Alpha adrenergic receptors are involved in the contractile activity of neuropeptide Y in the porcine isolated ovarian artery

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ABSTRACT: The objective of this study was to determine whether α-adrenergic receptors are involved in the contractile activity of neuropeptide Y (NPY) in the porcine isolated ovarian artery collected from immature pigs and from the animals on day 1–5, 8–13 and 17–20 of the estrous cycle. NPY increased ($P < 0.05$) blood pressure in preparations collected form the immature and mature pigs. NPY administration into prazosin pre-treated vessels increased ($P < 0.05$) blood pressure in preparations of the immature and mature animals with the highest potency in the vessels from days 17–20 of the cycle. Simultaneous methoxamine and NPY treatment caused an increase ($P < 0.05$) in blood pressure in the vessels from all the periods examined with the highest potency in the preparations from days 17–20 of the cycle. NPY administration at the time of the maximum response to rauwolscine increased ($P < 0.05$) blood pressure in the preparations from the immature and mature pigs with the highest changes in the preparations from days 17–20 of the cycle. In clonidine pre-treated ovarian arteries, NPY insignificantly increased ($P > 0.05$) blood pressure in the preparations collected from the immature pigs and on days 1–5, 8–13 of the cycle, and significantly increased ($P < 0.05$) blood pressure in preparations from the animals on days 17–20 of the oestrous cycle. The present study has revealed that NPY increases blood pressure in the isolated porcine ovarian artery and that α-adrenergic receptors are involved in the vosocontractile action of this peptide. Moreover, the changes in the blood pressure caused by NPY alone or administered after α-adrenergic receptor agonists or antagonists treatment are dependent on steroid hormone concentrations.

Keywords: neuropeptide Y; α-adrenergic agonists; α-adrenergic antagonists; norepinephrine; porcine ovarian artery; blood pressure

Since 1982, when extracts of two PP-like peptides named peptide YY (PPY) and neuropeptide Y (NPY) were isolated from the porcine brain and gut (Tatemoto *et al*., 1982), localisation and function of NPY has been widely examined in different organs of many mammalian species. NPY is a 36 amino acids molecule found in both the central and peripheral nervous system of mammals (Lundberg *et al*., 1982; Sienkiewicz *et al*., 1996). NPY, in addition to many other functions, participates in the regulation of blood pressure (Cortes *et al*., 1999; Yang and Chiba, 2000), central regulation of food intake (Narnaware and Peter, 2002), memory processing (Nakagawara and Sato, 1994), and mediation of analgesia and hyperalgesia (Wang *et al*., 2000).

The influence of NPY on the contractile activity of blood vessels has been studied in both *in vitro* and *in vivo* experiments. In experiments *in vitro* performed on dog (Chiba, 2001) and rabbit (Abel and Han, 1989) cerebral arteries, dog (Corder *et al*., 1987), cat (Lundberg *et al*., 1985) and pig (Roberts *et al*., 1999) spleen arteries, human (Franco-Cereceda, 1989), dog (Macho *et al*., 1989) and sheep (Kwan *et al*., 1990) coronary arteries, pig (Martling *et al*., 1990) and rabbit (Obara *et al*., 1989) pulmonary and bronchial arteries, human (Pernow and Lundberg, 1988) muscular arteries, and pig (Markiewicz *et al*., 1998a,b) ovarian artery it has been observed that NPY acts as a vasoconstrictor. The vasospastic effect of NPY has been also observed in experiments *in vivo* performed on rat (Cortes *et al*., 1999) and pig (Malmstrom, 2000) mesenteric arteries, rat renal interlobar arteries (Chen *et al*., 1997), dog spleen vessels (Yang and Chiba, 2000), rabbit ovarian ar-

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tery (Jorgesen and Sejresen, 1990) and cat renal and femoral arteries (Corder *et al*., 1986).

Wąsowicz *et al*. (1999) have shown, that the number of NPY-IR fibres and the concentration of NPY undergoes changes during the course of the oestrous cycle in the pig oviduct and uterus. In the oviduct, a number of NPY-positive nerve fibres was higher in both the early and late luteal phases than in the follicular phase. Distinct changes in the density of NPY-positive nerve fibre network occurred in the uterine cervix, where the nerve terminals were more numerous in the late luteal and early follicular phases of the oestrous cycle, when progesterone is a dominating sex hormone, than in the early luteal and late follicular phases, when oestradiol predominates. The authors suggested that these changes are most probably attributed to changes in circulating ovarian sex steroid hormones concentrations. An analogous relationship was observed for noradrenergic nerve fibre density in the porcine uterine cervix. Administration of exogenous oestradiol resulted in a decrease, while, administration of progesterone induced an increase in the number of noradrenergic nerve fibres supplying this part of the uterus as compared to that found in control animals (Kaleczyc, 1994).

The presence of numerous NPY-IR nerve fibres around blood vessels in the reproductive tract suggests that NPY participates in the regulation of blood flow through these organs (Hulshof *et al*., 1994; D'Albora *et al*., 2000). NPY is localised alone, or it is co-localised with norepinephrine (NE) in nerve fibres supplying blood vessels of the reproductive tract. It is suggested that NPY affects the contractile activity of these vessels, in part via its own receptors and in part by synergistic action with NE (Majewski *et al*., 1995; Markiewicz *et al*., 1998a,b).

Therefore, the aim of our study was to determine: (a) whether vosocontarctile effect of NPY depends on the phase of the oestrous cycle and (b) whether α-adrenergic receptors are involved in the vasocontractile action of this peptide.

