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Localization of substance P, calcitonin gene related peptide and galanin in the nerve fibers of porcine cystic ovaries

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Abstract: In a previous study, we showed that both the noradrenergic and cholinergic component of ovarian innervation is markedly changed in porcine cystic ovaries. The present study is aimed at elucidating the distribution pattern of substance P- (SP), calcitonin gene related peptide CGRP- and/or galanin (GAL)-containing nerve fibers within porcine cystic ovaries. The *status polycysticus* was induced by dexamethasone phosphate disodium salt i.m. injections performed from the 7th until the 21st day of the first studied estrous cycle. During the same period of time, gilts of the control group received saline. All animals were slaughtered on the expected 11th day of the second studied estrous cycle, and their ovaries were collected. When compared to control gonad, a distinct difference in the distribution pattern and the density of SP-, CGRP- and/or GAL-immunoreactive (GAL-IR) nerve fibers was observed. Thus, unlike in the control gonad, SP- and/or CGRP-IR perivascular nerve fibers were found to supply medullar blood vessels of polycystic ovary. Furthermore, the number of GAL-IR nerve fibers contributing to the ground plexus in polycystic ovaries was higher than that observed in the control gonads. Thus, as may be judged from the profound changes in the distribution pattern of differently chemically coded afferent terminals within polycystic gonads, it appears possible that neuropeptides released from these terminals may take part in the etiopathogenesis of this disorder. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 4, pp. 622–630*)

Key words: polycystic ovaries, sensory innervation, neuropeptides, nerve fibers, ovarian cysts, gilts

Introduction

Status polycysticus is a complex endocrine disorder occurring in women and female domestic animals that leads to anovulation and, eventually, to temporary or permanent infertility. However, the etiology and pathogenesis of ovarian cysts remains unclear. It is generally assumed that cysts are mainly caused by disturbances to the function of the hypothalamic-pituitary-ovarian axis leading to impairment of the synthesis, release and storage of various hormones of this functional unit [1–3].

It has recently been shown that not only is the density of sympathetic nerve fibers increased in the cystic ovaries of women [4], rats [5, 6] and pigs [7], but also the cholinergic innervation undergoes a profound remodeling in porcine ovaries treated with dexamethasone phosphate disodium salt (DXM) [8]. Furthermore, it has been found that laparoscopic resection or cauterization of the medullar part of cystic ovaries — i.e. the entry zone for ovarian nerve fibers — induced ovulation in women after ineffective hormonal therapy [4, 9, 10].

It is well known that the ovary is supplied by sensory subdivision of the peripheral nervous system. In ovaries, nerve fibers containing substance P (SP) [11, 12], calcitonin gene related peptide (CGRP) [13–15]

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or galanin (GAL) [12] — 'markers' of sensory neurons — have been observed in the vicinity of preantral and antral follicles as well as around blood vessels. Under physiological conditions, SP, CGRP and GAL play an important role in the regulation of ovarian function in women [16], rodents [17–19], cows [20] and pigs [12, 21, 22]. Moreover, all these neuropeptides participate in the modulation of ovarian steroidogenic activity, both directly (by influencing the activity of steroidogenic cells), and indirectly by regulating the extra- and intraovarian vascular bed [12, 16–22].

It should be stressed that there is a paucity of data concerning the sensory innervation of cystic ovaries. Until now, this topic has been addressed only in rats, in which a marked increase in the density of CGRP-IR nerve fibers was observed in dihydroepiandrosterone (DHEA)-induced polycystic ovaries [23]. Thus, in order to broaden our knowledge concerning the pattern of sensory innervation of cystic ovaries, we aimed the present study at determining the distribution and density of SP-, CGRP- and GAL-IR nerve fibers in porcine ovaries with *status polycysticus* induced by repeated DXM administration.

Material and methods

Animals and experimental procedure. During the experimental part of this study, we have followed the principles of animal care (NIH publication No. 86-23, revised in 1985), as well as the national laws on animal protection. The experiment was carried out during two controlled estrous cycles on 12 crossbred gilts (Large White x Landrace), aged 7–8 months and of 90–100 kg body weight. Behavioral estrus was detected using a boar-tester. The animals were then individually housed in stalls, under conditions of natural light and room temperature. They were fed with a commercial grain mixture and 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).

