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Glial Cell Activation in Pelvic Ganglia After Preganglionic But Not Postganglionic Lesions

Bengt Uvelius[1] and Martin Kanje[2]
[1]Department of Urology, Skane University Hospital, Malmo, Sweden; [2]Department of Biology, Lund University, Lund, Sweden
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ABSTRACT

INTRODUCTION: Pelvic surgery can result in injuries to the parasympathetic innervation of the pelvic organs. Such injuries can involve both preganglionic and postganglionic lesions. The purpose of the present study was to describe activation of the pelvic ganglion cells by the transcription factors ATF3 and p-c-Jun after preganglionic or postganglionic lesions in the rat.

METHODS: ATF3, p-c-Jun, and ED1 were monitored in rat pelvic ganglia 4 days after preganglionic and postganglionic lesions using immunocytochemistry.

RESULTS: Control ganglia exhibited weak nuclear staining for p-c-Jun but not ATF3. Postganglionic lesions induced ATF3 and p-c-Jun in neuronal nuclei. In contrast, preganglionic lesions induced ATF3 and p-c-Jun mainly in the nuclei of satellite cells and Schwann cells, although some neuronal nuclei were intensely p-c-Jun positive. No neurons expressing ATF3 were found. Staining by ED1 showed an increased number of macrophages in the ganglia after preganglionic lesions.

CONCLUSION: The authors hypothesize that the induction of nuclear ATF3 and p-c-Jun in Schwann cells and satellite cells is induced by degeneration, and that the expression of p-c-Jun and ATF3 in neuronal nuclei reflects activation of sprouting mechanisms.

INTRODUCTION

The pelvic ganglion that innervates the urogenital organs contains both sympathetic and parasympathetic neurons [1,2]. The sympathetic neurons are tyrosine hydroxylase (TH) positive. The parasympathetic neurons are mainly cholinergic and harbor vasoactive intestinal peptide (VIP) and nitric oxide (NO) as transmitters. The ganglion receives its input from the lumbar and sacral spinal cord.

Decentralization refers to cutting of the preganglionic nerve that contains preganglionic autonomic nerves from the spinal cord. This has been shown to induce intraganglionic sprouting of the VIP-containing postganglionic neurons [3] and formation of TH-positive baskets in the ganglion [2]. The sprouting VIP fibers form baskets around the denervated TH-containing neurons, and it has been suggested that this could inhibit appropriate reinnervation of the ganglion [3].

An interesting aspect of the previous experiments is that sprouting occurs from uninjured neurons. More profound intraganglionic sprouting is associated with injury to the axons of the postganglionic nerve fibers [4]. In humans, injuries to the preganglionic and postganglionic fibers are common and the unfortunate result of prostatectomy for prostate cancer. Therefore, an understanding of the mechanisms governing the plasticity of these neurons has a potential clinical impact.

KEYWORDS: Pelvic ganglion; p-c-Jun; ATF3; Denervation; Decentralization

CORRESPONDENCE: Bengt Uvelius, MD, Department of Urology, Skane University Hospital, SE-20502 Malmo, Sweden (bengt.uvelius@med.lu.se).

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Abbreviations and Acronyms

NO = nitric oxide
PBS = phosphate-buffered saline
TH = tyrosine hydroxylase
VIP = vasoactive intestinal peptide

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In the rat urinary bladder, there are virtually no intramural ganglion cells [5]. Therefore, the motor axons in the nerves from the ganglion to the bladder are truly postganglionic. Cutting of the postganglionic nerves that contain postganglionic autonomic nerves and sensory nerves from the urogenital organs is also associated with an increase in the VIP content of the ganglion. In addition, an upregulation of a variety of regeneration-related transcription factors like c-Jun is induced [6]. The same proteins are also upregulated in the neurons of the somatic nervous system as a result of axonal damage [7]. Because c-Jun is also upregulated in neurons of the pelvic ganglion after preganglionic injury, it has been suggested that c-Jun is a marker of sprouting rather than injured neurons [6].

In the dorsal root ganglion, Lindwall et al [8] demonstrated that nerve injury is associated with a rapid activation of c-Jun by phosphorylation. This precedes the induction of another transcription factor known as ATF3. It has been suggested that ATF3 is a general marker for regenerating neurons [9]. It is also upregulated in Schwann cells following sciatic nerve injuries [10].

The purpose of the present investigation was to determine how ATF3 and activation of c-Jun in the pelvic ganglion were affected by preganglionic and postganglionic injuries to the ganglion.

METHODS

Animals and Surgery

All experiments were approved by the local animal ethics committee at Lund University.

