Journal of Pediatric Surgery 51 (2016) 1321–1326

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Neurotrophin signaling in a genitofemoral nerve target organ during testicular descent in mice

Mary C. Cousinery [a,c], Ruili Li [a,c], Amanda Vannitamby [c], Jaya Vikraman [a,c], Bridget R. Southwell [a,c], John M. Hutson [a,b,c,*]

[a] F Douglas Stephens, Surgical Research Group, Murdoch Childrens Research Institute, Melbourne, Australia
[b] Department of Urology, The Royal Children's Hospital, Melbourne, Australia
[c] Department of Paediatrics, University of Melbourne, Melbourne, Australia

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 12 November 2015
Accepted 16 November 2015

Key words:
neurotrophin
ciliary neurotrophic factor
genitofemoral nerve
testicular descent

ABSTRACT

Background/Aim: It has been proposed that androgens control inguinoscrotal testicular descent via release of calcitonin gene-related peptide (CGRP) from a masculinised genitofemoral nerve (GFN). As there are androgen receptors in the inguinoscrotal fat pad (IFP) during the window of androgen sensitivity (E14-17 in mouse embryos), we tested the hypothesis that neurotrophins in the IFP may masculinise the sensory fibers of the GFN supplying the gubernaculum and IFP prior to gubernacular migration.
Methods: Androgen-receptor knockout (ARKO) and wild-type (WT) mouse embryos were collected at E17, with ethical approval (AEC 734). Sagittal sections of IFP, mammary area and bulbocavernosus (BC) muscle were processed for standard histology and fluorescent immunohistochemistry for ciliary neurotrophic factor (CNTF), ciliary neurotrophic factor receptor (CNTFR) and cell nuclei (DAPI).
Results: In the ARKO mouse CNTFR immunoreactivity (CNTFR-IR) was increased in the IFP but decreased in BC. Perinuclear staining of CNTF-IR was seen in mouse sciatic nerve but only weakly in IFP. In the mammary area, also supplied by GFN, there were no differences in IR staining.
Conclusion: This study found CNTFR-IR in the IFP was negatively regulated by androgen, suggesting that CNTF signaling may be suppressed in GFN sensory nerves to enable CGRP expression for regulating gubernacular migration in the male, but not the female. The indirect action of androgen via the GFN required for testicular descent may be one of the sites of anomalies in the putative multifactorial cause of cryptorchidism.
© 2016 Elsevier Inc. All rights reserved.

Cryptorchidism is a very common congenital anomaly, affecting 2–4% of newborn males [1], and the cause is likely to be multifactorial, as normal testicular descent is multistaged, involving numerous anatomical and hormonal factors [2,3]. To understand the cause of cryptorchidism it will be important to identify the precise mechanism of testicular descent in animal models, so that then we will know where to look for the etiology of human cryptorchidism, aiming to prevent its adverse effects on later fertility and malignancy [3,4].

Normal testicular descent occurs in 2 distinct steps, the transabdominal and inguinoscrotal phases [5]. The second phase occurs in humans at 25–35 weeks' gestation, and is a very complex process controlled mostly indirectly by androgen, which is thought to act via the genitofemoral nerve (GFN) to produce a specific neurotransmitter, calcitonin gene-related peptide (CGRP). In rodent models CGRP provides trophic and chemotactic stimulation of the gubernaculum to regulate its migration through the inguinoscrotal fat pad (IFP) to reach the scrotum [3].

Androgens act in fetal rodents during a narrow time window of sensitivity (embryonic day (E) E15-19 in rat and E14-17 in mouse) [6], but during this critical time no androgen receptors (AR) have been identified in the gubernaculum itself or the GFN sensory nerves in the dorsal root ganglion [7]. However, at this time there are AR in the mammary area and the IFP, both of which receive sensory fibers from the GFN [8]. This suggested that androgens may masculinise the GFN by stimulating release of neurotrophins from the GFN target organs, which are then taken up by the axons and transported centrally to the GFN cell bodies in the dorsal root ganglion (L1-2) [9]. A similar process has been found to occur in a nearby perineal structure, the bulbocavernosus (BC) muscle, which controls development of its own motor supply from the perineal nerve by 2 neurotrophins, ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) [10,11] which is known to regulate the GFN sensory fibers supplying the breast bud and mammary area. We aimed to determine whether the CNTF signaling pathway may have a role in masculinising the GFN sensory fibers supplying the IFP, knowing that the BC muscle produces CNTF in

* Corresponding author at: Surgical Research Laboratory, Murdoch Childrens Research Institute, The Royal Children's Hospital, Flemington Road, Parkville, Victoria, Australia, 3052. Tel.: +61 3 9345 5805.
E-mail address: john.hutson@rch.org.au (J.M. Hutson).

http://dx.doi.org/10.1016/j.jpedsurg.2015.11.009
0022-3468/© 2016 Elsevier Inc. All rights reserved.

Table 1
Primary antibodies used for IHC.

Primary Antibody	Company, Catalogue Number	Species of Animal, Clonality	Working Concentration (vol/vol)	Excitation Wavelength (nm), Color	Western Blot Positive for Specificity?
CNTF	Millipore, MAB338	Mouse, monoclonal	1/200	N/A	Yes
CNTFR-α	Abcam, ab175387	Rabbit, polyclonal	1/100	N/A	Yes
Mono β-Tubulin (Tuj 1)	Convance, MMS-435P	Mouse, monoclonal	1/100	N/A	NS
DAPI stain	Life Technologies, D1306	NS	1/50,000	405, blue	NS

*DAPI — 4′6-diamidino-2-phenylindole (labels nuclei of all cells).
*N/A = not applicable, NS = Not specified by antibody company.

response to androgen binding, and that the mammary area fat pad also contains GFN sensory fibers that respond to androgen.