In the DXM group, the polycystic status of the gonads was induced according to the protocol described earlier by Gee et al. [24] with the following modifications: the gilts included in the present study received DXM (3.3 μg/kg of body weight, in total volume of 6 ml; Dexasone®, Norbrook Laboratory, Newry, UK) every 12 hours (h), starting from the 7th day (1st day of the study) to the 21st day of the first studied estrous cycle (i.e. during 15 consecutive days). During the same period of time, animals of the CON group were injected with 6 ml of saline. Animals were then slaughtered by electric shock (ENZ 300 Metalowiec, Bydgoszcz, Poland) and exsanguinated on the 11th day of the second studied cycle (i.e. on the 26th day of the experiment). Ovaries were immediately dissected out and their weight, volume and diameters, as well as the number of ovarian follicular structures, were estimated. The follicles were divided into two size classes: 1–3 and 4–6 mm in diameter. Follicular structures exceeding 1.0 cm in diameter were classified as cysts [25]. Morphological examination of ovaries particularly focused on the results described previously by Kozłowska et al. [26], including on the number of partly luteinized follicular cysts (Figure 1), a decrease in the number of follicles measuring 1–3 mm in diameter, and a lack of follicles measuring 4–6 mm in diameter.

In the present study, the distribution and density of intraovarian SP-, CGRP- and GAL-immunoreactive (GAL-IR) nerve terminals were estimated around follicles, cysts, corpora lutea (CL), blood vessels, interstitial glands and within the ground plexus. In order to evaluate differences in the distribution pattern of perifollicular nerve fibers, ovarian follicles were, depending on their stage of development, microscopically classified according to Wulff et al. [27] and Barboni et al. [28] into the following classes:

— primordial — without granulosa cells;
— primary — surrounded by a single layer of cuboidal granulosa cells;
— secondary — with two or more granulosa cell layers without antral cavity;
— tertiary — with antrum.

Additionally, the tertiary follicles were divided into two size classes: up to 3 mm and 4–6 mm in diameter. Afterwards, blocks of ovarian tissue were processed for further immunochemical studies as follows: they were fixed by immersion in Zamboni's fixative for 30 min, washed in 0.1 M phosphate buffer, stored in 18% sucrose for several days and then frozen (–80°C) and stored until sectioning.

Double-labeling immunofluorescence. Ten-μm-thick cryostat sections (Reichert-Jung, Nußloch, Germany) of the ovaries were subjected to the double-immunofluorescence staining technique. The sections were air-dried at room temperature (RT) for 45 min, and rinsed (3 × 15 min) with PBS (phosphate buffered saline, pH 7.4). Next, sections were blocked with a blocking mixture containing 1% Triton X100, 0.1% bovine serum albumin, 0.05% thimerosal, 0.01% NaN_3 and 10% normal goat serum in 0.01 M phosphate-buffered saline for 1 h at room temperature to reduce non-specific background staining. After a wash, sections were incubated with a mixture of primary antisera raised in different species and recognizing SP (rat, working dilution 1:300; Biogenesis), CGRP (rabbit polyclonal, working dilution 1:8,000; ICN Cappel) or GAL (rabbit, working dilution 1:2,000; Bachem) overnight in the humid chamber at room temperature. Primary antisera were then visualized by a mixture of FITC-conjugated donkey anti-rat IgG-specific (working dilution 1:400; 1 h; Jackson Immunoresearch) and CY3-conjugated donkey anti-rabbit IgG-specific antisera (1 h; Jackson Immunoresearch); after the incubation, sections were

washed again and then coverslipped with carbonate-buffered glycerol (pH 8.6). The specificity of primary antisera was tested as follows: sections were incubated with antibody that had been preabsorbed with synthetic antigen (10 μg of antigen per ml diluted antiserum); the primary antibody was omitted from the incubation; or normal rabbit or rat serum was substituted for the primary antibody.

Double-immunolabeled nerve fibers were analyzed under an Olympus BX51 microscope equipped with epi-fluorescence and appropriate filter sets. The semiquantitative (arbitrary) evaluation of the SP-, CGRP- and GAL-IR nerve fibers density was based on the number of fibers found in the vicinity or within evaluated structures, as previously described by Majewski and Heym [29], with our modification. The results were categorized as: (–) — all studied sections were devoid of a particular class of fibers, (±) — studied fibers were absent from several of the studied sections, (+) — single fibers were observed in each of the studied sections; (+ +) — up to five fibers were observed in each of the studied sections; (+ + +) — six to 20 fibers were observed in each of the studied sections; (+ + + +) — > 20 fibers were found per section. This procedure was applied to nine randomly chosen ovarian sections from each studied animal and then pooled and presented as a mean value.

