Praca oryginalna *Original paper*

Cholinergic innervation of cystic porcine ovaries

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Summary

The aim of the study was to examine the changes in the density of VAChT (marker of acetylcholine present)-, NPY-, VIP-, SOM-, SP- and nNOS-immunoreactive (IR) nerve terminals and co-localization of VAChT with the above-mentioned neurotransmitters after the occurrence of dexamethasone (DXM)-induced ovarian cysts in gilts. DXM administration led to an increase in the density of VAChT/SP-, VAChT/nNOS- and NPY-IR nerve terminals around the cystic walls. In DXM-treated animals an elevated number of VAChT- and SP-IR nerve endings was found close to the tertiary follicles. Moreover, in the gilts receiving DXM the density of NPY-IR nerve endings (that simultaneously co-localized VAChT) was high near the interstitial gland. An increase in the number of VAChT/SP- and VIP-IR nerve fibers around the medullar arteries (A) was observed in cystic ovaries, while the number of VAChT-IR nerve endings near the cortical A was lowered after DXM application. Furthermore, nerve fibers containing VAChT were absent around veins in the whole ovary of DXM-treated animals. After DXM injections, an increase in the number of VAChT/SP- and VAChT/nNOS-IR nerve endings in the cortical, as well as VIP- and nNOS-IR (co-existing with VAChT), nerve terminals in the medullar part of the autonomic ground plexus (GP) was present. However, the administration of DXM led to a drop in the density of SOM-positive nerve endings (also VAChT-IR) in the medullar subdivision of the GP. The present study shows that in the porcine ovaries with DXM induced cysts the pattern of cholinergic innervation, as well as the co-localization of VAChT and NPY, VIP, SOM, SP or nNOS, were changed. Data obtained also suggest that acetylcholine and the above-mentioned neurotransmitters effecting the functioning (steroidogenic activity, blood flow) of the polycystic ovaries may have a significant influence on the course of this pathological status.

Keywords: acetylcholine, cholinergic innervation, cystic ovary, dexamethasone, pig

The cystic ovarian disease (COD) is a complex endocrine disorder associated with ovulatory dysfunction, leading to temporal or permanent infertility. However, the etiology and/or pathogenesis of COD are still obscure. As may be judged from data obtained in cows and pigs suffering of this disorder, the etiology of this syndrome is based on profound disturbances in the function of hypothalamo-pituitary-ovarian axis, causing impairment of the synthesis, release, and/or storage of various hormones of this functional unit (3, 8, 26, 30, 33).

Furthermore, it has also unequivocally been indicated that peripheral nerves supplying the ovary may also play an important role in the formation and/or maintenance of ovarian cysts. Namely, the resection of the ovarian medulla fragment containing a part of the nerves supplying the ovary (10) or laparascopic laser cauterizatin of this place (4) in women with COD, in which the hormonal therapy was ineffective, induced ovulation. It was also observed that both the density of adrenergic nerves, and the content of noradrenaline (NA) increased in cystic ovaries of women (25), rats (11, 20, 27) and pigs (13, 14, 17). Moreover, an elevation in the NA amount in the porcine ovaries with dexametasone (DXM)-induced cysts, accompanied simultaneously by distinct changes in the steroidogenic activity of the gonad, indicate that these events may be, at least partly, involved in the pathogenesis of COD (13, 14, 17).

It is generally known that the ovary is innervated also by fibres of the parasympathetic nervous system. In the porcine ovaries, these fibres were located around the preantral and antral follicles, corpora lutea (CL), blood vessels, as well as in the vicinity of the interstitial gland and within the cortical part of the autonomic ground plexus (19, 21). Numerous studies performed under physiological conditions support the important role of acetylcholine (ACh) – major choli-

nergic neurotransmitter and its cotransmitters – neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), somatostatin (SOM), substance P (SP) and the neuronal isoform of nitric oxide synthase (nNOS) in the regulation of ovarian function in women, rodents, cows and pigs. Thus, it has been found that these neurotransmitters participate in ovulation and in synthesis of steroids, modulating directly steroidogenic activity of cells and/or indirectly by influence on ovarian blood flow (5, 7, 23, 29, 32).