MATERIAL AND METHODS

Collection of the material for pharmacological studies

The genital organs were collected from the immature (n = 43) and mature (n = 129) sows in a local slaughterhouse. The days of the estrous cycle were defined by macroscopic observation of the ovaries and the uterus. In addition, to confirm the exact stage of the cycle, samples of the jugular vein blood were collected to determine the progesterone concentrations. The preparations including the ovarian artery and arterio-venous vascular network of the ovarian pedicle, together with the ovary were isolated from the genital tract. The ovarian arteries were cannulated and perfused with Krebs-Ringer's solution to remove the blood traces. Thereafter, the preparations were placed into Krebs-Ringer's solution (composed of mM: NaCl – 120.3, KCl – 5.9, $CaCl_2$ – 2.5, $MgCl_2$ – 1.2, NaH_2PO_4 – 1.2, $NaHCO_3$ – 15.5, glucose 11.5; 25°C, pH 7.4) and transported to the laboratory. Additionally, about 1 000 ml of the blood was collected to a heparinised bottle from the same pig. Then, preparations were placed into the chamber of organ bath. The cannulated ovarian artery was connected with the syringe pump (SEP21S, Medical Equipment Ascor, Poland) and the specimen was perfused with own oxygenated blood (5% CO_2 + 95% O_2, 37°C). The perfusion speed was calculated such a way that the final value of the pressure in the ovarian artery reached 80–100 mmHg. After the stabilization of the blood pressure, the administration of examined substances into the ovarian artery was started. The changes in the blood pressure were measured using physiological pressure transducer (type P23XL, Hugo Sachs Elektronik, Germany) and registered using multi-pen recorder type R-50 model 83 (Rikadenki, Japan).

Progesterone determination

Plasma progesterone concentrations in the blood plasma was assayed using a direct enzyme immunoassay (EIA) as described previously (Okuda *et al*., 1997). The standard curve ranged from 0.19 to 50 ng/ml and the effective dose for 50% inhibition (ID50) of the assay was 4.3 ng/ml. The sensitivity of the method was 0.4 ng/ml, and the intra- and inter-assay coefficients of variation were 5.5% and 9.5%, respectively.

The plasma concentrations of progesterone found in the immature pigs (n = 42; mean ± SEM) ranged from 0.332 ± 0.04 ng/ml to 0.464 ± 0.08 ng/ml. The concentrations of this hormone in blood samples collected from the animals on days 1–5, 8–13 and 17–20 of the oestrous cycle (n = 42 in each group;

mean ± SEM) ranged from 3.88 ± 1.13 ng/ml to 4.89 ± 0.86 ng/ml, from 28.01 ± 2.32 ng/ml to 36.32 ± 1.97 ng/ml and from 0.79 ± 0.06 ng/ml to 1.05 ± 0.11 ng/ml, respectively. The mean concentrations of progesterone in peripheral blood collected from the immature and mature pigs were similar with those previously described (Henricks *et al*., 1972) and confirmed the exact stage of the estrous cycle.

Pharmacological study

In the initial experiment, ovarian artery (from immature and mature sows on days 1–5, 8–13 and 17–20; n = 1 in each group) was treated with two different concentrations of NPY (10^{-8}–10^{-7} M). Based on the results obtained, in the further study only one concentration of NPY was applied. NPY was used alone or after pre-treatment with NPY antagonist and α-adrenergic agonists or antagonists. When NPY was combined with the other drug, it was administered at the moment of maximum response evoked by the agonists or antagonists. The scheme of the substances used, and the number of experiments performed in the each animal group are shown in Table 1. An effective doses of α-adrenergic receptor agonists and antagonists were established in our previous study (Dynarowicz *et al*., 1999).

In the proper study, following substances at final concentrations were used: porcine NPY (Penisula Lab. Inc.,) 10^{-7} M, prazosin hydrochloride (PRA; α_1-adrenergic receptor antagonist; Sigma Chemical Co) 10^{-5} M, rauwolscine hydrochloride (RAU; α_2-adrenergic receptor antagonist; Carl Roth GmbH+Co) 10^{-5} M, methoxamine hydrochloride (MET; α_1-adrenergic receptor agonist; Glaxo-Wellcome) 10^{-5} M, clonidine hydrochloride (CLO; α_2-adrenergic receptor agonist; Sigma Chemical Co) 10^{-5} M, PYX-1 (NPY receptor antagonist; Penisula Lab. Inc.) 10^{-6} M.

Statistical analysis

To show differences in the blood pressure between the non-treated and treated preparations obtained from the immature pigs and those collected in different days of the oestrous cycle as well as differences in blood pressure between the preparations collected from different periods of the oestrous cycle and the immature pigs, the mean (± SEM; n = 7 in each group) blood pressure measured during 3 minute periods before treatment was calculated for each experimental group and accepted as 100%. Thereafter, percentages of changes in blood pressure after treatment with the first substance and the final changes found after treatment using a combination of the two substances were calculated based on measurements performed during 3 minute periods of the maximum response after the substances administration. The statistical significance of the differences obtained were assessed by one-way analysis of variance ANOVA (Graphpad PRISM 2.0; Graphpad Software, San Diego, CA, USA) followed by Bonferroni's Multiple Comparison Test.