Results

Data concerning the localization and density of nerve fibers is presented in Table 1, while the co-localization patterns of SP and CGRP as well as SP and GAL within studied nerve fibers are summarized in Table 2.

Distribution and density of SP-, CGRP- and GAL-IR nerve fibers in the control and cystic ovaries

Control ovaries

Cortex

A moderate number of CGRP-IR nerve fibers (Figure 3), as well as single SP- (Figures 2, 14A) and GAL-IR (Figure 14B) nerve terminals were observed in the area of the ground plexus. Single CGRP-IR nerve fibers were found around the primary, secondary (Figure 5B) and tertiary follicles measuring up to 3 mm and 4–6 mm (Figure 6B), in the vicinity of CL and veins. However, SP- and/or GAL-IR nerve terminals were not observed in the proximity of primary, secondary (Figure 5A — SP), tertiary follicles measur-

Table 1. Arbitrary evaluation of the density of SP-, CGRP- or GAL-IR nerve fibers in the porcine ovaries of the control group (CON, n = 6) and the DXM-treated group (DXM, n = 6)

Ovarian tissue	SP		CGRP		GAL	
	Group					
	CON	DXM	CON	DXM	CON	DXM
Cortex						
Ground plexus	±	–	+ + B	+	+	+ + + + B
Follicles:						
Primordial	–	–	–	–	–	–
Primary	–	–	+	–	–	–
Secondary	–	–	+	–	–	–
Tertiary [diameter in mm]:						
to 3	–	–	+	+	–	–
4–6	–	LS	+	LS	–	LS
Cysts	LS	–	LS	+	LS	–
Corpora lutea	–	LS	±	LS	–	LS
Arteries	–	+	–	–	–	–
Veins	–	–	+	+	–	–
Interstitial glands	+	+	+	+	–	–
Medulla						
Ground plexus	±	+	±	+	+	+ +
Arteries	–	+ +	–	+ +	+ +	+ +
Veins	–	+	–	+	–	–

The number of fibers in the vicinity or within structure studied: (–) — the lack of fibers, ± — the lack or single nerve fibers, + — single; + + — from 2 to 5; + + + — from 6 to 20; + + + + — > 20; B — bunches of fibbers; LS — the lack of structure

Table 2. The co-localization of SP and CGRP, as well as SP and GAL in the porcine ovaries of the control group (CON) and the DXM-treated group (DXM)

Ovarian tissue		Group	SP and CGRP	SP and GAL
Cortex	GP	CON	–	*
		DXM	–	–
	PRF	CON	–	–
		DXM	–	–
	PF	CON	–	–
		DXM	–	–
	SF	CON	–	–
		DXM	–	–
	TF to 3 mm	CON	–	–
		DXM	–	–
	TF 4–6 mm	CON	–	–
		DXM	LS	LS
	C	CON	LS	LS
		DXM	–	–
	CL	CON	–	–
		DXM	LS	LS
	A	CON	–	–
		DXM	–	–
	V	CON	–	–
		DXM	–	–
	IG	CON	**	–
		DXM	**	–
Medulla	GP	CON	–	*
		DXM	**	*
	A	CON	–	–
		DXM	*	–
	V	CON	–	–
		DXM	**	–

GR — ground plexus; PRF — primordial follicles; PF — primary follicles; SF — secondary follicles; TF — tertiary follicles; C — cysts; CL — corpora lutea; A — arteries; V — veins; IG — interstitial gland; LS — the lack of structure; * — co-localization in part of the nerve fibers; ** — the co-localization in all nerve fibers and (–) — lack of co-localization of either SP and CGRP or SP and GAL

ing up to 3 and 4–6 mm (Figure 6A — SP), CL and veins. While the interstitial gland was innervated by single SP- and/or CGRP-IR nerve fibers, it was devoid of GAL-positive nerve fibers. It should be stressed that primordial follicles and arterial vessels were devoid of nerve fibers containing studied substances.

Medulla

Single SP- (Figures 9, 16A), CGRP- (Figure 10) or GAL-IR (Figure 16B) nerve fibers were found in the area of the ground plexus. While arteries were supplied by a moderate number of GAL-IR nerve fibers, they were devoid of SP- and CGRP-IR fibers. Furthermore, veins were also devoid of SP-, CGRP- or GAL-IR nerve fibers.