In the relationship with the complete lack of information concerning cholinergic innervation of the cystic ovaries, as well as with the possible important role of this subdivision of the autonomic nervous system in regulation of ovarian function determined under the physiological conditions, the present study was designed to reveal the cholinergic innervation of porcine cystic ovaries. In order to attain the goal of this study, both changes in the density the nerves terminals containing of vesicular acetylcholine transporter (VAChT; as marker of the ACh), NPY, VIP, SOM, SP and nNOS, as well as co-localization of VAChT with above-mentioned neurotransmitters in the porcine ovaries with DXM-induced cysts were determined.

Material and methods

Animals and experimental procedures. The experiment was performed on 12 crossbred adult gilts (Large White × Landrace), weighting 90-100 kg, with two controlled subsequent estrous cycles. Behavioural estrous was checked by using a boar-tester. The animals were then individually housed in stalls, under conditions of natural light and room temperature. They were fed a commercial grain mixture and given tap water ad libitum. The gilts were randomly assigned to one of two groups: control, receiving saline (Con, n = 6) and DXM-treated (DXM, n = 6). Principles of animal care (NIH publication No. 86-23, revised in 1985) as well as the specific national law on the protection of animals were followed.

In the DXM group, cysts were induced by i.m. injections of DXM (Dexasone®, Norbrook Laboratory, Newry, UK, 3.3 µg/kg b.w., in total volume of 6 ml), every 12 hours (h), from day 16 of the first estrous cycle to day 9 of the second studied cycle (i.e. by 15 consecutive days). During the same period of time animals of the Con group were injected with 6 ml of saline. The gilts were killed on the 20th day of the second studied cycle by an electrical shock (ENZ 300 Metalowiec, Bydgoszcz, Poland) and then exsanguinated. The ovaries were immediately dissected out and their weight, volume and measurements, as well as the number of ovarian structures were estimated. The follicles were divided into three size classes: 1-3, 4-6 and 7-10 mm in diameter. Follicular structures exceeding 1.0 cm in diameter were classified as cysts. Microscopically, stages of follicular development were classified according to Wulff et al. (34) and Barboni et al. (6) with some modifications. Primordial – without any granulosa cell; primary – surrounded by a single layer of cuboidal granulosa cells; secondary – with two or more granulosa cell layers without any antral cavity; tertiary – with antrum. Cryostat ovarian sections were subjected to routine single- and double--immunofluorescence technique, used to visualise the distribution of cholinergic population of intraovarian nerve terminals containing VAChT, NPY, VIP, SOM, SP or nNOS.

Single and double-labelling immunofluorescence. Tissues were fixed by immersion in Zamboni's fixative for 30 min, washed in phosphate buffer and cryoprotected in 18% sucrose until sectioning. Frozen fragments of the ovaries were cut in a cryostat (Reichert-Jung, Nußloch, Germany) into 10-µm-thick sections and then subjected to routine single- and double-immunofluorescence technique described by Majewski and Heym (22) in order to estimate the density of VAChT-, NPY-, VIP-, SOM-, SP- and nNOS--IR nerve endings, as well as the pattern of co-localization of VAChT and above-mentioned neurotransmitters, respectively. Briefly, sections were incubated in the humid chamber, overnight at room temperature, with primary antibodies against either VAChT (rabbit anti-C-terminal VAChT, diluted 1 : 10 000; Phoenix Pharmaceuticals, Inc., USA), NPY (rat polyclonal, diluted 1 : 400; Biomol, LP, UK), VIP (mouse, clone VIP-001, diluted 1 : 2000; Biogenesis, UK), SOM (rat monoclonal, reacts with SOM fragments, diluted 1 : 60; Biogenesis), SP (rat monoclonal, diluted 1 : 300; Biogenesis) and nNOS (mouse monoclonal, brain, diluted 1 : 1000, Sigma, UK). The antigen-antibody complex was then visualised by species-specific secondary antibodies conjugated to FITC (donkey anti--mouse and/or donkey anti-rat IgG, diluted 1 : 800) or CY3 (diluted 1 : 800), all from Jackson Immunoresearch, USA. Single- and double-immunolabelled nerve terminals were analysed under Olympus BX51 microscope equipped with epi-fluorescence and appropriate filter sets. The semiquantitative (arbitrary) evaluation of VAChT, NPY, SOM, SP, VIP and nNOS fibres density based on the number of nerve terminals found in the vicinity of structure evaluated – single; several (2-5); numerous (6-20); very numerous (> 20), as described previously in detail (22). This procedure was applied to 8 randomly chosen ovarian sections from each animal studied and then pooled and presented as a mean value.

