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DIFFERENTIAL EXPRESSION OF NEUROTROPHINS IN (DSS)-INDUCED COLITIS IN SMOOTH MUSCLE OF RAT COLON

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Graphical abstract

Abstract

There is an increasing recognition of the role of neurotrophins in the mature gastrointestinal tract both at the physiological and pathological levels. However, their expression and role in smooth muscle in the GIT system is under investigated. The aim of this study is to elucidate the expression of the four neurotrophins in smooth muscle tissue of the rat colon and to test the effect of dextran sodium sulphate (DSS) - induced colitis on the expression pattern of these factors. Using specific ELISA kits for each neurotrophin revealed that the four neurotrophins are differentially expressed in the longitudinal and circular muscle layers of the rat colon and that DSS-induced colitis alters this expression pattern. These results indicate that smooth muscle tissue contributes to the pool of neurotrophins in the GIT and might play a role in the pathogenesis of colitis. Understanding the interactions of neurotrophins produced from smooth muscle and colitis could provide new avenues to tackle the dysfunction associated with inflammatory bowel diseases such as colitis

Keywords: Neurotrophins, GIT system, rat colon

1.0 INTRODUCTION

Neurotrophins are a family of closely related dimeric peptides that were initially identified as neuronal survival factors secreted from target tissues. To date, they are implicated in a myriad of functions in the central and peripheral nervous systems, including regulation of neuronal differentiation, migration and activity-dependent synaptic plasticity. Four members of the mammalian neurotrophin family have been identified; nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). All these factors are chemically and structurally homologous to NGF [1-3]. Neurotrophins mediate their biological function by activating two distinct cell surface receptors with high and low affinity. Tropomyosin-related kinases (Trk) receptors include Trk A, Trk B, and Trk C [4]. They are activated specifically with high affinity by one neurotrophin. NGF interacts with TrkA, BDNF and NT-4 interact with TrkB, and NT-3 interacts with TrkC. NT-3 binds to TrkA and TrkB as well, but with less affinity [1, 2]. The low affinity NT receptor p75 belongs to the tumor necrosis family and binds all NTs [5].

There is increasing recognition that neurotrophins and their receptors are expressed in non-neuronal tissues and seems to be essential for many physiological functions in several systems [6]. One of these systems is the gastrointestinal tract where neurotrophins are under intense investigations. Neurotrophin system has been identified in several cell types in the mature gut; these include epithelial and enteroendocrine cells of the mucosa [7, 8], enteric neurons and glia [9, 10] and recently intestinal smooth muscle [9]. Moreover, several physiological and pathological functions attributed to neurotrophins revealed recently. For example, BDNF has an important role in gut motility. It enhances the peristaltic reflex by augmenting the release of sensory neuropeptides from enteroendocrine cells and enteric sensory neurons [11]. Moreover, BDNF enhanced the rate of colonic pellet propulsion, while

its immunoneutralization reduced the rate of pellet propulsion in rat colon. Furthermore, NGF, BDNF, and NT-3 stimulate colonic myoelectrical activity of rats [12]. Recently we have shown that BDNF is present in intestinal smooth muscle cells and exogenous BDNF enhances cholinergic contraction in smooth muscle strips [9, 13].

In addition to the physiological role of neurotrophins in the gut, they are involved in many aspects of the GIT inflammation [14, 15]. Neurotrophin expression is altered differentially during colitis and has been linked to changes in gut motility [8, 16, 17]. Additionally, up regulation of neurotrophins in spinal cord and dorsal root ganglion in response to inflammation plays a role in visceral hypersensitivity and the pathogenesis of inflammatory bowel disease and irritable bowel syndrome [15]

Little is known about neurotrophin in colon smooth muscle cells and the contribution of this potential source to gut inflammation; however, in other tissues, neurotrophins produced from smooth muscle of smooth muscle play important role at different stages of inflammation [18-20]. Moreover, neurotrophins modulate several characteristics of smooth muscle physiology such as contractility, proliferative ability and secretion of several cytokines and peptides [21, 22]. The relationship between neurotrophins and inflammation is particularly interesting especially when neurotrophins role is viewed at different stages of inflammation because they have a dual effect in the process of induction and repair of the disease. In this study, we show that colonal smooth muscle differentially express neurotrophins and experimentally induced colitis changes their expression pattern.

