of estrus were determined in the presence of intact boar and the beginning of their appearance in gilts was defined as day 0 of the estrous cycle. Two consecutive cycles were verified and pigs in the third estrous cycle were designated for the study (29). Collected pituitaries were frozen in liquid nitrogen, transported from the local slaughterhouse to the laboratory and stored at –80ºC until further analytical procedures; *i.e.* total RNA isolation and cDNA synthesis (to assess the expression of *Tac3* and *Tacr3* genes) and for preparation of homogenates (to determine concentration of NKB and TACR3 proteins).

In the second part of the research (Experiment II), pituitaries were isolated from gilts (weighting approx. 100 kg), slaughtered in the commercial abattoir during three chosen periods of the estrous cycle: 9 – 10 (mid-luteal phase), 15 – 16 (late luteal phase/early follicular phase) and 19 – 20 (late follicular phase). The stages of the estrous cycle were confirmed based on the ovarian morphology according to Akins and Morrissette (30). Collected pituitaries were sliced into smaller pieces, placed in ice-cold Dulbecco's Modified Eagle Medium (Sigma Aldrich, St. Louis, MO, USA) supplemented with antibiotics and transported to the laboratory for the anterior pituitary cell isolation.

The in vitro culture of anterior pituitary cells

The isolation of anterior pituitary cells was performed under sterile conditions according to the procedure described by Bogacka *et al.* (31) with some modifications previously published (28, 32, 33). The isolated pituitary cells were used in two concentrations: 2×10^6/2 ml medium (McCoy's 5A; Sigma Aldrich, St. Louis, MO, USA) for analysis of specific RNAs (n = 4) and 3×10^5/1 ml medium for determination of prolactin secretion (n = 5). The cells were pre-incubated for 72 h at 37ºC under controlled atmosphere, 5% CO_2 and 95% air. Following 48 h of pre-incubation, 1 ml fresh medium without serum was added to each well and at the end of pre-incubation all media were replaced by medium with the following factors: NKB (10^{-9}, 10^{-8} and 10^{-7} M) alone - for analysis of specific RNAs, but in the case of prolactin determination in culture media - NKB (10^{-9}, 10^{-8} and 10^{-7} M), dopamine (10^{-7} M) and TRH (10^{-7} M) alone, as well as NKB (10^{-9}, 10^{-8} and 10^{-7} M) in combination with dopamine (10^{-7} M) or TRH (10^{-7} M).

The cell incubation was essentially performed for 4 h, whereas in the case of prolactin secretion - the incubation was additionally extended to 24 h to observe effects of long-lasting treatments. Cells incubated without any treatment were considered as respective controls. Studied doses of experimental factors (purchased from Tocris Bioscience and BioChemika) were chosen based on the previous research (16, 26, 28). Media collected after cell incubation were stored at –20ºC for prolactin determination, whereas the cells placed in TRIZOL reagent (750 µl per well) were stored at –80ºC for analysis of mRNAs.

Total RNA isolation and reverse transcription

Isolation of total RNA from pituitary tissue and pituitary cells after *in vitro* culture was performed using RNeasy Mini Kit (Qiagen, Germantown, MA, USA) and TRIZOL reagent, respectively. The quality and quantity of RNA were spectrophotometrically measured using NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized in reverse transcription reaction using QuantiTect Reverse Transcription Kit (Qiagen, Germantown, MA, USA). Briefly, 1 µg of isolated RNA, 2 µl of gDNA Wipeout Buffer and 14 µl of RNase-free water were added to the reaction tube and incubated for 2 min at 42ºC. Then, 1 µl of Quantiscript Reverse Transcriptase, 4 µl of Quantiscript RT buffer (diluted × 5) and 1 µl of RT primers were added to each sample and incubated for 15 min at 42ºC and subsequently for 3 min at 95ºC.

