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Distribution of efferent neurones innervating the oviduct in the pig

Krzysztof Czaja, Jerzy Kaleczyc, Zenon Pidsudko, Amelia Franke-Radowiecka, Mirosław Łakomy

Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo II, Poland

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This study was aimed, by means of the retrograde tracing technique, at disclosing the distribution of efferent neurones innervating the porcine oviduct. The fluorescent retrograde tracer Fast Blue was injected into the wall of the right oviduct in six juvenile pigs during laparotomy performed under anaesthesia. After a recovery period of 3 weeks the animals were reanaesthetised, perfused with 4% buffered paraformaldehyde (pH 7.4) and different ganglia, thought to be potent sources of the efferent innervation, were collected. The occurrence and distribution of Fast Blue-positive neurones were studied in the sympathetic chain and prevertebral ganglia, including the coeliac-superior mesenteric ganglion complex, adrenal ganglion, aorticorenal ganglion, ovarian ganglion and inferior mesenteric ganglion. The labelled neurones were found only in the right, ipsilateral ganglia. The largest number of Fast Blue-positive neurones was found in the inferior mesenteric ganglion, ovarian ganglion and in the coeliac-superior mesenteric ganglion complex. In the inferior mesenteric ganglion, the Fast Blue-positive neurones showed a tendency to gather in the dorso-cranial and the dorso-caudal region of the ganglion, forming two discrete "oviductal centres". The aortico-renal and adrenal ganglion contained a smaller population of Fast Blue-positive nerve cell bodies. The smallest number of Fast Blue-positive neurones was found in the sympathetic chain ganglia (T_{14}-L5). The localisation of Fast Blue-positive neurones in the sympathetic chain ganglia and prevertebral ganglia suggests that these nerve structures play a fundamental role in the efferent innervation of the porcine oviduct.

key words: oviduct; efferent innervation, retrograde tracing, pig

INTRODUCTION

The mammalian genital organs are well supplied with autonomic and sensory nerve fibres utilising different biologically active substances as their messengers. Immunohistochemically, several subpopulations of these nerves have been disclosed with regard to their neuropeptide content [1, 2, 13–16, 20, 21]. However, sources of innervation of female reproductive organs in breeding animals did not attract too much attention. Nevertheless, some studies dealing with this problem were performed by means of routine anatomical [3, 6, 11, 12, 26] and extirpation methods. The extirpation studies were performed in the sheep [7, 8], pig [10, 23, 24] and cow [9, 25]. These studies involving extirpation (removal) of the reproductive organs and subsequent searching for degenerated nerve cell bodies revealed some nerve centres for the ovary, uterine tube, uterus and clito-

Address for correspondence: Dr Krzysztof Czaja, Department of Animal Anatomy, Faculty of Veterinary Medicine University of Warmia and Mazury, Bldg. 105 J., ul. Oczapowskiego 13, 10–917 Olsztyn-Kortowo II, Poland, fax: +48 89 523 49 86, e-mail: czajak@moskit.uwm.edu.pl

ris, but did not allow for a study of the detailed distribution of neurones supplying the organs. The retrograde tracing method is considered to be a more detailed and precise approach to study sources of origin of the internal organs' innervation. In the pig, Majewski and co-workers [17–19] studied projections from the inferior mesenteric ganglion (IMG) to the ovary, using the retrograde tracer FB. Furthermore, Czaja and co-workers investigated the distribution of primary afferent [4] and paracervical [5] neurones supplying the porcine oviduct by means of retrograde tracing. On the basis of the results of the tracing studies performed so far, the general picture of innervation of the genital organs was established. These organs receive their innervation from several sources including: sacral parasympathetic (pelvic), thoracolumbar sympathetic (hypogastric and from lumbar sympathetic chain) and somatic (pudendal ganglia). This arrangement of the innervation seems to be also found in the pig [23, 24]. However, very little is known about the efferent neurones supplying the porcine oviduct. To give some detailed data about this problem, we investigated the distribution of efferent neurones projecting to the oviduct in the pig using the retrograde tracer Fast Blue (FB).

MATERIAL AND METHODS

In the experiments, the principles of laboratory care as well as specific national laws on the protection of animals were followed.