Statistical analysis. Student t-test was used to compare the mean (± SEM) number of ovarian structures and weight, volume, as well as measurements of ovaries in the Con and DXM-treated groups (InStat GraphPad, San Diego, CA).

Results and discussion

Macroscopic evaluation of the ovaries. After DXM administration, cysts (1-2 cm in diameter, mean number of 1.8 ± 0.5 per ovary) were observed in both ovaries in all the DXM-treated gilts. Moreover, in ovaries of the gilts treated with DXM, the number of follicles with a diameter of 4-6 mm was higher ($P < 0.01$) than that found in the Con group. Follicles measuring 7-10 mm in diameter were not present in these animals. In this study the authors also observed an increase ($P < 0.05$) in the length of ovaries from animals treated with DXM when compared with Con animals. The

Tab. 1. Mean (± SEM) weight, volume and measurements of the ovaries, as well as the number of the follicles and cysts in the control (Con) and DXM-treated (DXM) group

Group	Weight (g)	Volume (ml)	Measurements (cm)			Number of follicles in diameter (mm):			Number of cysts
			length	width	height	1-3	4-6	7-10	1-2 cm
Con	5.1 ± 0.4	5.0 ± 0.6	2.9 ± 0.1	2.6 ± 0.3	1.9 ± 0.2	6.3 ± 2.3	2.3 ± 0.8	7.3 ± 0.4	l.s.
DXM	7.6 ± 1.2	8.7 ± 2.7	3.7 ± 0.2*	2.5 ± 0.2	1.5 ± 0.1	6.7 ± 0.9	14.3 ± 1.2**	l.s.	1.8 ± 0.5

Explanations: * – $P < 0.05$; ** – $P < 0.01$ indicate significant differences between the examined groups; l.s. – the lack of structure

number of small follicles (1-3 mm of diameter), as well as the weight, volume, width and height of the gonads were similar in both examined groups. Moreover, CL were not found in all animals (tab. 1).

Density of VAChT-, NPY-, VIP-, SOM-, SP- and nNOS-IR nerve terminals. In both the Con and DXM groups fibres containing VAChT (fig. E; F; G; H), NPY, VIP (fig. F; H), SOM, SP or nNOS (fig. E; G) were found in the cortical and medullar part of the autonomic ground plexus (GP; with exception of SP-IR fibres, which were not present in the medulla of ovaries treated with DXM). After DXM application, the number of VAChT- (fig. G), SP-, nNOS-IR (fig. G) nerve fibres in the cortical, as well as of NPY-, VIP- (fig. H) and nNOS-IR nerve terminals in the medullar part of the GP, was higher than that found in gonads of control animals. Moreover, in comparison to the Con group, administration of DXM lead to a drop in the number of SOM-IR nerves and, what is more, eliminated SP-IR nerve fibres from the medullar subdivision of the GP. In control animals, VAChT- (fig. A), NPY-, VIP-, SOM- and SP-IR fibres were present in the vicinity of all ovarian follicles, regardless of their stage of development. In contrast, in the gonads of gilts receiving DXM, SP--IR nerve terminals were absent near the primordial and primary follicles, while SOM-IR nerve endings were not present in the vicinity of tertiary follicles (TF) and cysts (C). Ovarian follicles, irrespective of their stage of development, were devoid of nNOS-IR fibres input