Female Sprague-Dawley rats weighing 200-250 g were anesthetized with ketamine (Ketalar®, Parke-Davis, Barcelona, Spain) 100 mg/kg and xylasine (Rompun®, Bayer AG, Leverkusen, Germany) via intramuscle administration. The lower abdomen was opened through a 1.5 cm longitudinal incision. The right major pelvic ganglion was exposed (Figure 1). In one group of animals, the preganglionic nerve was dissected free from its adjoining artery. It was then cut about 5 mm dorsal to the ganglion, leaving the vessel intact. In another group of animals, the nerves to the bladder and bladder neck were identified and cut through an oval incision on the surface of the cervix, at about 5 mm distance from the ganglion (see Figure 1). This left the nerves going to the uterus, urethra, and vagina intact. The left ganglion was dissected free from fat and exposed but left intact. The abdomen was closed in 2 layers. After 4 days, the animals were killed by carbon dioxide asphyxiation and the ganglia were dissected. Ganglia were also obtained from unoperated animals. The total number of animals used was 14.

Figure 1. Schematic Drawing of the Right Pelvic Ganglion and the Nerve Transection Sites.

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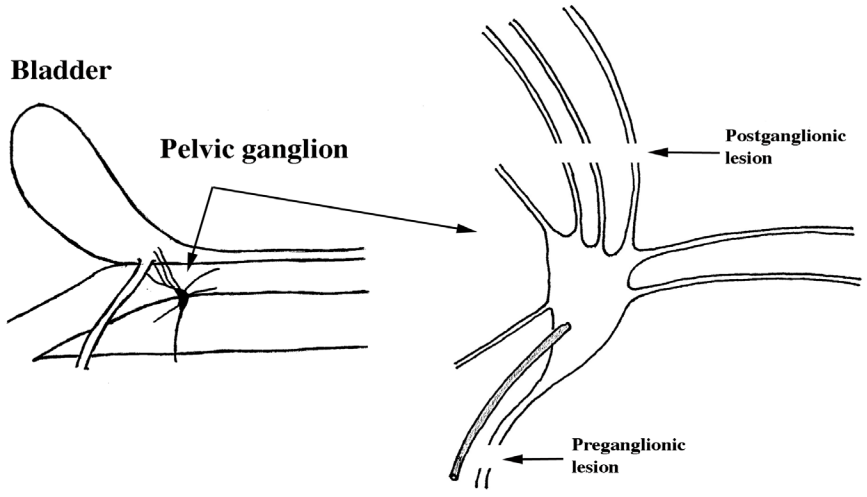

The ganglion is situated in the ridge between the uterine cervix and rectum. The preganglionic nerve(s) is accompanied by a small artery going to the ganglion.

Immunocytochemistry

The dissected ganglia were immersion in Stefanini's fixative over night. After washing in phosphate-buffered saline (PBS), the ganglia were cryoprotected in PBS containing 25% sucrose over night. The preparations were subsequently embedded in Tissue-tek® (Histolab, Sweden) and sectioned in a cryostat at 10 µm thickness.

The sections were collected onto Superfrost®plus (Thermoscientific, Germany) objective slides and allowed to air dry. After washing in PBS, the sections were exposed to primary antibodies dissolved in PBS containing 0.25% Triton-X, 0.25% serum albumin, and 0.05% sodium azide over night at 4°C. The sections were then washed and exposed to secondary antibody for 2 hours at room temperature, washed, mounted in glycerol:PBS 1:1, and coverslipped. Nuclei were counterstained with bisbenzimide.

Photography

Digital photographs of the preparations were obtained through an Olympus UMPH microscope equipped with Nomarski and fluorescence optics with an attached digital sight camera and a computer equipped with the software Nikon Nis elements Br 3.0. Adobe® Photoshop® 8.01 (Adobe Systems Inc., New York, NY, USA) was used to superimpose digital fluorescent images on Nomarski images in order to improve the accuracy of identifying satellite cells.

Antibodies

Primary antibodies were: (1) Rabbit anti-ATF3 (Santa Cruz Biotech Inc., USA), (2) Rabbit anti-p-c-Jun (Cell Signaling Tech, USA), and (3) Mouse anti-ED1 (Serotec, UK). Secondary antibodies were Alexa goat anti-mouse and Alexa goat anti-rabbit (Molecular Probes, USA). Dilutions were 1:200 to 1:500 for primary and 1:500 for secondary antibodies.

RESULTS

ATF3

Figure 2 (a) shows the contralateral control ganglia and (b and c) show immunoreactivity of ATF3, 4 days after preganglionic and postganglionic lesions. No immunoreactivity could be observed in contralateral control ganglia (Figure 2a) or ganglia dissected from unoperated animals (not shown). After postganglionic lesions (Figure 2b), ATF3 increased in the neuronal nuclei only. In contrast, preganglionic lesions caused an increase of ATF3 in the nuclei of satellite cells and in Schwann cells. The former were identified by their close association with neuronal cell bodies and by their ovoid shape. The latter were found in mainly in intraganglionic nerve bundles (Figure 2c) and had elongated nuclei.