Cystic ovaries

Cortex

A large number of GAL-IR nerve fibers (often forming small fascicles; Figure 15B) and single CGRP-positive fibers (Figure 4B) were found in the area of the ground plexus. However, SP-IR nerve terminals were not observed in this part of the plexus (Figures 4A, 15A). Single CGRP-IR nerve fibers were observed around small tertiary follicles and cysts (Figure 7B), while the SP- (Figure 7A) and GAL-IR nerve fibers were absent near these structures. Moreover, primordial, primary and secondary follicles were also devoid of SP-, CGRP- or GAL-IR nerve terminals. The periarterial and perivenous nerve plexuses were composed of single SP- (Figure 8A) or CGRP-IR nerve fibers, while both kinds of blood vessels were devoid of GAL-IR terminals. The interstitial gland was innervated by single SP- and/or CGRP-IR nerve fibers, but devoid of any GAL-IR input.

Medulla

While a moderate number of GAL-IR nerve fibers were present in the area of the ground plexus (Fig-

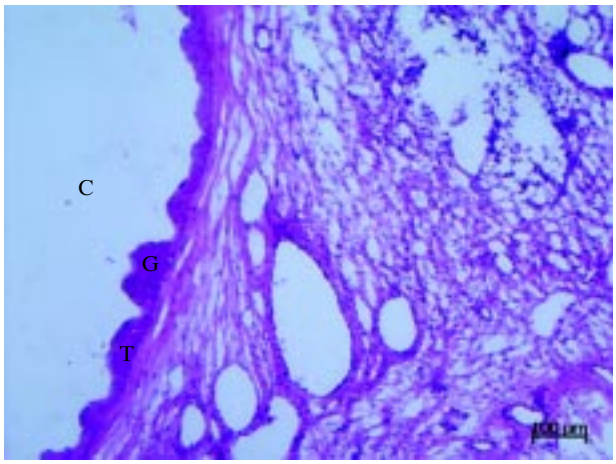


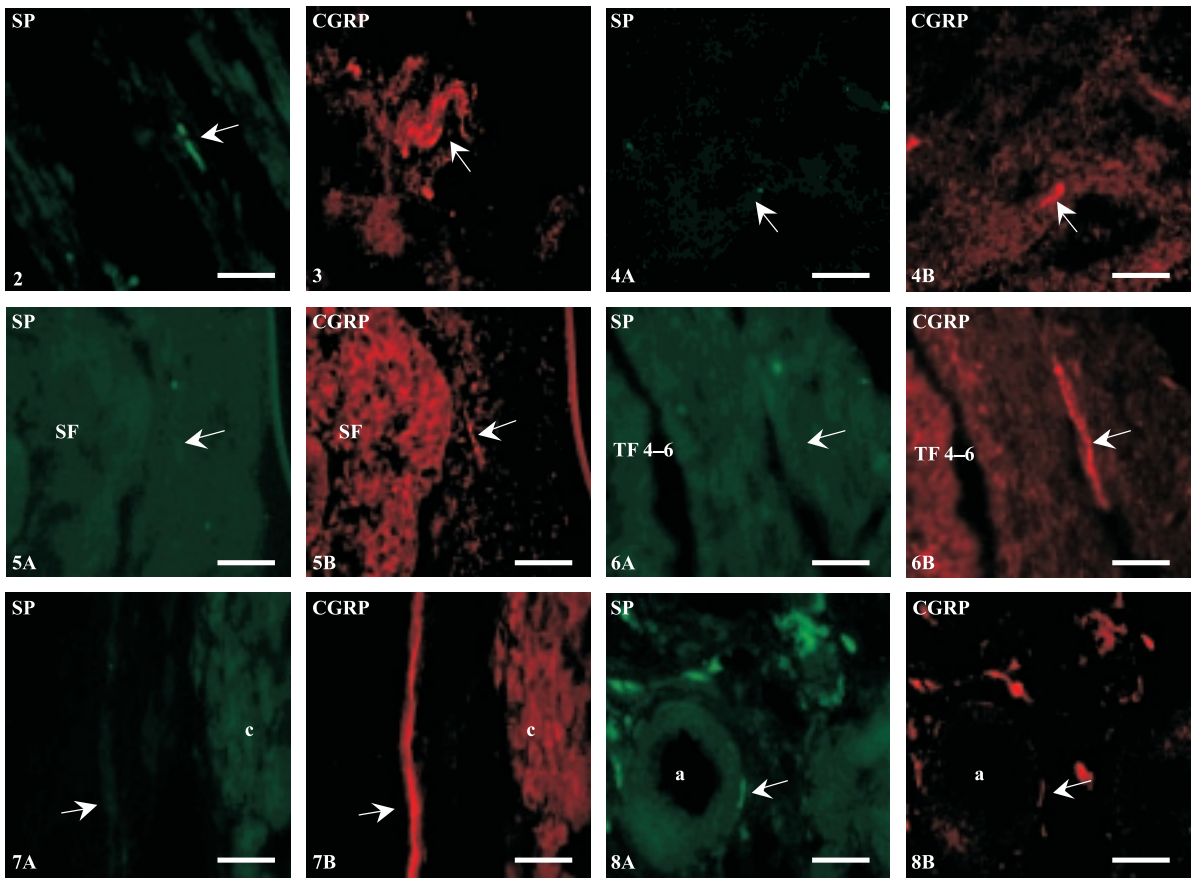
Figure 1. Light microscopic features of a newly formed cyst wall in polycystic ovary of the DXM-treated animal. C — cyst; *T* — theca, *G* — granulose cells (hematoxylin-eosin staining), × 10