2.0 MATERIAL AND METHODS

2.1 Induction of Colitis and Preparation of Tissue

The induction of colitis with dextran sulphate sodium (DSS) salt average molecular weight 40,000, (Sigma, St. Louis, MO) is a well-known model that mimics an inflammatory bowel disease [23]. The technique briefly, Adult Spraque -Dawley (S.D) rats were randomly divided into two groups; Control and DSS-colitis groups (weight: 200g, n: 6 per group). To induce colitis in DSS-colitis group, normal drinking water were replaced with autoclaved distill water containing 5 % DDS, prepared daily for 5 days. Age-matched rats treated with bottled water were constituted the control group. Then rats were euthanized on the sixth day. Animal weight, stool consistency, the presence of blood in feces and rectal bleeding were reported on daily bases to establish the mean daily disease activity index (DAI) to assess the disease progression. The criteria used to calculate the DAI based on Parameters such as weight loss (0 points = no weight loss to 5 points =more than 15% weight loss), stool consistency (0=normal to 5=watery diarrhoea) and bleeding (0=no bleeding, 2 points slight bleeding, 5 points gross bleeding).and recorded as a total of the three scores. DAI for DSS-induced colitis was 7±3. Moreover, the macroscopic changes in the distal colon were examined and compared with the DAI score of each animal.

Rats were euthanized by 100% carbon dioxide CO2 inhalation. The colon were dissected out, emptied of contents, and placed on cold smooth muscle buffer of the following composition (NaCl 120 mM, KCl 4 mM, KH2PO4 2.6 mM, CaCl2 2.0 mM, MgCl2 0.6 mM, HEPES (N-2-hydroxyethylpiperazine-N" 2-ethanesulfonic acid) 25 mM, glucose 14 mM, and essential amino mixture 2.1% (pH 7.4). 2-3 cm sections of the colon were removed and mounted onto glass rod, the fat and mesenteric attachments were removed and the longitudinal muscle were separated from the circular layer by radial abrasion with Kime wipe. The muscle layers were cleared from mucosal/submucosal layers by microdissection and was quickly frozen in liquid nitrogen and homogenized with a chilled pestle for protein

2.2 Neurotrophins ELISA

Total protein extracts were subjective to commercially available ELISA kits for BDNF, NGF, NT-3 and NT-4 according to the manufacturer instructions. Data were expressed as ng or pg per total protein extract and compared between control groups (circular and longitudinal) versus DSS-induced colitis groups (circular and longitudinal). The appropriate statistical tests were carried out in GraphPad (GraphPad Software, La Jolla, CA). A probability of $p < 0.05$ was considered significant. Values are reported as mean ± SEM. Each experiment was from at least three animals repeated three times.

3.0 RESULTS AND DISCUSSION

3.1 Expression of BDNF in Smooth Muscle Rat Colon and the Effect of DSS-Induced Colitis on the Expression Level

Total protein extract from the longitudinal and circular muscle layers of rat colon was subjected to specific BDNF ELISA. BDNF was present in both longitudinal and circular muscle layers. Comparing the protein levels in the two regions revealed a significantly 1.6-fold higher expression of BDNF in longitudinal muscle than circular muscle Figure 1.

There are very few studies of BDNF in gut smooth muscle however we recently Identified the expression of BDNF in both circular and longitudinal rabbit intestinal smooth muscle at the cellular and tissue levels [9] and the levels of expression was consistent with that of rat colon identified in this study.

To test the effect of experimentally induced colitis of the expression of BDNF in rat colon smooth muscle, we compared the levels of BDNF detected in total

protein extracts from the longitudinal and circular smooth muscle layers from rats treated with colitis with that of control rats. DSS-induced colitis resulted in significant upregulation of BDNF in both regions. The levels were 1.5-folds higher in the longitudinal layer of DSS-induced colitis than control rats while in circular muscle layer the expression increased about 2 folds compared to control Figure 1.

