Neuropeptide Y on allergic encephalomyelitis guinea pig morbidity and Th1/Th2 cell research on balanced effects

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ABSTRACT

Objective: To investigate neuropeptide Y (NPY) on EAE guinea pig Th 1 / Th 2 cell balanced influence, and explore NPY on allergic encephalomyelitis (EAE) morbid role in the immune regulatory mechanisms. Methods: 30 guinea pigs were randomly divided into normal control group, EAE control group, NPY intervention group. Observed three groups guinea pig serum interleukin 4 (IL-4), C interferon (IF N-C) levels, Th1/Th2 ratio, and the morbid condition of EAE control group and NPY intervention group. Results: peak serum IL-4 level of NPY intervention group were significantly increased, serum IFN-C level decreased, Th1/Th2 ratio decreased (P average<0.001). Conclusion: the lateral ventricle was injected into NPY can have a protective effect on EAE, protective mechanism may regulate Th1 / Th2 cell balance and promote IL-4 secretion, inhibit the production of IFN-C and play.

KEYWORDS
Encephalomyelitis; Experimental autoimmunity/pathology; Neuropeptide Y; Th1 cells; Th2 cells; Animal; Experiments; Muridae.

INTRODUCTION

Allergic encephalomyelitis (EAE) is recognized worldwide as the study of human multiple sclerosis (Multiple sclerosis, MS) animal model, is the immunological pathogenesis EAE are disproportion or dysfunction of T-cell subsets, Neuropeptide Y (Neuropeptide Y, NPY) is peptides that have a bioactivity and can participate in neural information transmission, it is for immunity to have obvious regulatory effect, can affect differentiation of Th cell. Current research of relationship between NPY and EAE, oversea study preliminarily showed that NPY can inhibit EE morbidity of MS animal models[1], but its specific impact mechanism is unclear, while the domestic research report of NPY and EAE is fewer. This experiment mainly explores NPY on protective effect of EAE morbidity and its immune regulatory mechanisms.

MATERIAL AND METHODS

Experimental material

Thirty female guinea pigs (250~350g); ten rats. These two kinds of animals were provided by the Experimental Animal Division Luzhou Medical College. Disposal of animal in experiments conform to animal ethics requirements. The main experimental reagents: Freund’s complete adjuvant (containing BCG 10mg/
Experimental group

Thirty guinea pigs were randomly divided into three groups: normal control group: lateral ventricle was injected into 10Ll normal saline; after one week the fake module (the same site was injected into same amount of Freund’s complete adjuvant). EAE control group: lateral ventricle was injected into 10Ll normal saline, after a week EA E module (spinal cord homogenate + Freund’s complete adjuvant). »NPY intervention groups: lateral ventricle was injected into 10Ll NPY, after a week the EA E modeling.

The micro NPY was injected into lateral ventricle. After 1% pentobarbital sodium (30mg / kg) was anesthetized abdominal cavity of guinea pigs, guinea pig will be fixed on the stereotactic instrument. Take the injection site sagittal suture junction with herringbone stitch after 1.2mm, right midline 2mm, subdural 4mm, vertical pierce with micro injector, when slowly draw out saw cerebrospinal fluid reflux NPY 10Ll (including NPY 6n mol) was injected, then injected methylene blue to identify the liquid is injected into the lateral ventricle.

The establishment of the EAE model

Rats were executed, the spinal cord was quickly removed, adopting glass homogenizer to homogenize, with 0.9% normal saline and 50% of the homogenate, mixed with the same amount Freund’s complete adjuvant (BCG 10mg/ml), using a syringe to whip oil-water emulsions, that is made of crude myelin basic protein (M BP) antigens. The crude MBP antigen was injected into subcutaneous tissue after paws of guinea pigs, each side of everyone 0.2ml manufactured EAE model.

The detection of Serum level of IFN, IL-4; the morbidity peak period of guinea pigs, each guinea pig through cardiac puncture and (or) through the orbital venous plexus take about 2~3ml blood, serum was separated by centrifugation, serum samples were placed in-20° refrigerator, batch determination adopted double-antibody sandwich ABC-ELISA to detect, operation in strict accordance with instructions.

The determination of Th1/Th2 ratio

Since Th1 cells secreting mainly IFN-C, Th2 cells secreted IL-4, the Th 1 / Th 2 ratio can be directly used IFN-C, and that the ratio of IL-4.

