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Distribution of ganglionic sympathetic neurons supplying the subcutaneous, perirenal and mesentery fat tissue depots in the pig

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Abstract. Previous morphological studies revealed that the adipose tissue is innervated by adrenergic nerve fibers. Furthermore, physiological studies showed that the metabolism of adipose tissue is controlled by the adrenergic component of the nervous system. However, nothing is known on the sources of innervation of different fat tissue depots. Therefore, we decided to study the distribution of ganglionic sympathetic neurons innervating adipose tissue in the pig by means of a retrograde tracing method. We used 9 male and 9 female pigs of approximately 50 kg body weight. The retrograde tracer, Fast Blue (FB), was injected into the subcutaneous, perirenal and mesentery fat tissue depots. Results of the present study showed that numerous centers of the sympathetic nervous system innervate adipose tissue in the pig. FB^+ neurons projecting to the subcutaneous fat tissue were placed in the thoraco-lumbar region of the sympathetic chain ganglia (SChG). However, neurons supplying perirenal and mesentery fat tissue depots were found in both the SChG and prevertebral ganglia (PVG). We conclude that different adipose tissue depots (subcutaneous, perirenal and mesentery) have different sources of innervation and that there is no significant difference in the distribution of neurons innervating adipose tissue in male and female pigs.

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Key words: fat tissue, sympathetic innervation, FB tracing, pig

INTRODUCTION

Adipose tissue has been referred to as "the adipose organ" to emphasize that it may be viewed as a discrete entity with defined functions beyond supporting or insulating other organs and serving as a depot for storage of the excess of energy. The discovery of the *ob* gene and anti-obesity effect of leptin, the *ob* gene product, was an important breakthrough in understanding the role of adipose tissue in regulation of food intake, body weight and endocrine function (Zhang et al. 1994). Furthermore, physiological studies showed that the metabolism of adipose tissue is controlled by the sympathetic nervous system (Havel 1965, Alexander and Stevens 1980, Egawa et al. 1990, Himms-Hagen et al. 1990, Bray 1993, Crandall et al. 1997, Rayner 2001). Morphological studies confirmed that the adipose tissue is innervated by noradrenergic nerve fibers (Ballard et al. 1974, Bernard et al. 1978, Hausman and Richardson 1987, Himms-Hagen et al. 1990). Noradrenaline has been found in nerve endings targeting blood vessels and cells of adipose tissue (Cannon et al. 1986). Recent extensive morphological studies (Lever et al. 1988, Norman et al. 1988, Nnodim and Lever 1988, Giordano et al. 1996) established the presence of nerve endings containing not only noradrenaline, but also tyrosine hydroxylase and neuropeptide Y on blood vessels, arterio-venous anastomoses and on adipose tissue cells themselves. These studies show that the adipose tissue is well supplied by sympathetic nerve fibers. However, the origin of nerve fibers supplying different fat tissue depots in adult animals has not been studied thoroughly. The retrograde tracing is commonly considered to be one of the most advanced and precise methods of localizing specific neuronal populations supplying any particular organ under study. Therefore, by means of the retrograde tracing method, in the present study we aimed to disclose the distribution of the ganglionic sympathetic neurons supplying the subcutaneous, perirenal and mesentery fat tissue in the pig. Results from this study may lead to elaboration of a practical method of controlling development of fat depots in the pig, which has an economic impact in the swine industry. In addition, they bring new anatomical data for the pig that continuously increases in value as a laboratory animal in the medical research (Swindle et al. 1992).