Table 1. Information on the experimental animals, combination of drugs administered and a number of the experiments performed

Drug treatment	Preparations from immature pigs	Preparations from mature pigs		
		1–5 d.c.*	8–13 d.c.*	17–20 d.c.*
	Number of experiments			
NPY	7	7	7	7
PRA + NPY	7	7	7	7
MET + NPY	7	7	7	7
RAU + NPY	7	7	7	7
CLO + NPY	7	7	7	7
PYX-1 + NPY	7	7	7	7

d.c.* = day of the oestrous cycle

The differences at $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

NPY at a concentration 10^{-8} M insignificantly changed ($P > 0.05$) blood pressure in the examined vessels, and at a concentration of 10^{-7} M increased ($P < 0.05$) blood pressure in the preparations obtained on days 1–5, 8–13 and 17–20 of the cycle and from the immature pigs (data not shown) as compared to that determined before NPY treatment. Based on these results, the concentration of 10^{-7} M was used in further examinations. NPY (10^{-7}) increased ($P < 0.05$) blood pressure in all the examined preparations as compared to values found before the peptide treatment (Figure 1). The highest increase in blood pressure was observed in the preparations obtained on days 17–20 of the cycle and this increase was significantly higher ($P < 0.05$) as compared to the values determined on days 1–5 and 8–13 of the oestrous cycle and in the immature animals (Table 2). The present study revealed that NPY caused an increase in blood pressure in the isolated ovarian artery obtained from the immature and mature pigs. Similar vasocontractile effect of NPY treatment was observed *in vitro* in the rabbit ovarian artery (Yao *et al*., 1996) as well as dog, cat and pig spleen arteries (Lundberg *et al*., 1984; Corder *et al*., 1987; Pernow and Lundberg, 1988), human and sheep coronary arteries (Franco-Cereceda, 1989; Kwan *et al*., 1990) and *in vivo* in vessels of rabbit (Jorgesen and Sejresen, 1990), rat (Zukowska-Grojec *et al*., 1986), cat (Corder *et al*., 1986), dog (Suzuki *et al*., 1988) and pig (Lundberg *et al*., 1988).

Administration of PYX-1, a nonispecific antagonist of NPY receptors alone as well as administration of NPY into PYX-1 pre-treated vessels did not cause any significant changes ($P > 0.05$) in blood pressure in all the groups examined as compared to the levels determined before the peptide administration (Figure 2). There were also no significant changes ($P > 0.05$) in the blood pressure after administration of PYX-1 alone to the preparations obtained from the immature pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle (Table 2). Application of NPY after PYX-1 administration caused the highest increase in blood pressure in the vessels collected on days 8–13 and it was insignificantly lower ($P > 0.05$) in the preparations obtained on days 1–5 of the oestrous cycle; in the vessels from the immature animals and those collected on days 17–20 of the cycle, an increase in blood pressure was significantly lower ($P < 0.05$) as compared to that found on days 8–13 (Table 2). Our results indicated that PYX-1 administered alone was not able to cause significant changes in blood pressure in all the vessels examined, however, it

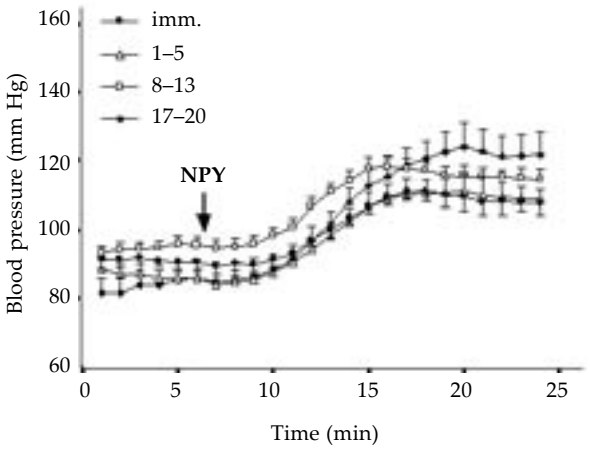

Figure 1. Mean blood pressure (± SEM; n =7 in each group) in the isolated ovarian artery collected from immature pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with neuropeptide Y (NPY) at a concentration of 10^{-7} M

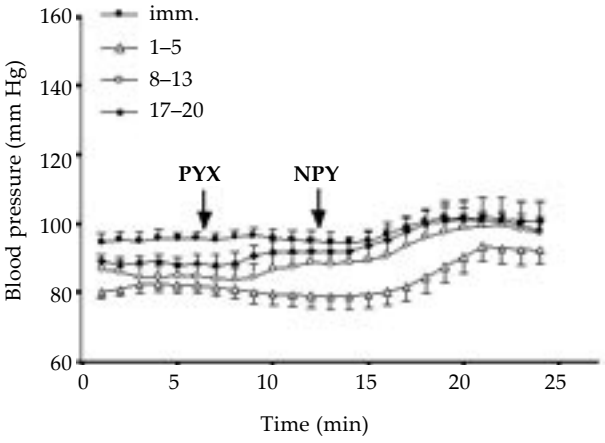

Figure 2. Blood pressure (mean ± SEM; n = 7 in each group) in the isolated ovarian artery collected from immature (imm.) pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with PYX-1 (PYX; 10^{-6} M), a non-selective antagonist of Y receptors and neuropeptide Y (NPY; 10^{-7} M). NPY was added at the time of maximum response to PYX

Table 2. Percentages of maximum changes in blood pressure measured in the isolated porcine ovarian arteries collected from immature pigs and animals in different days of the oestrous cycle, after treatment with NPY alone or administration of NPY (10^{-7} M) into the preparations pre-treated with prazosin hydrochloride (PRA; 10^{-5} M), rauwolscine hydrochloride (RAU; 10^{-5} M), methoxamine hydrochloride (MET; 10^{-5} M), clonidine hydrochloride (CLO; 10^{-5} M) and PYX-1 (NPY receptor antagonist; 10^{-6} M). To calculate the percentage of blood pressure increase, blood pressure values determined during 3 minute periods before the treatment were accepted as 100% and compared with those measured during 3 minute periods of maximum response after the administration of the substances examined