ure 17B), only single SP- (Figures 11A, 17A) and CGRP-IR (Figure 11B) nerve terminals contributed to this neural structure. Moreover, while arteries were innervated by a moderate number of SP- (Figure 12A), CGRP- (Figure 12B) or GAL-IR nerve fibers, veins were supplied by single SP- (Figure 13A) or CGRP- (Figure 13B), but not GAL-IR nerve fibers.

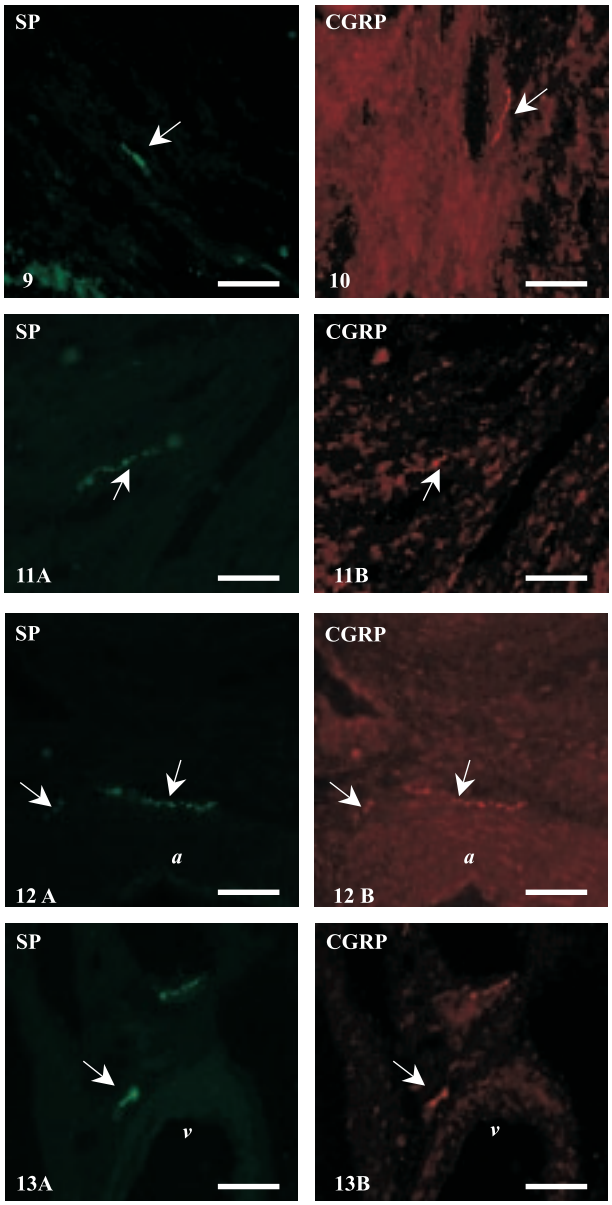
Co-localization patterns of SP and CGRP or SP and GAL within nerve fibers of the control and cystic ovaries