METHODS

Eighteen pigs of the Great Polish breed, approximately 50 kg body weight, were obtained from a commercial fattening farm and maintained under standard laboratory conditions. General standards of the laboratory care and the Polish law on protection of animals and animal experimentation were obeyed. Animals were divided into three groups, each consisting of 3 males and 3 females. Thirty minutes before the main anaesthetic was given, all 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. The 5% suspension of the fluorescent retrograde tracer, Fast Blue (FB; Dr. K. Illing GmbH, Groß--Umstadt, Germany), was injected into subcutaneous (S), perirenal (P) and mesentery (M) fat tissue depots using a Hamilton syringe with a 26 gauge needle. In the S pigs the tracer was injected into fifteen places through the cuts in the skin exposing the subcutaneous fat tissue. Places of injection were localized on the right side of the body and formed three lines (5 places in each line in the equal distance). First line started at the iliac spine and ended at the caudal angle of scapula, the second line was traced from the ischial spine in parallel to the first line and the third one from the side fold to the brachial joint. Five injections (2 μl each) distributed radially and penetrating about 2 cm under the skin were made in each place. In the P group the tracer was introduced in ten injections (2 μl each) evenly distributed on the surface of the right perirenal fat tissue. In the M group of pigs multiple injections of the tracer were made (10 injections, 2 μl each) into the fat tissue located close to the root of mesentery. A particular care was taken to minimize the possibility of contamination of the adjacent tissues with the dye. The surrounding tissues were washed thoroughly with isotonic saline after each injection. No leakage from the injection sites was visible, neither immediately after the injection nor just prior to closing the skin. After a survival period of three weeks, the animals were deeply anaesthetized (following the same procedure that was applied before laparotomies) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After laparotomy, the following autonomic ganglia were collected: coeliac superior mesenteric ganglion (CSMG), inferior mesenteric ganglion (IMG), intermesenteric ganglia (adrenal- ADG, aorticorenal- ARG and ovarian- OG or testicular- TG ganglion) and SChG. Because the CSMG was very large it was divided into three parts, the cranial (CP) and right and left caudal parts (SMPr and SMPl),

which were studied separately. The pieces of fat tissue depots with the sites of injections were collected too. All the tissue specimens were postfixed overnight by immersion in the 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 micrometer-thick cryostat sections of the ganglia were studied and photographed with a Leitz Orthoplan or a Zeiss Axiophot fluorescence microscope equipped with the epiillumination and an appropriate filter set for Fast Blue. To determine the number of the FB^+ neurons supplying the particular fat depot, the neurons were counted in every third section. This strategy eliminated the likelihood of counting the same neuron twice because the diameter of the neurons located in the studied ganglia varied from 20-30 μm. Only neurons with a visible nucleus were counted. The data obtained were statistically analysed (Student's t-test, $P \leq 0.05$). The statistical analysis was carried out using the Graphpad Prism computer program (GRAPHPAD PRISM v. 3.02, GraphPad Software Inc., San Diego, CA). Data are expressed as means ± SEM. Twenty-μm-thick cryostat sections of each fat tissue depot (S, P, and M) were checked under the fluorescence microscope for the correct injection sites of FB. No FB contamination of tissues adjacent to the particular fat tissue depot was found in any of the animals used.

RESULTS

FB^+ neurons projecting to the S fat tissue were observed in the ipsilateral thoraco-lumbar (Th_1-L_4) ganglia of the sympathetic chain (Fig. 1). The number of the labeled perikarya varied from 130 to 685 per ganglion. The largest number of the FB^+ neurons was localized in the SChG at the levels of Th_{11}, Th_{12} and Th_{14}. However, neurons supplying the P fat depot were found in both SChG and PVG. FB^+ neurons of this group were localized in CSMG and ipsilateral ADG, ARG, OG, TG and IMG as well as in the ipsilateral lumbar SChG at the level of L_1-L_3 (Fig. 2). The number of the FB^+ neurons supplying the P fat varied from 40 to 795 per ganglion. The largest number of these neurons was localized in CSMG and ADG as well as in SChG at the level of L_2 and L_3. There was no significant difference between the number of FB^+ neurons supplying P fat localized in right