Drug treatment	Preparations from immature pigs	Preparations from mature pigs		
		1–5 d.c.*	8–13 d.c.*	17–20 d.c.*
NPY	20.6 ± 0.50[a]	26.3 ± 0.74[b]	24.1 ± 0.23[b]	45.6 ± 0.51[c]
PRA	4.31 ± 0.59[a]	7.02 ± 0.83[ab]	7.34 ± 0.59[ab]	8.11 ± 1.03[b]
PRA + NPY	5.77 ± 0.50[a]	11.6 ± 0.34[b]	9.67 ± 0.13[c]	16.5 ± 0.53[d]
MET	19.22 ± 0.18[a]	35.7 ± 1.01[b]	23.0 ± 1.57[a]	46.0 ± 0.24[c]
MET + NPY	40.6 ± 0.16[a]	62.1 ± 0.09[b]	48.3 ± 0.29[c]	83.0 ± 0.25[d]
RAU	4.66 ± 0.61[ab]	6.03 ± 0.69[a]	4.58 ± 0.31[ab]	2.6 ± 0.87[b]
RAU + NPY	13.3 ± 0.12[a]	32.3 ± 0.56[b]	21.93 ± 0.26[c]	34.8 ± 0.45[d]
CLO	2.05 ± 1.17[a]	3.7 ± 0.46[a]	1.99 ± 0.41[a]	0.66 ± 1.1[a]
CLO + NPY	8.77 ± 0.1[a]	6.46 ± 0.16[b]	7.29 ± 0.3[b]	22.8 ± 0.23[c]
PYX-1	0.32 ± 0.26[a]	2.1 ± 0.47[a]	0.11 ± 0.97[a]	2.2 ± 0.84[a]
PYX-1 + NPY	6.15 ± 0.41[a]	13.7 ± 0.29[b]	16.8 ± 0.29[c]	14.12 ± 0.19[b]

d.c.*- day of the oestrous cycle

[a–d]different subscript letters indicate significant differences ($P < 0.05$), as determined by one way analysis of variance followed by Bonferoni's Multiple Comparison Test

inhibited vasocontractile action of NPY. These data are in agreement with results obtained in the study in which NPY or its analogue [Leu^{31},Pro^{34}]NPY were administered into PYX-1 pre-treated guinea pig uterine artery, *thoracic vena cava* (Morris and Sabesan, 1994), and rat mesenteric arteries (Moira *et al*., 1992). It was suggested that PYX-1 decreased an affinity of NPY to its receptors and inhibited an increase in intracellular calcium concentration induced by NPY (Tatemoto, 1990).

Although it has been shown that vasocontractile action of NPY is caused mainly by stimulation of postjunctional Y_1-receptors (Westfall *et al*., 1995) this peptide may also release NA by stimulation of prejunctional Y_2-receptors in blood vessels (Wahlestedt *et al*., 1990). It has been documented that α_2-adrenergic receptor may interfere with Y_2-receptor and that NPY may cause changes in the function of α_2-adrenergic receptor (Harfstrand *et al*., 1984). In our study, we showed that RAU (an antagonist of α_2-adrenergic receptors) insignificantly increased ($P > 0.05$) blood pressure in the preparations from days 17–20 of the cycle and insignificantly ($P > 0.05$) decreased it in the vessels from the immature pigs and in those collected on days 1–5 and 8–13 of the oestrous cycle as compared to the values found before RAU treatment (Figure 3). Administration of NPY at the time of maximum response to RAU caused an increase ($P < 0.05$) in blood pressure in the preparations collected from the immature and mature animals as compared to the levels observed before the peptide treatment (Figure 3). Administration of RAU alone caused significantly higher ($P < 0.05$) changes in blood pressure in preparations obtained from days 17–20 as compared to the values determined on days 1–5 of the cycle (Table 2). The highest increase in blood pressure after administration of NPY in RAU pre-treated vessels was observed in preparations collected on days 17–20 of the cycle and it was significantly lower ($P < 0.05$) in the preparations obtained on days 1–5, 8–13 of the oestrous cycle and in the immature pigs

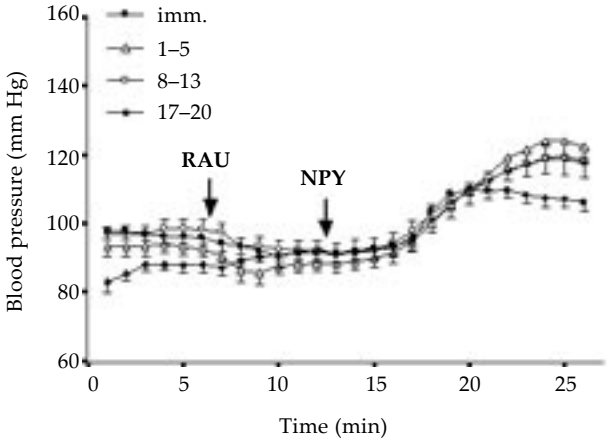

Figure 3. Mean blood pressure (mean ± SEM; $n = 7$ in each group) in the isolated ovarian artery collected from immature (imm.) pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with rauwolscine hydrochloride (RAU; 10^{-5} M) and neuropeptide Y (NPY; 10^{-7} M). NPY was added at the time of maximum response to rauwolscine

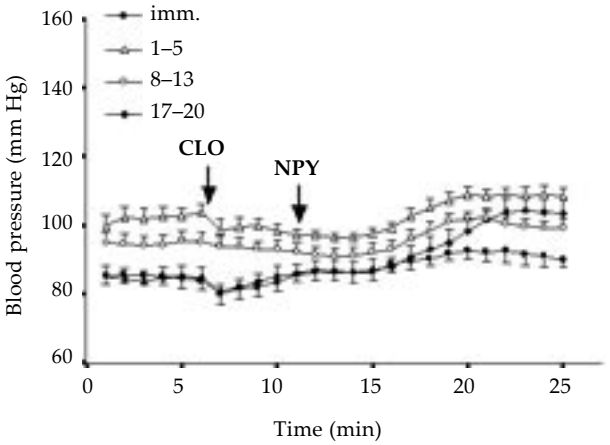

Figure 4. Mean blood pressure (mean ± SEM; $n = 7$ in each group) in the isolated ovarian artery collected from immature (imm.) pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with clonidine hydrochloride (CLO; 10^{-5} M) and neuropeptide Y (NPY; 10^{-7} M). NPY was added at the time of maximum response to clonidine

as compared to the levels observed on days 17–20 (Table 2). These results indicated that that administration of RAU, an antagonist of α_2-adrenergic receptors, into ovarian artery did not change blood pressure and administration of NPY into RAU pre-treated preparations caused an increase in blood pressure similar to that observed after addition of NPY alone. Similarly, lack of influence of RAU on vasocontractile action of NPY was observed in cat cerebral arteries (Edvinsson *et al*., 1983).

