223

Nomura M[1], Nishii H[1], Fujimoto N[1], Matsumoto T[1]
1. University of Occupational and Environmental Health

NEW METHODS FOR EVALUATION OF AFFERENT ACTIVITY FROM LOWER URINARY TRACT IN MICE

Hypothesis / aims of study

Accumulating evidence suggest that afferent activity from lower urinary tract (LUT) contributes to overactive bladder symptoms. Since decreasing afferent activity seems an attractive therapeutic approach, there is a need to develop methods for evaluating afferent activity. The aim of the present study is to develop new markers for the evaluation of the afferent activity. Since the afferent input from LUT is conveyed to the thalamus through the spinothalamic tract, in the present study, we examined the levels of *c-fos* mRNA, a neural activational marker, in the thalamus using a cyclophospamide (CP)-induced cystitis model. Since increased afferent activity from LUT causes stress response and behavioural alteration in an experimental animal model, we also examined the levels of corticotrophin-releasing hormone (CRH) mRNA, a major stress hormone, and homecage activity in a CP-induced cystitis model.

Study design, materials and methods

C57BL/6J male mice were used. In the first experiment, the mice were intraperitoneally injected with CP (200 mg/kg) or saline. In the second experiment, the mice were injected 3 different doses (50, 100, or 200 mg/kg) of CP and saline. In the third experiment, the mice were pretreated with capsaicin (125 mg/kg) injected subcutaneously 4 days before CP administration. The animals were decapicated and the brains were rapidly removed. Frozen sections of the brain were cut on a cryostat. An *in situ* hybridization histochemistry was performed to detect the *c-fos* mRNA in the thalamus and CRH mRNA in the hypothalamic paraventricular nucleus (PVN), an integrative site of stress responses. The homecage activity was examined by the Activity Sensor Unit for mouse system.

Results

In saline-treated group, no significant induction of *c-fos* mRNA in the thalamus were observed. In CP-treated group, the levels of *c-fos* mRNA in the thalamus increased significantly at 0.5 h, peaked at 1 h after CP administration, and decreased thereafter. In the CP-treated group, the CRH mRNA levels in the PVN increased significantly at 6 h, peaked at 12 h, and remained to increase significantly at 48 h after the CP administration in comparison to those in saline-treated group. Following CP administration, the *c-fos* mRNA levels in the thalamus were increased in a dose dependent manner and were suppressed by capsaicin pre-treatment. In CP-treated group, the homecage activity was significantly decreased in a dose-dependent manner.

Interpretation of results

The present findings demonstrated that increased afferent activity caused a significant induction of *c-fos* mRNA in the thalamus in a dose-dependent manner. The desensitization of C-fiber suppressed significantly the induction of *c-fos* caused by CP-cystitis suggesting activation of neurons in the thalamus is mediated at least in part via the activation of capsaicin sensitive C-fiber afferents. Increased afferent activity also caused a significant increase of CRH mRNA, a major stress-regulatory hormone, in the hypothalamic PVN and a significant decrease of homecage activity. These findings suggest that increased afferent activity from LUT cause stress responses and behaviour modification.

Concluding message

The levels of *c-fos* mRNA in the thalamus may be a direct marker for evaluation of afferent activity from LUT. The levels of CRH mRNA and the homecage activity can be a marker for evaluation of the impact of afferent activation from LUT.

FUNDING: Grants-in-Aid for Scientific Research (No.16790145)
DISCLOSURES: NONE
ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by The ethics committee of animal care and experimention