mRNA Expression on Neuropeptide Y (NPY) to Exercise Intensity and Recovery Time

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Abstract. [Purpose] The purpose of this study was to analyze the effect of exercise intensity and recovery times on the extension of Neuropeptide Y in mice hypothalamus. [Subjects] The mice (ICR) were housed per cage in a temperature (20–23°C) and light controlled environment with a 12:12h light-dark cycle. [Methods] ICR were divided into ten groups, 8 mice each group: rest time group, low-intensity exercise, moderate-intensity and high-intensity. The exercise was carried out on a treadmill adapted in order to permit 2 animals to run at the same. Neuropeptide Y transcript contents were estimated by RT-PCR and Real Time PCR. DNA laddering was used to detect DNA fragmentation as an indicator of Neuropeptide Y. [Results] The Neuropeptide Y mRNA relationship to exercise intensity showed significant differences immediately after exercise. The Neuropeptide Y mRNA relationship to exercise intensity showed differences on low-intensity, moderate-intensity and high-intensity. Neuropeptide Y mRNA showed differences between exercise levels of low-intensity to moderate-intensity and low-intensity to high-intensity. [Conclusion] It is noteworthy that the expression of NPY mRNA can be effected by stability time and the intensity of exercise. Neuropeptide Y mRNA expression is connected with recovery times at moderate-intensity and high-intensity. The present results suggest the possibility that exercise intensity and recovery times regulated of the desire for food.

Key words: mRNA Expression, Neuropeptide Y, Exercise intensity

(INTRODUCTION

A recent study reported that the control of food intake was activated by the difference of expression of neuropeptides mostly within the hypothalamus in respect to the central nervous system (CNS)1). The hypothalamus is a primary center in controlling food intake and energy metabolism, in which neuropeptides existing in the hypothalamus play essential roles in controlling weight. For instance, if electric stimulus is given to the ventromedial hypothalamic lesion (VMH) in hypothalamus regions, it causes an increase of the amount of food intake, and causes a decrease of energy expenditure, thus resulting in obesity2); if the lateral hypothalamus (LH) is stimulated by electricity, food intake decreases and energy expenditure increases, which causes nanocormia and anorexia3). In addition, studies on neuropeptides in the hypothalamus and neurotransmitters are conducted as vigorously as ever, and the neuropeptide Y (NPY) which is popularly researched among others is known as the material that stimulates food intake. NPY is a peptide existing around peripheral nerves and central nerves, and it was known as a pancreatic polypeptide-like substance which was reported to control food intake along with catecholamine4). In regard to the study on NPY’s control on food intake, when NPY was injected to the cerebral vehicle, it was found that the amount of the intake of food and water increased4,5), when it was injected to paraventricular nucleus (PVN) of hypothalamus, the intake of carbohydrate increased6). The study on relations between NPY and exercises which is closely related to food intake insisted that relations between the running wheel exercises during 18 weeks and NPY expression was not significant. Stress and exercise caused significance in NPY expression, but only running wheel exercise produced little significance7). On the contrary, as the study mentioned above, there is no evident relation between exercise and NPY, nor a clear standard to indicate how the increase and decrease of appetite was changed according to the method and intensity of exercises. Therefore, to verify the relations between NPY which has close ties with appetite and exercise, it needs to suggest a clearer standard on intensity of exercise. This study aims at explaining the relations between each intensity level of exercise and NPY, and thus finding the relations between the expression of NPY and the intensity of exercise.)
### Subjects and Methods

**Subjects**

5 through 7-week old female mice were obtained, which were ICR (Institute of Cancer Research) mice that were not changed in transgenic mutation from the maternal line. Experimental groups were raised in a plastic cage, the temperature was 20–30°C, humidity of 70–80% and the light was controlled by 12 hour cycles. They were fed with hard food and water. The food was made by Samtaco, Ltd, and made up of crude protein of 22.1%, crude fat of 3.5%, crude fiber of 5.0%, crude ash of 8.0%, calcium of 0.6%, phosphorus of 0.4% and soluble inorganic introgen of 60.4%.

