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Gender and gonadectomy influence on neurons in superior cervical ganglia of sexually mature rats

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HIGHLIGHTS

- Gender and gonadectomy influence on size of SCG neurons of rat.
- A density of neurons in SCG is the highest in female rats.
- A gender and gonadectomy influence on calretinin-IR presynaptic baskets in SCG.

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ABSTRACT

Gonadal hormones have a significant influence on both the number of neurons and the density of synapses in the superior cervical ganglion (SCG) during the early postnatal period. There are no studies reporting influence of the absence of these factors in sexually mature animals, although changes in SCG-neurons of the rat were observed up to 6 months of age. Hence, we investigated whether gonadectomy of sexually mature rats influences morphological properties of neurons in the SCG of the rat and if so, would it have a specific effect on neurochemically distinct subpopulations. Male and female Sprague-Dawley rats were gonadectomized at the age of two months. After 30 days, they were sacrificed and SCGs were harvested and processed immunohistochemically. The mean diameter of NPY− neurons was greater in male rats, in comparison to all other groups ($p < 0.05$). The number of NPY+, NPY− and total neurons per section area was significantly higher in female than in male, orchidectomized or the ovariectomized animals ($p < 0.05$). The share of the different neuronal populations in the SCG that were encircled with calretinin-positive baskets (c.b+) or c.b.− (NPY+ or NPY−) was significantly influenced by the gender of the animals and gonadectomy, with significantly more c.b.+ in male animals ($p < 0.05$). Results of the present study indicate that substantial changes in the SCG neurons of the rat occur after reaching sexual maturity, and are influenced by the gonadectomy.

1. Introduction

The superior cervical ganglion (SCG) is a paravertebral chain ganglion that supplies sympathetic innervations to blood vessels, smooth muscles and glands of the head and neck [1,7,8]. Neurons in the SCG could be distinguished according to their neurochemical coding. Neuropeptide Y (NPY) is present in a vast majority of vasomotor neurons, while the presence of calretinin immunoreactive presynaptic fibers around the NPY− somata marks a sub-population of secretomotor neurons that project into a submandibular salivatory gland [8].

Gender differences in the number of total neurons and different populations of the SCG neurons were observed in certain studies [17], while some groups of authors did not find these differences [5]. It was presumed, and partially proved, that these differences are the result of the influence of gonadal hormones, and that they develop during the early postnatal period [17,20,22]. Hence, most of the studies on the influence of gonadal hormones on the SCG neurons included gonadectomy of neonatal rats [19–21,23], assuming that only in the early postnatal development substantial changes in the SCG could take place.

Additionally, it was observed that neurochemical specificity and size of the SCG somata changes substantially during postnatal development until six months of age [13,14]. Recently, a significant increase in the number of neurons in the trigeminal (sensory) ganglion was observed postnataly between the third and eight month of age, due to a protracted maturation process [12], raising the possibility of a similar process happening in sympathetic ganglia.

Since there is no data on the influence of gonadectomy on development of the SCG in sexually mature rats, we wanted to find

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out if gonadectomy of adult rats has an influence on the diameter and density of SCG neurons. Also, we explored the possibility that gonadectomy (and consecutive lack of gonadal hormones) influences neurochemically specific sub-populations of SCG neurons.

2. Material and methods

All experimental procedures were performed according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Ministry of Agriculture (UP/I-322-01/11-01/117, 526-06-1-0255/11-1). A total of 16 Sprague-Dawley rats weighing 180–220 g were used. The animals were raised under controlled conditions (temperature 22 ± 1 °C, under 12/12 light/dark cycle). At the age of 8 weeks, animals were anesthetized with a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg). Female rats were ovariectomized through a bilateral dorsal incision, or sham operated. Male animals were orchidectomized through a bilateral incision of the scrotum, or sham operated. Each group consisted of four animals. The rats were fed ad libitum with standard laboratory chow (4RF21 GLP, Mucedola srl, Settimo Milanese, Italy) and housed individually in plastic cages with sawdust bedding.

Four weeks later, rats were again anesthetized and perfused transcardially with 0.9% saline followed by 300 mL of Zamboni's fixative (4% paraformaldehyde and 0.20% picric acid in 0.1 M phosphate-buffered saline (PBS) at pH 7.4). Sham operated female animals were sacrificed in proestrus, according to the cytology of the vaginal swab [6].

The superior cervical ganglia were removed and postfixed overnight in Zamboni's fixative.

To obtain data on peripheral distribution of neuronal fibers in the area of projection of SCG neurons, five male and four female 12-week-old rats were additionally sacrificed and submandibular glands were harvested.

After washing in PBS, tissues were embedded in paraffin, and 5 μm thick sections were obtained. Sections were placed on glass slides. After deparafinisation, immunohistochemical staining was performed.