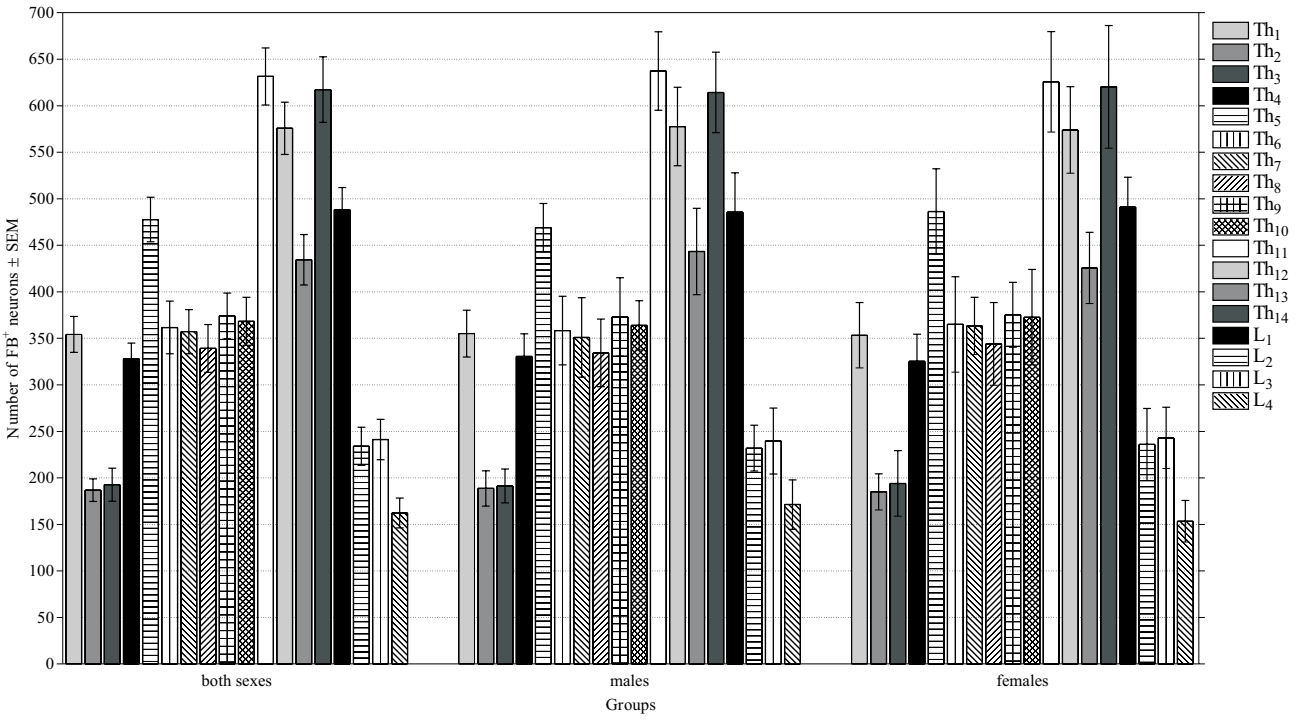

Fig. 1. Diagram showing segmental distribution of FB^+ neurons projecting to the subcutaneous- S fat tissue in the ipsilateral thoraco-lumbar (Th_1-L_4) ganglia of the sympathetic chain in the pig ($n = 6$; 3 male and 3 female). Bars represent mean number of neurons located in the particular ganglia. The error bars represent SEM.

