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Basic Study

Gut region-specific TNFR expression: TNFR2 is more affected than TNFR1 in duodenal myenteric ganglia of diabetic rats

Segment-specific TNFR expression in myenteric ganglia

Abstract

BACKGROUND

Cytokines are essential in autoimmune inflammatory processes that accompany type 1 diabetes. Tumor necrosis factor alpha plays a key role among others in modulating enteric neuroinflammation, however, it has a dual role in cell degeneration or survival depending on different tumor necrosis factor receptors (TNFRs). In general, TNFR1 is believed to trigger apoptosis, while TNFR2 promotes cell regeneration. The importance of the neuronal microenvironment has been recently highlighted in gut region-specific diabetic enteric neuropathy, however, the expression and alterations of different TNFRs along the gastrointestinal tract has not been studied.

AIM

To investigate the TNFR1 and TNFR2 expression in myenteric ganglia and their environment in different intestinal segments of diabetic rats.

METHODS

Ten weeks after the onset of hyperglycaemia, gut segments were taken from the duodenum, ileum and colon of streptozotocin-induced (60 mg/body weight kg i.p.) diabetic (n = 17), insulin-treated diabetic (n = 15) and sex- and age-matched control (n =

15) rats. Myenteric plexus whole-mount preparations were prepared from different gut regions for TNFR1/HuCD or TNFR2/HuCD double-labelling fluorescent immunohistochemistry. TNFR1 and TNFR2 expression was evaluated by postembedding immunogold electron microscopy on ultrathin sections of myenteric ganglia. TNFRs levels were measured by enzyme-linked immunosorbent assay in muscle/myenteric plexus-containing (MUSCLE-MP) tissue homogenates from different gut segments and conditions.

RESULTS

A distinct region-dependent TNFRs expression was detected in controls. The density of TNFR1-labelling gold particles was lowest, while TNFR2 density was highest in duodenal ganglia and a decreasing TNFRs expression from proximal to distal segments was observed in MUSCLE-MP homogenates. In diabetics, the TNFR2 density was only significantly altered in the duodenum with decrease in the ganglia (0.32 ± 0.02 *vs* 0.45 ± 0.04, $P < 0.05$), while no significant changes in TNFR1 density was observed. In diabetic MUSCLE-MP homogenates, both TNFRs levels robustly decreased in the duodenum (TNFR1: 4.06 ± 0.65 *vs* 20.32 ± 3.1, $P < 0.001$; TNFR2: 11.72 ± 0.39 *vs* 15.91 ± 1.04, $P < 0.01$), which markedly influenced the TNFR2/TNFR1 proportion both in the ganglia and in their muscular environment. Insulin treatment had controversial effects on TNFR expression.

CONCLUSION

These findings show diabetes-related region-dependent changes in TNFR expression and suggest that TNFR2 is more affected than TNFR1 in myenteric ganglia in the duodenum of type 1 diabetic rats.

Key Words: Tumor necrosis factor receptors; Myenteric ganglia; Enteric neurons; Neuronal environment; Diabetes; Insulin

Barta BP, Onhausz B, Doghmi AA, Szalai Z, Balázs J, Bagyánszki M, Bódi N. Gut region-specific TNFR expression: TNFR2 is more affected than TNFR1 in duodenal myenteric ganglia of diabetic rats. *World J Diabetes* 2022; In press

Core Tip: Our findings demonstrate an intestinal segment-specific expression of TNFRs in myenteric ganglia and their muscular environment in type 1 diabetic rats. TNFR2 density was significantly altered only in the duodenum of diabetics with decrease in the ganglia, while no significant changes in TNFR1 was observed. In diabetic muscle/myenteric plexus homogenates, the levels of both TNFRs decreased robustly in the duodenum, which markedly influenced the TNFR2/TNFR1 proportion both in the ganglia and muscular environment. These results highlight that TNFR2 is more affected than TNFR1 in myenteric ganglia in the duodenum of diabetic rats. Insulin treatment had controversial effects on TNFR expression.