Although, RAU did not affect NPY influence on blood pressure, our findings indicated that administration of CLO, an agonist of α_2-adrenergic receptors, did not influence blood pressure when administered alone but inhibited vasocontractile action of NPY. We examined that CLO did not cause any significant changes ($P > 0.05$) in blood pressure in all examined groups as compared to the values determined before CLO treatment. Application of NPY into CLO pre-treated ovarian arteries insignificantly increased ($P > 0.05$) blood pressure in the preparations collected from the immature pigs and in those obtained on days 1–5 and 8–13 of the cycle, and significantly increased ($P < 0.05$) blood pressure in the preparations collected from the animals on days 17–20 of the oestrous cycle as compared to the levels observed before the peptide treatment (Figure 4). There were no significant changes ($P > 0.05$) after administration of CLO alone in blood pressure in the preparations obtained from the immature and mature animals. Administration of NPY into the preparations pre-treated with CLO caused the highest increase in blood pressure in the vessels collected on days 17–20 of the cycle, and it was significantly lower ($P < 0.05$) in the vessels obtained on days 1–5 and 8–13 of the cycle and in the immature pigs as compared to the values found on days 17–20 of the oestrous cycle (Table 2). A diminish influence of NPY on the blood vessels examined observed after CLO pre-treatment may be a consequence of inhibition of NA and NPY release as a result of prejunctional stimulation of α_2-adrenergic receptors what was suggested previously by Pernow and Lundberg (1989) or Martire *et al*. (1995). Our results indicate, that changes in the activity of α_2-adrenergic receptors caused by their agonists or antagonists may change the final response of the porcine isolated ovarian artery on NPY action.

It has been also described, that NPY influences α_1-adrenergic receptor (Fallgren *et al*., 1993), the main receptor by which NE causes vasocontraction of arteries and subsequently increases blood pressure. In our study, we found that administration of PRA insignificantly decreased ($P > 0.05$) blood pressure and the subsequent administration of NPY caused an increase in blood pressure ($P < 0.05$) in preparations from the immature pigs (Figure 5) as compared to that observed before PRA treatment. In the preparations collected from mature pigs, addition of PRA decreased significantly ($P < 0.05$)

blood pressure in the vessels obtained on days 1–5 and 8–13 of the cycle and in the vessels collected on days 17–20 of the oestrous cycle the decrease was insignificant ($P > 0.05$; Figure 5). NPY applied at the time of maximum response to PRA increased ($P < 0.05$) blood pressure in preparations obtained from all tree examined periods as compared to the values found before the peptide treatment (Figure 5). The highest decrease in blood pressure caused by PRA treatment was observed in vessels obtained on days 17–20 of the cycle, insignificantly lower ($P > 0.05$) in the preparations from days 1–5 or 8–13, and significantly lower ($P < 0.05$) in vessels obtained from the immature pigs as compared to the levels observed on days 17–20 of the cycle. In PRA pre-treated vessels, NPY caused a significantly lower ($P < 0.05$) increase in blood pressure which was observed in preparations from days 1–5 and 8–13 of the cycle and in the immature animals as compared to the values obtained on days 17–20 (Table 2). The present results indicated that PRA decreased blood pressure in all the preparations examined and diminished vasocontractile action of NPY in PRA pre-treated vessels. These results are in agreement with those of the study performed on the rat mesenteric arteries in which PRA, administered at the peak of the NPY vasomotor response, elicited a gradual blockade of the vasoconstriction (Cortes *et al*., 1999). Moreover, Leu^{31}Pro^{34}NPY potentiation of the nerve-stimutaled vasoconstriction was completely inhibited by PRA in the perfused, isolated canine splenic arteries (Yang and Chiba, 2000).

In contrast to α_1-adrenergic receptors antagonist, the blood pressure in the preparations obtained from the immature animals increased significantly ($P < 0.05$) after MET administration and application of NPY at a moment of maximum response to MET caused the further significant increase ($P < 0.05$) as compared to the levels found before MET treatment (Figure 6). The significant elevation ($P < 0.05$) in the blood pressure was also observed after administration of MET with regard to the vessels obtained from the mature pigs on days 1–5, 8–13 and 17–20 of the oestrous cycle as compared to the values observed before MET treatment. NPY added at the time of maximum response to MET enhanced ($P < 0.05$) an increase in blood pressure in the preparations obtained in all three periods examined as compared to the levels determined before the peptide treatment (Figure 6). The highest increase in blood pressure caused by MET alone and the combined MET and NPY treatment was observed in the vessels obtained from animals on day 17–20 of the cycle, while it was significantly lower ($P < 0.05$) in preparations from days 1–5, 8–13 of the cycle and in the vessels from the immature pigs (Table 2). These results indicated that MET, an agonist of α_1-adrenergic receptors caused an increase in blood pressure in

