FERTILITY AND STERILITY®
VOL. 75, NO. 6, JUNE 2001

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries

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Objective: To quantify nerve fibers and mast cells in human ovaries at different functional stages.

Design: Retrospective study.

Setting: Research laboratory of the university.

Specimen(s): 8 human ovaries in the follicular (cyclic) phase, 7 polycystic ovaries, and postmenopausal ovaries with (n=5) or without (n=7) hyperthecosis.

Main Outcome Measure(s): Single- and double immunohistology for the S100 antigen in glial cells of autonomic nerve fibers, for chymase and tryptase in mast cells, and for the common leukocyte antigen on leukocytes. Histometric evaluation was also performed.

Intervention(s): None.

Result(s): Polycystic ovaries contained significantly more S100-positive nerve fibers in the corticomedullary region than did cyclic ovaries (mean ± SD per 2-mm^2 area, 476 ± 136 and 224 ± 133; $P<.01$). Postmenopausal ovaries with or without hyperthecosis had the highest density of nerve fibers. In cyclic and polycystic ovaries, more tryptase-positive mast cells than chymase-positive mast cells were found in the interstitial cortex and the medulla. In cyclic ovaries, areas with a moderate density of nerve fibers contained many mast cells. Hence, with increasing nerve fiber density in polycystic ovaries, the number of mast cells decreased strikingly compared with cyclic ovaries ($p<.001$). Almost no mast cells were seen in postmenopausal ovaries with and without hyperthecosis. The number of leukocyte antigen–positive leukocytes was similar in all groups.

Conclusion(s): The high density of nerve fibers in polycystic and postmenopausal ovaries, together with a conspicuous decrease in mast cells, indicates altered neuroimmune communication. (Fertil Steril® 2001;75: 1141–7. ©2001 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovaries, postmenopausal ovaries, ovarian innervation, mast cells, neuroimmunoendocrinology

Received July 11, 2000; revised and accepted February 14, 2001.

Supported by the Interdisciplinary Centre of Clinical Research at the University of Leipzig, project B6.

Presented at the Annual Conference of the Society for the Study of Fertility, Edinburgh, United Kingdom, July 31–August 2, 2000.

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0015-0282/01/$20.00
PII S0015-0282(01)01805-2

Strong evidence indicates that in the polycystic ovary syndrome (PCOS) (1–4), P450c17α, the enzyme for the catalyzing steps in androgen biosynthesis, and the aromatase complex for estrogen synthesis are deranged in thecal and granulosal cells, respectively (5, 6). It has therefore been hypothesized that disturbed steroidogenic production leads to increased androgen synthesis, which then induces growth of multiple cysts. A cofactor such as increased LH levels from a hypothalamic disorder is of secondary importance and induces the fully developed syndrome (1, 2). If increased ovarian androgen production represents the initial event, local factors that trigger the disturbed steroidogenic pathway must be defined.

Intraovarian nerve fibers may be considered modulators of steroid production in the ovary. This effect is exerted by β_2-adrenoreceptors that are located on thecal cells and that, when activated by catecholamines, stimulate androgen secretion (7, 8). Hyperactivation of the sympathetic innervation may play a role in the polycystic ovaries for two reasons. First, rats with estrogen-induced polycystic ovaries have been shown to have high uptake and levels of norepinephrine and a high degree of transmitter release after electrical stimulation of the ovary (9). In addition, polycystic ovaries from human patient with PCOS have more catecholaminergic nerve fibers than do normal ovaries (10).

It is unclear whether changes in nerve fibers first affect the cortical stroma, and thereafter

cause the formation of multiple cysts, in polycystic ovaries with or without clinically expressed PCOS. To address this issue by using a morphologic approach, we compared the density of nerve fibers in polycystic and postmenopausal ovaries; both have stromal hyperplasia, yet they differ according to presence or the absence of multiple cysts.

Mast cells are regularly found in ovaries; more of these cells are found in the medulla than the cortex, as we have recently shown in the bovine ovary (11). Mast cells are predominantly located adjacent to small blood vessels and to nerve fibers and can promote close contact between adrenergic and peptidergic nerve fibers, as is found in the skin and the gut (12, 13). Mast cells are involved not only in allergic and parasitic reactions, but also in inflammatory events because they have key immunoregulatory cytokines and can activate endothelial cells to recruit leukocytes (14, 15).