INTRODUCTION

In the last decade, the individual milieu of the different intestinal regions has been increasingly highlighted in health and disease[1, 2]. Numerous studies emphasize the key role of gut microbial dysbiosis[3, 4], involving regionally distinct alterations in the intestinal microbiota regarding the luminal content and also the mucosa-associated microbiota in type 1 diabetic rats[5, 6]. Diabetes-related gut region-dependent accumulation of reactive oxygen species and segment-specific activation of endogenous antioxidant defense system[7, 8] have clearly outlined the imbalance between antioxidative and oxidative mechanisms[9]. Strong shift in balance between anti-inflammatory and pro-inflammatory processes[10-12] has also been characterized in type 1 diabetes. All these region-dependent molecular dysfunctions contribute to the regional diabetic damage affecting the enteric nervous system and have functional consequences, like disturbed gut motility[13-15].

Type 1 diabetes is accompanied by autoimmune inflammatory processes, therefore different cytokines, as inflammatory mediators, also play an essential role in

enteric neuroinflammation[16]. Naturally, as cytokines have global effects on intestinal homeostasis in healthy and pathophysiological conditions[17-19], it may be of great interest to study the role and alterations of different cytokines from segment to segment along the gastrointestinal tract.

The variety of ligands and receptors that belong to tumor necrosis factor (TNF) superfamily is characterized by a conserved TNF homology domain at the C-terminal[20]. TNFα is mainly expressed by immune cells and macrophages[21], however, it is also produced by central and enteric neurons[11, 22, 23]. TNFα can bind to two functionally distinct transmembrane receptors, TNF receptor 1 and 2 (TNFR1 and TNFR2). TNFR1 is ubiquitously expressed on almost all cells. In contrast, TNFR2 expression is more restricted to some T cell subpopulations, endothelial cells, certain types of neurons and glial cells[24]. TNFRs have only 28% homology in their extracellular ligand-binding domains, and no homology between the cytoplasmatic domains[25], suggesting their capability to elicit distinct intracellular response.

Despite that TNFα is primarily thought to be a powerful pro-inflammatory cytokine[18], it has a dual role in cell survival/cell death depending on which TNFR it binds to. TNFR1 contains an intracellular death domain through which it triggers cell degeneration and apoptosis, while TNFR2 contributes to cell regeneration and survival[24, 26]. Therefore, selective TNFR1 inhibitors obstruct the pro-inflammatory TNFR1 signaling without losing the regulatory effect of TNFR2 in the treatment of autoimmune diseases[27]. Blocking the TNFR1-mediated TNF signaling pathway significantly protects rats from obesity and obesity-induced insulin resistance[28]. TNFR2 facilitates the differentiation of induced regulatory T cells, while TNFR1 mediates the inflammatory T cell differentiation in mice[29]. TNFR2 promotes oligodendrocyte regeneration and neuronal survival in an ischemic model[30] and provides protection against oxidative stress[31].

The primary aim of this study was to evaluate the effects of chronic hyperglycemia and immediate insulin treatment on TNFR1 and TNFR2 distribution in myenteric neurons along the duodenum-colon axis. Furthermore, we set out to

investigate whether the TNFR expression is affected in muscular environment of enteric neurons in different gut segments.