Quantitative real-time PCR

The expression of *Tac3* and *Tacr3* genes in pituitary tissue as well as *Prl*, *D2r* and *Trhr* genes in pituitary cells was assessed by real-time PCR method using 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture included 10 ng cDNA, specific forward and reverse primers, 12.5 µl SYBR Green PCR Master Mix (Life Technologies, Carlsbad, CA, USA) and RNase-free water addition in amount ensuring the final mixture volume 25 µl/well. The real-time PCR protocol started with initial denaturation (10 min, 95ºC) followed by 40 cycles, each including three steps: denaturation (15 s, 95ºC), primer annealing (60 s, at specific temperatures presented in *Table 1*) and elongation (60 s, 72ºC). At the end of each PCR reaction, melting curve analysis was acquired to confirm the specificity of amplified products. Negative control, containing nuclease-free water instead of cDNA template, was performed in each assay. Relative expression of examined genes was analyzed with $\Delta\Delta C_T$ method (34) and normalized using mean values for the expression of *Gapdh* and *Actb* reference genes.

Table 1. Characteristics of real-time PCR primers used for gene expression analysis. Studied genes and their products: *Tac3* - preprotachykinin-B; *Tacr3* - tachykinin receptor 3; *Prl* - prolactin; *D2r* - dopamine D2 receptor; *Trhr* - thyrotropin-releasing hormone receptor; *Gapdh* - glyceraldehyde-3-phosphate dehydrogenase; *Actb* - β-actin.

Gene symbol	Forward (F) Reverse (R) primer sequence; 5'→ 3'	Concentration of primers (nM)	Amplicon length (bp)	T_A (°C)	Accession number	Reference
Tac3	**F**: GTTGCTCCGGAGACTCTGTG **R**: TGATTCCTTAGCACCCACGC	400	91	63	NM_001007196.1	(Present study)
Tacr3	**F**: GCCAACTACTGTCGCTTCCA **R**: CCATATACCTGTCCACCGCAA	400	94	61	XM_003482466.3	(Present study)
Prl	**F**: ATAGTCGGCCAGGTCCATC **R**: GTAGGCAGTGGAGCAGGTTA	400	130	61	NM_213926.1	(35)
D2r	**F**: GCATCGACAGGTACACAGC **R**: GGCAGGAGATGGTGAAAGAC	400	120	61	NM_001244253.1	(35)
Trhr	**F**: TCCACCAAGTAGGTATGGGAGA **R**: TTGCCATCAGCTAAGGAGGG	400	75	61	NM_001177488.1	(28)
Gapdh	**F**: CCTTCATTGACCTCCACTACATGGT **R**: CCACAACATACGTAGCACCACGAT	500	183	59	NM_001206369.1	(36)
Actb	**F**: ACATCAAGGAGAAGCTCTGCTACG **R**: GAGGGGCGATGATCTTGATCTTC	500	366	61	U07786.1	(37)

