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DETERMINATION OF NERVE GROWTH FACTOR LEVELS INTO HUMAN BLADDER WALL BY MODIFIED ENZYME-LINKED IMMUNOABSORBENT ASSAY

Hypothesis / aims of study

Recent experimental observations showed that hypertrophic obstructed bladders produce high levels of Nerve Growth Factor (NGF), which plays a pivotal role in visceral afferent plasticity. In animal models NGF measurements have been performed in different tissues as bladder, dorsal root ganglia and spinal cord, and the crucial factor is the applied methodology of extraction to obtain consistent and reliable determinations of the neurotrophic factor. Thus, in detecting abnormal NGF tissue levels, it can be hypotized the presence of a cross reactivity with some proteins (i.e. IgG) present in the tissue.
The aim of the present investigation was to compare NGF bladder tissue levels obtained with standard enzyme-linked immunosorbent assay (ELISA) with levels obtained by a modified measurement methodology consisting of a immunoprecipitation reaction followed by ELISA.

Study design, materials and methods

Preliminary determination of NGF by ELISA (Promega, Madison-WI) in bladder samples (30 specimen) was performed according to the procedure provided by the producer: bladder specimens (1-2 mg) were homogenized by using a frosted glass potter in 800 L of lyses buffer: NaCl 137 mM, Tris-HCl 20 mM, pH 8,0, NP40 1%, glycerol 10%, PMSF 1 mM, aprotinin 10 g/ml, leupeptin 1 g/mL, sodium vanadate 0.5 mM (Promega, Madison-WI). The homogenate was centrifuged at 2000 rpm x 15 min at 4°C. NGF levels were measured in the soluble fraction after the determination of the total protein by Bradford assay. 22 L of each sample was diluted 1:2 with lyses buffer and diluted 1:5 with Dulbecco's PBS (DPBS) buffer. Samples were acidified to approximately pH 2,6 for 20 minutes and then neutralized to approximately pH 7.6.
Although the decrease of protein concentration of material were assayed for NGF content, the procedure revealed abnormally high absorbance at 450 nm. Thus, the standard procedure was modified by immunoprecipitating this immunoglobulin with Reumatoid Factor (IgM) before ELISA. The modified NGF assay was as follows: before ELISA assay, samples were treated with the addition of Reumatoid Factor (IgM, 1:10 by v/vol) for 10 min at 4°C and then centrifuged at 2000 rpm x 10 min. NGF levels were measured in 100 L of supernatant by ELISA assay (Promega, Madison-WI). The sensitivity was 15.6 pg/ml of NGF.

Results

After standard ELISA, mean value of NGF bladder tissue levels was 10198.815 pg/ml, and mean absorbance was 3,35 nm; after ELISA with previous immunoprecipitation of immunoglobulins with Reumatoid Factor, mean value of NGF was 2592 pg/ml and mean absorbance was 0,4 nm.

Interpretation of results

These results confirm the presence of a cross reactivity with IgG included in the examined tissue. As presence of IgG into the bladder wall and in other tissues is a common condition, it is necessary to eliminate this reactivity to obtain uncontamined measurements. After immunoprecipitating IgG, NGF concentration in bladder tissue shows a reduction of absorbance in identical samples higher than 70%, so to return within the ranges of a NGF standard curve.

Concluding message

The assessment of NGF bladder tissue levels, as performed by a common enzyme-linked immunosorbent assay, should take into account the addition of immunoprecipitation reactions to obtain consistent and reliable results.