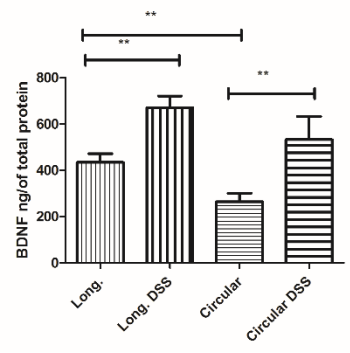

Figure1 BDNF expression in colonic smooth muscle cells in control and DSS-induced groups: BDNF level is expressed as ng/ of total protein. The figure shows basal expression of BDNF in the longitudinal and circular muscle layers. Comparing the BDNF level in the control vs. DSS-colitis group, there is a significant increase in BDNF level in DSS-colitis in both muscle layers. P**<0.005

In support of our results, several studies show that BDNF is upregulated during gut inflammation [24, 25]. Moreover, BDNF is present in smooth muscle of other tissues and is associated of inflammation [20, 21]. Upregulation of BDNF in smooth muscle of experimental colitis might explain the loss of innervation reported during inflammation [26-28]. Furthermore, BDNF expression is important for the development vagal sensory innervation [28-30] and interestingly BDNF conditional knockout in the intestinal smooth muscle of mice resulted in enhanced innervation by vagal sensory neurons which was especially obvious in the longitudinal muscle layer. Gut inflammation alters the contractile properties of the gut [31, 32]. Recently, we have revealed that exogenous BDNF enhances the cholinergic contraction of the longitudinal smooth muscle. The upregulation of BDNF reported here in smooth muscle of rat colon might explain motor function changes seen during colitis. However, the exact role of BDNF from smooth muscle during colitis needs further investigations.

3.2 Expression of NGF in Smooth Muscle Rat Colon and the Effect of DSS-Induced Colitis on the Expression Level

To test the expression levels of NGF in normal circular and longitudinal smooth muscle cells of rat colon, Total protein extracts from both regions were subjected to NGF specific ELISA. The expression levels of NGF protein was detected in both longitudinal and circular muscle smooth muscle cells and were significantly higher 1.5 fold in circular muscle than longitudinal muscle. comparing these results with DSS-induced colitis group revealed significant upregulations of NGF in both muscle layers. In the longitudinal layer, there was 1.4 fold increase in NGF levels while it was about 2 folds increase Figure 2.

The expression of NGF in smooth muscle is reported in several tissues such as airway [33] bladder [34] and vascular smooth muscle [35]. So it not surprising to find NGF in the smooth muscle of the colon.

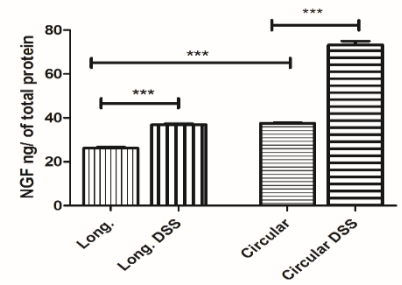

Figure 2 NGF expression in colonic smooth muscle layers in control and DSS-induced groups: NGF level is expressed as ng/ of total protein. The figure shows a baseline expression of NGF in longitudinal & circular smooth muscle layers of the colon. There is a significant increase in the expression of NGF in DSS-induced colitis in both muscle layers. ***p<0.0001

NGF upregulation is associated with inflammation and most of the most of its symptoms such as pain, induced hypersensitivity and caress sensitization [36]. Upregulation of NGF in smooth muscle of DSS-induced colitis could in part participate in these symptoms.

3.3 Expression of NT-3 In Smooth Muscle of Rat Colon and the Effect of DSS-Induced Colitis on the Expression Levels

Total protein extracts subjected to NT-3 specific ELISA revealed significantly higher expression of NT-3 in circular (80.78 ± 1.349 N=2) than longitudinal muscle layer. DSS-induced colitis did not significantly change the level of NT-3 in both muscle layers Figure 3.