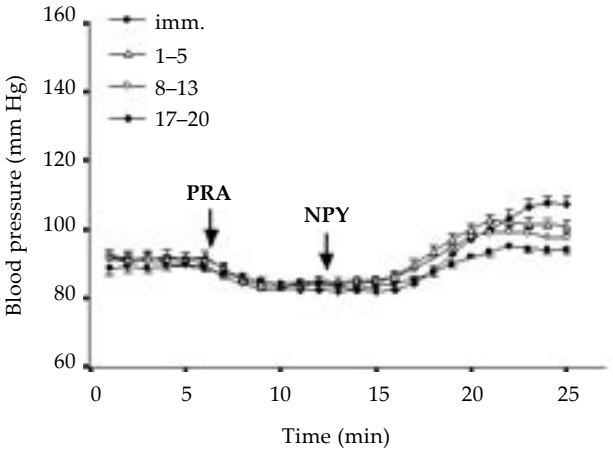

Figure 5. Blood pressure (mean ± SEM; $n = 7$ in each group) in the isolated ovarian artery collected from immature (imm.) pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with prozosin hydrochloride (PRA; 10^{-5} M) and neuropeptide Y (NPY; 10^{-7} M). NPY was added at the time of maximum response to prazosin

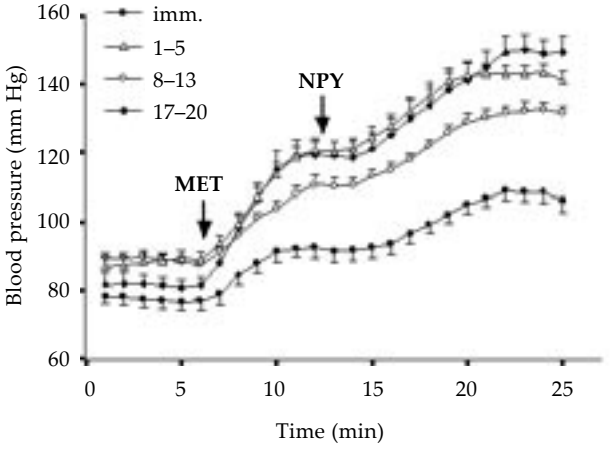

Figure 6. Blood pressure (mean ± SEM; $n = 7$ in each group) in the isolated ovarian artery collected from immature (imm.) pigs and animals on days 1–5, 8–13 and 17–20 of the oestrous cycle treated with methoxamine hydrochloride (MET; 10^{-5} M) and neuropeptide Y (NPY; 10^{-7} M). NPY was added at the time of maximum response to methoxamine

all the preparations examined. NPY administered at the peak of MET vasomotor response, elicited a further increase in blood pressure in the vessels examined. Similar potentiating effect of NPY on MET-induced vasoconstriction was observed in the rat *in vivo* (Bischoff *et al*., 1997). Our results are also in accord with previous findings (Lundberg *et al*., 1990) indicating that NPY causes vasoconstriction and enhances the effect of α_1-adrenergic receptors agonists. Moreover, these results indicate that in the pig ovarian artery, the final changes in blood pressure after inhibition or stimulation of α_1-adrenergic receptors and NPY treatment result from interaction between NE and NPY.

The present study was revealed for the first time that an increase in blood pressure caused by NPY in the porcine isolated ovarian arteries depends on the phase of the oestrous cycle. NPY administered alone or after pre-treatment with PYX-1 caused the highest increase in blood pressure in preparations from days 17–20 of the oestrous cycle as compared to that observed in the vessels from days 1–5 and 8–13 of the cycle and in the immature animals. Moreover, the phase dependent changes in blood pressure after administration of NPY into the vessels pre-treated with α_1-, and α_2-adrenergic receptors agonists and α_1-, and α_2-adrenergic receptors antagonists were observed. It has been shown, that NPY is co-localized with NE in nerve fibres supplying blood vessels of the reproductive tract and that it affects the contractile activity of these vessels, in part via its own receptors and in part by the synergistic action with NE (Hulshof *et al*., 1994; Majewski *et al*., 1995). The studies of (Dynarowicz *et al*., 1988) have revealed that ovarian steroids affect the reactivity of adrenergic and cholinergic receptors in the porcine ovarian and uterine arteries as well as they change the process of release, intake and elimination of NE. Therefore, it is possible, that changes in the response to NPY in the preparations obtained in different days of the cycle, observed in our study, could depend, in part, on changes in NE release and in the activity of NE receptors (Dynarowicz and Mortensen, 1986). However, it is possible that similar, steroid hormone dependent changes in the release of NPY and in the activity of NPY receptors may exist during the oestrous cycle in the pig. Therefore, the phase dependent changes in blood pressure in preparations observed in our study are probably a result of different action of both NPY and NE in different days of the cycle.

In summary the present study has revealed that NPY increases blood pressure in the isolated pig ovarian artery and that α-adrenergic receptors are involved in the vosocontractile action of this peptide. Moreover, it has been found that the contraction of the vessels examined, caused by NPY, depends on the phase of the oestrous cycle. The present findings, together with the literature data suggest that the mechanism of vasoconstriction induced by NPY is complex and involves a direct influence of this peptide on Y receptors and indirect influence on NA release and action, and is modified by ovarian steroid hormones.

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REFERENCES

Abel P.W., Han C. (1989): Effect of neuropeptide Y on contraction, relaxtion, and membrane potential of rabbit cerebral arteries. J. Cardiovasc. Pharmac., *13*, 52–63.

Bischoff A., Freund A., Michel M.C. (1997): The Y_1 antagonist BIBP 3226 inhibits potentiation of methoxamine-induced vasoconstriction by neuropeptide Y. Naunyn-Schmiedebergs Arch. Pharmac., *356*, 635–640.

Chen H., Bischoff A., Schafers R.F., Wambach G., Philipp T., Michel M.C. (1997): Vasoconstriction of rat renal interlobar arteries by noradrenaline and neuropeptide Y. J. Auton. Pharmacol., *17*, 137–146.

Chiba S. (2001): NPY as a periarterial sympathetic transmitter in isolated vessel. Nippon Yakurigaku Zasshi, *117*, 187–194.