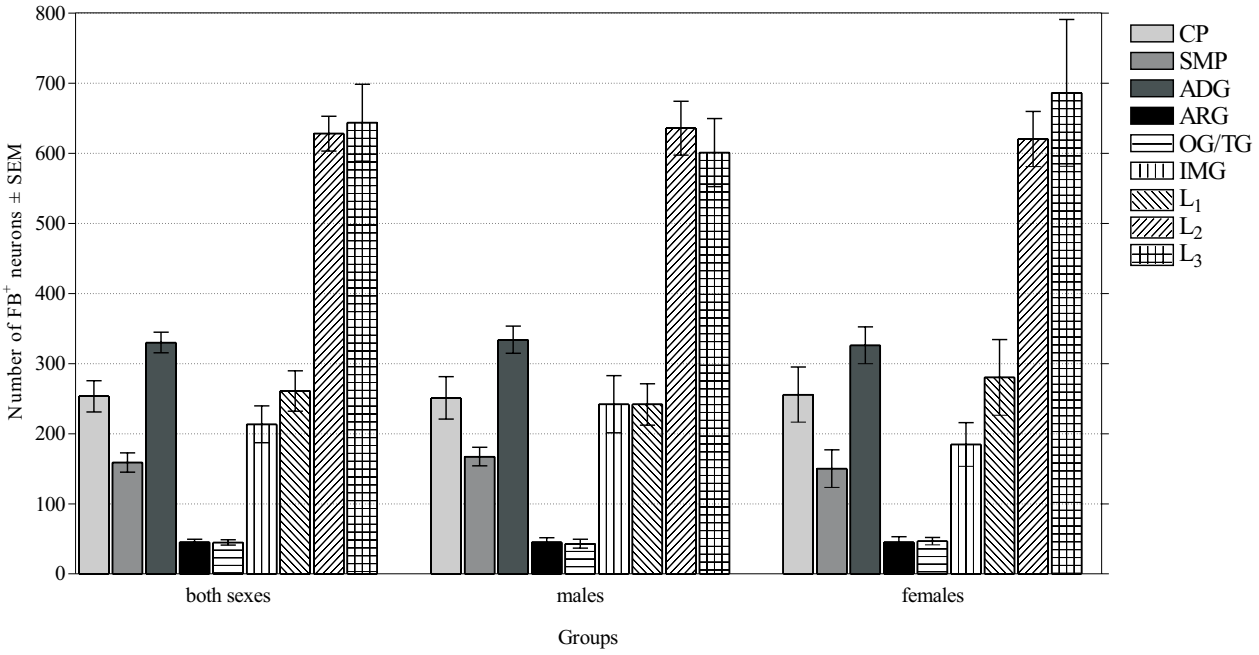

Fig. 2. Diagram showing segmental distribution of FB^+ neurons projecting to the perirenal fat depot-P localized in CSMG CP and SMP and ipsilateral ADG, ARG, OG, TG and IMG as well as in the ipsilateral lumbar SChG at the level of L_1-L_3. For indications, see Fig. 1.

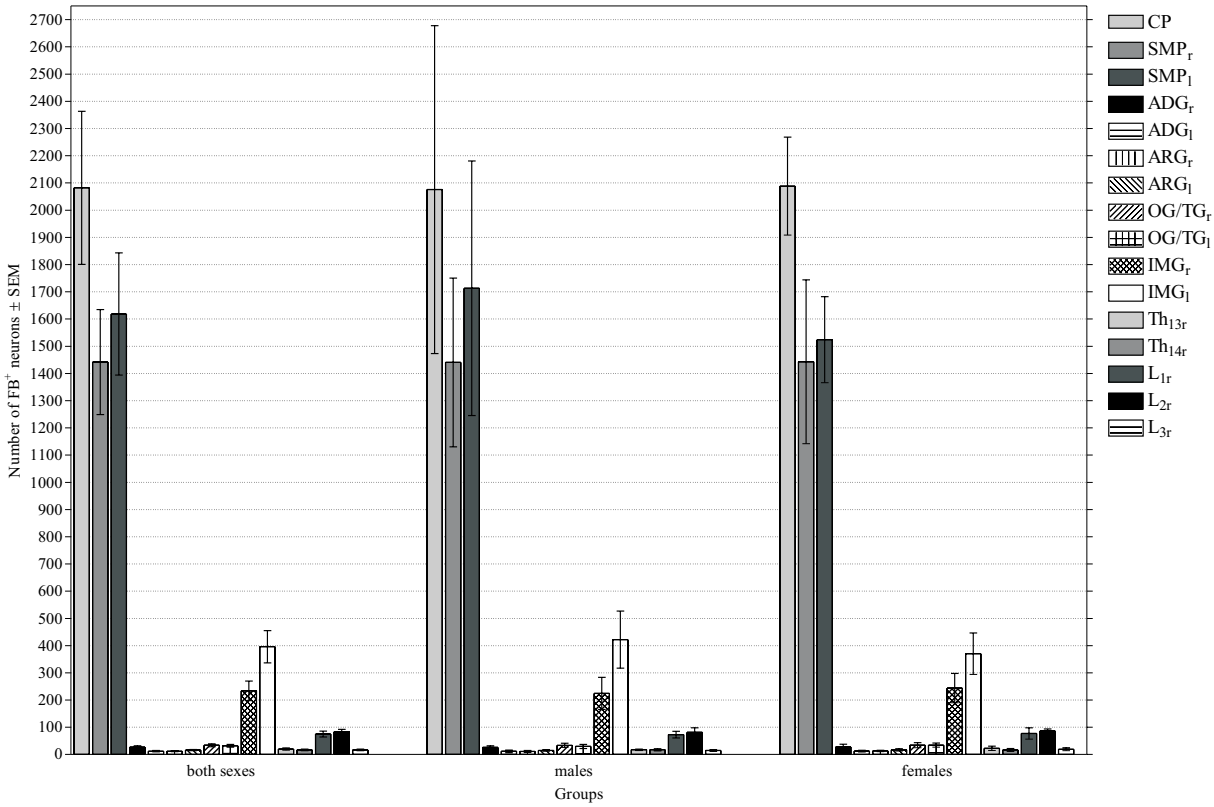

Fig. 3. Diagram showing segmental distribution of FB^+ neurons projecting to the mesentery fat depot-M localized in CSMG and both right and left ADG, ARG, OG / TG and IMG as well as in the right thoraco-lumbar (Th_{13}-L_3) SChG. For indications, see Fig. 1.

