Study on Elevating Sleep Efficacy of γ-Aminobutyric Acid

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Abstract. The purpose of this study is to investigate the effects of γ-aminobutyric acid on sleep improvement in mice. According to the method of Technical Standard for Health Food Inspection and Evaluation, animals were divided into three experimental groups. Group 1: Directly inducing sleep and prolonging sleep time experiment; Group 2: Subthreshold dose of sodium pentobarbital for hypnosis experiment; Group 3: Shortening sleep latency experiment. In each experimental group, 40 mice were randomly divided in four subgroups according to their body weight. After 30 days’ oral intake of different doses of γ-aminobutyric acid by mice, it is found that γ-aminobutyric acid can prolong the sleep time of mice hypnotized by pentobarbital sodium and shorten the sleep latency of mice hypnotized by barbital sodium. Meanwhile, there were no obvious abnormal changes in weight of mice. No side-effects were observed during the experiments. These results demonstrate that γ-aminobutyric acid produced by Bloomage Biotechnology Corporation Limited, has sleep aid function.

1. Introduction
Gamma aminobutyric acid (GABA) is a non-protein amino acid. It is widely distributed in plants and animals, such as tea, brown rice, fish, etc. It also exists in human body and plays an irreplaceable role for the regulation of the organisms’ life activities. It is known as an inhibitory transmitter compound in vertebrates and is present at a high concentration in the CNS [1] [2].

GABA is a natural calming and anti-anxiety ingredient. It is the most important inhibitory neurotransmitter in the brain. [3] [4]. Neurotransmitters are chemical messengers between neurons. GABA can prevent nerve cells from firing too often. As such, it has a calming and soothing effect. Having enough GABA in our brain is a guarantee of being relaxed and happy. Lack of GABA will cause a sleeping problem and anxious and tense feelings.

The effect of orally administered GABA on human ANS activities was also reported recently. The study [5] suggests that 30 mg GABA increases overall ANS and parasympathetic nervous system activities and thus may induce relaxation effects.

2. Materials and methods

2.1. Samples
Different concentrations of γ-aminobutyric acid (2.5g/L, 0.83g/L and 0.42g/L) provided by Bloomage Biotechnology Corporation Limited.
2.2. **Tested animals**

120 clean grade healthy male ICR mice, weight 12-22g, provided by Shanghai SLAC Laboratory Animal Co., Ltd.

In this study, animals were divided into three experimental groups.

- **Group 1**: Directly induced sleep and prolonged sleep time experiment;
- **Group 2**: Subthreshold dose of sodium pentobarbital for hypnosis experiment;
- **Group 3**: Shorten sleep latency experiment.

In each experimental group, 40 mice were randomly divided in four groups according to their body weight.

2.3. **Dosage**

Experimental group: Low, medium and high doses of $\gamma$-aminobutyric acid (8.33mg/kg BW, 16.67mg/kg BW, and 50mg/kg BW).

Control group: distilled water.

Route: Oral intake

Time: 30 days

2.4. **Instruments and reagents**

Electronic scales, stopwatch, pentobarbital sodium, barbital sodium

3. **Methods**

3.1. **Directly induced sleep experiment**

Observe whether the mice fall asleep after oral intake of samples. Righting reflex disappearance is taken as an indicator. When the mice were in the supine position, they can turn right immediately. If they can’t turn right in 60s, the righting reflex can be considered to have disappeared and the mice have fallen asleep. If the righting reflex recovered, the mice are awake again. The time from the disappearance to the recovery is the sleep time. Record the number of mice which fell asleep and the sleep time in control and experimental groups.

3.2. **Prolonged sleep time experiment**

The mice were injected intraperitoneal with 45mg/kg BW sodium pentobarbital 15min after oral intake of samples. Righting reflex disappearance is taken as a sleep indicator. Observe whether $\gamma$-aminobutyric acid can extend sleep time of mice hypnotized by pentobarbital sodium.

3.3. **Subthreshold dose of sodium pentobarbital hypnosis experiment**

The mice were injected intraperitoneal with 30mg/kg BW sodium pentobarbital 15min after oral intake of samples. Righting reflex disappearance for more than 1min is taken as a sleep indicator. Record the number of mice which fell asleep in 30min.

3.4. **Shorten sleep latency experiment**

The mice were injected intraperitoneal with 30mg/kg BW barbital sodium 15min after oral intake of samples. Righting reflex disappearance is taken as a sleep indicator. Observe whether $\gamma$-aminobutyric acid can shorten the sleep latency of mice hypnotized by barbital sodium.