Traditionally, mast cells have been thought to be a potent effector arm of the immune system only. However, mast cells are now thought to link the immune and the nervous system because of their responsiveness to neurotrophins and neuropeptides and their ability to synthesize and release neurotropic factors (16–18). Because this nerve pattern appears to be altered in polycystic ovaries, the communication between mast cells and nerve fibers may be disturbed. This question has not yet been addressed for the cyclic and the noncyclic ovary.

We carried out single and double immunolabeling of paraffin sections from cyclic human ovaries, polycystic ovaries, and postmenopausal ovaries to demonstrate S100-positive autonomic nerve fibers (16) and mast cells in these tissues. Postmenopausal ovaries were classified as without or with hyperthecosis (non-neoplastic proliferation of the ovarian stromal cells together with luteinized stromal cells) (20, 21) Compared with cyclic ovaries, noncyclic ovaries exhibited more nerve fibers, and mast cells were decreased in number or were missing.

MATERIALS AND METHODS

Paraffin-embedded ovaries from women of reproductive age (who had had surgery for nonmalignant and malignant uterine tumors in the past 10 years) were collected from the archives of Dr. M. Holzwarth, Institute of Pathology, Ulm, Germany. The functional stage of the ovaries was retrospectively classified according to the clinician's report and to the pathologist's diagnosis of the ovary and the endometrium.

The paraffin-embedded ovaries without corpora lutea (n = 2) were assigned the follicular phase—here, termed "cyclic ovaries." Large wedge resections (2 × 1 cm surface area) of polycystic ovaries (n = 4) were studied. These were obtained from patients with PCOS from multiple cysts lying beneath a superficially fibrotic cortex, as diagnosed by the clinician and confirmed by the pathologist's report. Polycystic ovaries (n = 3) were also obtained from the Institute of Forensic Medicine (see below). In addition, we studied ovaries from postmenopausal women 51 to 94 years of age who did (n = 5) or did not (n = 7) have morphologic signs of stromal hyperthecosis. The polycystic and postmenopausal ovaries were considered noncyclic.

Ovaries were also obtained from women 18 to 44 years of age within the first 2 days after the women had had a fatal car accident. The ovaries were fixed in 4% buffered formaldehyde. Parts of the cortex with antral follicles and the adjacent medulla, as well as parts of the corpora lutea, were dissected and embedded in paraffin wax.

The ovaries from women who died in a car accident were considered to be in the follicular phase (n = 6) because several antral follicles were larger than 1.5 cm in diameter and because regressing corpora lutea in the right or left ovary were present. Regression was histologically confirmed by degenerating luteal cells and the disappearance of capillaries. In addition ovaries (n=3) were roughly three times larger than cyclic ovaries. They were classified as polycystic ovaries because of the absence of corpora lutea, even though they had multiple cysts and even though it is known that roughly 20% of women at reproductive age have polycystic ovaries without expressing PCOS (17).

It was not necessary to obtain written consent from the patients for this retrospective study. The Faculty Committee for Ethical Approval of the University of Leipzig had no objections to this project.

Indirect single immunolabeling was done in 7-μm thick serial sections mounted on object slides that had been coated with paper glue for strong section adherence. For detection of all autonomic nerve fibers, polyclonal goat anti-S100 antibody was used (catalog no. 1971; Immunotech, Hamburg, Germany). This antibody detects human S100 protein A and B. The antibody labeled peripheral glial (Schwann) cells (13) and was used undiluted or at a 1:5 dilution in 0.01 M phosphate-buffered saline with 0.25% bovine serum albumin (A-4378; Sigma, Deisenhofen, Germany). Reproducible results for further localization of sympathetic and sensory fibers were not obtained with anitbodies against calcitonin gene–related peptide, vasointestinal peptide, substance P, tyrosinhydroxylase, or PGP 9.5 (a general marker for nerve fibers).

For successful mast cell staining with monoclonal mouse anitbodies, either against tryptase (diluted 1:500; code M 7052; DAKO, Hamburg, Germany) or chymase (diluted 1:2000; catalog no. MAB 1254; Chemicon International, Hofheim, Taunus, Germany), enzymatic pretreatment of the paraffin sections with 0.1% pronase E (type XIV; Sigma) in 0.5 M Tris-HCl, pH 7.6, at 37°C for 20 minutes was performed. This immunolabeling allowed us to differentiate mast cells of the mucosal type from those of the connective type (15). Mast cells were also revealed by using the classic staining technique—1% toluidine blue dissolved in distilled

water. To show all leukocytes, monoclonal mouse anitbody against the leukocyte common antigen (diluted 1:100; catalog n. MO 701; DAKO) was used.