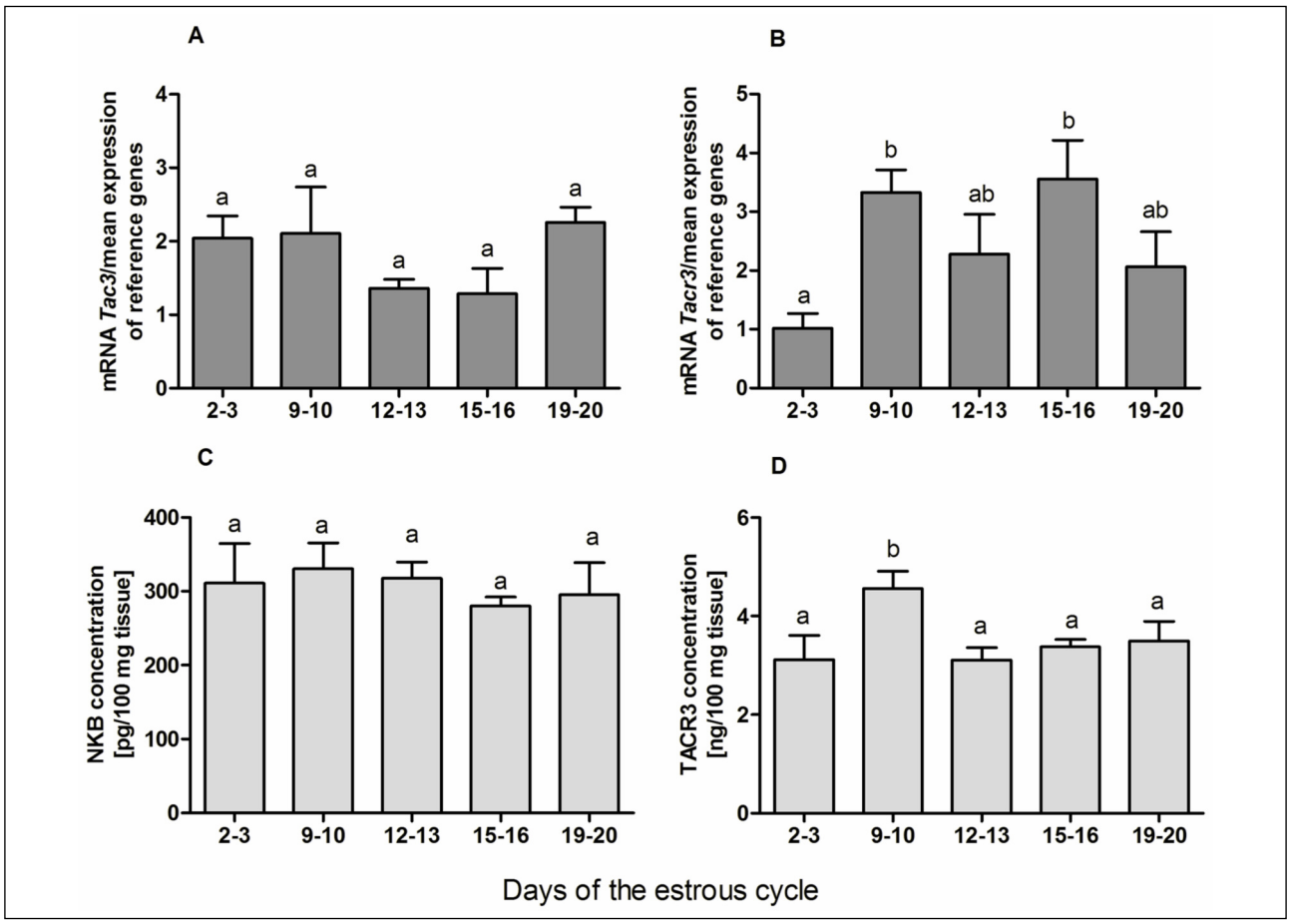

Fig. 1. Changes in the expression of *Tac3* and *Tacr3* mRNA (A, B; dark grey bars) and concentrations of NKB and TACR3 (C, D; light grey bars) proteins in the anterior pituitary of gilts during the estrous cycle. Data are presented as means ± SEM, n = 5 for each studied period. Various letters indicate significant differences ($P < 0.05$).

Enzyme-linked immunosorbent assay

The evaluation of NKB and TACR3 protein concentration in the anterior pituitary of gilts throughout the estrous cycle was performed using enzyme-linked immunosorbent assay (ELISA). The commercial porcine NKB and TACR3 ELISA kits (Cat. No: MBS037835 and MBS2500898, respectively; MyBioSource, San Diego, CA, USA) were used according to the manufacturer's instructions and the absorbance values were measured at 450 nm using Infinite M200 Pro Reader with the Tecan i-control software (Tecan, Switzerland). The data were linearized by plotting the protein concentration versus the optical

density. Results are shown on the graphs as pg (NKB) or ng (TACR3) per 100 mg tissue. Detection ranges were: 15.6 – 500 pg/ml for NKB and 0.16 – 10 ng/ml for TACR3, whereas the assay sensitivities were 5.0 pg/ml and 0.19 ng/ml for NKB and TACR3, respectively. The intra-assay coefficients of variation for conducted analyses were less than 5%.