and left SMP. In M subpopulation, FB^+ neurons were localized in CSMG and both right and left ADG, ARG, OG, TG and IMG as well as in the right thoraco-lumbar (Th_{13}-L_3) SChG (Fig. 3). The largest subpopulation of the M fat-projecting neurons was localized in the CSMG. Right and left IMG contained a moderate number of the labeled neurons while in other presently studied ganglia the number of the FB^+ neurons was smallest.

Approximately 85% of the FB^+ neurons projecting to the S fat tissue were fusiform or oval in shape and had the diameter of 25-30 μm (Fig. 4). FB^+ neurons that innervated the P fat tissue and were localized in PVG were multiform or oval in shape and had the diameter of 20-35 μm (Fig. 5), while labeled cells localized in the SChG resembled those found in the S group (Fig. 6). Both diameter and shape of the FB^+ neurons projecting to the M fat tissue were very similar to those of the previous groups (Figs. 7, 8 and 9).

There was a statistical difference in the number and distribution of labeled neurons among various experimental groups. FB^+ neurons did not show somatotopic organization. However, the majority of the neurons was placed at the periphery of the ganglia.

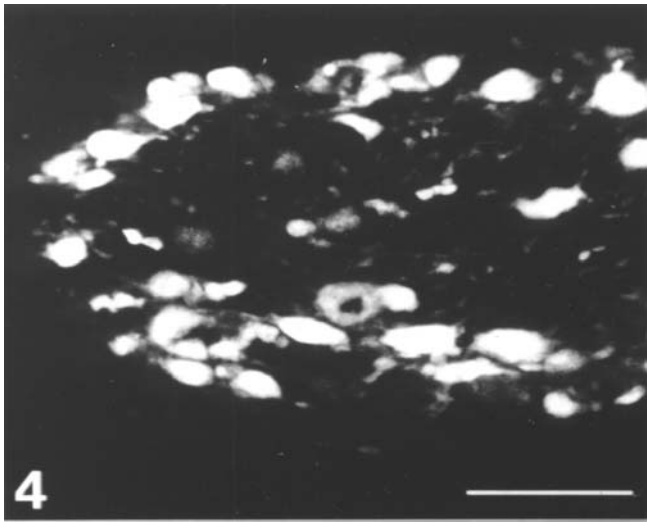

Fig. 4. Fusiform or oval in shape FB^+ neurons supplying the subcutaneous fat tissue in the ipsilateral Th_{11} SChG in the female pig (scale bar 50 μm).

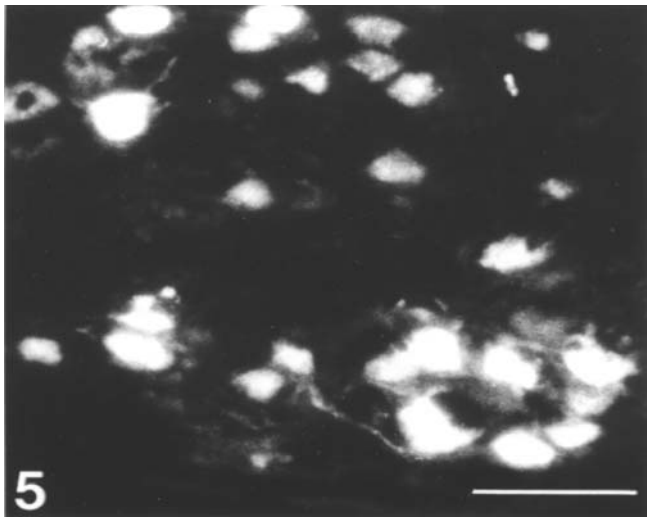

Fig. 5. Multiform or oval in shape FB^+ neurons of P group localized in CP of the male pig (scale bar 50 μm).

