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Intrinsic neuronal cell bodies in the rat ovary

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Abstract

The present study describes ganglia and isolated neurones in the ovary of the Wistar rat, employing histological and histochemical techniques. Four kinds of ganglia in the postpubertal and young adult rat were identified: the mesovarial, hilar, medullary and cortical ganglia. Isolated neurones were also found, being dispersed along blood vessels in the ovary medulla and near the follicles. The soma diameters of these neuronal cells ranged from 25 to 50 μm. In the prepubertal rat, only the mesovarial and hilar ganglia were observed. They contained small neurones with soma diameters ranging from 10 to 15 μm. NADPH-diaphorase activity was detected in some isolated neurones and in the cortical and hilar ganglia in all rats examined.

Keywords: Ovary; Ganglia; Neurones; Rat

The presence of intrinsic neurones in the mammalian ovary has long been a controversial issue. Although a ganglion was described in the medulla of the human ovary by the silver impregnation Golgi method, its presence was later denied [5]. Using silver impregnation methods, an ovarian ganglion was described in mice and rabbits, but not found in the dog [1,5]. In the cat ovary hilus, a vegetative microganglion was described on the basis of the findings obtained by the Nissl method [9]. More recent studies on rat ovary innervation only indicated the presence of nerve fibres showing adrenaline- and acetylcholine-, substance P (SP)-, neuropeptide Y (NPY)-, vasoactive intestinal peptide (VIP)- and other neuropeptides-like immunoreactivity [1,7,12]. These fibres appear to enter the ovary through the superior ovarian nerve to join the ovarian plexus, and reach the follicular theca, interstitial gland and vascular structures [2]. Neuronal tracer techniques indicated the sites of origin of these fibres to be in the dorsal root, paravertebral, prevertebral celiac–superior mesenteric, and nodose ganglia [2,11]. The aim of the present study was to examine the possible existence of neuronal cell bodies in the rat ovary. A preliminary report on the subject has been published [6].

Groups of 12 Wistar rats (10, 25, 45 and 50 days old), were sacrificed by cervical dislocation. Ovaries with mesovarium were quickly extirpated in toto and processed as follows: six ovaries from each group were fixed in Bouin's liquid, embedded in paraffin, serially sectioned at 5 μm, and stained with haematoxylin-eosin. Another six ovaries were fixed in Carnoy's fixative, embedded in paraffin, serially sectioned at 7 μm and stained with Nissl's method [4]. Six ovaries in each group were fixed in alcohol-ammonium and impregnated with silver nitrate, embedded in paraffin and serially sectioned at 10 μm [14]. The remaining six ovaries from each group were fixed in 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). Ovarian sections of 50 and 80 μm were obtained using a vibratome and then processed for histochemical detection of NADPH-diaphorase (NADPH-d) [15]. Neuronal body sizes were measured following the technique described by Burke et al. [3]. Photographic records were obtained with a Nikon Optiphot microscope.

In the ovaries of 25, 45, and 50 days old rats four ganglia were observed: mesovarial (Fig. 1), hilar (Fig. 2), medullary (Fig. 3) and cortical (Fig. 4). The mean number of neurones was 20 ± 2 in the medullary ganglion, 16 ± 3 in the hilar and mesovarial ganglia, and 8 ± 3 in the cortical ganglion. The average diameter of the neuronal bodies in mesovarial, hilar and medullary ganglia was 45 μm, while in the cortical ganglion it was 25 μm.

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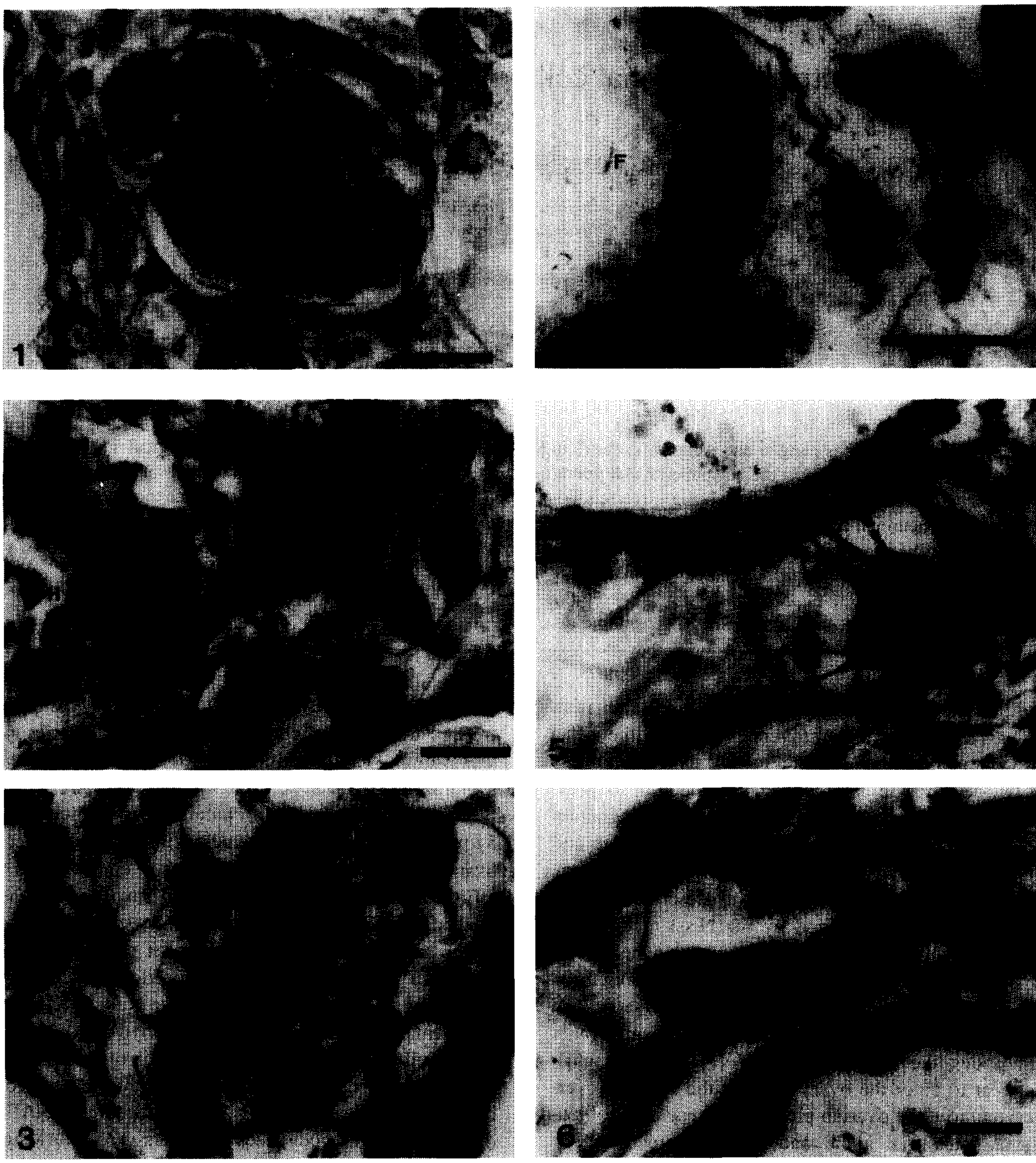