MATERIALS AND METHODS

Animal model

Adult male Wistar rats (Toxi-Coop Zrt., Hungary) weighing 200–300 g, kept on standard laboratory chow (Innovo Kft., Hungary) and with free access to drinking water, were used throughout the experiments. The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 °C, 12 h/12 h light/dark, 50% humidity) for 2 wk prior to experimentation. The rats were divided randomly into three groups: streptozotocin (STZ)-induced diabetics (diabetics; n = 17), insulin-treated STZ-induced diabetics (insulin-treated diabetics; n = 15) and sex- and age-matched controls (n = 15). Hyperglycaemia was induced as described previously[7, 11, 13]. The animals were considered diabetic if the non-fasting blood glucose concentration was higher than 18 mmol/L. From this time on, the insulin-treated group of hyperglycaemic rats received a subcutaneous injection of insulin (Humulin M3, Eli Lilly Nederland, Netherlands) each morning (2 IU) and afternoon (3 IU). Equivalent volumes of saline were given subcutaneously to the diabetic and the control rats. The blood glucose level and weight of each animal were measured weekly. Those diabetic animals which recovered spontaneously, or their blood glucose level decreased below 18 mmol/L during the 10-week experimental period were excluded from the study. In all procedures involving experimental animals, the principles of the National Institutes of Health (Bethesda, MD, USA) guidelines and the EU directive 2010/63/EU for the protection of animals used for scientific purposes were strictly followed, and all the experiments were approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), with the license number XX./1636/2019.

Tissue handling

Ten weeks after the onset of hyperglycaemia, the animals were killed by cervical dislocation under chloral hydrate anaesthesia (375 mg/kg i. p.). The gut segments of diabetic, insulin-treated diabetic and control rats were dissected and rinsed in 0.05 M phosphate buffer (PB; pH 7.4). Samples were taken from the duodenum (1 cm distal to the pylorus), the ileum (1 cm proximal to the ileo-caecal junction) and the proximal colon, and processed for fluorescent immunohistochemistry (n = 5 animals per group), quantitative electron microscopy (n = 5 animals per group) and enzyme-linked immunosorbent assay (ELISA, n = 10 animals - control group, n = 12 animals - insulin-treated diabetic group, n = 10 animals - diabetic group). For double-labelling fluorescent immunohistochemistry, the intestinal samples from different gut segments were cut along the mesentery, fixed in 4% paraformaldehyde and whole-mount preparations were made. For post-embedding electron microscopy, small pieces (2–3 mm) of the gut segments were fixed in 2% para-formaldehyde and 2% glutaraldehyde solution and then further fixed for 1 h in 1% OsO_4. After rinsing in buffer and dehydrating in increasing ethanol concentrations and acetone, they were embedded in Embed812 (Electron Microscopy Sciences, USA). For the ELISA, the 3-cm-long gut segments were cut along the mesentery and pinched flat. After removing the layer of mucosa and submucosa, the residual tissue containing the intestinal smooth muscle layers and the myenteric plexus in between were snap-frozen in liquid nitrogen and stored at -80 °C until use.

Fluorescent immunohistochemistry

For double-labelling immunohistochemistry, whole-mount preparations derived from different gut segments were immunostained with HuCD and TNFR1 or TNFR2. HuCD as a pan-neuronal marker is suitable for labelling of neuronal cell body without the projections. Briefly, after blocking in tris(hydroxymethyl)aminomethane-buffered saline (TBS) containing 1% bovine serum albumin and 10% normal goat serum, the samples were incubated overnight with pan-neuronal anti-HuCD (mouse monoclonal IgG; A-21271, Invitrogen, USA; final dilution 1:50) and anti-TNFR1 (rabbit polyclonal, SAB4502988, Sigma-Aldrich, Hungary; final dilution 1:100) or anti-TNFR2 (rabbit poly-

clonal, SAB4502989, Sigma-Aldrich, Hungary; final dilution 1:200) primary antibodies at 4 °C. After washing in TBS with 0.025% Triton X-100, whole-mounts were incubated with anti-mouse Cy™3 (Jackson ImmunoResearch Laboratories, USA; final dilution 1:200) and anti-rabbit Alexa Fluor 488 (Life Technologies Corporation, Molecular Probes, USA; final dilution 1:200) secondary antibodies for 1 h at room temperature. Negative controls were performed by omitting the primary antibody when no immunoreactivity was observed. Whole-mount preparations were mounted on slides in Fluoromount™ Aqueous Mounting Medium (Sigma-Aldrich, Hungary), observed and photographed with a Zeiss Imager Z.2 fluorescent microscope equipped with an Axiocam 506 mono camera.