Corder R., Lowry P.J., Wilkinson S.J., Ramage A.G. (1986): Comparison of the haemodynamic actions of neuropeptide Y, angiotensin II and noradenaline in anaesthetised cats. Eur. J. Pharmac., *121*, 25–30.

Corder R., Lowry P.J., Withrington P.G. (1987): The actions of the peptides, neuropeptide Y and peptide YY, on the vascular and capsular smooth muscle of the isolated, blood-perfused spleen of the dog. Brit. J. Pharmac., *90*, 785–790.

Cortes V., Donoso M.V., Brown N., Fanjul R., Lopez C., Fournier A., Huidobro-Toro J.P. (1999): Synergism between neuropeptide Y and norepinephrine highlights sympathetic cotransmission: studies in rat arterial mesenteric bed with neuropeptide Y, analogs, and BIBP 3226. J. Pharmacol. Exp. Ther., *289*, 1313–1322.

D'Albora H., Lombide P., Ojeda S.R. (2000): Intrinsic neurons in the rat ovary: an immunohistochemical study. Cell Tissue Res., *300*, 47–56.

Dynarowicz I., Mortensen A. (1986): The influence of adrenomimetic agents upon the blood vessels of the reproductive organ of the sow during the oestrous cycle (in Polish). Pol. Arch. Wet., *26*, 75–86.

Dynarowicz I., Mortensen A., Watkowski T. (1988): Participation of adrenergic receptors in the regulation of blood flow through the porcine reproductive organs during estrous cycle (in Polish). Pol. Arch. Wet., *28*, 115–127.

Dynarowicz I., Jaroszewski J.J., Markiewicz W. (1999): Interaction of the rat C-terminal fragment [Tyr^0] CGRP (28–37): with noradrenaline and acetylcholine in isolated uterine artery of the pig. Pol. J. Vet. Sci., *2*, 71–75.

Edvinsson L., Emson P., McCulloch J., Tatemoto K., Uddman R. (1983): Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. Neurosci. Lett., *43*, 79–84.

Fallgren B., Arlock P., Edvinsson L. (1993): Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction by an intracellular calcium-dependent mechanism. J. Auton. Nerv. Syst., *44*, 151–159.

Franco-Cereceda A. (1989): Endotheli- and neuropeptide Y-induced vasoconstriction of human epicardial coronary arteries *in vitro*. Brit. J. Pharmac., *97*, 968–972.

Harfstrand A., Fuxe K., Agnati L.F., Ganten D., Eneroth P., Tatemoto K., Mutt V. (1984): Studies on neuropeptide-Y catecholamine interactions in central cardiovascular regulation in the alpha-chloralose anaesthetized rat. Evidence for a possible new way of activating the alpha-2 adrenergic transmission line. Clin. Exp. Hypertens., *6*, 1947–1950.

Henricks D.M., Guthrie H.D., Handlin D.L. (1972): Plasma estrogen, progesterone and luteinizing hormone levels during the estrous cycle in pigs. Biol. Reprod., *6*, 210–218.

Hulshof S.C., Dijkstra G., Van der Beek E.M., Bevers M.M., Figueiredo J.R., Beckers J.F., Van den Hurk R. (1994): Immunocytochemical localization of vasoactive intestinal peptide and neuropeptide Y in the bovine ovary. Biol. Reprod., *50*, 553–-560.

Jorgesen J.C., Sejresen P. (1990): Neuropeptide Y reduces ovarian blood flow in the rabbit. Peptides, *11*, 451–454.

Kaleczyc J. (1994): Effect of estradiol and progesterone on noradrenaline content in nerves of the oviduct, uterus and vaginal in ovariectomized pigs. Folia Histochem. Cytobiol., *32*, 119–126.

Kwan Y.W., Wadsworth R.M., Kane K.A. (1990): Effects of neuropeptide Y and calcitonin gene-related peptide on sheep coronary artery rings under oxygenated, hypoxic and stimulated myocardial ischaemic conditions. Brit. J. Pharmac., *99*, 774–778.

Lundberg J.M., Terenius L., Hökfelt T., Martling C.R., Tatemoto K., Mutt V., Polak J., Bloom S., Goldstein M. (1982): Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effect of NPY on sympathetic function. Acta Physiol. Scand., *116*, 477–480.

Lundberg J.M., Saria A., Anggard A., Hokfelt T., Terenius L. (1984): Neuropeptide Y and noradrenaline interaction in peripheral cardiovascular control. Clin. Expl. Theory. Pract., *6*, 1961–1972.

Lundberg J.M., Torssell L., Sollevi A., Pernow J., Theodorsson Norheim E., Anggard A., Hamberger B. (1985): Neuropeptide Y and sympathetic vascular control in man. Regul. Pept., *13*, 41–52.

Lundberg J.M., Hemsen A., Larsson O., Rudehill A., Saria A., Fredholm B.B. (1988): Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. Eur. J. Pharmac., *145*, 21–29.

Lundberg J.M., FrancoCereceda A., Hemsen A., Lacroix J.S., Pernow J. (1990): Pharmacology of noradrenaline and neuropeptide tyrosine (NPY)-mediated sympathetic cotransmission. Fund. Clin. Pharmacol., *4*, 373–391.

Macho P., Perez R., Huidobro-Toro J.P., Domenech R.J. (1989): Neuropeptide Y (NPY): a coronary vasoconstrictor and potentiator of catecholamine-induced coronary constriction. Eur. J. Pharmacol., *167*, 67–74.

Majewski M., Kaleczyc J., Sienkiewicz W., Łakomy M. (1995): Existence and co-existence of vasoactive substances in nerve fibres supplying the abdomino-pelvic arterial tree of the female pig and cow. Acta Histochem., *97*, 235–256.