P-c-Jun

Figure 2 (d-f) shows immunoreactivity of p-c-Jun. There was a faint immunoreactivity of the nuclei in many nerve cell bodies in both control (Figure 2d) and contralateral ganglia. Postganglionic lesions increased p-c-Jun in the nuclei of nerve cell bodies but in no other cells (Figure 2e). Preganglionic lesions increased p-c-Jun in the nuclei of Schwann cells and in a limited number of satellite cells (Figure 2f). Similar to the control and contralateral ganglia, many nerve cell nuclei showed a faint p-c-Jun; a few ganglion cells were found where the nuclei showed a pronounced increase of p-c-Jun (Figure 2f).

ED1

Figure 2 (g-i) show the distribution of macrophages in the pelvic ganglion, as revealed by ED1 staining. The staining was punctate in the macrophages. In control and contralateral ganglia, ED1 positive staining was found in a small population of cells between the neurons and in intraganglionic nerve bundles, consistent with distribution of resident macrophages. Preganglionic lesions (Figure 2i) but not postganglionic lesions (Figure 2h) increased the number of ED1 positive structures.

DISCUSSION

The main finding in the present study was that preganglionic and postganglionic injuries resulted in different activation of cells in the pelvic ganglion. Preganglionic but not postganglionic injury induced an upregulation of the transcription factor AFT3 in the nuclei of Schwann cells and satellite cells. The transcription factor c-Jun was likewise activated in satellite cells after preganglionic injuries, but it was activated in neurons mainly after postganglionic injuries. An association between c-Jun activation and an induction of ATF3 has previously been observed in somatic neurons such as the sensory cell bodies in the dorsal root ganglia [8]. The present results suggest that this is also the case for autonomic neurons. c-jun belongs to the AP1 transcription factors and the ATF3 gene also contains AP1 binding elements. Lindwall and Kanje [11] previously suggested that p-c-Jun is associated with survival and regeneration of sensory neurons.

It is noteworthy that both preganglionic [3] and postganglionic [4] injury have been reported to result in intraganglionic sprouting of adrenergic and cholinergic VIP-containing nerve fibers within the pelvic ganglion, although the sprouting response is more elaborate after postganglionic injuries. The present authors found neither activation of c-Jun nor induction of ATF3 in satellite cells after postganglionic lesions, suggesting that the genes regulated by these transcription factors in glia

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Figure 2. Immunoreactivity of ATF3 (a-c), p-c-Jun (d-f), and ED1 (g-i) in Rat Pelvic Ganglia (see legend on next page). doi: 10.3834/uij.1944-5784.2010.06.18f2

