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Changes in the expression of tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), galanin (GAL), vasoactive intestinal polypeptide (VIP) and substance P (SP) in the uterine cervix-projecting neurons located in the lumbar paravertebral ganglia of the pig

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The uterine cervix-projecting neurons located in the lumbar paravertebral ganglia were identified by retrograde tracing. These contained immunoreactivity to TH and DBH. No immunoreactivity to GAL, VIP and SP was found in the neurons. Extirpation of the uterus reduced the expression of TH and induced the expression of GAL in the neurons. Expression of other substances studied was unchanged.

key words: neuropeptides, sympathetic chain, axotomy, uterus, pig

INTRODUCTION

The influence of axotomy on the expression of biologically active substances in sympathetic neurons was studied in the rodent paravertebral ganglia, especially the superior cervical ganglion (SCG) [2]. It was found that the axotomised neurons react to injury with a reduction in the synthesis of TH, DBH and neuropeptide Y (NPY) [3] as well as with a rise in the synthesis of GAL, VIP and SP [10]. Studies on the influence of axotomy on the biology of the uterus-projecting neurons located in the porcine inferior mesenteric ganglion (IMG), a prevertebral ganglion, revealed a significantly different response. The expression of TH was down-regulated [8] and the expression of GAL was up-regulated in the neurons [9]. No changes in the expression of DBH, VIP and SP were detected. The results of the present paper will deliver evidence as to whether the porcine para- and prevertebral sympathetic neurons react differently to axotomy from neurons studied in rodents [5].

MATERIAL AND METHODS

The study was performed in 8 gilts at the age of 10 weeks. The paravertebral neurons were identified with retrograde fluorescent tracer Fast Blue (FB; Dr Illing, Germany) injected as 5% aqueous suspension into the porcine uterine cervix during laparotomy. After 3 weeks the whole uterus with the uterine cervix was extirpated in 4 animals (MCX group). The remaining 4 animals were sham-operated and served as the control group (MC0 group). 1 week later all the animals were deeply anaesthetised and perfused transcardially with 4% paraformaldehyde solution in phosphate buffer. The 4^{th} and 5^{th} lumbar paravertebral ganglia were dissected out, cut with a cryostat into 12 μm sections, put on chrome alum-gelatine-coated slides and processed for double indirect fluorescence. Primary antibodies against TH (mouse, 2/40/15, Boehringer Mannheim, D), DBH (rabbit, DZ1020, Affinity, USA), GAL (rabbit, RIN-7153, Peninsula, USA), VIP (rabbit, 20077, Incstar, USA) and

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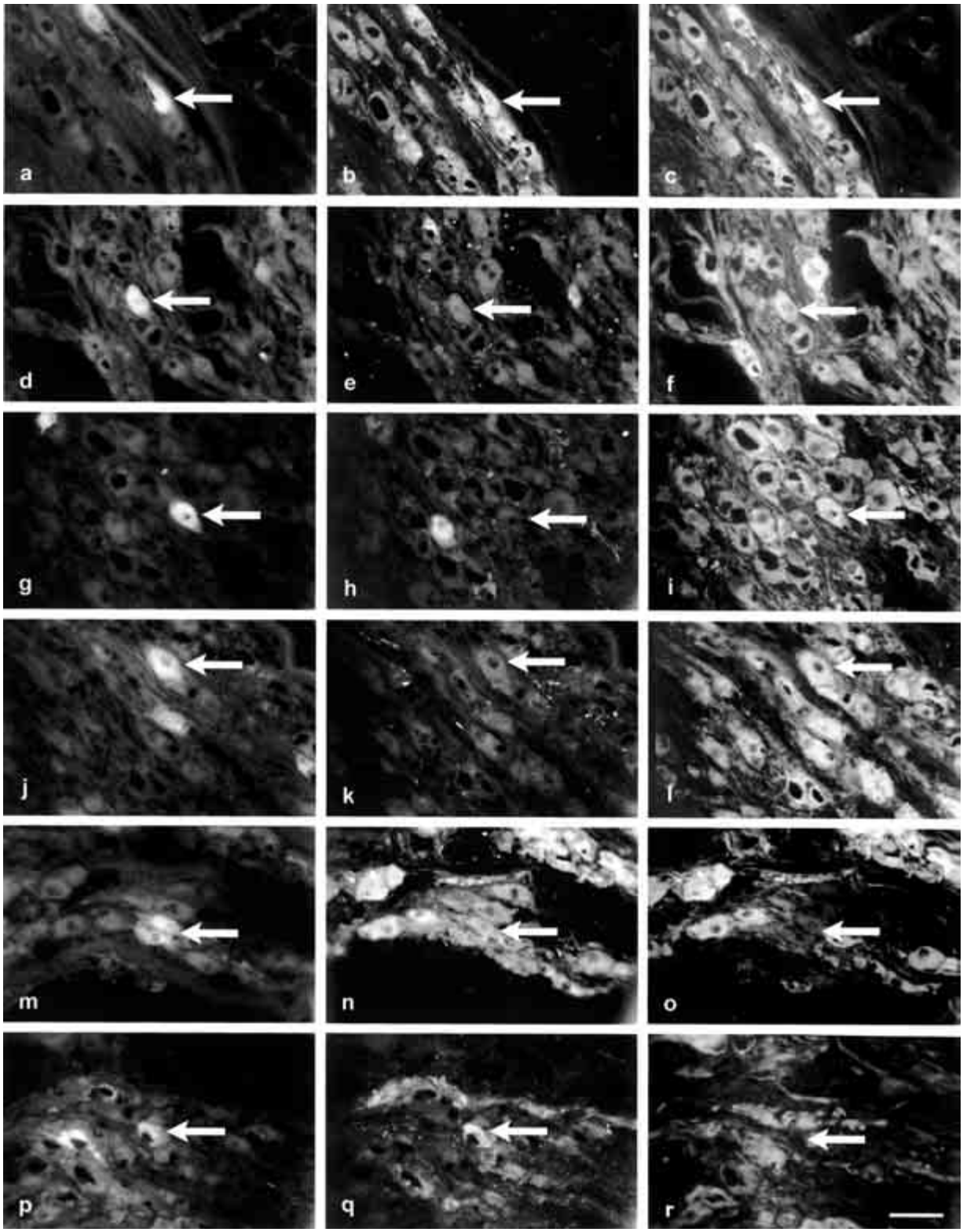

