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Neurite outgrowth in cultured mouse pelvic ganglia - Effects of neurotrophins and bladder tissue

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ABSTRACT

Neurotrophic factors regulate survival and growth of neurons. The urinary bladder is innervated via both sympathetic and parasympathetic neurons located in the major pelvic ganglion. The aim of the present study was to characterize the effects of the neurotrophins nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) on the sprouting rate of sympathetic and parasympathetic neurites from the female mouse ganglion. The pelvic ganglion was dissected out and attached to a petri dish and cultured in vitro. All three factors (BDNF, NT-3 and NGF) stimulated neurite outgrowth of both sympathetic and parasympathetic neurites although BDNF and NT-3 had a higher stimulatory effect on parasympathetic ganglion cells. The neurotrophin receptors TrkA, TrkB and TrkC were all expressed in neurons of the ganglia. Co-culture of ganglia with urinary bladder tissue, but not diaphragm tissue, increased the sprouting rate of neurites. Active forms of BDNF and NT-3 were detected in urinary bladder tissue using western blotting whereas tissue from the diaphragm expressed NGF. Neurite outgrowth from the pelvic ganglion was inhibited by a TrkB receptor antagonist. We therefore suggest that the urinary bladder releases trophic factors, including BDNF and NT-3, which regulate neurite outgrowth via activation of neuronal Trk-receptors. These findings could influence future strategies for developing pharmaceuticals to improve re-innervation due to bladder pathologies.

1. Introduction

In recent years there has been a great interest in the plasticity of the pelvic ganglia, because of the implications to clinical urology. First, surgical treatment of prostate cancer (radical prostatectomy) often leads to erectile dysfunction, largely as a result of injury to the postganglionic nerves (Hannan et al., 2015). Second, prostate enlargement often leads to bladder overactivity, which has been coupled to changes in pelvic nerve function (Ochodnicky et al., 2012).

The pelvic ganglia in the rat contain both parasympathetic and sympathetic neurons which innervate the lower urinary tract and the internal reproductive organs (Alm et al., 1995). The sensory nerve fibers from the urinary bladder pass through the ganglion.

The neurons within the ganglion can be discriminated based on the presence of transmitter, transmitter-synthesizing enzymes or on their content of neuromodulators. The adrenergic, sympathetic nerves contain tyrosine hydroxylase (TH). In the pelvic ganglion of the male rat, about 1/3 of the neurons are TH positive (Keast et al., 1995) whereas < 10% are TH positive in the female rat (Vera and Nadelhaft, 1992). The number of neurons in the female pelvic ganglia is only about 40% of that in the male (Greenwood et al., 1985). The parasympathetic nerves contain choline acetyltransferase and make up for the remaining part (non TH positive neurons) (Keast et al., 1995). Keast and Kepper (2001) further subdivided the male pelvic ganglion cells into noradrenergic, containing noradrenaline and NPY (Neuropeptide Y), and cholinergic, containing acetylcholine and VIP (vasoactive intestinal peptide) or acetylcholine and NPY.

Neural plasticity and sprouting are influenced by the release of trophic factors from the innervated organs (Purves et al., 1988). The neurotrophic factor family comprises nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5), glial-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF) (Gunn-Moore and Tavare, 1998). Neurotrophic factors are expressed in rodent bladder tissue but the expression levels varies during postnatal development (Kawakami et al., 2002; Lin et al., 2010; Stewart et al., 2008; Vizzard et al., 2000) Changes of neurotrophin levels have been described after nerve injury and in pathological conditions of the bladder (Hannan

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et al., 2015; Vizzard, 2000) but the physiological consequences of these changes are not fully understood.

Binding of neurotrophins to their cognate Trk receptors increases tyrosin kinase activity and drives intracellular signaling pathways, including the ERK1/2, PI3K and PLC (Gunn-Moore and Tavare, 1998) and JAK/STAT (Bella et al., 2007) pathways. Neurotrophins are normally produced by the target tissue and the neurons, their neurites and dendrites, and the synthesis rate is continuously modified to meet the demands of the innervated organs. In the male rat, the noradrenergic and the cholinergic, NPY-containing, subgroups usually express the receptors for NGF, Trk A, but only a small fraction of the of cholinergic VIP-containing pelvic neurons expressed TrkA (Keast and Kepper, 2001). TrkB and TrkC were also found to be expressed in pelvic neurons of the rat (Lin et al., 2006). The receptors for BDNF (TrkB) and NT-3 (TrkC) are found in most ganglion cells (Lin et al., 2006), and are thus present in most cholinergic ganglion cells. The low-affinity neurotrophin receptor p75 is expressed in satellite cells (Keast and Kepper, 2001; Lin et al., 2006) but also in most sympathetic ganglion cells (Keast and Kepper, 2001).

The aim of the present study was to establish a co-culture model of neurite outgrowth, and to investigate the effects of neurotrophins on neurite outgrowth and identify neurotrophin receptors in the adult female mouse pelvic ganglion. We used female mice since there is less information on sprouting phenomena in this gender. In addition, we wanted to characterize neurotrophin expression in one target organ (the urinary bladder).