Smooth muscle tissues contribute to the pool of NT-3 in the gastrointestinal track which might elucidate the reported roles of NT3 in the gut. For example, NT-3 accelerates colonic transit and increases stool frequency in patients with constipation [37]. Moreover, loss of NT-3 in developing gastrointestinal smooth muscles resulted in disruption of vagal gastrointestinal afferents and affected satiation [38]. In support of our findings, NT-3 is expressed in airways [39] and vascular smooth muscle cells [40]. But the role of NT-3 present in smooth muscle of the colon reported in this study still waits for investigation.

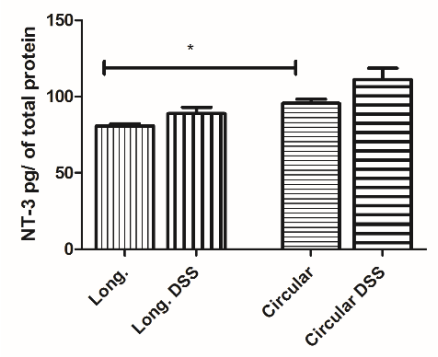

Figure 3 NT-3 expression in colonic smooth muscle layers in control and DSS-induced groups: NT-3 level is expressed as pg/µg of total protein. The figure shows basal expression of NT-3 in the longitudinal and circular muscle tissues. The expression is significantly more in the circular muscle tissue. Comparing the NT-3 level in both groups, there is no significant difference in the longitudinal and circular SMC of DSS induced group versus control group.*p< 0.05

3.4 Expression of NT-4 In Smooth Muscle of Rat Colon and the Effect of DSS-Induced Colitis on the Expression Levels

NT-4 was identified in both longitudinal and circular normal rat colon muscle layers in total protein extract subjected to NT_4 specific ELISA. The expression levels were significantly higher in longitudinal compared to the circular muscle layer. Comparing NT-4 between the normal and DSS-induced colitis revealed a significant reduction of NT-4 in the longitudinal muscle layer while the levels in the circular layer remained unaffected Figure 4.

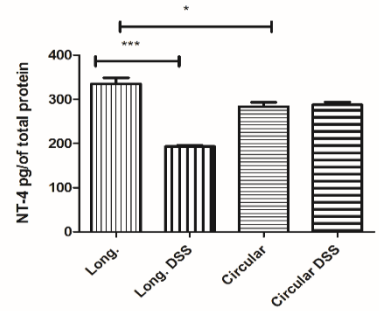

Figure 4 NT-4 expression in colonic smooth muscle cells in control and DSS-induced groups: NT-4 level is expressed as pg/ of total protein. The figure shows basal expression of NT-4 in the longitudinal and circular muscle tissues. NT-4 level is significantly more in the longitudinal muscle tissue compared to the circular. There is a significant reduction in the expression of NT-4 in longitudinal smooth muscle cells in DSS-induced group compared to expression in longitudinal smooth muscle cells of control group. However, no significant difference is shown between control & DSS-induced groups regarding circular smooth muscle cells of the colon. *p< .05, ** p<0.0001

Little is known about the role of NT-4 in the gut. NT-4 knock-out mice have a selective vagal afferent loss [41] which indicates the importance of NT-4 in the development survival of the neurons. Therefore the reduction of NT-4 is smooth muscle of DSS-induced colitis in this study might explain the loss of innervation reported in gut inflammation. Moreover, outside the GIT, NT-4 plays a role if inflammation and the interaction between the cells and the immunosystem [42, 43]. Further investigations are needed to explore the function of NT-4 in the physiology and pathophysiology of the GIT, especially in smooth muscle cell.

4.0 CONCLUSION

Theses result indicate that smooth muscle of rat colon contains neurotrophins at different levels and that DSS-induced colitis alters their expression pattern. The role of neurotrophins produced from smooth muscle at normal and pathological states needs further investigations.

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