Our detection system consisted of biotinylated secondary antibodies and the avidin–biotin horseradish peroxidase complex (Vectostain Elite ABC kit; Vector Laboratories, Alexis, Grünberg, Germany). We used 0.01 M phosphate-buffered saline and 0.05 M Tris-HCl–buffered saline (pH 7.6) for antibody dilution and buffer rinses, respectively. Buffer rinses were done first with Tris-HCl–buffered saline and 0.125% Tween 20 (two rinses of 5 minutes) and then with Tris-HCl–buffered saline only.

The method used for single immunolabeling has been described elsewhere (8): First, to inactivate endogeneous peroxidase activity and reduce background staining, sections were treated with 3% H_20_2 in absolute methanol for 30 minutes, rinsed in Tris-HCl–buffered saline three times for 5 minutes each time, and incubated with 2% normal goat serum in phosphate-buffered saline for 20 min. Second, without buffer rinse, sections were incubated with the primary antibody (polyclonal from rabbit or monoclonal from mouse) in a humid chamber at 4°C overnight. Third, sections were treated with the second goat-antimouse antibody or goat-antirabbit antibody (BA-9200 and BA-1000, respectively, Vector Laboratories), both diluted 1:200 in Tris-HCl–buffered saline with 1.5% normal goat serum; incubation was done at room temperature for 20 minutes. Fourth, sections were treated with the avidin–biotin horseradish peroxidase complex (50 drops of kit solution into 50 mL of Tris-HCl–buffered saline) for 30 minutes.

Finally, 0.02% 3′3′-diaminobenzidine (DAB; Aldrich, Basel, Switzerland) was prepared in 0.05 M Tris-HCl, pH 7.6, and 5 μL of an 8% $NiCl_2$ stock solution per mL DAB solution plus 0.03% H_20_2 was added. Histochemical peroxydase detection was carried out at room temperature for 10 minutes. Sections were rinsed in distilled water, counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted in Canada balsam. Control sections that were run with normal goat or mouse immunoglobulin of the same subclass as the primary antibody remained negative, and mast cells did not bind the avidin–biotin complex.

Double immunolabeling was done for colocalization of tryptase-positive mast cells and nerve fibers. For procedure 1, paraffin sections were digested with pronase, and tryptase-positive mast cells were labeled as described above. After the DAB reaction, a brown reaction product indicated the positive cells. For procedure 2, nerve fibers first stained with the anti-S100 antibody and avidin–biotin immunocomplex were then as red-stained structures detected by using 3-amino-9-ethylcarbazole (AEC; Sigma) (11). Sections were treated with the AEC solution at 37°C for 10 minutes. After sectioned were rinsed with distilled water and counterstained with Ehrlich hematoxylin, they were embedded in water-soluble medium (Crystal Mount; Natutec GmbH, Frankfurt, Germany). Pictures were taken by using an Axioplan 2 light microscope (Zeiss, Jena, Germany) on Agfapan 25 (AGFA, Leverkusen, Germany) and EPY64T film (Kodak, Stuttgart, Germany).

Nerve fibers (regardless of their length), mast cells, and leukocytes were counted in serial sections stained with single immunolabeling for S100-positive nerve fibers, for tryptase-positive mast cells, or for leukocyte antigen–positive leukocytes, respectively. S100-positive nerve fibers and tryptase-positive nerve fibers were also determined by using double immunolabeling. The sections were evaluated with an ocular square grid at objective magnification of ×20.

The inner zone of the cortex was examined just adjacent to the medulla (i.e., the corticomedullary region), which was characterized by thick-walled arterioles. Because the nerve fibers differed in density between cyclic and in polycystic ovaries, only areas with a high density of nerve fibers were screened. Five separate fields, each 0.4 mm^2 in area, were counted, and the number was summarized for a final area of 2 mm^2.

Data were calculated as the means ±SD of each group. Counting the structures twice on two different days by the same observer produced variations of up to 5%. Two observers also evaluated the sections in a blinded manner and most often correctly attributed the sections to the different ovarian groups. The Mann–Whitney *U* test was used to test for statistically significant differences between the groups.