The experiment was performed on six sexually immature female pigs of the Great Polish race, about 15 kg of body weight (b.w.), obtained from a commercial fattening farm. The animals were kept under standard laboratory conditions. Thirty min. before the main anaesthetic was given, all the animals were pre-treated with atropine (Polfa, Poland; 0.04 mg/kg b.w., s.c.) and propionyl-promasine (Combelen, Bayer, Germany; 0.4 mg/kg b.w., i.m.). The main anaesthetic, sodium pentobarbital (Vetbutal, Biovet, Poland; 30 mg/kg b.w.) was given intravenously. During laparotomy the right oviduct was gently removed from surrounding tissues and the fluorescent retrograde tracer Fast Blue (FB; Dr K Illing GmbH, Groβ-Umstadt, Germany) was injected into the wall of the ampullar (n = 3) and the isthmic (n = 3) part of the organ. A total volume of 10 μl of FB was injected into each part (5 injections per part) of the organ using a Hamilton syringe. Particular care was taken to minimise the contamination with the dye of adjacent tissues by washing the pelvic organs thoroughly with isotonic saline after each injection. No leakage from injection sites was visible, either immediately after the injection or just prior to closing the abdomen. After a survival period of three weeks, the animals were deeply reanaesthetised (following the same procedure as applied before the laparotomies) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After perfusion, the following tissues were collected: all bilateral sympathetic chain ganglia (SCG), coeliac-superior mesenteric ganglion complex (CSMG) and bilateral adrenal ganglia (ADG), aortico-renal ganglia (ARG), ovarian ganglia (OG) and IMG. Because the CSMG was found to be very large it was divided into two parts, the cranial (CP) and the caudal part (SMP), which were studied separately. All the tissue specimens were overnight postfixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and then stored at 4°C in 0.1 M phosphate buffer containing 18% sucrose and 0.01% NaN_3 until sectioning. Ten μm-thick cryostat sections (about 20–160 per ganglion, depending on the size of the ganglion) of the ganglia were studied and photographed with a Zeiss-Axiophot microscope, equipped with epi-illumination fluorescence and an appropriate filter for FB. Labelled neurones were counted in every fourth section. This strategy was to eliminate the likelihood of counting the same neurone twice.

RESULTS

The retrogradely labelled efferent neurones were found in both the SCG and prevertebral ganglia (PVG). In the SCG, FB^+ neurones were distributed in T_{14}-L_5. Most of the neurones projecting to the isthmus occurred in the ganglia located more caudally, while those supplying the ampulla, in turn, occupied more cranially found ganglia (Fig.1). The FB^+ neurones occurred in the medial and/or cranial region of the ganglia, forming a few cell-containing groups (Fig. 2A). In the prevertebral subdivision of the sympathetic ganglia, FB-containing neurones were observed in all the ganglia studied, including CSMG as well as ipsilateral ADG, ARG, OG and IMG. The majority of FB^+ cells were located in OG and IMG but, in the case of neurones projecting to the isthmus, also in SMP (Fig. 1). In the CSMG, a significant difference in the number of FB^+ neurones was found between those supplying the isthmus and ampulla. It appeared that the SMP contained many more neurones supplying the isthmus than neurones innervating the ampulla. In contrast to the SMP, the CP was found to contain more neurones supplying

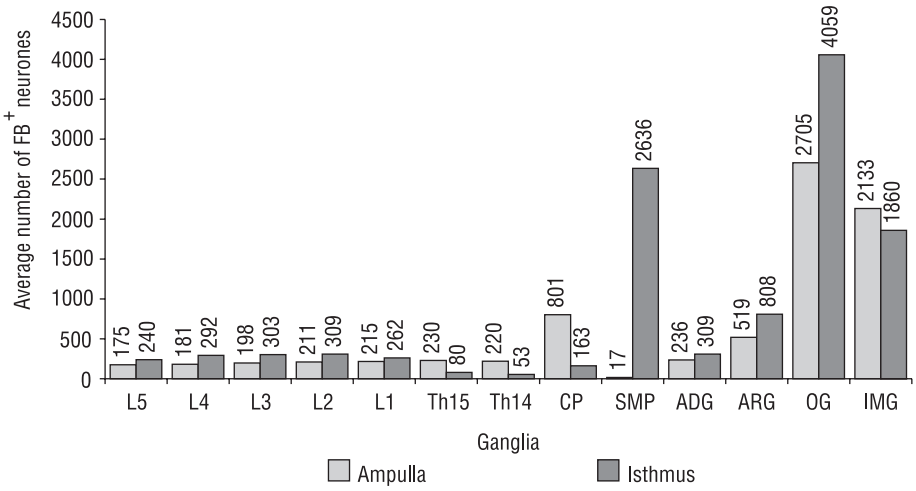

Figure 1. Distribution of FB^+ neurones innervating the oviduct in the pig.