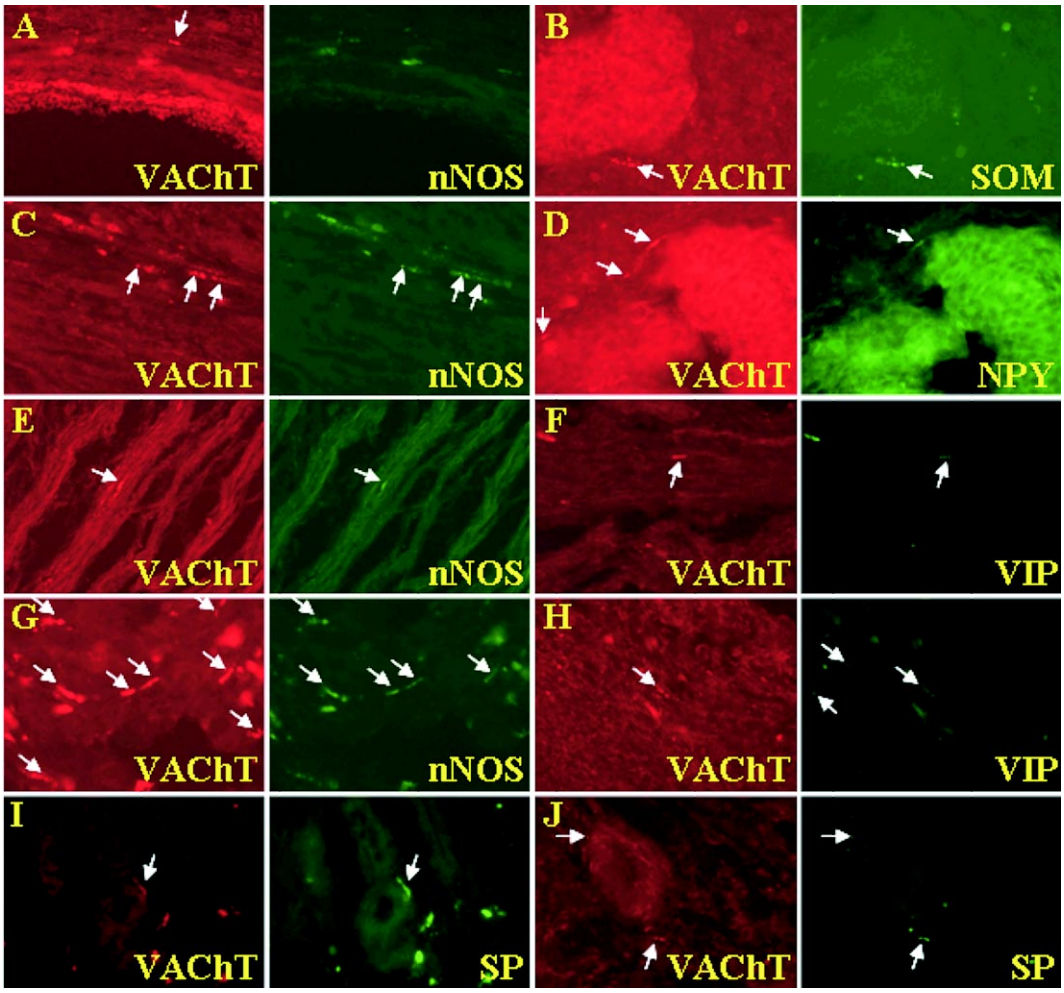

Fig. 1. Immunocytochemical localization of VAChT-, NPY-, VIP-, SOM-, SP- and nNOS-IR nerve fibres (↑) and co-localization patterns of VAChT with above-mentioned neurotramitters in the porcine ovaries of the control (Con; fig. A, E, F, I) and DXM-treated (DXM; fig. B, C, D, G, H, J) animals. In the Con gilt, single VAChT-IR nerve fibres were visible near the tertiary follicle (TF), while nNOS were not found enclose this structure (fig. A). After DXM application, the middle number of VAChT/nNOS-IR fibres was present around the cyst (fig. C). Moreover, in the gilt receiving DXM the co-localization VAChT and SOM, as well as VAChT and NPY was found in vicinity of the secondary (fig. B) and TF (fig. D), respectively. In the Con animal, single VAChT/nNOS-IR nerve terminals in the cortical (fig. E), as well as VAChT/VIP-IR nerve endings in the medullar part of the ground plexus (GP; fig. F) were observed. In DXM-treated gilt, the numerous of VAChT/nNOS-IR nerve terminals were visible in the cortical GP (fig. G). Furthermore in this animal, a moderate number of VAChT/VIP-IR nerve fibres was present in the medullar of the GP (fig. H). In the Con group, single VAChT/SP-IR nerves were found around the artery (A) in the ovarian medulla (fig. I). After DXM injections several VAChT/SP-IR nerve endings were visible near the medullar A (fig. J); 200 ×