RESULTS

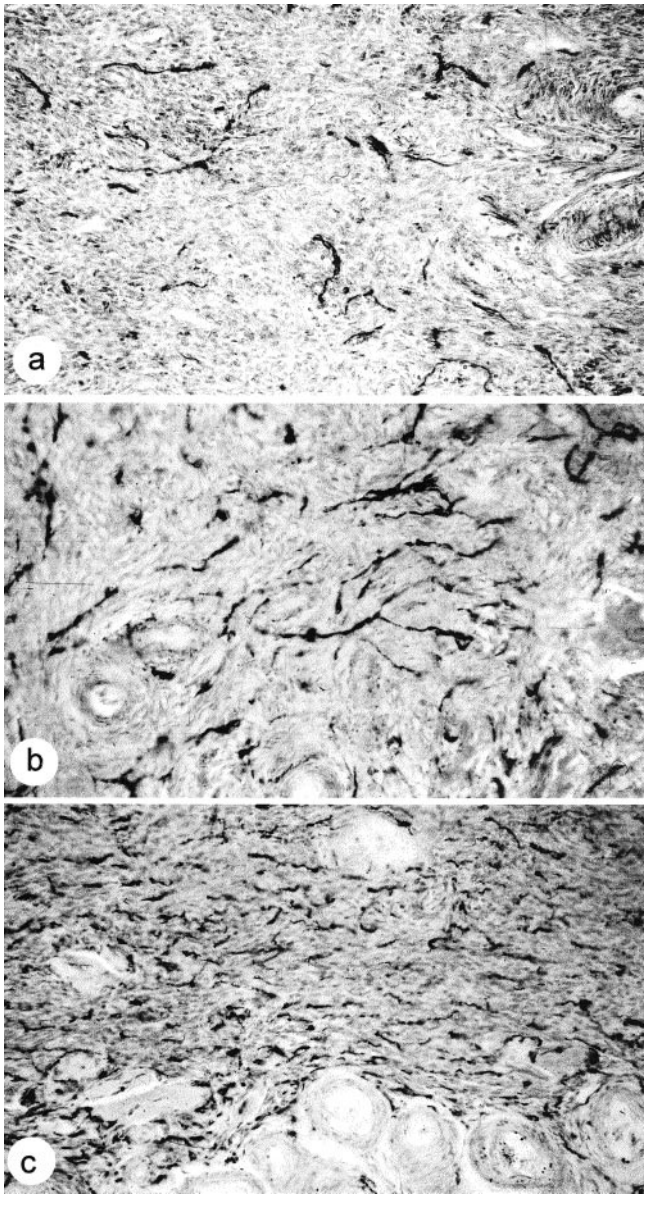
Fewer S100-positive nerve fibers were noted in cyclic ovaries than in polycystic ovaries (Figure 1, a and b). Because the number of nerve fibers seemed to be similar in polycystic ovaries from patients without and those with clinically expressed PCOS, the ovaries were evaluated together in the polycystic group. In ovaries with hyperthecosis, characterized by nodular or diffuse stromal hyperplasia of the cortex as well as by luteinized interstitial gland cells, S100-positive fibers appeared to be higher in density than in polycystic ovaries (Figure 1, c). The postmenopausal ovaries without hyperthecosis showed diffuse stromal proliferation and were similar in appearance to ovaries with hyperthecosis. Both exhibited a similar amount of S100-positive nerve fibers.

Ovaries in the follicular phase had the lowest number of nerve fibers (mean [±SD] per 2-mm^2area, 224 ± 133) and the polycystic ovaries had an intermediate number (476 ± 136) compared with postmenopausal ovaries with (601 ± 156) and those without (695 ± 100) hyperthecosis (Figure 2).

We compared the number of toluidine blue–stained mast cells with the number of tryptase- or chymase-positive mast cells as detected by single immunolabeling. The best results

