Neuroendocrine cells in eutopic endometrium of women with endometriosis

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BACKGROUND: Endometriosis is a common gynaecological disease, but the pathogenesis of endometriosis and pathophysiological basis for endometriosis-associated painful symptoms are still uncertain. Little is known about neuroendocrine (NE) cells in the uterus.

METHODS: For this study, 38 premenopausal women with histologically diagnosed ovarian endometrioma or peritoneal endometriosis and 24 women without endometriosis were selected. Biopsy samples from eutopic endometrium were used for immunohistochemical staining to detect synaptophysin (SYN) and neuron-specific enolase (NSE) expression in women with and without endometriosis.

RESULTS: There were substantially more NE cells of eutopic endometrium stained with SYN and NSE in women with endometriosis than in those without endometriosis (3.8 ± 1.8 versus 0.5 ± 0.7/mm², P < 0.001, and 2.8 ± 2.1 versus 0.4 ± 0.6/mm², respectively, P < 0.001). These cells were scattered in the epithelium of endometrial glands. At all stages of the menstrual cycle, the densities of NE cells stained with SYN and NSE were greater in women with endometriosis than in those without endometriosis (P < 0.05).

CONCLUSIONS: These results suggest that NE cells in eutopic endometrium probably play some role in the pathogenesis or symptoms of endometriosis.

Key words: endometriosis / neuroendocrine cells / pathogenesis / nerve regeneration / pain

Introduction

Endometriosis occurs in 10–15% of women of reproductive age worldwide. Endometriosis is a chronic, benign, estrogen-dependent multifactorial, gynaecological disease, with pain being the most common and specific symptom. The pathogenesis of endometriosis and pathophysiological basis for endometriosis-associated pain are still unclear.

Some researchers have identified nerve fibres in endometriotic lesions in women with endometriosis (Tamburro et al., 2003; Tokushige et al., 2006, 2007). Berkley et al. (2004, 2005) have reported that endometriotic implants develop a sensory and sympathetic nerve supply both in rats and in humans. We have recently shown that there was a much greater density of nerve fibres in deep infiltrating endometriosis than in peritoneal endometriotic lesions, and the density of nerve fibres was highest in rectal lesions (Wang et al., 2009). These nerve fibres in endometriotic lesions probably play an important role in the pathogenesis of pain and hyperalgesia. Some researchers have reported that nerve invasion by means of perineural and endoneural invasion, as well as the presence of degranulating mast cells near nerve structures could be responsible for the neuropathic pain and hyperalgesia (Anaf et al., 2004, 2006).

The neuroendocrine (NE) system is collectively defined as cells present either in endocrine organs or dispersed throughout the human body (diffuse NE system), which can produce neurotransmitters, neuregulators, or neuromodulators, or neuroneptide hormones or paracrine regulators (Lai et al., 2006). NE cells or ‘paraneurons’ producing different bioactive substances are present in the epithelium of many organs (Russo and Vittoria, 2006). A very small number of studies have shown that different subpopulations of NE cells are present in the urogenital organs of some mammalian species (Cecio and Vittoria, 1989; Vittoria et al., 1989; Czaja et al., 1996). Some researchers think that a small number of NE cells may possibly be contained in the normal human endometrium, and in proliferative conditions, such as endometrial carcinomas, they could increase in number (Vittoria et al., 1989).

We studied the presence of NE cells in eutopic endometrium of women with and without endometriosis by immunohistochemistry...
using specific markers. The most commonly used immunohistochemical markers of NE cells are neuron-specific enolase (NSE), synaptophysin (SYN), chromogranin A, and cytokeratin (Dursun et al., 2004). SYN is regarded as the most specific markers of NE differentiation, and manifests greater sensitivity than chromogranin A or NSE (Kasprzak et al., 2007). We have therefore used SYN and NSE antibodies to differentiate NE cells.

Materials and Methods

Collection of tissue samples

This study was approved by the Human Ethics Committees of the Sydney South West Area Health Service and the University of Sydney. Endometrial samples were obtained from women attending our operating theatre lists with pelvic pain or infertility, or from clinically characterized and archived tissues in the Department of Anatomical Pathology, Royal Prince Alfred Hospital, Sydney.