1. Methods

Androgen receptor knockout (ARKO) male mice (n = 16) and littermate wild-type (WT) males, and some transscrotal (TS) mutant rats and C57 black/6 (C57bl/6) mice were used. In ARKO mice the third exon of the androgen receptor (AR) gene, which contains the DNA-binding domain, had been targeted by the Cre/loxP system to remove 1114 base pairs, rendering the animal completely insensitive to androgens [12]. All animals were bred and maintained in the institutional animal research laboratory. Animals were housed in Techniplast IVC cages or filter-top caging with irradiated FibreCycle bedding and environmental enrichment (seeds and cardboard homes), with 14 hours light, 10 hours dark, at 23 °C and 44% humidity. The animals were fed with irradiated Ridly Agri Mouse breeder cubes ad libitum. All experiments (AEC734), were approved by the institutional ethics committee.

For embryonic specimens, pregnant dams were sacrificed and fetuses collected at embryonic day 17 (E17) (vaginal plug = EO). All dams were anesthetized with 5% isoflurane gas, followed by intraperitoneal injection of Avertin (0.4 ml, 2.5%, 2,2,2-tribromoethanol, Sigma-Aldrich, #48402-5G) and then decapitation. Fetuses removed from the uterus were left on ice for 15–20 minutes and then decapitated before tissue collection. The caudal half of the abdominal cavity and trunk was dissected and fixed in 4% paraformaldehyde at 4 °C overnight before being processed through a graded series of alcohol and xylene and embedded in paraffin blocks. A 2 mm tail clipping was collected for genotyping as previously described [13]. Rat and C57bl/6 mouse sciatic nerves were collected for testing the primary antibody. The sciatic nerve was dissected out from the lower end of the sacral plexus to the popliteal region.

Paraffin blocks were sectioned on a microtome (Leica Microsystems, Wetzlar, Germany) at 5 μm and some sections were stained with hematoxylin and eosin to enable clear visualization of the 3 areas for study of neurotrophin expression: the BC muscle, the mammary fat pad and the IFP.

Slides containing the BC, IFP and mammary area were selected for immunohistochemistry (IHC). Slides were single labeled with either antibodies against CNTF or CNTFR (Tables 1 and 2) as described previously [13].

A Leica LSM-2 confocal microscope (Leica Microsystems, Wetzler, Germany) was used to acquire images. The wavelength settings (range properties) used for labeled secondary antibodies were DAPI: 412–482 nm, 488 (green): 500–552 nm, 568 (red): 567–781 nm, and 594 (red): 597–850 nm. Images were merged with use of Leica Fluorescence Analysis Suite software (Leica Microsystems, Wetzler, Germany) which resulted in multiple color overlays. Two magnifications were used (×40 and ×63 objectives), with zoom function between one and four. Confocal images were obtained and processed using ImageJ, including the addition of scale bars (ImageJ version, 1.48 V, Wayne Rasband, National Institutes of Health, USA).

Quantitative measurements on confocal microscope images were made using ImageJ, where the percentage of total nuclear area (DAPI stained) and CNTF/CNTFR positive area was measured (in microns) in the BC, IFP and mammary area and tabulated using GraphPad Prism (version 5.02, USA). The '% area' value for positive fluorescence of each antibody versus DAPI (nuclei) was tabulated and the area of positive labeling was calculated and graphed (Fig. 1). Data were managed in Excel 2010 (Microsoft Office). Independent sample t tests were applied to each set of data that were parametric and a Mann–Whitney test was applied for nonparametric data. Significance was determined when $p < 0.05$.

2. Results

In eight ARKO and WT littermates, H + E staining identified the 3 areas selected for IHC, namely BC, IFP and mammary area. The BC was larger in WT males than ARKO males. In the WT fetus the IFP consisted of loose mesenchyme in the scrotum but appeared to be densely packed in the equivalent labial region in the ARKO fetus. The anogenital (AG) distance was significantly greater in WT males (785 μm) compared with ARKO males (266 μm) ($p < 0.05$). The gubernaculum was already remodeling and appeared smaller in preparation of inguinoscrotal descent in WT males. By contrast, in ARKO males the gubernacular bulb remained prominent (results not shown).

The rat sciatic nerve was used as a positive control for CNTF and CNTFR IHC [14]. In the sciatic nerve the mouse monoclonal anti-CNTF antibody labeled axonal cytoplasm as well as perinuclear staining in presumed glial supporting cells, while the CNTFR antibody (rabbit polyclonal) showed punctate labeling consistent with CNTFR located on the cell membrane of axons. The negative control (with exclusion of primary antibody) showed complete lack of labeling.

CNTF immunoreactivity (IR) was found in both rat and mouse sciatic nerves in the perinuclear region of supporting (presumed glial) cells, but there was no evident cytoplasmic in labeling the BC or mammary area, and only very weak cytoplasmic labeling in the IFP (Fig. 2).

Table 2
Secondary antibodies used for IHC.

Secondary Antibody	Company, Catalogue Number	Species of Animal/Against	Excitation Wavelength (nm), Color	Primary Antibody Labeled
Alexa 568	Invitrogen A-11019	Goat/Mouse	568, red	CNTF
Alexa 594	Invitrogen A-21207	Donkey/Rabbit	561, red	CNTFR
Alexa 488	Invitrogen A-21202	Donkey/Mouse	488, green	Tuj1

*All secondary antibodies were used at a 1/500 dilution.
*nm = nanometer.

Download English Version:

https://daneshyari.com/en/article/4154756

Download Persian Version:

https://daneshyari.com/article/4154756

Daneshyari.com