(fig. A) in both studied groups. It should also be stressed that in the DXM group, an increase in the density of NPY-IR nerve fibres in the region of secondary follicles (SF), as well as of VAChT- (fig. D) and SP-IR in the vicinity of TF were found. Furthermore, a higher number of VAChT- (fig. C), NPY-, SP- and nNOS-IR (fig. C) nerve terminals around the cystic walls were observed in the DXM-treated gilts compared to the TF in the Con animals. The VAChT- (fig. I, J), NPY-, VIP- or SP-IR (fig. I, J) perivascular nerve fibres were determined around both the cortical and medullar arteries (A) in the gonads of Con and DXM animals. The number of NPY-IR nerves was elevated, while the number of VAChT-IR nerve endings was lowered around cortical A after DXM treatment. However, arterial vessels in the cortex were devoid of SOM-IR nerve terminals in both groups. In addition, the administration of DXM caused a lack in nNOS-positive nerve fibres around A within the whole ovary. Regarding the innervation of the cortical and medullar veins (V) in the control gilts, all studied neurotransmitters (with the exception of SOM-IR nerves in the cortex and nNOS in the whole ovary) were present in nerves supplying these V. In comparison, administration of DXM either leads to an increase, as well as to a drop in density of NPY-IR nerve fibres around the cortical and medullar V, respectively. In the DXM group, there was a lack of VAChT- and nNOS-IR nerve terminals near the V in the whole ovary, as well as of VIP- and SP-IR terminals around medullar V. Moreover, while single SOM-IR nerves were observed near these structures in the cortex of DXM-treated gonads, they were not observed in ovaries of control animals. Nerves supplying the interstitial gland (IG) in the ovaries of both groups contained all the studied neurotransmitters. However, the number of NPY-IR nerve endings near these structures was higher following DXM application than that found in the Con animals (tab. 2).

The co-localization patterns of VAChT and NPY, VIP, SOM, SP or nNOS. VAChT-IR nerve fibers contributing to the cortical part of the GP, were simultaneously immunopositive for VIP, SP or nNOS (fig. E, G) in both the Con and DXM groups. Furthermore, the VAChT/VIP- (fig. F, H) and VAChT/nNOS-IR nerve terminals were also present in the medullar GP of both groups. In the animals receiving DXM, VAChT/NPY- and VAChT/SOM-IR, nerve endings in the cortical part of GP, as well as VAChT/SOM-IR nerve fibers in the medullar part of GP were also observed. In addition, a subset of VAChT-positive nerve terminals in the medullar part of GP in the Con gilts were simultaneously SP-IR. In the DXM group the co-localization of VAChT and SOM (fig. B), VAChT and NPY (fig. D), VAChT and SP, as well as VAChT and nNOS (fig. C), were observed in nerve endings supplying the SF, TF and C. In the DXM-treated gilts, VAChT-IR nerve endings supplying the cortical A were simultaneously VIP-IR, while those supplying the medullar A contained SP instead (fig. J). In the Con animals periarterial VAChT-IR nerve endings co-expressed nNOS- or SP-IR (fig. I). The IG was supplied by VAChT/NPY-, VAChT/VIP-, VAChT/SOM- and VAChT/nNOS-contained nerves of both groups (tab. 3).

The present study shows that DXM application leads to the formation of ovarian cysts and derangement in the follicular development. Generally, it was accompanied by changes in the density of VAChT-, NPY-, VIP-, SOM-, SP- and nNOS-IR nerve endings, as well as by changes in the pattern of co-localization of VAChT and the above-mentioned neurotransmitters.

In this experiment DXM injections resulted in the creation of cysts in the ovaries. In the cystic gonads, a higher number of medium-sized follicles (4-6 mm in diameter) was paralleled by a lack of large follicles (7-10 mm in diameter) and by an increase in the length of the ovaries. These results are in agreement with earlier studies performed on sows receiving adenocorticotropic hormone (15) or DXM (26).