There were 38 eutopic endometrium specimens collected from women with surgically and histologically confirmed endometriosis (mean age: 31.9 years; range: 20–48 years): menstrual phase (n = 11); proliferative phase (n = 12); secretory phase (n = 15). Of the 38 patients, 20 had peritoneal endometriosis and 18 had ovarian endometriomas.

The endometriosis patients all complained of dysmenorrhea and a range of other related pain symptoms. In all patients, the extent of endometriosis was assessed according to the revised American Fertility Society (AFS) score (Revised American Society for Reproductive Medicine classification of endometriosis, 1997). None of the women had received medical therapy for endometriosis in the 3 months before laparotomy or laparoscopy for excision of endometriotic lesions and endometrial sampling. All patients with endometriosis had not previously presented with other diseases that could explain the presence or increased number of NE cells.

The control group consisted of 24 normal endometrium specimens from women in whom endometriosis had been excluded laparoscopically (mean age: 31.7 years; range: 22–44 years): eight women had unexplained infertility; eight women had intramural uterine fibroids; two women had peritoneal endometriosis. These cells were also scattered in the epithelium of glands. The cytoplasm around the nucleus was stained with SYN (Fig. 1A) and NSE (Fig. 1C). NE cells were not identified in the stroma. The mean densities of NE cells stained with SYN or NSE were 3.8 (range: 1.6–9.2) ± 1.8/mm² and 2.8 (range: 1.2–5.4) ± 2.1/mm², respectively, in women with endometriosis (Table I). In contrast, there were only 7 samples which showed distinct staining in normal endometrium out of the 24 samples from women without endometriosis. These cells were also scattered in the epithelium of glands (Fig. 1B and D), and the mean densities of NE cells stained with SYN or NSE were only 0.5 (range: 0–1.8) ± 0.7/mm² and 0.4 (range: 0–1.5) ± 0.6/mm² in women without endometriosis (Table I). The density of NE cells stained with SYN or NSE in eutopic endometrium from women with endometriosis was significantly greater than that from those without endometriosis (P < 0.001) (Table I).

In women without endometriosis, there were no SYN- and NSE-positive NE cells identified in proliferative phase endometrium. The densities of NE cells stained with SYN or NSE in normal secretory phase endometrium were 1.2 (range: 0–2.2) ± 0.9/mm² and 0.9 (range: 0–1.7) ± 0.7/mm² (Table I), respectively; and the densities of NE cells stained with SYN or NSE in normal menstrual phase endometrium were 0.4 (range: 0–1.1) ± 0.9/mm² and 0.5 (range: 0–1.1) ± 0.6/mm² (Table I), respectively; there were no differences in the density of NE cells stained with SYN and NSE across the menstrual cycle phases in women without endometriosis (P > 0.05) (Table I).

In women with endometriosis, the densities of NE cells stained with SYN or NSE in proliferative phase endometrium were 3.4 (range: 1.6–7.4) ± 1.8/mm² and 3.0 (range: 1.1–5.6) ± 1.7/mm² (Table I), respectively; in the secretory phase, the densities were 4.1 (range: 1.8–9.2) ± 1.3/mm² and 3.9 (range: 1.7–7.4) ± 1.5/mm² (Table I), respectively; during menstruation, the densities were 3.3 (range:
Figure 1  Neuroendocrine (NE) cells stained for synaphophysin (SYN) and neuron-specific enolase (NSE) (with fast red chromogen) in eutopic endometrium from women with and without endometriosis. (A) Eutopic endometrium stained with SYN from women with peritoneal endometriosis, secretory phase; magnification, ×400. (B) Normal endometrium stained with SYN from women with intramural uterine fibroids, but no endometriosis, secretory phase; magnification, ×400. (C) Eutopic endometrium stained with NSE from women with ovarian endometrioma, proliferative phase; magnification, ×400. (D) Normal endometrium stained with SYN from women with ovarian cyst, but no endometriosis, secretory phase; magnification, ×400. Scale bars represent 20 μm. Black arrows represents NE cells stained with SYN and NSE. EG, endometrial gland.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Proliferative</th>
<th>Secretory</th>
<th>Menstrual</th>
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<tr>
<td><strong>SYN</strong></td>
<td></td>
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<tr>
<td>Normal</td>
<td>0.52 ± 0.7 (n = 24)</td>
<td>0 (n = 8)</td>
<td>1.2 ± 0.9 (n = 8)</td>
<td>0.4 ± 0.9 (n = 8)</td>
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<tr>
<td>Endometriosis</td>
<td>3.8 ± 1.8 (n = 38)</td>
<td>3.4 ± 1.8 (n = 12)</td>
<td>4.1 ± 1.3 (n = 15)</td>
<td>3.3 ± 0.8 (n = 11)</td>
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<td><strong>NSE</strong></td>
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<tr>
<td>Normal</td>
<td>0.4 ± 0.6 (n = 24)</td>
<td>0 (n = 8)</td>
<td>0.9 ± 0.7 (n = 8)</td>
<td>0.5 ± 0.6 (n = 8)</td>
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<tr>
<td>Endometriosis</td>
<td>2.8 ± 2.1 (n = 38)</td>
<td>3.0 ± 1.7 (n = 12)</td>
<td>3.9 ± 1.5 (n = 15)</td>
<td>2.6 ± 1.1 (n = 11)</td>
</tr>
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Data are represented by mean density (cells/mm²) ± SD.