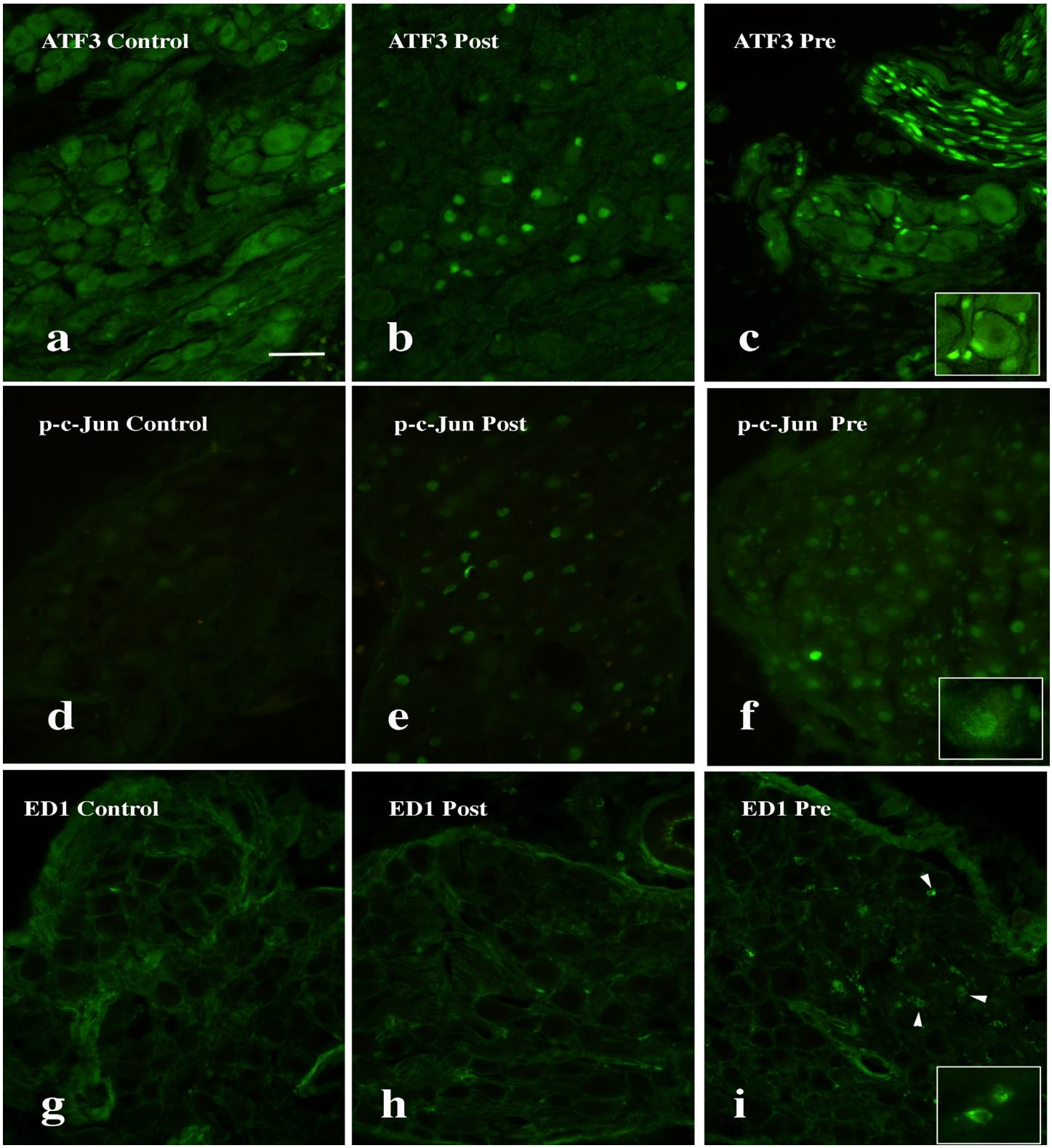

Figure 2 legend:

Left column: contralateral ganglia or ganglia from unoperated animals.

Middle column: ganglia 4 days after postganglionic nerve lesion.

Right column: ganglia 4 days after preganglionic lesions. Inserts are from separate ganglia.

ATF3 increased in neurons after postganglionic lesion (b) and in Schwann cells (c) and satellite cells (c and its insert) after preganglionic lesions. Ganglia in (a) and (c) are from the same animal.

A faint p-c-Jun neuronal nuclear immunoreactivity in the ganglion in (d) was from a control animal. Neuronal nuclear immunoreactivity of p-c-Jun increased (e, f) after both type of lesions. p-c-Jun also increased in the nuclei of Schwann cells (f) and satellite cells (f and its insert) after preganglionic lesions.

ED1 in macrophages increased only after preganglionic lesions (i:arrowheads, insert). Ganglia in (g) and (i) are from the same animal as in (f).

Scale bar in (a) corresponds to 30 µm in panels (a)-(i), and to 12 µm in the inserts.

cells are not involved in sprouting. What could be responsible for the selective glial activation? A major difference between the 2 experimental lesions is that only the preganglionic lesions lead to nerve degeneration within the ganglion. In this case, the preganglionic motor nerves reaching the ganglion and the sensory nerves passing the ganglion are prone to degradation because they have lost contact with their cell bodies. In contrast, there should be few or no degenerating elements in the ganglion following postganglionic lesions. The degenerative events should lure macrophages into the ganglion because these cells are engaged in Wallerian degeneration and the removal of degradation products. Furthermore, such cells are known to release a variety of inflammatory agents such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) alpha. Indeed, the present authors found more macrophages after preganglionic than after postganglionic lesions using ED1 immunostaining. It is tempting to suggest that the degenerative events that trigger an inflammatory response could be linked to the activation of the glial cells after preganglionic lesions.

The present results indicate that ATF3 is a marker for axonal injury because it is only demonstrated in the neuronal nuclei after postganglionic lesions. In contrast, a basal activation of c-Jun occurred in many neurons from control or contralateral ganglia. This activation was very pronounced in some uninjured neurons that had been denervated. The occurrence of a basal activation of c-Jun is known in the superior cervical ganglion [12] and might reflect an ongoing plasticity of innervation. Based on preganglionic and postganglionic lesions, Nangle and Keast [6] suggested that induction of c-Jun was related to sprouting rather than nerve injury. The present results lend support to this idea and add that activation of this transcription factor by phosphorylation is related to sprouting.

CONCLUSION

The induction of nuclear ATF3 and p-c-Jun in Schwann cells and satellite cells in the pelvic ganglion after preganglionic lesions might be induced by degeneration, and the expression of p-c-Jun and ATF3 in neuronal nuclei after postganglionic lesions might reflect activation of sprouting mechanisms.

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Conflict of Interest: none declared

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