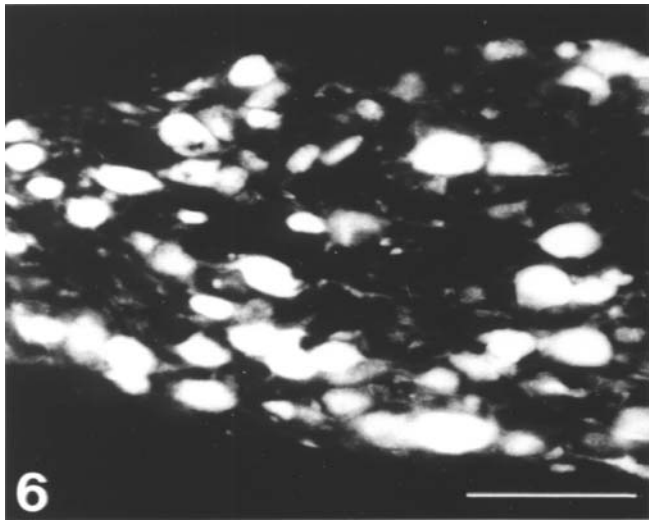

Fig. 6. Fusiform or oval in shape FB^+ neurons projecting to the perirenal fat depot localized in right L_2 SChG of the female pig (scale bar 50 μm).

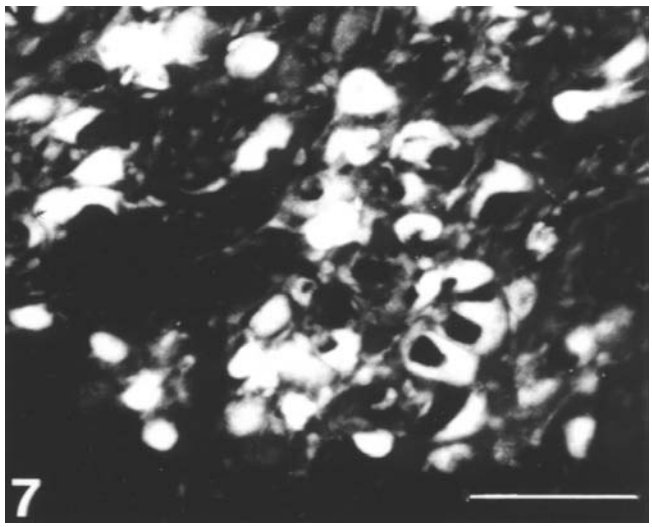

Fig. 7. Multiform or oval in shape FB^+ neurons supplying mesentery fat depot localized in the left SMP of the female pig (scale bar 50 μm).

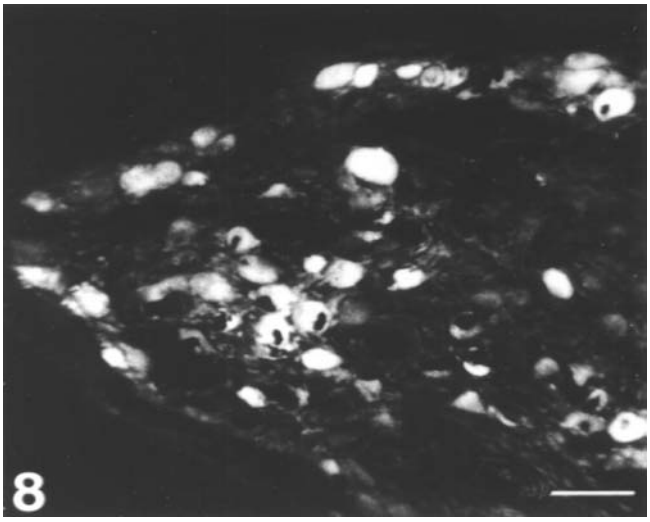

Fig. 8. Oval in shape FB^+ neurons supplying mesentery fat depot localized in the cranial part of the left IMG in the male pig (scale bar 50 μm).