Control ovaries

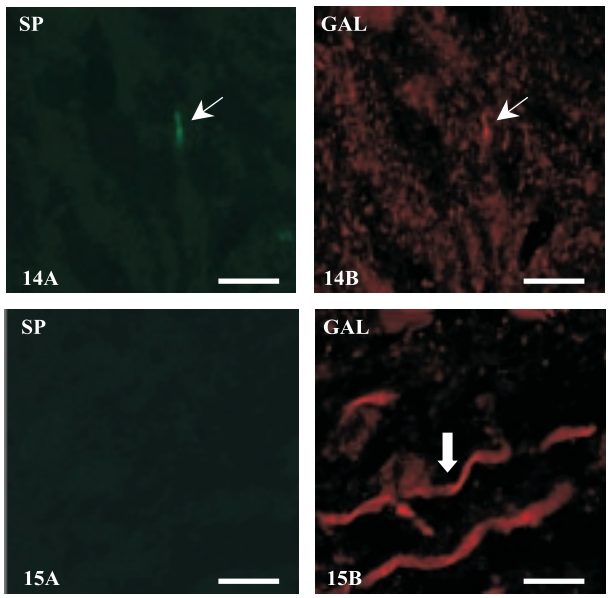
While virtually all SP-IR nerve fibers within the cortical part of the ground plexus were simultaneously GAL-positive (Figures 14A, B), co-localization of both substances was observed in all nerves forming



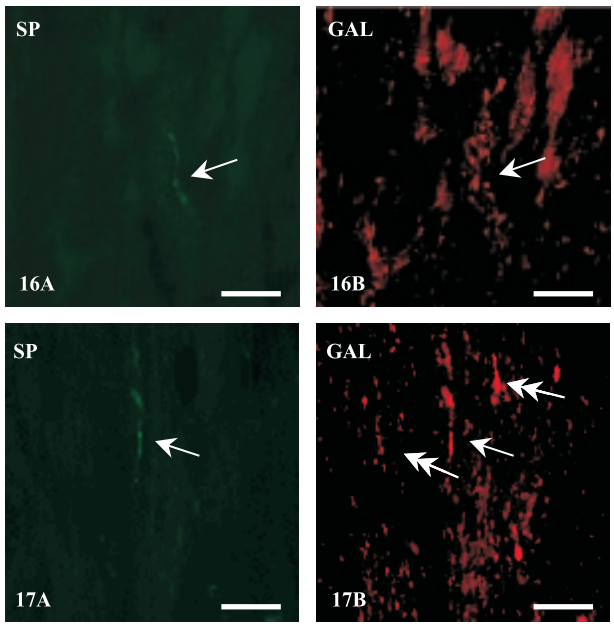
Figures 2–8. Immunohistochemical localization of SP- and/or CGRP-IR nerve fibers in the ovarian cortex of control (CON) and DXM-treated animals (DXM). Typical distribution pattern of SP- (**Figure 2**, usually single, varicose nerve terminals) and CGRP-IR nerve fibers (**Figure 3**, usually bundles of either preterminal, smooth and/or terminal, varicose nerve fibers) within the ovarian cortex of control animals. Please note that the DXM treatment led to a drastic reduction in the number of single SP-IR terminals within the ovarian cortex (**Figure 4A**), while single CGRP-IR nerve fibers were still present in this ovarian domain (**Figure 4B**). In the CON animals, secondary (**Figure 5**) and tertiary follicles (diameter 4–6 mm; **Figure 6**) were innervated by SP-immunonegative (**Figures 5A** and **6A**, respectively) but CGRP-IR varicose nerve fibers (**Figures 5B** and **6B**, respectively). After DXM treatment, nerve fibers observed around newly formed cysts (**Figure 7**) were SP-immunonegative (**A**) but CGRP-IR (**B**), while periarterial terminals (**Figure 8**) expressed usually SP- (**A**), but not CGRP-immunoreactivity (**B**). Arrows — nerve terminal(s); *SF* — secondary follicle; *TF 4–6* — tertiary follicle, diameter 4–6 mm; *c* — cyst; *a* — ovarian artery; scale bars in all figures = 25 μm



Figures 9–13. Immunohistochemical localization of SP- and/or CGRP-IR nerve fibers in the ovarian medulla of control (CON) and DXM-treated animals (DXM) by single- (**Figures 9** and **10**) and double-immunolabeling techniques (**Figures 11–13**). In animals of the CON group, single SP- (**Figure 9**) or CGRP-IR nerve fibers (**Figure 10**) were found within the medullar stroma. A similar picture was observed after DXM treatment within the medullar stroma (**Figures 11A, B**) as well as around arterial (**Figures 12A, B**) and venous vessels (**Figures 13A, B**). Arrow(s) — nerve terminal; *a* — artery; *v* — vein; scale bars in each picture = 25 μm