Quantitative post-embedding immunohistochemistry

Five Embed blocks originating from each intestinal segment and condition were used to prepare ultrathin (70 nm) sections, which were mounted on nickel grids and processed for TNFR immunogold labelling. Ultrathin sections (three grids per block) were incubated overnight in anti-TNFR1 (rabbit polyclonal, SAB4502988, Sigma-Aldrich, Hungary; final dilution 1:100) or anti-TNFR2 (rabbit polyclonal, SAB4502989, Sigma-Aldrich, Hungary; final dilution 1:200) primary antibodies, followed by colloidal gold conjugated anti-rabbit IgG (conjugated to 18 nm gold particles; Jackson ImmunoResearch, USA; final dilution 1:20) secondary antibody for 3 h. The specificity of the immunoreaction was assessed in all cases by omitting the primary antibodies in the labelling protocol and incubating the sections only in the gold conjugated secondary antibody. Sections were counterstained with uranyl acetate (Merck, Germany) and lead citrate (Merck, Germany), and were examined and photographed with a JEOL JEM 1400 transmission electron microscope. The quantitative features and the subcellular distributions of the gold particles labelling TNFR1 or TNFR2 were determined in the myenteric ganglia. Fifty digital photographs of ten myenteric ganglia per intestinal segment per condition were made at a magnification of 20,000x with the AnaySIS 3.2 program (Soft Imaging System GmbH, Germany). The intensity of the labelling was expressed as the total number of gold particles per unit area (μm^2).

Measurement of tissue TNFR concentrations

The intestinal tissue samples, including the intestinal smooth muscle layers with the myenteric plexus in between (MUSCLE-MP), were frozen in liquid nitrogen, crushed into powder in a mortar and homogenized in 500 µl homogenizing buffer (100 µl Protease Inhibitor Cocktail (Sigma-Aldrich, Hungary) in 20 mL 0.05 M PB). Tissue homogenates were centrifuged at 5000 rpm for 20 min at 4 °C. The TNFR1 and TNFR2 Levels of the MUSCLE-MP tissue samples were determined by means of quantitative ELISA according to the manufacturer's instructions (TNFR1: GA-E3995RT, TNFR2: GA-E3997RT, GenAsia Bio-tech Co., China). Optical density was measured at 450 nm (Benchmark Microplate Reader; Bio-Rad, Hungary). Tissue TNFR concentrations were expressed as ng/mg protein.

Bradford protein micromethod for the determination of tissue protein content

A commercial protein assay kit was used for the determination of protein content in tissue samples. Bradford reagent was added to each sample. After mixing and following 10 min incubation, the samples were assayed spectrophotometrically at 595 nm. Protein level was expressed as mg protein/mL.

Statistical analysis

Statistical analysis was performed with one-way analysis of variance (ANOVA) and Newman-Keuls test, Kruskal-Wallis test and Dunn's multiple comparisons test. All analyses were carried out with GraphPad Prism 6.0 (GraphPad Software, USA). A probability of $P<0.05$ was set as the level of significance. All data were expressed as means±SEM.

4 RESULTS

Disease characteristics of type 1 diabetic rats

The general characteristics of the diabetic, insulin-treated diabetic and control rats were monitored during the 10-week experiment and are summarized in Table 1. Diabetic rats were characterized by a long-lasting chronic hyperglycaemia, their blood glucose concentration was 5 times higher than that of the control group (25.64±0.73 mmol/L *vs*

5.89±0.13 mmol/L). Immediate insulin treatment inhibited extremely high glucose levels, however, the values were still higher than in the control group (10.29±0.76 mmol/L). All the animals gained weight during the experimental period, but the final body weight of diabetic rats was significantly lower as compared to insulin-treated diabetic and control animals.

