1-(1,3-Benzodioxol-5-yl-carbo-nyl) Piperidine, a Modulator of α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid Receptor, Ameliorates Exercise-Induced Fatigue in Mice

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Received May 23, 2013; accepted October 7, 2013; advance publication released online October 19, 2013

The current study was designed to investigate the effects of 1-(1,3-benzodioxol-5-yl-carbonyl) piperidine (1-BCP) on swimming endurance capacity which as one indicator of fatigue in the weight-loaded forced swimming mice. Mice were given either vehicle or 1-BCP (0.1, or 0.2 mmol/kg body weight daily) by intraperitoneal injection once daily for 2 weeks. The 1-BCP groups showed a significant increase in swimming time to exhaustion compared with the control group. 1-BCP increased the liver glycogen (LG) and muscle glycogen (MG) contents significantly, while decreased the lactic acid (LA) and blood urea nitrogen (BUN) levels notably compared with control group. Besides, 1-BCP treatment also significantly improved the endogenous cellular antioxidant enzymes in mice by increasing the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Therefore, this study demonstrated for the first time that the supplementation of 1-BCP, as a positive allosteric modulator of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, could enhance the endurance capacity of mice and facilitated them recovery from fatigue. Thus, we provide a new effective therapeutic strategy for fatigue.

Key words 1-(1,3-benzodioxol-5-yl-carbonyl) piperidine; fatigue; endurance capacity; weight-loaded forced swimming; α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor modulator

Fatigue is best defined as the difficulties in initiating or sustaining voluntary activities, and can be classified into mental and physical subcategories. Fatigue is usually accompanied by deterioration in performance, which is resulted from severe stress and hard physical or mental work. 1,2 It is one of the most frequent symptoms and most disabling nonmotor problems in the modern society and would generate severe negative consequences on cognition, physical performance function, and quality of life in patients with the Parkinson’s disease. 3 Unfortunately, there have not been few medications to treat fatigue, and the current therapeutic strategies for relieving fatigue have not been effective enough. 3

Persons at risk for impaired performance and health-related issues resulting from fatigue would benefit from agents capable of reducing these detrimental effects during they are fatigue. These agents could produce anti-fatigue effects by supplying energy substances, accelerating the elimination of metabolic products, adjusting the internal environment, enhancing body immunity or directly stimulating the nervous system. 4 A series of alertness-enhancing compounds that have been utilized to combat the fatigue, including methamphetamine, amphetamine, caffeine, and modafinil. However, a number of literatures reported their side effects, such as the potential to addiction and potent stimulant actions, which can distort cognitive and sensory processes at doses required to counteract the effects of fatigue. 5,6 In addition, selective serotonin reuptake inhibitors have been utilized to treat chronic fatigue syndrome. The usefulness of these agents, however, may be limited due to serotonergic hypersensitivity. 7 Therefore, it is of critical importance to search a safe and effective antifatigue compound that can relieve fatigue and promote behavioral performance.

1-(1-Benzodioxol-5-yl-carbonyl) piperidine (1-BCP) as a positive modulator of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, can modulate AMPA receptor gated ionic currents. It allosterically binds to glutamate receptor 1 (GluR1)–GluR4 subunits and limits desensitization, thus provoking an increase in amplitude and/or duration of ionic current though the glutamate-gated channel. 8 AMPA receptor is a subtype of ionotropic glutamate receptor, which plays important roles in neurotransmission in the central nervous system and in synaptic plasticity that underlies learning processes and memory. 9,10 Besides, the involvement in fatigue and wakefulness of AMPA receptor has been addressed in several studies. 11,12 Hence, AMPA receptor has been identified as the targets for drugs designed to enhance memory and cognition in the treatment of neurodegenerative disorders. 13 However, the anti-fatigue effects of these drugs have not been demonstrated. Positive allosteric modulators of AMPA receptor are superior to AMPA receptor agonists or agents that might increase glutamate release, in which they are able to increase glutamatergic tone without the obvious liability of excitotoxicity. 14 However, poor physicochemical properties such as poor aqueous solubility and inadequate lipophilicity needed to cross the blood–brain barrier, have undermined their successful development as viable therapeutic agents target to AMPA receptor. 15–17 1-BCP attracted our particular attention because it has moderate lipophilicity to cross the blood–brain barrier. 18 As a positive allosteric modulator of AMPA receptor, 1-BCP has been launched to Phase II clinical trials for the treatment of Alzheimer’s disease and other age-related learning and memory disorders in order to observe its proposed improved cognitive and behavioural function in dementia. 19 However, the anti-fatigue effect of 1-BCP has...
not been demonstrated. In our previous studies, we reported a new synthesis method of 1-BCP and its crystal structure.\textsuperscript{15} Hence, in the present study, we evaluated endurance capacity and anti-fatigue property of 1-BCP, all these based on the the model of forced swimming mice.