Radioimmunoassay of prolactin

Concentration of prolactin was determined in media, collected after cell culture, by radioimmunoassay (RIA) using a rabbit primary antibodies against porcine prolactin (Harbor UCLA Medical Centre, Torrance, CA, USA) and secondary precipitating antibodies (38) according to the procedure described by Dusza and Krzymowska (18). Porcine prolactin purchased from A.F. Parlow (Harbor UCLA Medical Centre, Torrance, CA, USA) was used for preparation of standards and for labeling with I^{125} (Perkin Elmer, Waltham, MA, USA). Intra- and inter-assay coefficients of variation were less than 5% and 10%, respectively and the analytical sensitivity of the assay was 0.039 ng per sample.

Statistical analysis

Statistical analysis of the results was performed using Statistica 12.0 software (StatSoft Inc., Tulsa, OK, USA). The one-way ANOVA with subsequent NIR Fisher's *post-hoc* test was used to compare the expression of genes and proteins in pituitary tissue, whereas the effects of NKB on the expression of studied genes in pituitary cells and prolactin secretion by these cells were analyzed by one-way ANOVA followed by Dunnett's *post-hoc* test. Data are presented as means ± SEM and values with $P < 0.05$ were considered to be statistically significant.

RESULTS

The expression of Tac3 and Tacr3 mRNAs and neurokinin B and neurokinin B receptor proteins in the porcine anterior pituitary during the estrous cycle

No significant changes in the expression of *Tac3* gene were observed in porcine anterior pituitary throughout the estrous cycle (*Fig. 1A*). In turn, the expression of *Tacr3* gene was decreased ($P < 0.05$) on days 2 – 3 in comparison to values on days 9 – 10 and 15 – 16 of the estrous cycle (*Fig. 1B*). The highest mean value of *Tacr3* gene expression was noted in the late luteal/early follicular phase of the estrous cycle (days 15 – 16). In turn, the NKB protein concentration in the anterior pituitary ranged from 280.28 ± 11.99 to 330.45 ± 35 pg/100 mg tissue and did not vary significantly between studied days of the estrous cycle (*Fig. 1C*), whereas TACR3 protein concentration fluctuated within the range 3.10 ± 0.26 – 4.56 ± 0.35 pg/100 mg tissue and was the highest ($P < 0.05$) on days 9 – 10 of the estrous cycle in comparison to other examined periods of the estrous cycle (*Fig. 1D*).

Changes in the expression of Prl, D2r and Trhr genes in the porcine pituitary cells in response to neurokinin B

The influence of NKB on the expression of studied genes was dependent on the phase of the estrous cycle as well as examined dose of this neuropeptide. The expression of *Prl* gene was stimulated ($P < 0.05$) by NKB at concentration 10^{-9} M only on days 15 – 16 of the estrous cycle (*Fig. 2A*). Significant increase ($P < 0.05$) in the expression of *D2r* mRNA was observed on days 9 – 10 of the estrous cycle following NKB treatment at concentration 10^{-9} M (*Fig. 2B*). In turn, evident stimulatory ($P < 0.05$) effect of NKB (at concentrations 10^{-8} and 10^{-7} M) on the expression of *Trhr* gene was noted during the late follicular phase of the estrous cycle (*Fig. 2C*).