FIGURE 1

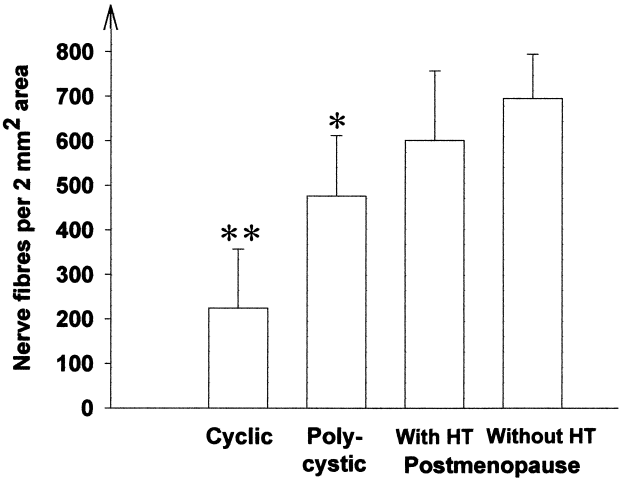
S100-positive nerve fibers in the cortico-medullary region of human ovaries on immunohistology. (**a**), Polycystic ovary. (**b**), Cyclic ovary. (**c**), Postmenopausal ovary. More nerve fibers are seen in the polycystic ovary than in the cyclic ovary, and the highest nerve density is found in postmenopausal ovaries with hyperthecosis. Magnification in all panels, ×130.



Heider. Nerve fibers and polycystic ovary. Fertil Steril 2001.

FIGURE 2

The number of S100-positive nerve fibers in cyclic ovaries, polycystic ovaries, and postmenopausal ovaries with and those without hyperthecosis (*HT*). Corticomedullary areas with high nerve fiber density were examined in immunolabeled sections. Data are means ±SD. $^{**}P<.01$ vs. polycystic ovaries; $^{*}P<.05$ vs. postmenopausal ovaries.



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were obtained with immunolabeling for tryptase, because only 60% chymase-positive mast cells and 69% toluidine blue stained cells were counted in relation to tryptase-positive mast cells.

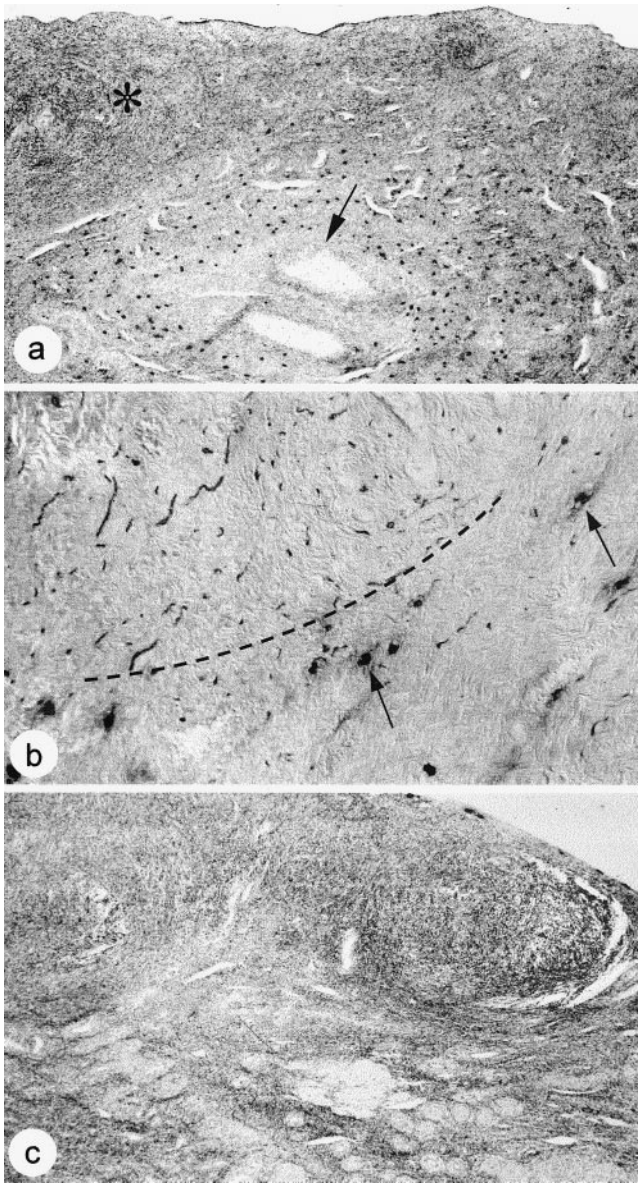
In cyclic ovaries, mast cells were found neither in the outer cortex nor in the wall of intact or regressing preantral and antral follicles. However, mast cells were distributed in the hilus, the medulla, and the inner cortex (Figure 3, a). Cortical areas with many mast cells alternated with areas containing few mast cells. These areas with few mast cells distinctly exhibited more S100-positive nerve fibers in serial sections of cyclic ovaries and on double immunolabeling (Figure 3, b). This opposite relationship was the reason why the close contacts between nerve fibers and mast cells were rarely seen in cyclic ovaries. In polycystic ovaries, an irregular distribution of nerve fibers and mast cells was also evident in the corticomedullary region, yet the areas with increased nerve fiber density were more extended.