MATERIALS AND METHODS

1-BCP 1-BCP was synthesized in our laboratory (Purity: 99.6%, HPLC, methanol : water=60:40, 1 mL/min), and dissolved in 0.2 mL vehicle (Tween: saline=1:20, v/v).

Animals All the in vivo tests were carried in the Department of Pharmacology, The Fourth Military Medical University (Xi’An, China), which obtained the permission for performing the research protocols and all animal experiments conducted during the present study from the ethics committee of The Fourth Military Medical University. Six-week-old male Kunming mice (18–22 g, specific pathogen-free grade, SPF) were purchased from the Academy of Experimental Animal Center of The Fourth Military Medical University. They were housed in standard cages (21.5×32×14 cm, 5 mice/cage) under controlled conditions of temperature (24±1°C), humidity (50±12%), with a 12/12-h light-dark cycle (lights on from 08:00 to 20:00). They were fed with the balanced murine diet provided by the Academy of Experimental Animal Center of The Fourth Military Medical University.

Experimental Design After adaptation for at least 1 week, 60 mice were randomly divided into six groups: two control groups, two 1-BCP treatment groups at a high dose (1-BCP-H), and two groups at a low dose (1-BCP-L), with 10 mice each. One group from each set was used for the exhaustive swimming test. The other group was used for collecting the blood to determine biochemical parameters related to fatigue, after swimming for 30 min. For 2 weeks, the 1-BCP-H groups were given 0.2 mmol/kg body weight of 1-BCP, and the 1-BCP-L groups were given 0.1 mmol/kg body weight of 1-BCP by intraperitoneal injection every day at 1:00–3:00 p.m. The control groups received vehicle. After each treatment, all groups of the mice were allowed to rest 30 min and were forced to swim for 10 min to become accustomed to swimming.

Measurement of the Forced Swimming Capacity The swimming exercise was carried out in a tank (50×50×40 cm depth and maintained at a temperature of 30±1°C. The current in the pool was generated by circulating water with a pump, and the strength of the current was adjusted to 8L/min with a water flow metre (type F45500, Blue White Co., Westminster, CA, U.S.A.). The water was agitated to make the mice limbs keep moving.\textsuperscript{16} After the final treatment with 1-BCP or vehicle, the mice were allowed to rest for 30 min. Each of the mice had a weight attached by intraperitoneal injection every day at 1:00–3:00 p.m. The control groups received vehicle. After each treatment, all groups of the mice were allowed to rest 30 min and were forced to swim for 10 min to become accustomed to swimming.

Analysis of Biochemical Parameters After the final treatment with 1-BCP or vehicle, the mice were allowed to rest for 30 min. They were placed in the swimming tank (50×50×40 cm with 30 cm deep of water (temperature: 30±1°C). After swimming for 30 min and anesthetization with pentobarbital sodium, whole blood samples were collected from the orbital sinus to determine the levels of lactic acid (LA), blood urea nitrogen (BUN), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT).\textsuperscript{16} Each mouse was sacrificed with anesthesia after the final experiment. All of the blood biochemical parameters were determined with commercial kits (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China).

Analysis of Tissue Glycogen Contents Immediately after the blood had been collected, liver and gastrocnemius muscle were quickly dissected out, washed with saline, blotted dry with filter papers, and accurately weighed. Each tissue sample was homogenised in 8 mL of homogenisation buffer from commercial kits Assay Kit A043 (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China). Frozen in liquid nitrogen, and kept at −70°C until analysis for glycogen content. Briefly, portions of the muscle and liver were put into a tube containing 1.5 mL of 30% KOH saturated with Na₂SO₄, and immersed in a boiling water bath for 30 min before glycogen was assayed. The levels of tissue glycogen contents were determined with commercial kits Assay Kit A043 (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China).