Malmstrom R.E. (2000): Neuropeptide Y Y1 receptor mediated mesenteric vasoconstriction in the pig *in vivo*. Regul. Pept., *95*, 59–63.

Markiewicz W., Jaroszewski J.J., Dynarowicz I. (1998a): Changes in vessel resistance of the immature pig ovarian artery bed after neuropeptide Y (NPY) and noradrenaline (NA) treatment. Pol. J. Pharmacol., *50* Suppl., 179.

Markiewicz W., Jaroszewski J.J., Dynarowicz I. (1998b): Changes in vessel resistance of the pig ovarian artery bed after neuropeptide Y (NPY) and noradrenaline (NA) treatment during the ovarian cycle. Pol. J. Pharmacol., *50* Suppl., 180.

Martire M., Pistritto G., Mores N., Agnati L.F., Fuxe K. (1995): Presynaptic α_2-adrenoceptors and neuropeptide Y Y_2 receptors inhibit [3H]noradrenaline release from rat hypothalamic synaptosomes via different mechanisms. Neurosci. Lett., *188*, 9–12.

Martling C.R., Matran R., Alving K., Hökfelt T., Lundberg J.M. (1990): Innervation of lower airways and neuropeptide effects on bronchial and vascular tone in the pig. Cell Tissue Res., *260*, 223–233.

Moira A., McAuley M.A., Westfall T.C. (1992): Possible location and function of neuropeptide Y receptor subtypes in the rat mesenteric arterial bed. J. Pharmacol. Exp. Therap., *261*, 863–868.

Morris J.L., Sabesan S. (1994): Comparison of the NPY receptors mediating vasoconstriction of the guinea-pig uterine artery and *thoracic vena cava* using a range of NPY analogues. Neuropeptides, *26*, 21–28.

Narnaware Y.K., Peter R.E. (2002): Influence of diet composition on food intake and neuropeptide Y (NPY) gene expression in goldfish brain. Regul. Pept., *15*, 75–83.

Nakagawara M., Sato Y. (1994): Neuropeptide Y: psychopharmacological and clinical aspects. Yakubutsu Seishin Kodo, *14*, 117–127.

Obara H., Kusunoki M., Mori M., Mikawa K., Iwai S. (1989): The effects of various peptides on the isolated pulmonary artery. Peptides, *10*, 241–243.

Okuda K., Uenoyama Y., Fujita Y., Iga K., Sakamoto K., Kimura T. (1997): Functional oxytocin receptors in bovine granulosa cells. Biol. Reprod., *56*, 625–631.

Pernow J., Lundberg J.M. (1988): Neuropeptide Y induces potent contraction of arterial vascular smooth muscle via an endothelium-independent mechanism. Acta Physiol. Scand., *134*, 157–158.

Pernow J., Lundberg J.M. (1989): Modulation of noradrenaline and neuropeptide Y (NPY) release in the pig kidney *in vivo*: involvement of alpha 2, NPY and angiotensin II receptors. Naunyn Schmiedebergs Arch. Pharmacol., *340*, 379–385.

Roberts R.E., Kendall D.A., Wilson V.G. (1999): Alpha(2)-adrenoceptor and NPY receptor-mediated contractions of porcine isolated blood vessels: evidence for involvement of the vascular endothelium. Brit. J. Pharmacol., *128*, 1705–-1712.

Sienkiewicz W., Majewski M., Kaleczyc J., Łakomy M. (1996): Distribution of catecholamine-synthesizing enzymes and some neuropeptides in the median eminence-arcuate nucleus complex (MEARC) of the immature female pig. Acta Histochem., *98*, 419–434.

Suzuki Y., Shibuya M., Ikegaki I., Satoh S.I., Takayasu M., Asano T. (1988): Effects of neuropeptide Y on canine cerebral circulation. Eur. J. Pharmacol., *146*, 271–277.

Tatemoto K. (1990): Neuropeptide Y and its receptor antagonists. Use of an analog mixture-screening strategy. Ann. NY Acad. Sci., *611*, 1–6.

Tatemoto K., Carlquist M., Mutt V. (1982): Neuropeptide Y: a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature, *296*, 659–660.

Wahlestedt C., Grundemar L., HÍkanson R., Heilig M., Shen G.H., Zukowska-Grojec Z., Reis D.J. (1990): Neuropeptide Y receptor subtypes, Y1 and Y2. Ann. NY Acad. Sci., *611*, 7–26.

Wang J.Z., Lundeberg T., Yu L.C. (2000): Antinociceptive effects induced by intra-periaqueductal grey administration of neuropeptide Y in rats. Brain Res., *859*, 361–363.

Wąsowicz K., Majewski M., Kaleczyc J., Łakomy M., Zięcik A., Kraeling R. (1999): NPY and VIP innervation of the oviduct and uterusof the pig at different stages of the oestrous cycle. Pol. J. Vet. Sci., *2*, 137–143.

Westfall T.C., Yang C.L., Curfman-Falvey M. (1995): Neuropeptide Y-ATP interactions at the vascular sympathetic neuroeffector junction. J. Cardiovasc. Pharmacol., *26*, 682–687.

Yang X.P., Chiba S. (2000): Dissociation of potentiation of Leu31 Pro34 neuropeptide Y adrenergic and purinergic transmission in isolated canine splenic artery. Jpn. J. Pharmacol., *83*, 197–205.

Yao W., Sheikh B., Ottesen B., Jorgensen J.C. (1996): Vascular effects and cyclic AMP production produced by VIP, PHM, PHV, PACAP-27, PACAP-38, and NPY on rabbit ovarian artery. Peptides, *17*, 809–815.

Zukowska-Grojec Z., Haass M., Bayorh M.A. (1986): Neuropeptide Y and peptide YY mediate nonadrenergic vasoconstriction and modulate symphatetic responses in rats. Regul. Pept., *15*, 99–100.

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