Presence of TNFR1 and TNFR2 immunoreactivity in myenteric ganglia

Double-labelling fluorescent immunohistochemistry revealed TNFR immunoreactiv-ity in myenteric ganglia. Both the TNFR1- and TNFR2-immunoreactive myenteric neurons were clearly visible on whole-mount preparations (Figure 1).

Subcellular localization and quantification of TNFRs expression in myenteric ganglia

Quantitative evaluation of TNFRs expression was carried out by post-embedding immunogold electron microscopy on ultrathin sections of myenteric ganglia (Figure 2). The 18 nm gold particles labelling TNFR1 or TNFR2 were often located at the plasma membranes or intracellular membranes in all the investigated gut segments and experimental conditions (Figure 3 and 4).

In healthy controls, the baseline density of TNFR1-labelling gold particles was higher than that of TNFR2 in all investigated gut segments. In contrast, TNFRs distribution differed in intestinal regions: TNFR1-labelling gold particle density was lowest in the duodenum (0.88±0.08) and highest in the ileum (1.26±0.09), while TNFR2 density was highest in the duodenum (0.45±0.03) and exhibited a significant decreasing tendency along the duodenum-colon axis (Figure 5).

TNFR1 density did not change significantly neither in the diabetic nor in the insulin-treated animals in any investigated gut segments (Figure 5A). However, TNFR2 density decreased significantly only in myenteric ganglia of the diabetic duodenum ($p<0.05$; Figure 5B). Insulin treatment did not prevent this decrease and resulted in a significantly lower TNFR2 density in the ileal ganglia relative to controls (Figure 5B).

Consequently, in the duodenum of diabetics, the proportion of TNFR2/TNFR1 was decreased by more than 30% (Figure 6). This ratio remained unchanged in the ileum and decreased also in the colonic ganglia of diabetics. In insulin-treated rats, the TNFR2/TNFR1 ratio was close to the control level in the duodenum, and decreased in ileal and colonic ganglia (Figure 6).

TNFR1 and TNFR2 Levels in tissue homogenates

In controls, the level of both TNFRs in MUSCLE-MP homogenates, which included the circular and longitudinal smooth muscle layers of the gut wall as well as the myenter-ic plexus, displayed a definite decrease from duodenum to colon. While the TNFR1 Level was 20.32±3.1 ng/mg in the duodenum, the ileum contained only a tenth of this value (1.85±0.04; $p<0.05$) and the colon even less (0.85±0.11; $p<0.0001$; Figure 7A). The TNFR2 tissue level was also highest in the duodenal homogenates of controls (15.91±1.04) and significantly lower in the ileum (12.76±0.86; $p<0.05$) and colon (9.38±0.21; $p<0.0001$; Figure 7B).

In diabetic homogenates, both TNFR levels decreased markedly in the duodenum relative to control samples (Figure 7). The TNFR2 Level was decreased by 25% in these samples (11.72±0.39 *vs* 15.91±1.04; $p<0.01$), but the greatest decrease was observed in the case of TNFR1, which was only a fifth of the control value (4.06±0.65 *vs* 20.32±3.1; $p<0.001$). Therefore, the proportion of TNFR2/TNFR1 was quadrupled in duodenal tissue homogenates of diabetics as compared to controls (Figure 8). However, there were no changes in either the TNFR1 and TNFR2 tissue level or in their proportion in other diabetic gut segments (Figure 7 and 8). Insulin treatment only prevented the robust TNFR1 de-crease in the duodenum, but not the TNFR2 change. As well as the insulin significantly altered the TNFR levels in the other intestinal segments (Figure 7).