Fig. 1. Mesovarial ganglion. Spherical neuronal bodies in the periphery; nerve-fibres emerging from one side of the ganglion, contributing to form a bundle of nerve-fibres (arrow); 25 days-old rat. Silver impregnation. Scale bar = 50 μm.

Fig. 2. Hilar ganglion, found beside one branch of the Helician artery (HA); 25 days-old rat. Silver impregnation. Scale bar = 50 μm.

Fig. 3. Medullary ganglion, situated in the connective vascular tissue of the medulla; 45 days-old rat. Silver impregnation. Scale bar = 50 μm.

Fig. 4. Cortical ganglion. NADPH-d positive neurones among the follicles (F); one nervous prolongation near the granulose cells; 50 days-old rat. Scale bar = 50 μm.

Fig. 5. Isolated neurones, found in the perivascular tissue of the cortex; 45 days-old rat. Silver impregnation. Scale bar = 50 μm.

Fig. 6. Isolated neurone, located in the loose connective tissue of the medulla. Long dendritic processes radiate to opposite sides; 45 days-old rat. Silver impregnation. Scale bar = 50 μm.

Only two ganglia were observed in the 10 days old rat ovaries, mesovarial and hilar. Each ganglion contained 12 ± 3 neurones, with neuronal bodies ranging from 10 to 15 μm.

In 25 days old animals isolated neurones in the cortex and medulla (Figs. 5–6), scattered near the follicles or along the blood vessels, were observed. These neurones exhibited the largest cell diameter (50 μm) observed. The mean number of isolated neurones was 15 ± 2 in each ovary.

Cell bodies had a typical euchromatic nucleus with prominent nucleolus and showed conspicuous Nissl's bodies in the cytoplasm. A predominantly multipolar neuronal shape was observed.

Ganglionar cytoarchitecture exhibited two different aspects. Medullary, cortical and hilar ganglia displayed an undefined distribution of the neurones. By contrast, neuronal bodies in the mesovarial ganglion were distributed in the periphery, with nerve processes radiating to the centre and contributing to form a bundle of fibres. Neuronal morphology varied also, depending on the particular ganglion examined. The mesovarial ganglion only had spherical neurones, without extensions on the side facing the ganglionar capsule. Hilar and cortical ganglia showed, in addition to spherical neurones, multipolar cell bodies. The medullary ganglion had only multipolar neurones with prolongations in all directions. Isolated neurone bodies were arranged in a chain-like fashion through the connective tissue, their processes forming a network in both medulla and cortex.

In 25, 45 and 50 days old rats NADPH-d positive neurones were found located in the cortical (Fig. 4) and hilar ganglia. Some isolated neurones also showed the same activity. Axons of the hilar cells exhibited a close spatial relation with blood vessels. Dendritic processes of the cortical neurones were distributed near the granulose cells, but their endings could not be identified.

The number and size of ganglia and neurones increased with animal age. In addition, the largest size and higher number of neurones were observed around puberty.

It has been previously proposed that the peripheral nervous system may participate in the functional regulation of the ovary [8,13]. The results presented here add further support to this statement.

A new anatomical substratum is described for the origin of some afferent and/or efferent nervous pathways of the ovary. These structures may be participating in the modulation of ovarian functions, although its overall relevance is still unclear. Our results also suggest that the postnatal maturation of the intrinsic neuronal elements in the ovary is likely to be a necessary step for the functional maturity of the organ. Moreover, the presence of NADPH-d positive neurones might imply a direct participation of NO in the regulation of some of the physiological ovarian processes [10].

Previous studies showing alterations of the ovarian function in young animals after denervation of the gonad, were based on the assumption that interruption of the nervous afference suppressed the neural regulation. In view of the present findings, this concept should be re-evaluated.

We are now trying to identify the time of appearance of neurones and ganglia during the rat ontogeny and the neurotransmitters used by these cells.

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