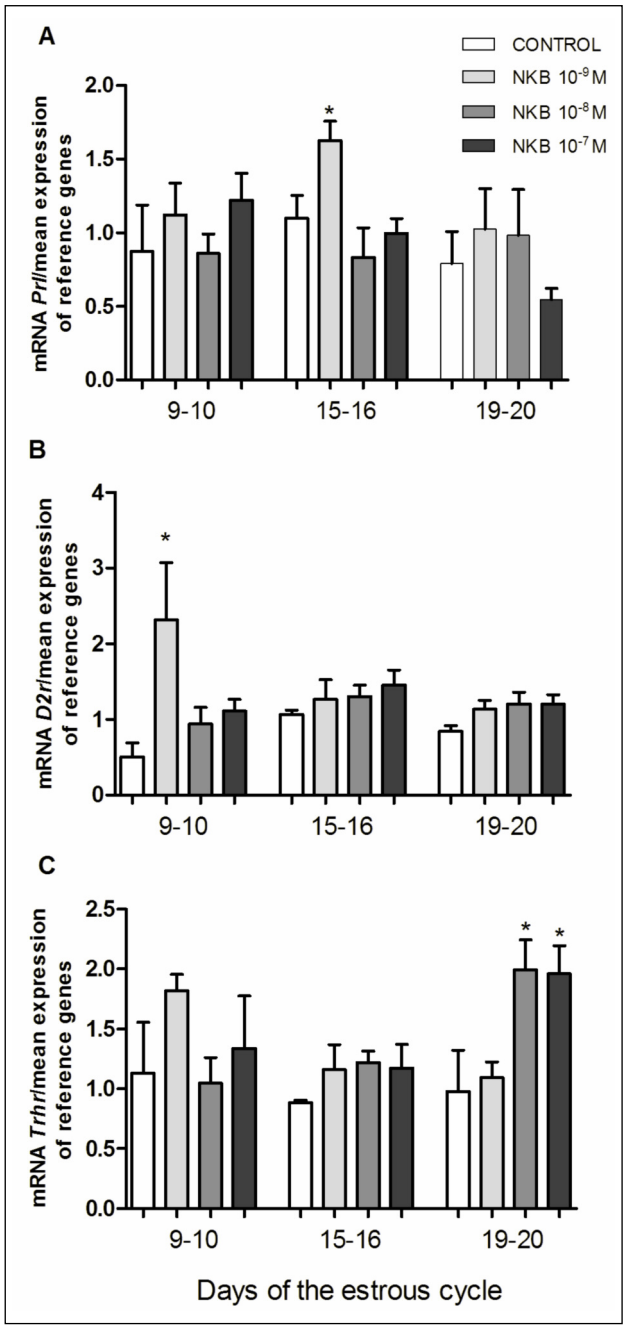

Fig. 2. The effect of NKB (10^{-9}, 10^{-8} and 10^{-7} M) on the expression of *Prl* (A), *D2r* (B) and *Trhr* (C) mRNAs in the porcine pituitary cells following 4-h incubation on days 9 – 10, 15 – 16 and 19 – 20 of the estrous cycle. Data are presented as means ± SEM, n = 4 for each studied period. Significant differences in comparison to the respective control value are marked with asterisks ($P < 0.05$).

The influence of neurokinin B in vitro on prolactin secretion by the porcine pituitary cells

Neurokinin B alone and in combination with dopamine or TRH had no effect on prolactin secretion by anterior pituitary