A distinct loss of tryptase-positive mast cells was observed in polycystic ovaries compared with cyclic ovaries when mast cells were counted in areas with many nerve fibers. Mast cells were almost absent in the inner cortex and the medulla of postmenopausal ovaries with and those without hyperthecosis (Figure 3, c). However, mast cells were retained in the hilus, which was defined by the presence of large blood vessels. Loss of mast cells did not signify an absence of leukocytes because the corticomedullary region was populated by leukocyte antigen–positive leukocyte, as demonstrated in serial sections.

This loss in mast cells was confirmed by counting tryptase-positive mast cells (Figure 4). When mast cells in areas with many nerve fibers were counted, the most mast cells were found in cyclic ovaries (mean ±SD per 2-mm^2

FIGURE 3

Mast cells in human ovaries shown by single immunolabeling for tryptase and by double immunolabeling, which also locates S100-positive nerve fibers. (**a**), On single immunolabeling, the cyclic ovary shows tryptase-positive mast cells in the cortical stroma, with the exception of the two regressing follicles (*arrow*) and parts of the cortex (*asterisk*). Magnification, ×35. (**b**), On double immunolabeling, the cyclic ovary shows mast cells (*arrows*) in an area with a negligible number of nerve fibers (below the dashed line). Magnification, ×70. (**c**), In hyperthecosis, mast cells are missing in the cortex and the medulla. Magnification, ×35.



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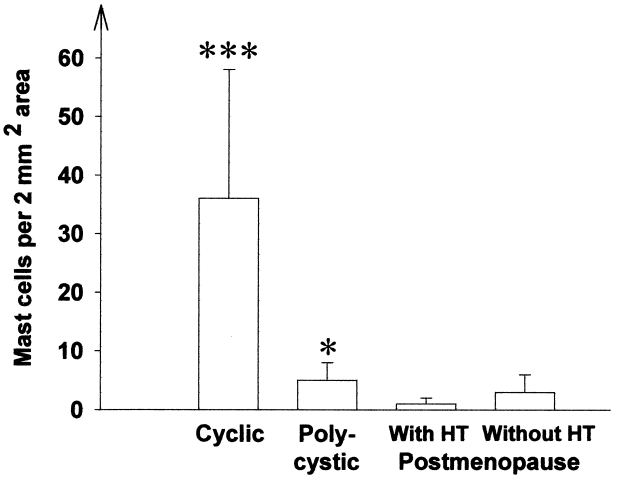
area, 36 ± 22) fewer were found in the polycystic ovaries (5 ± 3), and very few were found in postmenopausal ovaries with (1 ± 1) and without (3 ± 3) hyperthecosis.

FIGURE 4

The number of tryptase-positive mast cells has been counted in cyclic and non-cyclic ovaries and in postmenopausal ovaries. Cortico-medullary areas with high nerve fiber density were examined. Data are means ± SD. ***p<.001 vs polycystic, postmenopause with and without hyperthecosis (HT); *p<.05 vs postmenopause with HT.



Heider. Nerve fibers and polycystic ovary. Fertil Steril 2001.

We then evaluated the number of leukocyte antigen–positive leukocytes without selecting particular cortico-medullary areas. No statistically significant differences in leukocyte count were apparent in cyclic and noncyclic ovaries (mean ± SD per 2 mm^2 area, 49 ± 8 for cyclic ovaries, 49 ± 6 for polycystic ovaries, and 67 ± 32 for postmenopausal ovaries). When mast cells were counted in the same areas, the percentage in comparison with leukocyte antigen-positive leukocytes decreased strikingly: 89% of mast cells in the cyclic ovaries, 35% in the polycystic ovaries, and 3% to 6% in postmenopausal ovaries.

DISCUSSION

Thirty years ago, Semenova (10) showed an increase in catecholaminergic fibers in ovaries from patients with PCOS. We confirm this observation for polycystic ovaries, even though it is difficult to obtain adequately large wedge resections from patients with PCOS. Nonetheless, our observation allows to suggest that the high density of nerve fibers represents the first step in the appearance of multiple cysts. In the second step, PCOS manifests itself in heterogeneous clinical symptoms, such as obesity; hyperinsulinemia (with or without insulin resistance); and high levels of LH, prolactin, and androgen (1–3).