4. **Results**

4.1. **Directly inducing sleep experiment**

The number of mice which fall asleep in the test is 0.
Table 1. The direct effect of GABA on inducing sleep ($\bar{X} \pm S$)

| Group       | Number of mice | Initial weight (g) | Final weight (g) | Number of mice which fell asleep | Sleep time (s) | $P$  |
|-------------|----------------|--------------------|------------------|----------------------------------|---------------|------|
| Control     | 10             | 19.0±1.1           | 37.0±1.6         | 0                                | /             | 0    |
| Low-dose    | 10             | 19.2±1.3           | 37.1±2.3         | 0                                | >0.05         | 0    |
| Medium-dose | 10             | 19.0±1.1           | 36.6±2.2         | 0                                | >0.05         | 0    |
| High-dose   | 10             | 19.1±1.3           | 37.7±2.1         | 0                                | >0.05         | 0    |

4.2. Prolonging sleep time experiment

GABA can extend the sleep time of mice hypnotized by pentobarbital sodium in the test.

Table 2. The effect of GABA on prolonged sleep time ($\bar{X} \pm S$)

| Group       | Number of mice | Initial weight (g) | Final weight (g) | Sleep time (s) | $P$  |
|-------------|----------------|--------------------|------------------|---------------|------|
| Control     | 10             | 19.0±1.1           | 37.0±1.6         | 2153±159      | /    |
| Low-dose    | 10             | 19.2±1.3           | 37.1±2.3         | 2235±179      | 0.284|
| Medium-dose | 10             | 19.0±1.1           | 36.6±2.2         | 2354±178*     | 0.011|
| High-dose   | 10             | 19.1±1.3           | 37.7±2.1         | 2319±153*     | 0.033|

* Statistically different with the control group, $P<0.05$

4.3. Subthreshold dose of sodium pentobarbital hypnosis experiment

Table 3. The improving sleep effect of GABA on the mice with subthreshold dose of sodium pentobarbital ($\bar{X} \pm S$)

| Group       | Number of mice | Initial weight (g) | Final weight (g) | Number of mice which fell asleep | Sleep percentage (%) | $P$  |
|-------------|----------------|--------------------|------------------|----------------------------------|----------------------|------|
| Control     | 10             | 19.5±1.3           | 37.2±2.1         | 1                                | 10%                  | /    |
| Low-dose    | 10             | 19.5±1.3           | 37.6±1.6         | 1                                | 10%                  | 1.000|
| Medium-dose | 10             | 19.5±1.4           | 36.7±2.1         | 3                                | 30%                  | 0.264|
| High-dose   | 10             | 19.5±1.3           | 37.8±2.3         | 2                                | 20%                  | 0.531|

4.4. Shortening sleep latency experiment

GABA can shorten the sleep latency of the mice hypnotized by barbital sodium.

Table 4. The effect of GABA on shortening the sleep latency ($\bar{X} \pm S$)

| Group       | Number of mice | Initial weight (g) | Final weight (g) | Sleep time (s) | $P$  |
|-------------|----------------|--------------------|------------------|---------------|------|
| Control     | 10             | 19.8±1.2           | 37.4±2.0         | 2416±132      | /    |
| Low-dose    | 10             | 19.7±1.3           | 37.1±1.8         | 2349±140      | 0.284|
| Medium-dose | 10             | 19.9±1.2           | 37.8±1.8         | 2223±205*     | 0.011|
| High-dose   | 10             | 19.8±1.3           | 37.0±2.3         | 2320±130      | 0.033|

* Statistically different with the control group, $P<0.05$

4.5. The influence of GABA on the body weight of mice

GABA has no influence on the body weight of mice.

5. Conclusion

Stress is a normal part of our life since we are facing a variety of stress from work, finances, family, and traffic in our daily life. For some people, the effects can be overwhelming, leading to nervousness and insomnia. Managing stress can exert beneficial effects on the cardiovascular, immune, and neuroendocrine systems. It is proved by clinical studies that the mental symptoms such as sleeplessness, somniphathy and depression are improved in more than 65% of the people with such symptoms with oral intake of GABA for 8 weeks [6]. Several animal experiments have demonstrated that the administration
of GAGA can increase the concentrations of plasma growth hormone and the rate of protein synthesis in the brain [7], improve many brain functions such as memory and study capability, and lower the blood pressure of spontaneously hypertensive rats [8, 9, 10, 11].

GABA, the major inhibitory neurotransmitter in the central nervous system [12, 13, 14], is an ingredient that has been shown to have an acute psychological stress-reducing effect in humans [15] and a tranquilizing effect on sleeplessness, depression and autonomic disorder observed during the menopausal or presenile period [16].

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