**Methods**

The experimental mice underwent tests after being taken from the laboratory of a C university and went through a 1 week adaptation period. Following the adaptation period, they were randomly classified into two groups with high intensity exercises group (n=8), a middle intensity exercise group (n=8) and a low intensity exercise group (n=8) and a stability time group (n=8). For the exercise stress test, tread mills were used; the intensity exercise group (n=8) and a stability time group went through exercise for 30 minutes at the speed of 8m/min (40–45% of VO2max) on low intensity exercise group went through exercise for 30 (n=8). For the exercise stress test, tread mills were used; the intensity exercise group (n=8) and a stability time group went through exercise for 30 minutes at the speed of 25m/min (80% of VO2max) on the angle of inclination of 0%8). All the white mice groups went through a 2-day adaptation training period (10m/min, 0%, 20min) and experienced a 1-time exercise after one day passed. After the tread mill exercise, they were killed by cervical decapitation, and their tissues were taken immediately and analyzed. The Reverse Transcription-Polymerase Chain Reaction method which was first designed by Mullis, who worked for Cetus, US, in 1983, is a method to obtain the DNA in a test tube by forming, repeating and synthesizing three processes: denaturation of DNA into single chain by using two primers against a certain DNA part; annealing with single chain of the primer and extension of the primer by the DNA polymerase. Tissues will be removed and homogenized in the stability chamber for 10 minutes. The RNA pellet was obtained after the supernatant was removed following the process of centrifugation at 4°C at 15,000 rpm for 10 minutes. After being washed off by 80% of EtOH, the pellet was completely dissolved by tapping it with the third distilled water which was processed with DEPC of 10 µL.

From these processes, a sample of 1 µL was taken, and RNA was quantified through the 260nm wavelength. To identify the expression effect of NPY gene using PCR reaction, a primer of Table 1 was utilized, which was designed to amplify a fragment of 138bp. For B-Actin which was used as an internal control, a primer of Table 1 was utilized.

The PCR reaction cycle condition was processed with 33 cycles at predenaturation, 95°C, for 5 mins., denaturation 94°C for 30 secs., annealing 57°C for 30 secs., extension 72°C for 30 secs., and postextension 72°C for 5 mins. The amplified cDNA was found to be a 1% TAE agarose.

The Real time PCR method, the last step of PCR technology ever, is the skill that can be released and quantified on a real-time basis by adding fluorescence-emitting probes to PCR mixture and emitting fluorescence which was accumulated onto the mixture. This test used the Real time PCR method, and not only quantified and compared them by checking the intensity of exercise and its consequential amount of NPY expression, but also quantified and compared the amount of NPY expression according to the difference between resting times by the intensity of exercises.

**Table 1. Nucleotide sequences of PCR primers**

<table>
<thead>
<tr>
<th></th>
<th>Sequences</th>
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<tbody>
<tr>
<td>NPY</td>
<td>5'-AGGGAGTGGCAGCATTTA-3'</td>
</tr>
<tr>
<td></td>
<td>5'-GAGATAGTTCAGAAGAAACCATG-3'</td>
</tr>
<tr>
<td>B-actin</td>
<td>5'-TTGTGGCCCTTCTTTGAGTTG-3'</td>
</tr>
<tr>
<td></td>
<td>5'-CTCCTTAATGTCACGCAGATTTC-3'</td>
</tr>
</tbody>
</table>

Demographic data of difference NPY mRNA were recorded. Post hoc analyses by using the Tuckey test was performed for the between-groups comparison. All statistical analysis were performed using SPSS, version 15.0. A p value<0.05 was considered significant.

**Results**

The difference of expression amount of post-exercise NPY mRNA per intensity showed the ratio of 0.002 which means a significant difference (pc<0.01). It can be said that post-exercise NPY mRNA per intensity has a meaningful difference in each stability time, low intensity, middle intensity and high intensity. To verify the difference of post-exercise NPY mRNA per intensity in each group, a posteriori test was used. Immediately following exercise, stability time and low intensity showed little significance, but NPY mRNA showed 0.008 and 0.001, indicating significant differences at stability time, middle intensity and high intensity, and it showed significant difference of 0.016.
increases in the plasma, appetite increases 9), and that the
He also insisted that if the amount of free fatty acid
concentration reached a normal level, the appetite curved.
and that BDA acted as a food intake signal, and if the
of blood glucose concentration (BDA) promoted appetite,
was suggested by Mayer in 1952 proposed that the decrease
been vigorously conducted. For instance, a theory which
hypothalamus are very popular these days. They found that
domains, and revealed that each domain had its own role.
neurotransmitters which are involved in appetite control
manner. Studies of the distribution domains of
accompanied by exercises by the token of NPY which is a
intensity and high intensity.
Table 2. Means and SD of NPY mRNA expression

