Taltirelin Improves Motor Ataxia Independently of Monoamine Levels in Rolling Mouse Nagoya, a Model of Spinocerebellar Atrophy

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To examine the relationship between motor ataxia and monoamine levels in the central nervous system, the contents and concentrations of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) in the cerebellum, brain stem and spinal cord were measured in rolling mouse Nagoya (RMN), a murine model of spinocerebellar atrophy. The tissue weight of the cerebellum and spinal cord, but not that of the brain stem was significantly lower in RMN than in the control group. In RMN, the NA content of the brain stem and spinal cord, but not the cerebellum were decreased relative to the control, and the concentration of NA in the spinal cord was also lower, but not significant. The DA and 5-HT contents in each tissue did not differ from those of the control, but the concentrations of monoamines, except for DA, were elevated in the brain stem and spinal cord in RMN. In particular, the concentrations of NA, DA and 5-HT in the cerebellum were significantly increased in RMN. Repeated administration of tartilerin hydrate, an analog of thyrotropin-releasing hormone, improved the ataxia of RMN, and elicited no obvious changes in either monoamine content or concentration of cerebellum, brain stem and spinal cord. These results indicate that the concentration of DA, as well as NA and 5-HT, increased in the RMN cerebellum, and that tartilerin improves the motor function of these mice via mechanisms other than changes in the levels of NA, DA and 5-HT in the central nervous system.

Key words spinocerebellar atrophy; tartilerin hydrate; dopamine; noradrenaline; serotonin (5-HT); rolling mouse Nagoya

The major symptom of spinocerebellar atrophy (SCA) is cerebellar or spinal ataxia. Noradrenergic and serotonergic fibers project to the cerebellum and the spinal cord, and regulate the motor systems. Furthermore, dopamine (DA)-containing immunoreactive fibers and some dopaminergic receptor subtypes have been found in rat cerebellar cortex, and microinjection of dopaminergic agonists and antagonists into the rat cerebellum reportedly decrease locomotor activity. In patients with olivo-ponto-cerebellar atrophy, which is a type of spinocerebellar atrophy, the DA releaser amantadine improves their ataxia.

Rolling mouse Nagoya (RMN) has been extensively studied morphologically and physiologically as an animal model of SCA. This mouse is shown to have the mutation in the gene encoding the P/Q-type voltage-dependent Ca\(^{2+}\) channel \(\alpha_{1A}\) subunit and changes of voltage sensitivity in cerebellar Purkinje cells. And the apoptotic cell death of cerebellar granule cells was increased in RMN, which is considered as an example of delayed cerebellar maturation. Although some reports have indicated that the metabolism of noradrenaline (NA) or serotonin (5-HT) is altered in ataxic mice including RMN, no study has investigated the DA level in their cerebellum, brain stem and spinal cord.

Thyrotropin-releasing hormone (TRH) and its stable analogs produce a number of physiological and behavioral effects in both animals and humans. Several studies have shown that these agents enhance locomotor activity and ameliorate cerebellar ataxia in rats and mice. In addition, these agents exert neuroprotective effects in rodents with spinal cord injury and brain trauma and anticonvulsant effects in amygdaloid-kindled rats. Clinical studies have also shown that TRH, and its synthetic analog taltilerin hydrate, are effective for treatment of hereditary ataxias, including olivo-ponto-cerebellar atrophy and Machado-Joseph disease, which is a type of spinocerebellar atrophy. Taltirelin hydrate and another TRH analog, YM-14673, have been shown to alter the metabolism of acetylcholine and DA, and activate the dopaminergic system. In the present study, we measured the contents and concentrations of DA as well as NA and 5-HT, and the effects of the synthetic TRH analog taltilerin hydrate on these monoamine levels and locomotor activity, in the murine ataxia model RMN.

MATERIALS AND METHODS

Preparation of Ataxic Model Mice All the experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University and were conducted in accordance with the guidelines of the National Institute of Health and The Japanese Pharmacological Society.