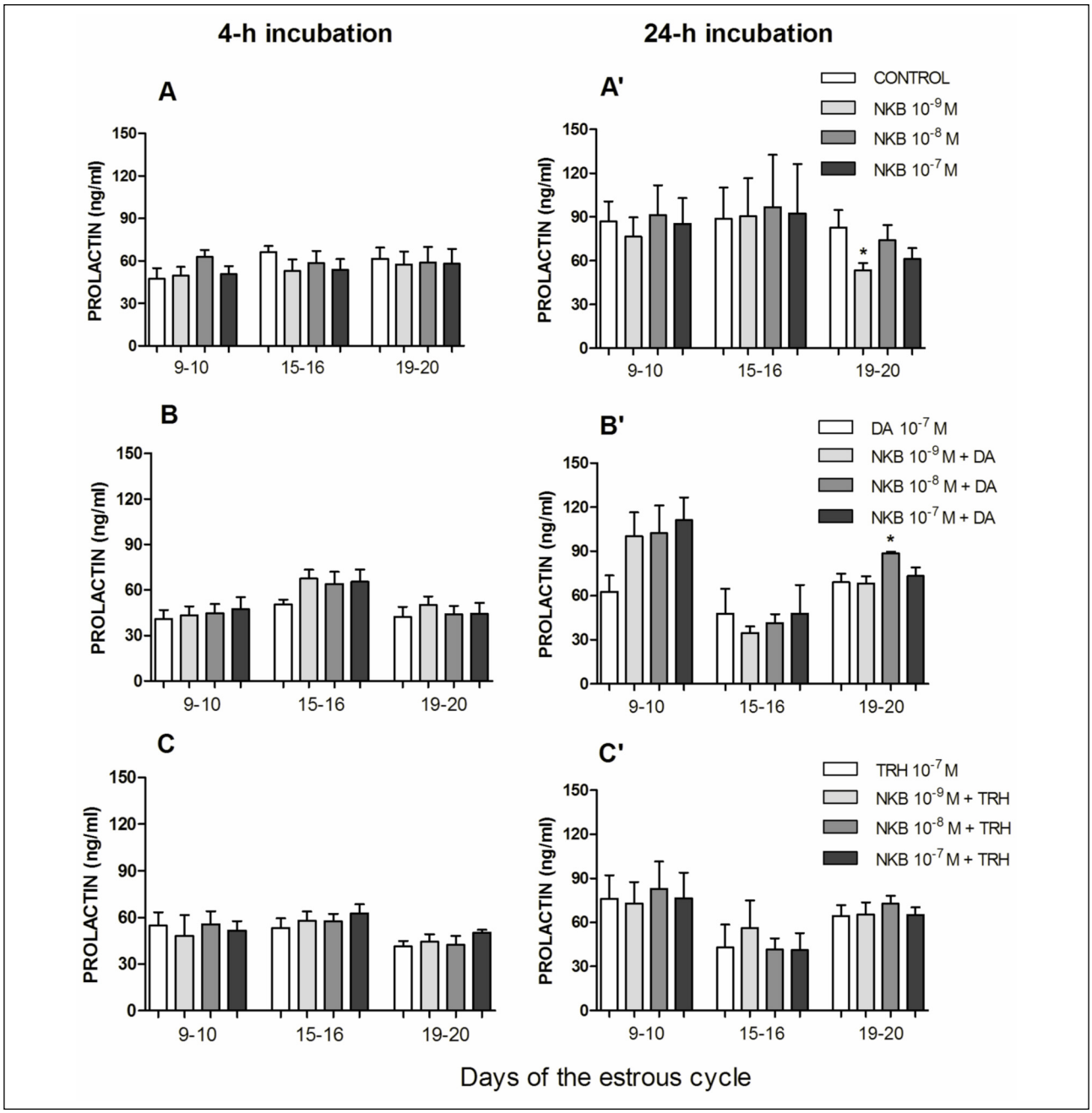

Fig. 3. The effect of NKB (10^{-9}, 10^{-8} and 10^{-7} M) alone (A, A') and in combination with dopamine (DA; B, B') or TRH (C, C') on prolactin secretion by the porcine pituitary cells on days 9 – 10, 15 – 16 and 19 – 20 of the estrous cycle after 4-h (left panel: A, B, C) and 24-h (right panel: A', B', C') incubations. Data are presented as means ± SEM, n = 5 for each studied period. Significant differences in comparison to the respective control value are marked with asterisks ($P < 0.05$).

cells on any studied days of the estrous cycle following 4-h incubation (*Fig. 3A-3C*). In turn, following 24-h incubation, NKB alone at concentration 10^{-9} M decreased ($P < 0.05$) prolactin secretion by the pituitary cells on days 19 – 20 of the estrous cycle (*Fig. 3A'*). In contrast, NKB (10^{-8} M) in combination with dopamine (10^{-7} M) stimulated ($P < 0.05$) prolactin secretion on days 19 – 20 of the cycle (*Fig. 3B'*). Prolactin concentration in culture media was not affected at all in response to co-treatment with NKB and TRH (*Fig. 3C'*). In addition, it should be noted that dopamine alone (B/B') decreased prolactin secretion in comparison to respective controls (A/A'), whereas TRH alone was not effective in stimulating this hormone secretion.