Figure 1. The immunohistochemical characteristics of Fast Blue-positive (FB+) neurons of the 5th lumbar paravertebral ganglion of the control (**a–l**) and the hysterectomised (**m–r**) animals; DBH (**b**) and TH-immunoreactivity (**c**) in FB+ (**a**) neuron (arrow); FB+ (**d**) neuron (arrow) is GAL– (**e**) and TH+ (**f**); FB+ (**g**) neuron (arrow) is VIP– (**h**) and TH+ (**i**); FB+ (**j**) neuron is SP– (**k**) and DBH+(**l**); FB+ (**m**) neurons (arrow) are DBH+ (**n**) and TH– (**o**); FB+ (**p**) neuron is GAL+ (**q**) and TH– (**r**). Bar 50 μm.

SP (rat, RPN 1572, Amersham, UK) as well as appropriate secondary antibodies conjugated with FITC and Texas Red were used as described elsewhere [8, 9]. The slides were observed using a Zeiss Axiophot epifluorescence microscope and filters specific for FB, FITC and Texas Red.

RESULTS AND DISCUSSION

Moderately numerous FB-positive (FB+) neurons were found in the 4th and 5th lumbar paravertebral ganglia of the control animals (Fig. 1a, d, g, j). All these neurons were TH+ (Fig. 1c, f, i) and DBH+ (Fig. 1b, l). No immunoreactivity to GAL (Fig. 1e), VIP (Fig. 1h) and SP (Fig. 1k) was found in these nerve cells. After axotomy the majority of FB+ neurons (Fig. 1m, p) displayed a profound reduction in TH-immunoreactivity (Fig. 1o). However, the DBH immunofluorescence remained at the same level as that seen in the control animals (Fig. 1n). On the other hand, the majority of FB+ neurons displayed strong immunoreactivity to GAL (Fig. 1q). All these neurons were TH– (Fig. 1o, r). No immunoreactivity to VIP and SP was found in the FB+ neurons in the axotomised animals.

A reduction in the expression of TH and DBH and a rise in the expression of GAL, VIP and SP [4] was found to be the typical axotomy-related response of rodent neurons located in the paravertebral ganglia. This is seen not only on the protein level, but also at the level of mRNA [6]. The changes in the expression of the above-mentioned substances are triggered by trophic factor (NGF, nerve growth factor) deprivation [2] and substances produced by injured Schwann cells, such as leukaemia inhibitory factor (LIF) [7]. However, very little is known about the response to axotomy of sympathetic neurons located in the prevertebral ganglia. Studies on the reaction to axotomy of porcine uterus-projecting neurons located in the IMG, one of the prevertebral ganglia, revealed that axonal injury does not change the level of expression of DBH, VIP and SP [8, 9]. The phenomenon of the varying post-axotomy response may be attributed to the species-related differences in the biology of the rodent and porcine sympathetic neurons, or to the differences in the biology of sympathetic neurons located in the para- and prevertebral ganglia. The data presented indicate clearly that the axotomised porcine prevertebral sympathetic neurons do not change the expression of DBH, VIP and SP, at least during the one-week post-axotomy period. This finding points clearly to species-related differences in the adaptational response of rodent and porcine sympathetic neurons to axotomy. The most striking phenomenon is the lack of induction of VIP, which is regarded as a potent neuroprotective agent [1]. VIP may protect injured sympathetic neurons from degeneration. It would be interesting to learn whether injured porcine sympathetic neurons also produce neuroprotective substances. However, further studies are required to answer this question.

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