DISCUSSION

In this study, an intestinal segment-specific TNFR distribution has been demonstrated both in healthy control and type 1 diabetic rats. Enteric neuronal TNFR1 and TNFR2

expression was also shown by others[32, 33]. The presence of TNFRs on myenteric neurons is supportive of their modulatory impact on neuronal activity, which is triggered by cytokines[33]. Moreover, the regionality of their base level suggests distinct significance in cell fate regulation in different gut segments. Though TNFR1 displayed higher expression than TNFR2 in myenteric ganglia in all gut segments, this does not necessarily prioritise the TNFα-TNFR1 signalling pathway, because the receptors' affinity to different forms of TNFα is not equal. Recent studies have shown that the transmembrane form of TNFα has higher affinity for TNFR2, while the released soluble form has higher affinity for TNFR1[34, 35]. Considering the regional differences along the duodenum-colon axis, the TNFR2 expression was highest in duodenal ganglia, while the TNFR1 density was the lowest there, which suggests that TNFR2 may have stronger influence on duodenal myenteric neurons than TNFR1. Furthermore, control duodenal MUSCLE-MP homogenates displayed the highest TNFR levels, which greatly differed from that of other segments, suggesting a privileged role for TNFRs also in the duodenal microenvironment around myenteric ganglia.

In the colon and ileum of diabetics, neither the TNFR1 nor the TNFR2 density was found to be significantly different in myenteric ganglia. Furthermore, tissue levels of different TNFRs and their proportion also remained unchanged in the MUSCLE-MP homogenates from these segments. Since the myenteric and mucosal/submucosal TNFα expression has been shown to decrease in the distal gut regions of diabetic rats [11] presumably as a result of heme oxygenase induction[7, 36], we suppose that the crucial mechanism involved in diabetic loss of myenteric neurons [13] and environmental damage[5, 6, 8, 37, 38] in the colon and ileum is not the result of TNFα-TNFRs signalling. Therefore, further investigation is necessary regarding the impact of other cytokines in diabetic enteric neuropathy. Immediate insulin treatment was not preventive, but rather it had confusing effect on TNFR expression. Studies have shown that increased TNFα is fundamentally involved in the disturbed insulin signalling and contributes to the development of insulin resistance[39, 40]. Direct exposure to TNFα induces insulin resistance in myocytes and brown adipocytes due to impairment of the insulin receptor

substrate proteins[41]. High TNFR1 and TNFα levels are also related to insulin resistance and impaired β-cell function in pregnant women[42]. Diabetes-induced insulin insensitivity is reversed by aryl-hidrocarbon receptor ligands, such as tryptophan, which also decreased the TNFα expression in Kupffer cells and plasma and lead to reversed intestinal barrier function in insulin-dependent diabetic mice[43].

In myenteric ganglia of the diabetic duodenum, a decrease in the number of TNFR2-labelling gold particles was revealed without any significant alterations in TNFR1 density. This suggests that from the two TNFRs, the TNFR2 and therefore the TNFα-TNFR2 signalling is more affected by the diabetic state in the duodenum. Recently, regionally distinct TNFα production has been demonstrated in myenteric ganglia of different gut segments and intestinal layer-dependent differences in TNFα levels have been revealed in type 1 diabetic rats[11]. A significantly increased TNFα expression has been observed only in the duodenal ganglia of diabetics[11]. TNFR2-deficient mice have decreased sensitivity to TNFα or greater resistance to TNFα-induced cell death[44], therefore decreased TNFR2 Levels may protect neurons. This is in agreement with our earlier findings that the duodenum was the only gut segment where diabetic loss of myenteric neurons has not been demonstrated[7, 13], despite of the increased TNFα expression in the duodenal ganglia of type 1 diabetic rats[11]. TNFR2 downregulation increased Schwann cell apoptosis in culture, but also triggered the apoptosis-induced proliferation of Schwann cells[45]. In human and murine macrophages, the TNF-induced TRAF2 degradation was blocked in the absence of TNFR2[46]. Renal TNFR2 deficit protects from glomerulonephritis by reducing macrophage infiltration[47]. Furthermore, TNFR2 deficiency reduces the production of immunosuppressive factors, like nitric-oxide and interleukin 6[48]. TNFα/TNFR2 signalling is a crucial checkpoint in immunomodulation[49], it influences the T cell responses in auto-immunity[29, 50-52]. TNFR2 signalling has an important role in protecting neurons against glutamate-induced excitotoxicity through the nuclear factor-kappa B (NF-kappa B)-dependent pathway[23, 53]. It has been shown that TNFα can be neurotrophic for enteric neurons since it promotes the glial-derived neurotrophic factor (GDNF) expression of intestinal