Tab. 2. Arbitrary evaluation of the density of VAChT and NPY, VIP, SOM, SP and nNOS nerve terminals in the porcine ovaries of the control (Con) and DXM-treated (DXM) group

Neurotransmitter	VAChT		NPY		VIP		SOM		SP		nNOS	
Group	Con	DXM	Con	DXM	Con	DXM	Con	DXM	Con	DXM	Con	DXM
Ovarian tissue	Cortex											
Ground plexus	+	+++	+++	+++	++	++	++	++	++	+++	+	++
Primordial follicles	+	+	+	+	+	+	+	+	+	–	–	–
Primary follicles	+	+	+	+	+	+	+	+	+	–	–	–
Secondary follicles	+	+	+	++	+	+	+	+	+	+	–	–
Tertiary follicles	+	++	+	+	+	+	+	–	+	++	–	–
Cysts	l.s.	++	l.s.	++++	l.s.	+	l.s.	–	l.s.	++	l.s.	++
Arteries	++	+	++	+++	+	+	–	–	+	+	++	–
Veins	+	–	+	++	+	+	–	+	+	+	–	–
Interstitial gland	+	+	++	+++	+	+	+	+	+	+	+	+
	Medulla											
Ground plexus	+	+	+++	++++	+	++	++	+	+	–	+	++
Arteries	+	++	+++	++	+	++	+	+	+	++	+	–
Veins	++	–	+++	++	+	–	+	+	++	–	–	–

Explanations: The number of fibres in the vicinity or within structure studied: (–) – the lack of fibres; + – single; ++ – from 2 to 5; +++ – from 6 to 20; ++++ – > 20; l.s. – the lack of structure

Tab. 3. The co-localization of VAChT and/or NPY, VIP, SOM, SP and nNOS in the porcine ovaries of the control (Con) and DXM-treated (DXM) group

Ovarian tissue		Group	Neurotransmitters					
			VAChT	NPY	VIP	SOM	SP	nNOS
Cortex	GP	Con	–	–	*	–	*	*
		DXM	–	*	*	*	*	*
	PF	Con	–	–	–	–	–	–
		DXM	–	–	–	–	–	–
	PRF	Con	–	–	–	–	–	–
		DXM	–	–	–	–	–	–
	SF	Con	–	–	–	–	–	–
		DXM	–	–	–	*	–	–
	TF	Con	–	–	–	–	–	–
		DXM	–	*	–	–	–	–
	C	DXM	–	–	–	–	*	*
	A	Con	–	–	–	–	–	*
		DXM	–	–	*	–	–	–
	V	Con	–	–	–	–	–	–
		DXM	–	–	–	–	–	–
	IG	Con	–	*	*	*	–	*
		DXM	–	*	*	*	–	*
Medulla	GP	Con	–	–	*	–	*	*
		DXM	–	–	*	*	–	*
	A	Con	–	–	–	–	*	–
		DXM	–	–	–	–	*	–
	V	Con	–	–	–	–	–	–
		DXM	–	–	–	–	–	–

Explanations: GR – ground plexus; PF – primordial follicles; PRF – primary follicles; SF – secondary follicles; TF – tertiary follicles; C – cysts; A – arteries; V – veins; IG – interstitial gland; * – the co-localization; (–) – the lack of co-localization