The settlement of statistics

Data is expressed by x-± s, sample average comparison between the two groups were used t-test, samples among multi-group were conducted one-way ANOVA, pairwise comparisons adopting LSD test (when variance was irregular using t’ test), counting data using Fisher’s exact test, the settlement of statistics is completed by the SPS S11.5 software.

RESULTS

The morbidity of NPY intervention group, EAE control group and the normal control group, Ill animal manifested as hair loss, loss of appetite, or poor feeding, weight was significantly decreased, appear different degrees with limb weakness, ataxia, paralysis, about half of the animals with urinary incontinence. The morbidity latency of NPY intervention group guinea is 25.4±12.59 (P<0.05), mortality is 10%; while the morbidity latency of EAE control group is 10.0±4.83 (P<0.05), mortality is 50%.

NPY intervention group, EAE control group and the normal control group, the ratio comparison of serum IL-4, IFN-C andTh1/Th2, see attached list.

Annotation; compared with the control group EAE*P<0.001, compared with the normal control group aP <0.05, bP <0.05, cP<0.001, dP<0.011, eP<0.027, fP<0.001.

<table>
<thead>
<tr>
<th>grouping</th>
<th>IL-4( pg/ml)</th>
<th>IFN-C(ng/ml)</th>
<th>Th1/Th2 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY intervention group</td>
<td>14.57±1.66*a</td>
<td>12.45±3.49*b</td>
<td>0.85*c</td>
</tr>
<tr>
<td>EAE control group</td>
<td>9.04±0.48d</td>
<td>25.51±0.84e</td>
<td>2.82f</td>
</tr>
<tr>
<td>Normal control group</td>
<td>24.01±3.94</td>
<td>1.87±0.22</td>
<td>0.07</td>
</tr>
</tbody>
</table>

DISCUSSION

Experimental EAE is due to its immunity and pathological features and has striking similarities with human
multiple sclerosis acute disseminated encephalomyelitis; it has become recognized as animal model that study human multiple sclerosis. NPY is the most widely distributed of the central nervous system neurotransmitter peptides, which acts widely, can affect Th cells.

In this experiment, the intervention group guinea pigs compared with the EAE control group, the intervention group’s morbid symptoms were light, latency was long, neurological dysfunction were not obvious, mortality is low, statistical analysis conducted, P<0.05, and had statistical difference. It showed that lateral ventricle was injected into NPY can inhibit EAE incidence and shorten latency, reduce its symptoms, it has a significant protective effect for EAE incidence, consistent with overseas research[1]. It is also found in experiment, NPY intervention group guinea pig serum IFN-C levels were decreased compared with EAE control group, serum IL-4 EA E values than the control group was significantly higher, Th1/Th2 ratio decreased (P<0.001). It is showed that NPY inhibits IFN-C secretion Th1-type cytokine, and promote IL-4 secretion of Th2 type cytokine, resulting in the conversion of the Th2, thereby regulating Th1/Th2 balance. Since IF N-C and I -4 respectively were the main representative factor Th1 and Th2, the abnormal expression of two types of cytokine were connected with imbalance of Th1/Th2 cell function[2,3], so IFN-C and the ratio level of serum IL-4 were used in the study to represent Th1/Th2 ratio.

CD4 Th cell is the main effect cell in the pathogenic process of MS, according to the secretion of cytokines differently, which were divided into Th1 and Th2 subsets. Th1 mainly secreted IFN-C, I -2, tumor necrosis factor A (TNF-A), etc., and Th2 secreted IL-4, IL-10 and so on. Previous experiments show that EAE was the pathogenic disease of Th1[4-6], IFN-C that Th1 secreted promote the incidence of MS, and IL-4 that Th2 secreted can inhibit morbidity of MS[7]. Under normal circumstances, the dynamic balance of body Th1 and Th2 is the guarantee of body in normal state[8,9]. Under EAE disease states, Th0 cells differentiate into Th1 direction by specific antigen stimulation, resulting in balance disorders of Th1/Th2, and generate immune drift phenomenon. NPY can reduce the ratio of Th1/Th2, making the Th1 cytokine levels drop, Th2 cytokine levels rise, which prompted Th1/Th2 balance that have

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[9] Li wang, Yuzhang Wu; CT and Th1/Th2 polarization; Third military medical university journal, 22(10), 985-988 (2000).