The increase in nerve fiber density that we observed may be involved in non-neoplastic proliferation of stromal cells, which is known to produce up to a fivefold increase in

cortical volume in polycystic ovaries compared with cyclic ovaries (2, 20). Most postmenopausal ovaries display varying degrees of nodular or diffuse stromal hyperplasia, being connatural with stromal hyperthecosis (21). Because the postmenopausal ovaries that we studied developed stromal hyperplasia together with the highest density in nerve fibers, this finding is not observed in polycystic ovaries only. We can therefore deduce that nerve fiber changes first reach the cortical stroma and thereafter induce polycystic formation if follicles are still present.

Adrenergic hyperactivation has been reported in estrogen-induced polycystic ovaries in the rat (9). This is likely to be related to increased production of nerve growth factor (NGF), as suggested by the studies of Dissen et al. (22). These investigators implanted NGF-producing progenitor cells into the rat ovary to induce chronically high NGF levels, which interrupted the estrous cycle and caused precystic follicles and high androgen production. They also suggested that NGF may indirectly affect androgen production via low- and high-affinity receptors expressed on thecal cells (22). Recently, increased synthesis and expression of NGF and its low-affinity receptor were reported in estrogen-induced polycystic ovaries in the rat (23). The dense supply of nerve fibers in polycystic and postmenopausal ovaries that we observed may be caused by a similar mechanism.

Because NGF as well as its low- and high-affinity receptors have been located at the protein and messenger RNA level in antral follicles, they are regarded as the source of NGF production (8). Ovarian mast cells that produce and respond to NGF (17, 24) represent another, presumably essential source. In our study, the number of tryptase-postive mast cells was higher in cyclic ovaries than in chymase-positive mast cells. Thus, ovarian mast cells do not behave like characteristic connective tissue mast cells that produce both tryptase and chymase (19). Similar to the mast cell distribution in the bovine ovary (8), mast cells favored particular compartments (apart from follicles and corpora lutea) in the human ovary: the medulla and the interstitial cortex. Because close contacts between mast cells and nerve fibers were too few in number both in the cyclic and the polycystic ovaries, we omitted the tedious process of evaluating extended series of sections double-stained for mast cells and nerve fibers.

The density of nerve fibers and mast cells appears to be inversely related. Fewer S100-positive nerve fibers appeared in cyclic ovaries that had more mast cells; in comparison, polycystic ovaries contained more nerve fibers and fewer mast cells. Postmenopausal ovaries with and those without hyperthecosis had the highest density of nerve fibers and often lacked mast cells. However, leukocytes were numerous in the postmenopausal ovaries, raising the question of whether mast cells were absent or whether they lacked protease-positive granules. If lack of protease-positive granules, the immunohistologic technique is inadequate and an ultrastructural study is recommended.

Absence of granules in mast cells may be from a subacute degranulation produced by changes in the local milieu or recently immigrated and still-immature mast cells that are unable to develop granules. Mast cells without granules may still synthesize and release NGF for growth and maintenance of intraovarian nerve fibers by an alternate, IgE-independent secretory mechanism (26). We favor this possibility because in postmenopausal ovaries, antral follicles are excluded as source of NGF production (8). In addition, the presence of granule-containing mast cells in the hilus of postmenopausal ovaries may indicate that not immigration but maturation of mast cells is disturbed.

In summary, we report that nerve fiber density is increased and the number of mast cells is decreased in polycystic and postmenopausal ovaries. This finding indicated a still-unknown neuroimmune connection and altered function between intraovarian nerve fibers and mast cells in polycystic ovaries.

Acknowledgments: The authors thank Dr. R. Spanel for review of the paraffin-embedded samples when working at the Institute of Pathology and Dr. M. Holzwarth, Head of the Institute of Pathology, Ulm, Germany; Sylvia Spenner for secretarial assistance; and Albrecht Rast for help with the computer program.