Figures 14–15. Immunohistochemical localization of SP- and/or GAL-IR nerve fibers in the ovarian cortex of control (CON) and DXM-treated animals (DXM). In the control animals, single SP- (**Figure 14A**) and GAL-IR (**Figure 14B**) nerve fibers were observed in the cortical stroma, while in DXM-treated gilts SP-IR nerve fibers were not present (**Figure 15A**); in contrast, fairly numerous bunches of GAL-IR nerve terminals were observed in this ovarian compartment (**Figure 15B**). Arrow(s) — nerve terminal; scale bars in each figure = 25 μm



Figures 16–17. Immunohistochemical localization of SP- and/or GAL-IR nerve fibers in the ovarian medulla of control (CON) and DXM-treated animals (DXM). In the CON group, single SP- (**Figure 16A**) and GAL-IR (**Figure 16B**) nerve fibers were observed in the medullar part of the ovary, while in the DXM-treated animals in addition to single SP- and GAL-IR nerve terminals (arrows), a moderate number of GAL-IR but SP-immunonegative fibers were also found (**Figures 17A, B**; double arrows). Scale bars in each figure = 25 μm

the medullar part of the ground plexus (Figures 16A and B). Virtually all SP-IR nerve fibers supplying the interstitial gland were simultaneously CGRP-IR.

Cystic ovaries

Double-labeling revealed that virtually all the SP-IR nerve fibers contributing to the medullar part of the ground plexus (Figures 11A and B), as well as those supplying medullar veins (Figures 13A and B) and the interstitial gland were simultaneously CGRP-positive. In turn, only some of the fibers supplying medullar arteries contained simultaneously SP and CGRP (Figures 12A and B). In addition, in the area of the ground plexus of the medulla, all SP-IR nerve fibers were also GAL-positive (Figures 17A and B).

Discussion

This is the first report demonstrating changes in the distribution pattern and number of SP-, CGRP- and/ /or GAL-IR nerve fibers supplying porcine ovaries suffering from an experimentally evoked *status polycysticus*.

In the control ovaries, a moderate number of CGRP-IR nerve fibers, often forming small fascicles, were found within the cortical part of the ground plexus, while nerve terminals expressing SP and/or GAL were distinctly less numerous. Furthermore, single CGRP-IR nerve fibers were observed around all classes of ovarian follicles, CLs, cortical veins as well as the interstitial gland; the latter fibers often coexpressed immunoreactivity to SP. Within the medullar part of the gonad, fairly numerous GAL-IR fibers were found around medullar arteries, while CGRP--IR terminals were sparse in this region. Although our findings are in part consistent with earlier observations coming from immature animals [12, 15] there were, however, also striking differences found, especially concerning the SP- and/or GAL-IR perifollicular nerves. While such chemically coded terminals were present in the vicinity of both the pre- and antral follicles in immature ovaries, this subpopulation of presumably sensory terminals was not observed in proximity to any class of ovarian follicles in the present study. Additionally, the density of periarterial CGRP- and SP-IR fibers was generally higher in the ovaries of immature gilts than in gonads of adult animals studied in this experiment.

It appears possible that the differences in both the distribution and density of SP-, CGRP- and/or GAL-IR nerve fibers observed between immature and mature gonads (present study) may result from the stage of gonad development which is reflected in a remodeling of both the density and the chemical coding of sensory terminals contributing to the ascending limb of neural circuits controlling the maturation (immature gonads) and maintaining the proper function of a fully developed ovary.

We have found that both the distribution pattern and the density of SP/CGRP- and SP/GAL-IR nerve fibers in the cystic ovaries were changed, and that these changes depended on the chemical coding and the target tissue of these terminals. Thus, in the present study, we found that in the gonads challenged with DXM, cortical arteries were innervated by single SP-IR nerve fibers, and that medullar arteries and veins were supplied by SP/CGRP-IR nerve processes. Such a pattern of perivascular innervation was, however, not observed in the ovaries of control animals. It is very interesting that SP-IR nerve terminals, observed in the present study near medullar veins, were not found in the ovaries of the gilts receiving DXM from the 16th day of the estrous cycle [8]. This difference may be a consequence of the different hormonal status of studied animals during the DXM administration period (especially the hormonal milieu at the starting day), or could be a 'snapshot picture' of a remodeling process on the day when the ovaries were collected. This issue needs clarification.