DISCUSSION

Previous studies have suggested an influence of NKB on the activity of tuberoinfundibular dopaminergic (TIDA) pathway and prolactin secretion (39-40), however its engagement in the modulation of anterior pituitary secretory activity is not sufficiently elucidated (13, 27). The undertaken studies aimed to evaluate the modulatory potential of NKB in relation to prolactin secretion *in vitro* by the porcine anterior pituitary cells. They revealed some changes that occur in the pituitary NKB system throughout the estrous cycle in gilts. These studies also delineated an influence of NKB on the expression of *Prl*, *D2r*

and *Trhr* genes in the pituitary cells and its limited potential to affect prolactin secretion by these cells.

First of all, the presence of *Tac3* and *Tacr3* mRNAs as well as NKB and TACR3 proteins in the anterior pituitary tissue of cyclic gilts has been found in the current study. Previously, *Tacr3* mRNA has also been identified in the porcine pituitary by Jakimiuk *et al.* (14). Moreover, the expression of *Tac3* and *Tacr3* genes was detected in the central nervous system (14, 41) as well as in peripheral reproductive tissues in pigs (10, 42). Interestingly, the amounts of NKB and TACR3 proteins in the porcine anterior pituitary appeared to be lower than NKA and its receptor (TACR2) proteins (28). The average contents of NKB and TACR3 were approximately 50% and 80% of those established for NKA and TACR2, respectively. In contrast to TACR2 receptors, which have been detected on male rat lactotrophs (43), there is no information in literature concerning cellular distribution of TACR3 in the anterior pituitary gland.

In the present study, the expression of *Tac3* gene and NKB protein in the anterior pituitary did not significantly fluctuate during the estrous cycle. Surprisingly, it seems that steroidogenic milieu typical of various phases of the cycle did not affect above parameters. Conversely, differentiated expression of NKB precursor was observed by Acuna *et al.* (10) in porcine oviduct during the estrous cycle, which was upregulated in the preovulatory phase, but downregulated in the luteal phase. In addition, in our previous study (28), the expression of *Ppt-a* mRNA and NKA has demonstrated some changes in the pituitary tissue of cyclic gilts. In turn, the expression of *Tacr3* mRNA and TACR3 protein in the porcine pituitary has shown some alterations during the estrous cycle, encompassing increased expression of *Tacr3* gene on days 9 – 10 and 15 – 16 and the highest TACR3 protein content on days 9 – 10 of the cycle. Herein, it might be mentioned that changes in the expression of NKB receptor in the anterior pituitary have not been studied yet. For comparison, in our previous study, the highest concentration of TACR2 was also noted on days 9 – 10, whereas that of *Tacr2* mRNA was increased on days 2 – 3 and 9 – 10 of the estrous cycle (28). The above data collectively imply that expression of tachykinin receptors (both TACR2 and TACR3) in the pituitary is influenced by ovarian steroids, as it has been stated for hypothalamus (44, 45). On the basis of present results, it might be generally assumed that the impact of NKB on secretory activity of the anterior pituitary in cyclic pigs mainly depends on an abundance of its receptors. In addition, it has to be mentioned that final effect of any secretagogue action on the release of particular pituitary hormones also depends on the intracellular events taking place in target cells (46).