References

1. Strauss JF III, Dunaif A. Molecular mysteries of polycystic ovary syndrome. Mol Endocrinol 1999;13:800–5.
2. Homburg R. Polycystic ovary syndrome—from gynaecological curiosity to multisystem endocrinopathy. Hum Reprod 1996;11:29–39.
3. Jahanfar S, Eden JA. Genetic and non-genetic theories on the etiology of polycystic ovary syndrome. Gynecol Endocrinol 1996;10:357–64.
4. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, et al. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci U S A 1999;96:8573–8.
5. Barnes RB. Pathophysiology of ovarian steroid secretion in polycystic ovary syndrome. Semin Reprod Endocrinol 1997;15:159–68.
6. Pierro E, Andreani CL, Lazzarin N, Cento R, Lanzone A, Caruso A, et al. Further evidence of increased aromatase activity in granulosa luteal cells from polycystic ovary. Hum Reprod 1997;12:1890–6.
7. Dyer CA, Erickson GF. Norepinepherine amplifies human chorionic gonadotropin-stimulated androgen biosynthesis by ovarian theca-interstitial cells. Endocrinology 1985;116:1645–52.
8. Ojeda SR, Dissen GA, Malamed S, Hirshfield AN. A role for neurotrophic factors in ovarian development. In: Hsueh AJW, Schomberg DW (eds). Ovarian cell interactions: genes to physiology. New York: Springer-Verlag, 1993:181–202.
9. Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR. Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology 1993;133:2690–5.
10. Semenova II. Adrenergic innervation of ovaries in Stein-Leventhal syndrome. Vestn Akad Med Nauk SSSR 1969;24:58–62.
11. Reibiger I, Spanel-Borowski K. Difference in localization of eosinophils and mast cells in the bovine ovary. J Reprod Fertil 2000;118:243–9.
12. McKay DM, Bienenstock J. The interaction between mast cells and nerves in the gastrointestinal tract. Immunol Today 1994;15:533–8.
13. Botchkarev VA, Eichmüller S, Peters EMJ, Pietsch P, Johansson O, Maurer M, et al. A simple immunofluorescence technique for simultaneous visualization of mast cells and nerve fibers reveals selectivity and

hair cycle-dependent changes in mast cell-nerve fiber contacts in murine skin. Arch Dermatol Res 1997;289:292–302.
14. Kubes P, Granger DN. Leukocyte-endothelial cell interactions evoked by mast cells. Cardiovasc Res 1996;32:699–708.
15. McNeil HP. The mast cell and inflammation. Aust N Z J Med 1996; 26:216–25.
16. Blennerhassett MG. Nerve and mast cell interaction: cell conflict or information exchange. In: Fischbach GD (ed). Molecular neurobiology: mechanisms common to brain, skin, and immune system. New York: Wiley-Liss, 1994:225–41.
17. Purcell WM, Atterwill CK. Mast cells in neuroimmune function: neurotoxicological and neuropharmacological perspectives. Neurochem Res 1995;20:521–32.
18. Dines KC, Powell HC. Mast cell interactions with the nervous system: relationship to mechanisms of disease. J Neuropathol Exp Neurol 1997;56:627–40.
19. McNutt NS. The S100 family of multipurpose calcium-binding proteins. J Cutan Pathol 1998;25:521–9.
20. Clement PB. Nonneoplastic lesions of the ovary. In: Kurmann RJ (ed). Blaustein's pathology of the female genital tract. New York: Springer-Verlag, 1994:597–645.
21. Clement PB. Anatomy and histology of the ovary. In: Kurmann RJ (ed). Blaustein's pathology of the female genital tract. New York: Springer-Verlag, 1994:563–95.
22. Dissen GA, Lara HE, Leyton V, Paredes A, Hill DF, Costa ME, et al. Intraovarian excess of nerve growth factor increases androgen secretion and disrupts estrous cyclicity in the rat. Endocrinology 2000;141:1073–82.
23. Lara HE, Dissen GA, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, et al. An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. Endocrinology 2000;141:1059–72.
24. Leon A, Buriani A, Dal Toso R, Fabris M, Romanello S, Aloe L, et al. Mast cells synthesize, store, and release nerve growth factor. Proc Natl Acad Sci U S A 1994;91:3739–43.
25. Mécheri S, David B. Unravelling the mast cell dilemma: culprit or victim of its generosity. Immunol Today 1997;18:212–5.
26. Lorenz D, Wiesner B, Zipper J, Winkler A, Krause E, Beyermann M, Lindau M, Bienert M. Mechanism of peptide-induced mast cell degranulation. J Gen Physiol 1998;112:577–91.