*Significant increase compared with women without endometriosis (P < 0.001).

+Significant increase compared with women without endometriosis (P < 0.01).

cSignificant increase compared with women without endometriosis (P < 0.05).

1.6–7.4) ± 0.8/mm² and 2.6 (range: 1.1–4.8) ± 1.1/mm² (Table I), respectively. There were no significant differences in the density of SYN- and NSE-positive NE cells in the glandular epithelium of the endometrium across the menstrual cycle phases in women with endometriosis (P > 0.05) (Table I).

At all stages of the menstrual cycle, the densities of NE cells (stained with SYN or NSE) were significantly greater in women with endometriosis than in those without endometriosis (P < 0.05) (Table I).

**Discussion**

We have demonstrated a greater density of NE cells stained with SYN in the eutopic endometrium of women with endometriosis compared...
with that from those without endometriosis (3.8 versus 0.5/mm², \( P < 0.001 \)). These cells were scattered irregularly in the epithelium of endometrial glands. At all stages of the menstrual cycle, the densities of NE cells stained with SYN were greater in women with endometriosis than in those without endometriosis (\( P < 0.05 \)). In women without endometriosis, no NE cells were identified in the proliferative phase, and there were no significant differences in the low densities of NE cells across the menstrual cycle in these women (\( P > 0.05 \)). In women with endometriosis, there were also no significant differences in the densities of NE cells across the menstrual cycle (\( P > 0.05 \)).

NE cells can be considered as the medium by which the organism can transform an external stimulus into an internal message regulating the function of the organ. They may be directly involved in the regulation of a number of physiological processes in normal and pathological conditions (Kasacka, 2004). Uterine NE cells probably regulate several functions of the uterus by means of their hormones and/or amines (Vittoria et al., 1989), such as the regulation of the cyclical morphological changes (Stroband et al., 1986). They may also play a role in the embryo nutrition and development (Sinowitz and Friess, 1983). In our study, a small number of NE cells stained with SYN in endometrial glands in the secretory and menstrual phases from women without endometriosis, and it is postulated that these cells could, by analogy to other species, be related to coordination of endometrial morphological changes during the menstrual cycle. For example, the morphology of the endometrium of the secretory phase is coordinated to prepare to receive the implanting embryo. Subsequent menstruation is then a transition phase where the endometrium starts a new proliferative phase. A small number of NE cells probably appear to be capable of contributing to the coordination of these changes.

NE cells have sometimes been described as ‘taste cells’, and a receptor-secretory function has been claimed for them in some situations. These cells are able to receive an apical (luminal) stimulus and to transform it into a secretory response (Russo and Vittoria, 2006). Some researchers have proposed that NE cells producing 5-HT can release the amine onto the surface of the lining epithelium and into the organic lumen of the organ (Nilsson et al., 1987). The smooth musculature of the gut is one of the main targets of 5-HT, which can strongly stimulate its contraction (Czaja et al., 1996). 5-HT may induce contraction of the smooth muscle in the urogenital tract of dogs, with consequent expulsion of the urine (Hanyu et al., 1987). There were more NE cells in eutopic endometrium from women with endometriosis, thus these cells have the potential to contain amines to induce the contraction of smooth muscle to cause pain symptoms.