We also found that DXM treatment led to a profound rearrangement of the sensory component of the ground plexus within the ovary: while under these experimental conditions an increase in the density of GAL-IR nerve fibers was observed, we found a simultaneous decrease in the number of CGRP-IR nerve fibers and a total loss of SP-IR terminals from the cortical subdivision of this structure. Furthermore, both the primary and secondary follicles were virtually devoid of CGRP-IR nerve fibers. As has been shown in the rat (a solitary report available so far focuses on this topic), an induction of *status polycysticus* by DHEA treatment led to an increase in the density of CGRP-IR nerve fibers within the gonad [23]. Thus, it appears reasonable to assume that differences observed between this and our study are probably related to different hormones, i.e., most probably different mechanisms underlying cyst formation, as well as the species-specific characteristics of gonad innervation pattern.

Explaining the mechanism(s) leading to changes observed in the sensory innervation of cystic ovaries is difficult due to the lack of available data from other studies concerning this issue. It can be assumed that the changes in the pattern of sensory innervation may be attributable either to the mechanisms underlying cysts formation, or may result from the changed hormonal/trophic factors milieu in the cystic gonads. Furthermore, it appears possible that the lack of SP-IR terminals and reduction in the number

of CGRP-IR nerve fibers within the ground plexus of the cystic ovaries, as well as the lack of CGRP-IR nerve fibers in the vicinity of preantral follicles, were caused by the DXM *per se*. This assumption is indirectly supported by the fact that, in the rats, DXM led to a decline in the immunoreactivity of SP and CGRP in normal dental nerves [30], uninjured pulp [31], as well as in mRNA CGRP expression in the human medullary thyroid carcinoma cell line [32]. Thus, DXM may influence the distribution and density of sensory nerve fibers in the cystic ovaries acting directly on ovary-projecting afferent neurons. This supposition is based on the finding that a subset of SP-, CGRP- and/of GAL-expressing neurons within the lumbar dorsal root ganglia (DRG) is simultaneously bearing nuclear glucocorticoid receptors [33].

On the other hand, changes observed in the chemical coding of studied neurons may also be attributable to direct effects of steroid hormones on these cells. It is known that estrogen receptors (ERs) are expressed in rat DRG, paracervical ganglion neurons and sympathetic neurons projecting to the ovaries and uterine cervix [34–36]. Furthermore, the neurons possessing androgen receptors in the DRG of male rats also contained CGRP [37]. The expression of ERs in rat DRG neurons changed in the estrous cycle in a phase-related manner [34]. Exogenous estrogens administration reduced the number of ERs-containing ovarian neurons in the rat celiac ganglion [38]. Also, long-term estrogen treatment led to a decrease in the number of both noradrenergic and ERs-expressing ovarian neurons in the caudal mesenteric ganglion of adult gilts [39]. It is worth noting that in the gilts from which cystic ovaries were collected for the present study, the levels of estrogens and androstendione increased in the peripheral blood (unpublished data), and the intraovarian expression of steroidogenic enzymes also changed [26]. It is possible that changes in steroid metabolism in cystic ovaries partly result from DXM, as has been indicated previously [40].

As mentioned above, DXM injections led to an appearance of SP- and/or CGRP-IR nerve fibers in the vicinity of the blood vessels in the cystic ovaries. These findings suggest that blood flow through cystic gonads may be regulated by SP and CGRP released from these perivascular nerve endings, as it has been shown that under physiological conditions, SP [16] and CGRP [22] are able to control the amount of blood supplying the ovary by influencing the contractibility of porcine ovarian arteries.

In conclusion, the present study shows that the profound changes in the distribution and number of SP-, CGRP- and/or GAL-IR nerves within the porcine gonad with DXM-induced *status polycysticus* appear to be dependent on the chemical coding of the affected nerve terminal and the kind of its target tissue, thus implying its susceptibility to changed hormonal/trophic factor milieu challenged by cyst formation. Whether the observed changes are simply the result of a changed microenvironment of affected nerve terminals, or whether nerves expressing a changed neurochemical phenotype are playing a role in the active maintenance of cysts formation, remains to be elucidated.

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