In the next step of the study, NKB selectively affected the expression of *Prl*, *D2r* and *Trhr* genes in the porcine anterior pituitary cells *in vitro*, depending on its concentration and stage of the estrous cycle. Only the lowest concentration of NKB (10^{-9} M) increased the expression of *Prl* and *D2r* on days 9 – 10 and 15 – 16 of the cycle, respectively. These effects indicate a potential participation of NKB in the induction of prolactin pulses occurring in the late luteal/early follicular phase as well as in the stabilization of prolactin plasma concentration at relatively low level during the luteal phase by stimulating the expression of *D2r* gene. The likelihood of such NKB action under physiological conditions might be supported by efficacy of the lowest its concentration (10^{-9} M). In our previous study, NKA at the lowest dose (10^{-9} M) similarly elevated *D2r* mRNA content in isolated pituitary cells representing the mid-luteal phase (28). Moreover, an influence of NKB on the expression of *Prl* gene has been also demonstrated in the pituitary cells of grass carp (15). The upregulation of *Prl* gene by NKB - observed in our study and reported by Hu *et al.* (15) - suggests that this neuropeptide may belong to a numerous group of prolactin-releasing factors, which exert their action under certain physiological conditions (20, 23). In addition, it was found that higher concentrations of NKB (10^{-8} and 10^{-7} M) stimulated the expression of *Trhr* gene in studied cells only on days 19 – 20 of the estrous cycle. Theoretically, based on this action of NKB, a participation of the peptide in evoking a periovulatory increase in prolactin secretion might be expected. In the earlier study, NKA upregulated *Trhr* gene in all considered periods of the cycle, however it did not significantly affect prolactin secretion (28). The presence of dopamine and TRH receptor on lactotrophs has been well documented (47, 48). Nevertheless, a question whether NKB - affecting the expression of *Prl*, *D2r* and *Trhr* genes - acts directly on lactotrophs (and/or in a paracrine manner) still remains open.

The effect of NKB on prolactin secretion by the porcine anterior pituitary cells *in vitro* was generally weak. NKB alone and in combination with dopamine or TRH did not significantly change prolactin concentration in media following 4-h incubation of the cells. Despite the stimulation of *Prl* gene expression by NKB (during 4-h incubation) on days 15 – 16 of the cycle, prolactin secretion was not concomitantly affected by this peptide, excluding insignificant elevations observed after co-treatment with dopamine. Possibly, the effect of NKB on prolactin secretion was not strong enough, therefore it would be masked by other pituitary factors acting in a paracrine way, *e.g.* endogenous opioid peptides (26). On the other hand, the effectiveness of NKB action might be limited due to a shortage of other co-factors under *in vitro* conditions, *i.e.* of hypothalamic or peripheral origin (13, 22, 25, 26). The extension of incubation from 4 to 24 h has resulted in a reduction and elevation of prolactin secretion by NKB alone and in connection with dopamine, respectively. In our previous study (28), NKA exerted similar effects - following 24-h incubation - on prolactin secretion by the porcine pituitary cells, as NKB did in the present experiment. Namely, NKA alone inhibited prolactin secretion (on days 19 – 10 of the cycle) and in co-treatments with dopamine - stimulated (on days 9 – 10), but with TRH - had no effect. In studies of Mau (49), SP (other tachykinin peptide) in combination with dopamine also effectively elevated prolactin secretion *in vitro* from the male rat lactotrophs. Although our observations suggest possible influence of NKB on prolactin secretion in cyclic pigs, further experiments are needed to better clarify its involvement in this process.

In summary, the present study has provided new information concerning potential implication of NKB in modulation of prolactin synthesis and/or secretion by the anterior pituitary in cyclic gilts. Some changes in the pituitary abundance of NKB receptor mRNA and protein have been revealed, while the tissue content of NKB peptide and its precursor mRNA remained unaltered throughout the estrous cycle. Moreover, NKB was capable of changing the *in vitro* expression of *Prl*, *D2r* and *Trhr* genes in the porcine pituitary cells on particular days of the estrous cycle. However, these effects have been only partially reflected in prolactin secretion *in vitro* in response to NKB alone and in the presence of dopamine. Collectively, our results indicate an involvement of NKB in the modulation of processes potentially connected with prolactin synthesis and/or secretion by the porcine anterior pituitary and suggest specific objectives of further studies. It seems that they should aim to elucidate cellular localization of NKB receptors in the anterior pituitary as well as conditions of its efficient action (*e.g.* effects of co-treatment with steroid hormones) on prolactin secretion in cyclic pigs.

Acknowledgments: This research was funded by National Science Centre, Poland (Project No. 2016/21/N/NZ9/02301). Pituitaries used in the *Experiment I* were collected in earlier study also supported by National Science Centre (Grant No. 2012/07/D/NZ4/04177).

Conflict of interest: None declared.

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Received: March 6, 2020
Accepted: April 30, 2020

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