The RMN model was produced at Nagoya City University, but originally obtained from Dr. S. Oda, the Laboratory of Animal Management and Resources, Division of Biosphere Dynamics, Department of Biosphere Resources Science, Nagoya University Graduate School of Bioagricultural Sciences. Mice homozygous for the mutation were obtained by intercrossing heterozygous pairs. The homozygous mutants were easily recognized by their ataxic gait. In this paper, the term RMN refers to the homozygous mutants (rol/rol). To compare the native neurotransmitter levels in RMN, wild type (+/+) and heterozygous (+/rol) mice were regarded as controls, since Muramoto et al. observed no major differ-
ences in tyrosine hydroxylase activity between the heterozygous and wild type.\textsuperscript{29} Animals were allowed free access to food and water and maintained under a 12 h light/dark cycle in temperature- and humidity-controlled rooms.

**Measurement of Motor Activity** The spontaneous motor activity of each mouse was measured for 30 min with an Animex counter (Animex III; Shimadzu, Kyoto, Japan), and the number of falls was counted by behavioral observation at the same time. Fall index was estimated as: fall index = number of falls/spontaneous motor activity×100.

**Drug Administration** In the experiments to evaluate the effects of taltirelin on the contents of monoamines and fall index of RMN, taltirelin hydrate (1 mg/kg) or vehicle was administered daily for 7 d after the measurement of motor activity in 9-week-old RMN. Taltirelin hydrate (Tanabe Seiyaku Co., Ltd.) was dissolved in 0.9% w/v physiological saline and administered daily intraperitoneally (i.p.) at 0.1 ml/10 g. Control mice received the same volume of vehicle. At 27 h after the last administration, their spontaneous motor activity and number of falls were measured again.

**Measurement of NA, DA, and 5-HT** Immediately after the measurement of spontaneous motor activity, the mice were killed by inhalation of ether. The cerebellum, brain stem and spinal cord were rapidly removed and kept at −80 °C. The dissected samples were weighed, homogenized in 0.4 ml of 0.2 M perchloric acid including 0.1 mM ethylenediamine-N\textsubscript{2}N\textsubscript{2}N\textsubscript{2}N\textsubscript{2}′-tetraacetic acid disodium salt (Na2EDTA) and 0.1 mg/ml 3,4-dihydroxybenzylamine (DHBA) as an internal standard with a Handy Sonic (Tomy Seiko Co., Ltd.), and centrifuged at 10000×g for 15 min. The supernatants were collected into microtubes containing 1 ml sodium acetate and analyzed by HPLC with electrochemical detection. The mobile phase was composed of 83% acetonitrile and 17% methanol. The flow rate was maintained at 0.23 ml/min and the samples were injected into the column (2.1 mm×150 mm; EICOMPAC CA-5ODS; Eicom, Kyoto, Japan) in 10-ml aliquots. The substances were oxidized with a graphite electrode at a potential of +450 mV relative to an Ag/AgCl reference electrode, and the electrochemical detector (ECD-300; Eicom) was set at a gain of 0.5 nA full scale. The peaks generated were recorded and their height was measured.

**Statistical Analysis** All data are expressed as mean± S.E.M. To compare the contents and concentrations of monoamines in each tissue, Student’s t-test was used. To evaluate the effect of taltirelin on the fall index, paired t-test was used. Differences at \(p<0.05\) (two-tailed) were considered to be significant.

**RESULTS**

**Changes in Monoamines in the Central Nervous System of RMN** The body weight of RMN was lower than that of control mice (Table 1). In homozygous RMN, the weight loss of the cerebellum and spinal cord, but not that of the brain stem, was significant. The contents and concentrations of monoamines in each tissue were then compared between RMN and controls (Table 2). Since there was no difference in the cerebellar contents of NA, DA and 5-HT between RMN and the control mice, their concentrations were significantly increased in RMN due to the loss of tissue weight; the increase in DA concentration was particularly marked (+41.4%). There was a slight, non-significant difference of brain stem weight between RMN and controls. However, as the NA content of the brain stem in RMN was lower than that of the control, the concentration of NA in this area was not increased in RMN. As the brain stem contents of DA and 5-HT were similar between RMN and the control mice, the concentrations of DA and 5-HT were elevated in RMN, although the elevation of the DA concentration was not significant. In the spinal cord, the NA content was markedly decreased. As the contents of DA and 5-HT did not differ significantly between the groups, the concentrations of these substances were increased in this area in RMN, and the elevation of 5-HT concentration (+30.7%), in particular, was significant.