<table>
<thead>
<tr>
<th>Exercise intensity</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.01</td>
<td>0.205</td>
</tr>
<tr>
<td>B</td>
<td>1.03</td>
<td>0.205</td>
</tr>
<tr>
<td>C</td>
<td>1.33</td>
<td>0.854</td>
</tr>
<tr>
<td>D</td>
<td>2.08</td>
<td>1.325</td>
</tr>
</tbody>
</table>


Table 3. Difference of NPY mRNA to exercise intensity

<table>
<thead>
<tr>
<th>Exercise intensity</th>
<th>Mean Difference</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>−0.205</td>
<td>0.221</td>
</tr>
<tr>
<td>C</td>
<td>−0.854</td>
<td>0.261**</td>
</tr>
<tr>
<td>D</td>
<td>−1.325</td>
<td>0.221**</td>
</tr>
</tbody>
</table>

| B                  |                |            |
| C                  | 0.205          | 0.266      |
| D                  | 0.798          | 0.285*     |
| C                  | 1.053          | 0.293**    |
| D                  | 0.854          | 0.221**    |

| C                  |                |            |
| D                  | 0.798          | 0.281*     |
| C                  | 0.255          | 0.202      |
| D                  | 1.325          | 0.218**    |

Exercise intensity M SD
A 1.01 0.205
B 1.03 0.205
C 1.33 0.854
D 2.08 1.325

*p<0.05 **p<0.01 A: rest B: low intensity exercise C: moderate intensity exercise D: high intensity exercise.

and 0.002 in low intensity, middle intensity and high
intensity (p<0.05). However, there is little difference
between stability time and low intensity as well as middle
intensity and high intensity.

DISCUSSION

Studies on a mechanism which generates appetite have
been vigorously conducted. For instance, a theory which
was suggested by Mayer in 1952 proposed that the decrease
of blood glucose concentration (BDA) promoted appetite,
and that BDA acted as a food intake signal, and if the
concentration reached a normal level, the appetite curved.
He also insisted that if the amount of free fatty acid
increases in the plasma, appetite increases 9), and that the
theory that the change of blood amino acids takes effect on
food intake argued, after checking the relations between the
change of body temperature and the amount of food intake,
that the temperature is very important in controlling food
intake10,11). Further, it reported that when stress and exercise were
activated together, the expression of NPY presented a
faster increase12). This study tried to investigate the appetite
by disclosing relations between exercise and NPY as follows15,16). In the result of the study on the difference of NPY mRNA according to the intensity of exercise, NPY mRNA was remarkably expressed in the order of high intensity, middle intensity and low intensity at stability time and immediately after exercise. There were little significance found between stability time and exercise groups, and stability time and low intensity, but significant difference were found between stability time and middle intensity, and stability time and high intensity by 0.008 and 0.001 respectively. Post-exercise difference between low intensity and middle intensity showed 0.016, satisfying p<.05, and difference between low intensity and high intensity showed 0.002, satisfying p<0.01 of significant level. However, the difference between middle intensity and high intensity showed little significance.

In conclusion, it is worth mentioning that the expression
of NPY mRNA can be different by stability time and the
intensity of exercise. Moreover, it can be found that the
larger the difference of intensity of exercise grew, the larger
the expression of NPY mRNA became, and the stronger the
intensity of exercise appeared, the larger the expression
of NPY mRNA became. But, there was no relation between 18
week running wheel exercise and the expression of NPY.
Further, it reported that when stress and exercise were
activated together, the expression of NPY presented a
significant difference1. In comparison with previous
findings, the difference of NPY expression can be explained
depending on what kind of exercise was performed and its
intensity, and which level of exercise causes stress. The per-
intensity amount of NPY mRNA expression at stability time
and after exercise showed a significant difference. In
addition, NPY mRNA showed a significant difference at
stability time, low intensity, middle intensity and high
intensity immediately after each level of exercise was
performed, and it also showed a significant difference at
stability time and middle intensity, stability time and high
intensity, low intensity and middle intensity, and low
intensity and high intensity immediately after immediately
following exercise. This finding indicates that the intensity
of exercise affects NPY mRNA expression immediately
after exercise. The low intensity exercise showed little
difference from stability time, but middle and high intensity
exercise showed gradual increase of NPY expression as the
intensity of exercise became stronger. These results mean
that the stronger the intensity of exercise becomes, the larger the expression of NPY grows. On the basis of this study, the relationship between exercise and appetite should be clear; a reasonable intensity level of exercise needs to be set, and a new attention to food intake after exercise needs to be controlled according to exercise goals.

REFERENCES