In the present study the innervation pattern of the ovaries of the Con gilts by VAChT-, NPY-, VIP-, SOM-, SP- and nNOS-IR nerve fibres was similar to the innervation described earlier by Majewski (21) in the gonads of immature gilts. In turn, in the gilts receiving DXM the authors observed an increase in the number of VAChT/SP-, VAChT/nNOS- and NPY-IR nerve endings around the C, VAChT-, SP- or NPY-IR near the TF and SF, respectively, as well as of NPY--positive nerve terminals (also VAChT-IR) supplying the IG. The explanation of the increased density of VAChT/SP-, VAChT/nNOS- and NPY-positive nerve endings around the C, F and IG is difficult, as in the available literature there is a complete a lack of data concerning the cholinergic innervation, and, on the other hand, data dealing with the distribution of NPY-IR nerve terminals in such ovaries are also very limited. Thus, as of yet, it has previously been only reported that the density of NPY-IR nerve terminals increased near the C and F in the gilts receiving DXM (13). It should be stressed that an increase in the number of VAChT-, NPY-, SP- and nNOS-IR nerve fibers around the C, F and IG may indicate that neurotransmitters released from these nerve terminals may affect the steroidogenic activity of these structures. It has been discovered that ACh and NPY stimulated the secretion of progesterone (P_4) in the human (7) and rat (5) granulosa cells. An increase in the concentration of P_4 was also observed in the cystic fluid and wall in the pigs (17) and in the cystic fluid in the cows (9). However, NPY in the nerve fibres located near the C may also inhibit andronstendione secretion from these structures. A decrease in the content of this steroid in the cysts was detected in the gilts after DXM injections (17). It has previously been found that SOM hampers LH- and FSH-stimulated aromatase activity, as well as the P_4 production in rat granulosa cells (1). Moreover, nitric oxide (NO), synthetised by nNOS, also suppressed the estradiol-17β (E_2) and P_4 release from the porcine granulosa cells (24). A diminution in the E_2 concentration in the cystic fluid was found in the gilts (3) and cows (9). Furthermore, a decrease in the content of P_4 in the medium-sized follicles was also determined in the cystic porcine ovaries (17). It is very interesting that in the ovaries of the gilts receiving DXM, SP-IR nerve terminals around the small F, as well as SOM-IR nerves near the C and TF, were completely eliminated. It is most probable that a lack of these nerve endings may be the consequence of DXM administration. A similar phenomenon was detected earlier in the normal dental nerves (16) and in the hypothalamic neurons in rats (2) in response to DXM.

The present study demonstrated an increase in the density of VAChT/SP- and VIP-IR nerve fibers around the medullar arteries (A), of NPY-IR nerve terminals near the cortical A and veins (V), as well as single SOM-IR nerve fibers enclose the cortical V, in the DXM-treated gilts. This is in line with the authors' earlier study where we found the increase in the number of NPY-IR nerve endings near the blood vessels in the cystic ovaries (13). It is known that ACh (12), NPY (23), VIP (18) and SP (31) regulating the ovarian of blood flow can change the quantity of lipoproteins (the source of cholesterol for the steroid synthesis) that reach the ovary. In the present study, DXM application also resulted in a drop in the number of VAChT-IR nerve fibers supplying cortical A, as well as the density of NPY-positive nerve fibers around the medullar A and V. A decrease in the number of NPY-IR nerve fibers is in accordance with results described by Paredes et al. (27). After DXM injections the authors also observed a lack of nerves containing VAChT around the V and nNOS near the A in the whole ovary, and VIP- and SP-IR nerve endings enclosing the V in the medulla. Most probably the elimination of these fibers was associated with DXM application (2, 16), which was suggested by us earlier, in reference to the F.

Furthermore, in our experiment, the administration of DXM caused an increase in the density of VAChT/SP- and VAChT/nNOS-IR nerve fibers in the cortical, as well as of NPY-IR nerve terminals and nNOS-, VIP--positive nerve endings (also VAChT-IR) in the medullar part of the autonomic GP. A high content of VIP, as mentioned above in reference to the blood vessels, was also present in rat cystic ovaries (28). Moreover, an increase in the number of VAChT-, VIP-, SP- and nNOS-IR nerve fibers in the cortical and/or medullar part of the autonomic GP may provide evidence for a rich supply of nerve fibers to the cystic porcine ovaries. In contrast, an application of DXM led to a decrease in the density of SOM-IR nerve fibers (co-existing with VAChT) and also eliminated SP-IR nerve endings within the medullar part of the GP. A drop of SOM- and the absence of SP-IR fibers in the medullar subdivision of the GP was probably caused by DXM injections (2, 16), similarly as was the case of the F and blood vessels in the present study.

In conclusion, this data show that the density of VAChT-, NPY-, VIP-, SOM-, SP- and nNOS-IR nerve fibers and co-localization of VAChT with the above-mentioned neurotransmitters change in the porcine polycystic ovaries, that were induced by DXM injections. Moreover, these results may suggest an important role of ACh and the other neurotransmitters in the course of this pathological state.

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