the ampulla than those projecting to the isthmus (Fig. 1). FB^+ neurones located in the CSMG did not show somatotopic organisation and were unevenly dispersed throughout the ganglion (Fig. 2B). The ARG and ADG contained a small number of FB^+ nerve cells. Most of the FB^+ neurones found in ADGs were located in the caudal regions of the ganglia (Fig. 2C), while neurones in ARGs, in turn, occupied peripheral parts of the ganglia (Fig. 2D). The OG contained the greatest number of neurones supplying the porcine oviduct (Fig.1). They were relatively evenly distributed throughout the ganglion (Fig. 2E). In the IMG, the FB^+ neurones showed a tendency to gather in the dorso-cranial (near the output of the intermesenteric nerve) and in the dorso-caudal (close to the origin of the hypogastric nerve) regions of the ganglion forming two distinct "centres" (Fig. 2F). Single FB^+ neurones occurred between the oviductal centres. The vast majority of the oviduct-projecting neurones were found in PVG. A distinctly smaller number of efferent neurones supplying the organ studied was observed in the SCG (Fig. 1). The majority of labelled neurones localised in all the ganglia studied were of 20–35 μm in diameter and multiform or oval in shape. FB^+ cells innervating the ampulla or isthmus comprised about 40.8% or 59.2%, respectively, of the population of all efferent neurones supplying the porcine oviduct.

DISCUSSION

The present study has shown that the efferent innervation of the porcine oviduct originates from both the SCG and PVG. Considering domestic animals, sources of the efferent innervation of the oviduct have previously been investigated in the cow [9, 25], sheep [7, 8] and pig [10, 23, 24] but using the extirpation method. This method involves a surgical removal of the organ studied (extirpation) and then, after a survival period, systematic investigations of peripheral and/or central nerve structures to reveal degenerated neurones, assuming that cutting out endings of processes of the neurones terminating within the extirpated organ produces regressive changes in their perikarya. However, the extirpation method does not allow for the performing of detailed quantitative investigations of the neurones including those innervating the oviduct. Retrograde tracing studies dealing with sources of the innervation for other female genital organs have been carried out mainly in laboratory animals. In the pig, only Majewski et al. [17–19] and Wąsowicz et al. [22] have investigated the efferent innervation of the ovary and uterus, but not the oviduct. The present study has shown that the efferent innervation of the porcine oviduct originates from T_{14}-L_5 sympathetic chain ganglia and prevertebral ganglia, including CSMG as well as ipsilateral ADG, ARG, OG and IMG. Previous studies using routine anatomical preparations have revealed that efferent nerve fibres supplying female genital organs including the oviduct originate from intermesenteric ganglia, IMG, OG and sometimes from hypogastric plexus ganglia [3, 6, 11, 26] as well as from L_1-L_5 SCG [12]. The above-mentioned studies have provided no information on the pattern of the distribution of efferent neurones innervating the genital tract. The present findings corroborate in general results of previous studies dealing with sources of the efferent innervation of the