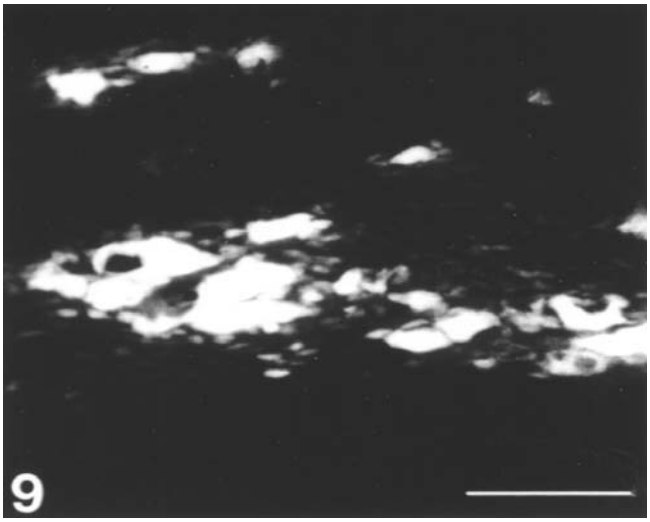

Fig. 9. Fusiform in shape FB^+ neurons projecting to the mesentery fat depot localized in the right L_2 SChG of the male pig (scale bar 50 μm).

DISCUSSION

This is the first study demonstrating the source of the sympathetic peripheral nerve fibers supplying different adipose tissue depots in the pig. These results are in general agreement with morphological and physiological findings in several species. Morphological studies showed that the adipose tissue is innervated by sympathetic nerve fibers (Ballard et al. 1974, Bernard et al. 1978, Hausman and Richardson 1987, Himms-Hagen et al. 1990). Furthermore, physiological studies showed that metabolism of the adipose tissue is controlled by the sympathetic component of the nervous system (Havel 1965, Alexander and Stevens 1980, Egawa et al. 1990, Himms-Hagen et al. 1990, Bray 1993, Crandall et al. 1997, Lawrence and Coppack 2000). The present results showed that the FB^+ neurons projecting to the subcutaneous fat tissue were placed in the ipsilateral thoraco-lumbar (Th_1-L_4) ganglia of the sympathetic chain. The term "subcutaneous fat tissue" used in this paper should not be understood literally, but as an expression referring to one of the layers of general integument of the body. Therefore it encompasses all structures found within this layer: adipocytes, blood vessels as well as some structures of pili (*arrector pili* muscles) that can penetrate as deep as into the external layer of the tissue. Providing that all these structures are supplied by the autonomic neurons, it is possible that the population of FB-positive nerve cells found in this study included some subpopulations of neurons projecting to the particular structures mentioned above. Neurons supplying the perirenal fat depot were found in both SChG and PVG. Perirenal fat- projecting neurons were localized in CSMG and ipsilateral ADG, ARG, OG, TG and IMG, as well as in the ipsilateral lumbar SChG at the level of L_1-L_3 whereas FB^+ neurons of the mesentery group were localized in CSMG and both right and left ADG, ARG, OG, TG and IMG, as well as in the right thoraco-lumbar SChG at the level of Th_{13}-L_3. These differences may result from the different topography and extent of the studied fat tissue depots. Previous experiments carried out by means of the retrograde tracing method in the pig (Majewski and Heym 1991a, Majewski et al. 1991b, 1995, 1996, Wąsowicz et al. 1998, Czaja 2000, Pidsudko et al. 2001) showed organ-related differences in the distribution of neurons supplying female genital organs and gut. Differences in the distribution of neurons innervating the oviduct, ovary, uterus, and gut may be a result of the different pathways used by the axons of those neurons to reach the target tissues. This hypothesis may also explain differences in localization of the sympathetic nerve sources supplying subcutaneous, perirenal and mesentery fat depots found in the present study. The number of labeled neurons in particular ganglia seems to confirm the topographical relationship between various fat tissue depots and ganglia innervating these tissues. The largest number FB^+ neurons projecting to the subcutaneous fat tissue was localized in SChG at the level of Th_{11}, Th_{12} and Th_{14}. The largest number of neu-

rons projecting to the perirenal fat was localized in CSMG and ADG, as well as in SChG at the level of L_2 and L_3. The most numerous subpopulation of mesentery fat neurons were those projecting from the CSMG.

CONCLUSION

In conclusion, present results are in agreement with the results of earlier morphological and physiological investigations concerning innervation of the fat tissue depots in several other species. However, further morphological and physiological studies are needed to establish the physiological relevance of the sympathetic neurons in the neural control of the fat tissue metabolism.

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The skillful technical assistance of Mr. Aleksander Penkowski is greatly acknowledged. This study was supported by the grant Early Career Cooperative Research Awards 2/99, sponsored by the United States Department of Agriculture and the Ministry of Agriculture and Food Economy of Poland.

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