2. Material and methods

2.1. Animals

Animal experiments were conducted in conformity with national and international guidelines and were approved by the Lund/Malmö animal ethics committee (M114-15). Female NMRI mice weighing 20–25 g were killed by cervical dislocation, and the pelvic ganglia were immediately dissected out under a microscope and transferred to cold Hepes buffered Krebs solution. The location of the ganglion is outlined in Fig. 1A. A supratrigonal section of the urinary bladder and a section of the muscular part of the diaphragm were dissected out for co-culture experiments. For some experiments, the prostate and pelvic ganglia were dissected from male mice and used in the co-culture set-up (see below in 2.3 Culture set-up).

2.2. Bladder wholemounts

Bladders with proximal urethra were dissected from female mice, and transferred to Krebs-buffered solution. The ureters were ligated, the urethra was cannulated and the bladders were filled with 0.3 ml Krebs-buffer solution followed by transfer to 4% formaldehyde solution. After 15 min the bladders were cut open longitudinally along their dorsal side and after 1 h the specimens were rinsed and trimmed, and then transferred to buffer containing hyaluronidase (0.33 mg per 100 ml) and ISO-OMPA (0.1 μM) overnight in a 4 °C. The bladders were then rinsed and stained for acetylcholinesterase as described by Uvelius and Gabella (1998). After the incubation, bladders were flattened by making radial cuts from the dorsal to the ventral side. Specimens were then dehydrated, and mounted in araldite on slides. Photographs were taken with a macro lens, with the slides placed on a light board.

2.3. Culture set-up

The tissues (pelvic ganglia, urinary bladder, diaphragm and prostate tissue) used for culture were mounted with Matrigel® (Collaborative research Inc., USA) and cultured in 35-mm plastic culture dishes containing 2 ml RPMI 1640 medium (Sigma-Aldrich Co LTD, UK). In the first set of experiments (used for results in Figs. 2 and 3), the pelvic ganglion was attached to center of the culture dish. Added substances (see Table 1) were diluted in the RPMI-medium. When the ganglion was co-cultured with tissue (experiments used for results in Figs. 5, 7 and 8), 6 tissue pieces (size: app. 1 mm^3) were attached in a circle 3 mm from the ganglion (Fig. 5A). Tissue cultures were maintained at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2 in a cell culture incubator. The culture dishes were taken out from the incubator once a day. The length of the longest sprouting neurites were identified and measured under a light microscope (Olympus SZ61). Measurements were done on live images using a calibrated scale bar in eyepiece of the microscope. At least 10 different neurites from one ganglion were identified and measured at each time point. The medium was never exchanged during the culture time.

2.4. Immunohistochemistry

After 5 days of culture, the organ-cultured tissues were fixed in 4% paraformaldehyde over night at 4 °C, washed with PBS and put in 20% sucrose solution at 4 °C. The tissues were cut out from the culture dishes and embedded in Tissue tek® (Miles, USA) and cut into 10-μm sections

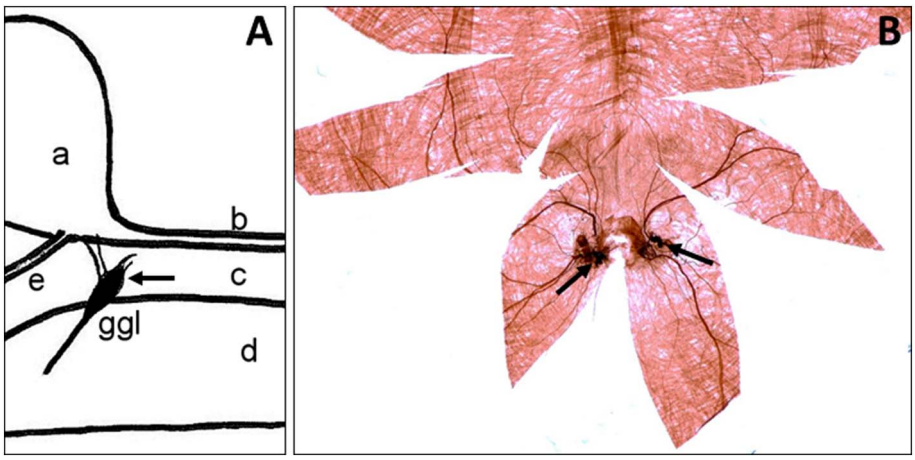

Fig. 1. A schematic drawing of the location of the pelvic gangion (A). a = urinary bladder, b = urethra, c = vagina, d = rectum, e = right ureter, ggl = right pelvic ganglion. Wholemount of a mouse urinary bladder (B). Nerve distribution in the caudal half of the urinary bladder is visualized (black) using acetylcholinesterase staining. A few small accessory ganglia are seen close to the ureteral orifices (marked with arrows), but no ganglia are found distal to that. The faint unspecific staining (brown) of the smooth muscle cells shows that they are arranged in a crisscross pattern. The base of the illustration corresponds to 20 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)