Endometriosis shows many manifestations of an inflammatory condition, including disturbed cell-mediated immunity and autoantibody production (Ploski et al., 2009). We have demonstrated greater numbers of macrophages and immature dendritic cells in the eutopic endometrium and ectopic lesions of women with endometriosis (Berbic et al., 2009; Schulfke et al., 2009). These may contribute to pain and infertility which are the main symptoms in women with endometriosis. However, bleeding disturbances from the uterus and bowel are often involved (Ekoukou et al., 2005). In view of their potential for local release of biogenic amines and often active regulatory molecules, the greatly increased numbers of NE cells in endometrium may contribute to these bleeding, pain and fertility disturbances.

Some researchers have shown intraepithelial axons, which are closely applied to NE cells, in the vestibular gland duct of patients with vestibulitis. This close association may play a role in the symptomatology, including pain, of vestibulitis (Warner et al., 1996). In the vulvo-vestibular syndrome, the serotonin produced by local NE cells is thought to stimulate vestibular non-myelinized nociceptor nerve fibres, which induce changes leading to the central pain sensation (Warner et al., 1996; Slone et al., 1999). In moderate-to-severe inflammation of vulvar vestibulitis, there was a statistically significant increase in the number of NE cells (Slone et al., 1999). In other systems, such as the gastro-duodenal system, many diseases are also related to NE cells. Precursors of duodenal ulceration are characterized by hyperplasia of NE cells in the duodenal bulb mucosa (Panfilov et al., 1989). It was found that the number of serotonin-, histamine- and melanin-producing NE cells of the gastric mucosa increases in patients with gastro-duodenal haemorrhage (Ratner et al., 1990).

We have shown the presence of unmyelinated nerve fibres in the functional layer of eutopic endometrium from women with endometriosis, but no nerve fibres in normal endometrium (Tokushige et al., 2006). We have also demonstrated that there are more nerve fibres in the lesions of deep infiltrating endometriosis than in superficial peritoneal endometriotic lesions (Wang et al., 2009). The increased NE cells in eutopic endometrium of endometriosis may well have the ability to produce some neuro-active molecules capable of stimulating the nerve fibres to contribute to pain, and these cells could also be related to the pathogenesis and other symptoms of endometriosis. Much further research is required to define possible roles of NE cells in endometriosis. Such future research should involve studies with a range of additional NE markers.

SYN is involved in multiple aspects of synaptic vesicle endo- and exocytosis (Valtorta et al., 2004). Neurotransmitter release is the main mechanism of information transfer among neurons, and neurotransmitter molecules are stored in specialized organelles, the synaptic vesicles, from which they are released at the arrival of the nerve impulse (Valtorta et al., 2004). Some researchers have shown that antibodies to SYN could inhibit neurotransmitter release from motor nerve terminals (Alder et al., 1992). We postulated that the intense expression of SYN in NE cells in eutopic endometrium of endometriosis may stimulate nerve fibres and nociceptors to induce pain signals.

NSE is the \( \gamma \)-isoform of the glycolytic enzyme 2-phospho-\( \Delta \)-glycerate hydrolase. It is contained in relatively large amounts in neurons, peripheral nervous system tissue and NE cells (Casmiro et al., 2008). NSE is a sensitive marker of neuronal damage in several central nervous system diseases, including hypoxic brain injury, acute ischaemic stroke, head injury and epilepsy (Casmiro et al., 2008). Endometriosis has been proposed to be a consequence of neurological dysfunction, and possibly involved in a process of denervation and reinnervation (Quinn, 2004). We have demonstrated an abnormal sprouting of nociceptors in deep infiltrating endometriosis (Wang et al., 2009). The higher density of NE cells with intense expression NSE may be related to the injury and regeneration of nerve fibres in endometriosis.

To the best of our knowledge, this is the first demonstration of SYN- and NSE-positive NE cells in the human endometrium, and of a substantial increase in the presence of these cells in the glands of eutopic endometrium from women with endometriosis compared
with that from those without endometriosis. The functions of NE cells in normal or ectopic endometrium remain unclear. Increased NE cells in women with endometriosis may play some roles in endometriotic-related symptoms and pathogenesis.

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