**Amelioration of Motor Disturbance by Taltirelin without Change in Monoamine Contents or Concentrations** Administration of taltirelin hydrate (1 mg/kg/d, 7 d) ameliorated the degree of motor disturbance, as evidenced by a significant decrease in the fall index of RMN (Fig. 1), while having no effect on body weight, weight loss of central nervous system regions. At the same time, however, neither contents nor concentrations of NA, DA and 5-HT in RMN were altered by taltirelin administration (Table 3).

**DISCUSSION**

Whole body weight, and the weights of the cerebellum, brain stem and spinal cord in RMN, were lower than those of
the RMN cerebellum, suggesting that cerebellar DA metabol- 
only NA and 5-HT but also DA was significantly increased in 
abnormal expression of tyrosine hydroxylase, which is a rate-
largest DA receptor protein immunoreactivity in the cerebel-
the cerebellum projecting to cerebellar nuclei, exhibit the 
weight loss.7,8) In the present study, the concentration of not 
cluded to the degree of weight loss of these tissues. In particu-
concentration increases age-dependently, while there are a few tyro-
in cerebellar Purkinje cells at 2 weeks-old and this expres-
ment increases age-dependently, while there are a few tyro-
TRH have been shown to have a neuroprotective effect in an-
administration of taltirelin may bring about changes in the me-
with a decrease in the fall index, the monoamine contents and concentrations were unaltered. In normal rats, systemic administration of DA receptor antago-
Taltirelin hydrate (1 mg/kg) was administered for 7 d. Each value represents the fall 
control mice. The most noteworthy finding of the present 
Nigrostriatal dopaminergic pathway by 6-hydroxydopamine reportedly reduce the lco-
several previous studies have demonstrated that TRH and 
administration of taltirelin ameliorated the ataxia of RMN, 
concentration in RMN may reflect the increasing of the tyrosine 
Several previous studies have demonstrated that TRH and 
results of all the monoamines measured 
did not change markedly in cerebellum, while their concentra-
tions were significantly increased. These differences be-
tween contents and concentrations are most likely attribut-
tion increases age-dependently, while there are a few tyro-
In conclusion, this is the first report to have demonstrated 
REFERENCES

Fig. 1. Effects of Taltirelin Hydrate (1 mg/kg/d) on Fall Index of RMN

Table 3. Effects of Repeated Administration of Taltirelin Hydrate (1 mg/kg/d) on the Contents and Concentrations of NA, DA, 5-HT in Cerebellum, Brain Stem and Spinal Cord of RMN (n=12)

<table>
<thead>
<tr>
<th>Contents (ng)</th>
<th>Cerebellum</th>
<th>Brain stem</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA Saline</td>
<td>25.1±1.4</td>
<td>36.1±1.4</td>
<td>31.1±2.5</td>
</tr>
<tr>
<td>Taltirelin</td>
<td>25.9±0.9</td>
<td>36.1±1.1</td>
<td>34.2±1.3</td>
</tr>
<tr>
<td>DA Saline</td>
<td>0.35±0.02</td>
<td>1.42±0.06</td>
<td>2.39±0.19</td>
</tr>
<tr>
<td>Taltirelin</td>
<td>0.36±0.03</td>
<td>1.31±0.11</td>
<td>2.48±0.11</td>
</tr>
<tr>
<td>5-HT Saline</td>
<td>6.5±1.0</td>
<td>37.6±1.5</td>
<td>58.9±3.6</td>
</tr>
<tr>
<td>Tartirelin</td>
<td>6.2±0.5</td>
<td>37.2±1.5</td>
<td>60.9±1.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (pg/mg wet tissue)</th>
<th>Cerebellum</th>
<th>Brain stem</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA Saline</td>
<td>544±25</td>
<td>703±15</td>
<td>435±21</td>
</tr>
<tr>
<td>Taltirelin</td>
<td>568±24</td>
<td>715±19</td>
<td>433±12</td>
</tr>
<tr>
<td>DA Saline</td>
<td>7.6±0.3</td>
<td>27.6±0.8</td>
<td>31.5±1.7</td>
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<tr>
<td>Tartirelin</td>
<td>7.9±0.6</td>
<td>26.1±1.9</td>
<td>31.3±0.8</td>
</tr>
<tr>
<td>5-HT Saline</td>
<td>140±19</td>
<td>732±1.6</td>
<td>778±26</td>
</tr>
<tr>
<td>Tartirelin</td>
<td>135±11</td>
<td>736±2.8</td>
<td>775±18</td>
</tr>
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