Statistical Analysis Data were analyzed using SPSS 13.0 version. The results were expressed as the mean±S.E.M. The data on body weight were assessed using two-way repeated ANOVA followed by Fisher partial least squares difference (PLSD) post-hoc analysis. The other data were subjected to one-way ANOVA followed by Tukey–Kramer post-hoc analysis. The level of \( p<0.05 \) was used as the criterion of statistical significance.

RESULTS

Effects on Body Weight Change The change of body weight is shown in Table 1. Body weight was recorded before experiment (initial) and after 2 weeks (terminal). In the present study, both 1-BCP-H and 1-BCP-L group had no significant effect on the body weight compared to the control group (\( p>0.05 \)).

Effects on the Forced Swimming Time The swimming time to exhaustion of the mice was measured to investigate the anti-fatigue activity of the 1-BCP. As shown in Fig. 1, there are significant differences between the control group and each treatment group. The swimming time of the mice were 35% and 56% higher, repectively, than that in the control group, after supplementation for 2 weeks with 0.1, or 0.2 mmol/kg body weight of 1-BCP (\( p<0.01 \)).

Effects on Biochemical Parameters. 1-BCP Decreased LA and BUN in the Blood The contents of LA and BUN were measured to investigate the accumulation of metabolites caused by fatigue. As shown in Fig. 2, mice in 1-BCP-H and

Table 1. Effects of 1-BCP on Body Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial (g)</th>
<th>Terminal (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.2±1.5</td>
<td>28.2±2.6</td>
</tr>
<tr>
<td>1-BCP-L</td>
<td>19.8±1.2</td>
<td>27.3±1.1</td>
</tr>
<tr>
<td>1-BCP-H</td>
<td>19.6±0.9</td>
<td>27.6±1.5</td>
</tr>
</tbody>
</table>

The values are expressed as mean±S.E.M. (n=10). \( p>0.05 \) compared with the control group.
1-BCP-L group showed a significantly reduction in the levels of LA and BUN after exercise. The LA levels in 1-BCP-H and 1-BCP-L mice were both significantly lower than that of the control group ($p<0.01$), decreased by 22% and 17%, respectively. Also, the BUN values of mice from these two groups had a similar trend in the LA levels, which were significantly lower than that in control groups ($p<0.01$).

**1-BCP Enhanced the Antioxidative Enzymes in Mice**

The effect of 1-BCP on primary antioxidant enzymes include SOD, GSH-Px and CAT were showed in Table 2. The activities of GSH-Px and CAT in 1-BCP-H group were increased by 3.2% and 9.7%, both were without statistical significance compared to the control ($p>0.05$). The activities of SOD in the 1-BCP treatment groups (both high and low doses) were significantly higher than that of the control (41% and 52% greater, respectively) ($p<0.05$).

**Effects on the Contents of LG and MG**

The contents of liver and gastrocnemius muscle glycogen were measured to investigate the energy sources including glycogen storage. As shown in Fig. 3, there are significant differences between the control group and each treatment group. After swimming, the contents of liver glycogen in 1-BCP-L and 1-BCP-H groups were increased 63% and 78%, respectively, compared to the control group ($p<0.01$), after supplementation for 2 weeks with 1-BCP. In addition, there is a dose-dependent effect between 1-BCP-H and 1-BCP-L ($p<0.05$). Similarly, the content of gastrocnemius muscle glycogen was also enhanced by 43% and 50% in contrast with that of the control group ($p<0.01$).

**DISCUSSION**

In the present study, we examined the effects of 1-BCP on swimming time to exhaustion in mice. Our results demonstrated for the first time that the supplementation of 1-BCP could significantly improve the endurance capability of mice during exhaustion swimming test, regardless at low-dose (0.1 mmol/kg) or high-dose (0.2 mmol/kg) treatment. Several biochemical parameters and glycogen storages were measured to reveal potential mechanisms for this increased endurance capacity after the mice exhaustive swimming immediately.