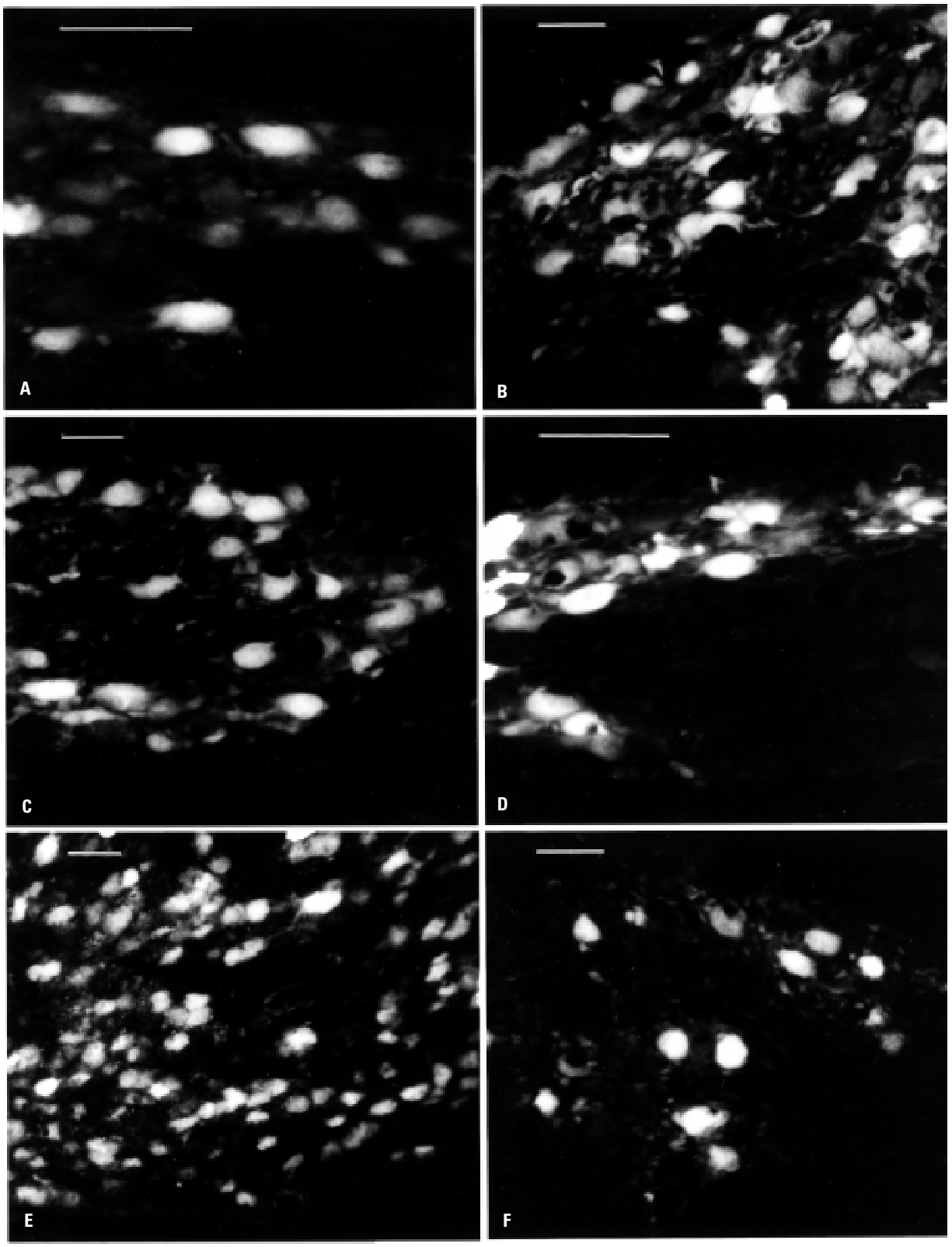

Figure 2. A. Oval in shape FB^+ neurones supplying the ampulla located in the cranial region of the ipsilateral Th_{14} SCG; **B.** Irregular in shape neurones located in SMP innervating the isthmus of the porcine oviduct; **C.** FB^+ neurones projecting to the ampulla, found in the caudal region of the ADG; **D.** FB^+ neurones supplying the isthmus, found in peripheral region of ARG; **E.** Round and oval in shape FB^+ neurones projecting to the ampulla, found in OG; **F.** The dorso-cranial (near the output of intermesenteric nerve) region of ipsilateral IMG. FB^+ neurones supplying the ampulla; scale bar — 50 μm.

porcine oviduct obtained with the extirpation method [23, 24]. However, as already mentioned, these studies revealed no detailed data dealing with the number of efferent neurones innervating the oviduct. In contrast to the findings of Welento et al. [23, 24], the present results have shown the presence of labelled neurones in L_5 SCG and in CSMG. These inconsistencies may result from the fact that different methods of investigation were used. Previous experiments carried out by means of the retrograde tracing method [17–19, 22], as well as the present study, have revealed organ-dependent differences in the distribution of efferent neurones supplying different female genital organs. In addition, results of the present study have revealed significant differences in the distribution between neurones in CSMG supplying the ampulla and isthmus. The variations in the distribution between efferent neurones innervating the oviduct, ovary and uterus, and also those supplying the ampulla and isthmus, may result from the fact that different pathways are used by processes of these nerve cells to reach the target tissues. We also found that FB^+ neurones in the IMG showed a tendency to gather in the dorso-cranial and in the dorso-caudal regions of the ganglion, forming two distinct "centres". Similar organisation of IMG has also been described by Majewski et al. [17] and Wąsowicz et al. [22] with regard to the ovary- and uterus-projecting neurones. These observations suggest the morphological organisation of IMG neurones with respect to their functions. The retrograde tracing performed in the present study has shown that the most intensive projections were found from OG, IMG and CSMG. In the light of both the present and previous [4, 5] studies it can be stated that these ganglia may control the vast majority of the oviductal functions in the pig.

The distribution of FB^+ neurones found in the SCG and PVG suggests that these ganglia play a fundamental role in the efferent innervation of the porcine oviduct. The present study has also revealed the ordered organisation of the efferent neurones innervating the porcine oviduct. It also provides the basis for further studies on the histochemical and physiological characterisation of these neurones.

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