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Title: THE RELEVANCE OF NERVE GROWTH FACTOR ON THE BLADDER OF A RAT WITH DIABETES MELLITUS INDUCED BY STREPTOZOTOCIN

Aims of Study

It is well known that the nerve growth factor(NGF) is produced within the bladder. However, the effects of NGF for bladder function remain unclear. With a hypothesis that NGF has some roles for diabetic neurogenic bladder, we investigated the relation between urine volume, frequency and tissue NGF concentration in a model of a streptozotocin induced diabetic rat.

Methods

(1) Streptozotocin treatment

A total of 60 female Wistar rats weighing between 150-250 g were divided into four groups. Group 1 includes the rats one week after the induction of diabetes (one week diabetic) and group 2 the rats four weeks after the induction (four week diabetic) and two groups of age-matched control. The animals were individually housed with access to food and water ad libitum. Following the fasting of rats for 24hours, diabetes was induced with a single injection of streptozotocin into a tail vein (STZ in 0.1 mM citrate-buffer saline, 60 mg/kg). Controls matched for age received the same volume of vehicle alone. Only rats with a blood glucose concentration of 250 mg/dl or higher were used in the diabetes group in the following experiments.

(2) Preparation of ELISA Sample

Rats were sacrificed and the bladder body (from the dome to the entrance of the ureters) was immediately removed out, weighed, and stored at -70 until processing. Individual bladders were solubilized in 100 times of lysis buffer 20 mM Tris, 137 mM NaCl, 10% glycerol, 1 mM PMSF, 10 mg/ml aprotinin, 1 mg/ml leupeptin, 0.5 mM sodium vanadate . Bladder tissue was disrupted with a homogenizer for 3 min and then centrifuged (10,000g 15min). The supernatants were used for NGF quantification.

(3) Principle of the NGF E_{MAX} Immunoassay System (Promega)

Polyclonal antibodies against NGF were absorbed to microtiter plates. After addition of the NGF sample or standard solution, the secondary antibody was applied. Three to four wells were used for each sample and the standard solution. This antibody complex was detected with a horseradish peroxidase-labeled immunoglobulin. Enzyme activity was quantified by the change in optical density, using tetramethyl benzidine as substrate. The NGF standard provided with this system generated a linear standard curve from 7.8 to 500 pg/ml. Absorbance values of standards and samples were corrected by subtraction of the background value. Samples were diluted to bring the absorbance valers onto the linear portion of the standard curve. No samples fell below the minimum detection limits of the assay. Curve fitting of standards and evaluation of NGF content of sample was performed using a least squares fit.

(4) Frequency, tidal and total volume of urine were recorded.

Results

No significant difference in tidal volume of urine was observed between diabetic groups (one week 0.52±0.13 ml,

four weeks 0.47±0.2 ml) and control groups (one week 0.42±0.2 ml, four weeks 0.48±0.3ml). On the contrary, urine frequency was significantly different being frequent in diabetic groups (one week 30±5 times/24h, four weeks 24±4 times/24h) than in control groups (one week 14±4 times/24h, four weeks 16±4 times/24h).

Bladder NGF level was significantly increased in diabetic rats after one week of STZ induction. When compared with the controls (6.5±1.2 ng/w.w), NGF level in diabetic rats rose with a peak at one week (24±2.4 ng/w.w) , then began to fall. Four weeks after induction, the level fell to 16.5±3.1 ng/w.w but still remained greater than control.

Conclusions

These results suggest the relation between the tissue concentration of NGF and urine frequency in a model of diabetic rat.