A direct measure of anti-fatigue effect is the enhance in exercise endurance. Forced swimming to exhaustion is just an experimental exercise model which works well for evaluating the endurance capacity and gives a high reproducibility. Reduced susceptibility to fatigue is correlated with longer swimming times.17) Our studies found that mice after supplementation with 0.1, or 0.2 mmol/kg body weight of 1-BCP showed a significant increase in swimming time to exhaustion compared with the control group, indicating that 1-BCP possesses an anti-fatigue effect.

Lactic acid is produced by anaerobic glycolysis, which can be further degraded via the tricarboxylic acid cycle for the production of ATP by oxidative phosphorylation under normal circumstances. High-intensity exercise leads to the accumulation of LA, which maybe an cause of fatigue.18) However, Pedersen et al.19) reported that intracellular acidosis of muscle has protective effects during muscle fatigue. In there opinion,
intracellular acidosis of skeletal muscles is commonly thought to contribute to muscle fatigue. However, intracellular acidosis also acts to preserve muscle excitability when muscles become depolarized, which occurs with working muscles. So, besides lactic acid, several another biochemical parameters and glycogen storages related to fatigue were measured to reveal potential mechanisms. Blood urea nitrogen, a product of energy metabolism, is another sensitive index of fatigue status. There was a positive correlation between nitrogen in vivo and exercise tolerance. The worse an animal is adapted to exercise, the more the urea nitrogen level increases. In this study, LA and BUN levels in the 1-BCP groups were significantly lower than the control group, which indicated that 1-BCP facilitated mice recovery from fatigue due to reduce the accumulation of fatigue-related metabolites after its were supplemented with 0.1 or 0.2 mmol/kg body weight for 2 weeks.

Among the various fatigue mechanisms, the radical theory has attracted increasing interests. Harman’s classical “radical theory” suggests that intense exercise can cause increased free radical production and induce disturbance of oxidant-antioxidant homeostasis, such that leads to an oxidative stress state, which may be involved in fatigue. The primary antioxidant enzymes include SOD, GSH-Px and CAT. SOD catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide. GPH-Px is an enzyme responsible for reducing hydrogen peroxide or organic hydroperoxides to water and alcohol, respectively. CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen. These antioxidant defense mechanisms become weaker during chronic fatigue and other disease conditions. So, the enhance in the activities of these defense mechanisms can help to fight against fatigue. Our results showed that 1-BCP significantly improved the endogenous cellular antioxidant enzymes in mice by increasing the activities of SOD, CAT, and GSH-Px, which suggested that 1-BCP facilitated mice recovery from fatigue by removing oxygen free radicals rapidly.

The amount of glycogen storage is of great significance in determining the capacity for prolonged strenuous exercise. Liver glycogen and muscle glycogen levels depletion might be an important factor in the development of fatigue because as glycogen is depleted during high-intensity exercise, there is an inability to maintain blood glucose level, which result in fatigue and exhaustion. Results from this study showed that mice in 1-BCP groups significantly increased their liver glycogen and muscle glycogen levels, compared with control group, indicating 1-BCP enhance the exercise endurance may owing to increased the storage of liver glycogen. Moreover, there is a dose-dependent effect between 1-BCP-H and 1-BCP-L (p<0.05), which is another confirmation that 1-BCP has the anti-fatigue effects. However, it still needs the further studies to measure the possible reason that 1-BCP increased the contents of liver and muscle glycogen of mice postexercise by improving glycogen reserve, or by reducing the consumption of glycogen during exercise, or together.

In addition to the above mechanisms, positive modulators of AMPA receptor can potentially enhance cognition and combat fatigue probably by, firstly, offsetting losses of glutamatergic synapses; secondly, promoting synaptic plasticity; thirdly, increasing the production of trophic factors; and lastly, promoting the recovery of central nervous system cells. Our next studies will fucus on these hypotheses to determine central anti-fatigue effects of 1-BCP and explain its mechanisms of central anti-fatigue effects.

In conclusion, the present study demonstrated for the first time that 1-BCP, as a positive allosteric modulator of AMPA receptor, exhibited significant anti-fatigue effect by elevating endurance capacity and facilitating mice recovery from fatigue. 1-BCP is promising to be developed as a novel anti-fatigue compound which is beneficial for athletes or other occupations with heavy manual labor or long duration. Thus, we provide a possibility that positive allosteric modulators of AMPA receptor may have potential to be a new avenue to ameliorate fatigue probably.

REFERENCES