smooth muscle cells and facilitates the neurite outgrowth also *via* NF-kappa B-related pathway[33]. Therefore, evaluating the possible involvement of NF-kappa B in region-dependent diabetic enteric neuropathy may be an interesting future prospective. Both TNFR1 and TNFR2 tissue levels decreased in diabetic duodenal MUSCLE-MP homogenates. As the intestinal smooth muscle represents in a larger amount in these tissue samples, therefore differences in the results of electron microscopic and ELISA methods can reflect different alterations of TNFRs in the myenteric neurons than the muscular environment. It should also be noted, that TNFR1 and TNFR2 tissue levels decreased to a very different extent in diabetic duodenal MUSCLE-MP samples. This results in the complete reversal of the TNFRs proportion in the neuronal muscular environment compared to myenteric ganglia. Shift in the TNFR2/TNFR1 balance may be essential in TNF signalling and considering that TNFR2 in different cell types may be endowed with opposing functions[54], the importance of environmental changes on neuronal loss or survival is further strengthened.

CONCLUSION

In conclusion, the present study revealed an intestinal segment-specific pattern of TNFRs expression in myenteric ganglia and their muscular environment in type 1 diabetes. Our results support that TNFR2 is more affected than TNFR1 in myenteric ganglia in the duodenum of diabetic rats. The diabetes-related regional changes in TNFRs expression may contribute to region-dependent myenteric neuropathy, however, further experiments are required to conclude its functional consequences.

ARTICLE HIGHLIGHTS

Research background

Tumor necrosis factor alpha plays an essential role in inflammatory modulation of enteric neurons, however, it can promote apoptosis or cell survival depending on its different receptors (TNFR1 and TNFR2).

Research motivation

Distinct neuronal microenvironment is critical in gut segment-specific diabetic enteric neuropathy, therefore, the region-dependent expression and alterations of different TNFRs may also be important in therapy regarding motility disturbances in type 1 diabetes.

Research objectives

To study the TNFR1 and TNFR2 expression in myenteric ganglia and their environment in different intestinal regions of diabetic rats.

Research methods

Double-labelling fluorescent immunohistochemistry, post-embedding immunogold electron microscopy and enzyme-linked immunosorbent assay were applied to evaluate the TNFR1 and TNFR2 expression in myenteric ganglia and muscle/myenteric plexus tissue homogenates from different gut segments of streptozotocin-induced diabetic, insulin-treated diabetic and control rats.

Research results

TNFRs expression displayed a strictly region-specific pattern even in controls animals. However, among all the investigated gut segments, only the duodenum represented significant alterations of TNFR expression in diabetic rats. Here, the TNFR2 density was decreased in the myenteric ganglia, while no significant changes in TNFR1 density was revealed. Moreover, the TNFR2/TNFR1 proportion was markedly influenced both in the ganglia and in their muscular environment of diabetics. Insulin had controversial effects on TNFR expression.

Research conclusions

Present findings show diabetes-related region-specificity in TNFRs expression pointing out that TNFR2 is more affected than TNFR1 in duodenal myenteric ganglia of type 1 diabetic rats.

Research perspectives

Nuclear factor –kappa B means a key point in TNFR2 signalling pathway. Therefore, evaluation of the involvement of nuclear factor-kappa B in region-dependent diabetic enteric neuropathy may be important in the future.

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