The following primary antibodies were used: polyclonal sheep anti-NPY antibody (ab6173, Abcam, Cambridge, UK; at dilution of 1:1000); a polyclonal rabbit anti-calretinin antibody (ab702, Abcam, Cambridge, UK; 1:300); a monoclonal mouse anti-PgP 9.5 antibody (480012, Invitrogen, Camarillo CA, USA; 1:500) and a polyclonal rabbit Anti-Ki-67 (AB9260, Cemicon, Temecula CA, USA; 1:100). Detection was performed using Rhodamine Red TM-conjugated donkey anti-rabbit or anti-mouse and FITC-conjugated donkey anti-sheep secondary antibodies (711-295-152; 715-295-151 and 713-095-147, all Jackson ImmunoResearch Laboratories, Inc., Baltimore, PA, USA). After final rinsing in PBS, all slides were mounted, air-dried, and cover slipped (Immu-Mount, Shandon, Pittsburgh, PA, USA). Staining controls included omission of primary antibody from the staining procedure, which resulted in no staining in tissue.

A microscope (BX61, Olympus, Tokyo, Japan) with a cooled digital camera (DP71, Olympus, Tokyo, Japan) was used to obtain images (under 10× and 40× objective) for further analyses. Measurements were performed using Image J software (National Institutes of Health, Bethesda, MD, USA). Only neurons with visible nuclei were taken into consideration for analysis.

The number of NPY+, NPY− and the total number of neurons were counted in two longitudinal sections (at least 25 μm apart from each other) and the surface of sections was measured by manual outlining using freehand selection function. The size of neurons was measured in two fields of view obtained by photographing stained sections using a 40× objective. The average of the longer and shorter axis was considered as a 'cell diameter' [4].

Analysis for determination of the percentage of NPY+ or NPY− neurons that were surrounded by pericellular calretinin-ir

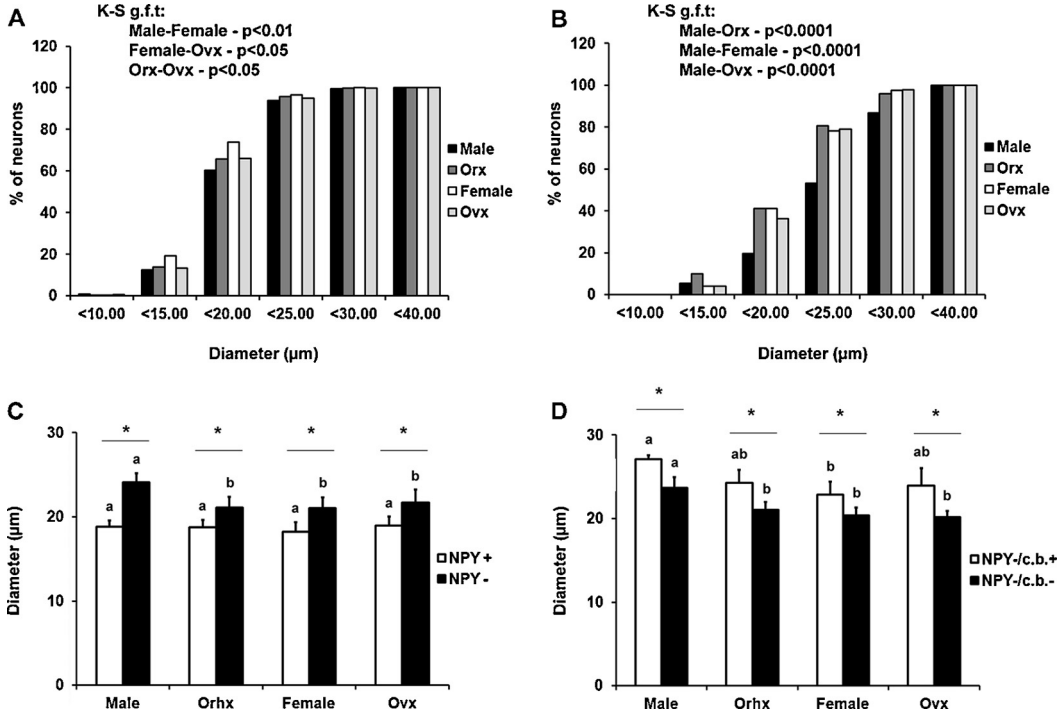

Fig. 1. Gender and gonadectomy influence on size of neurons in superior cervical ganglia (SCG) of rats. Cumulative distribution in size of NPY+ (A) and NPY− neurons (B) in SCGs of male, female, orchidectomized (Orx) and ovariectomized (Ovx) rats. K–S g.f.t. – Kolmogorov–Smirnov goodness-of-fit test. (C) Mean diameter of NPY+ and NPY− neurons and sub-populations of NPY−/c.b.− and NPY−/c.b.+ neurons (D). Different letters (a, b) denote $p < 0.05$ between experimental groups; $^*p < 0.05$ between two (sub)populations of neurons. $N = 4$ for each group.