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Effect of Oral γ-aminobutyric Acid (GABA) Administration on Sleep and its Absorption in Humans

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Abstract The effects of γ-aminobutyric acid (GABA) on sleep and its levels in blood after oral administration were investigated in humans. A randomized, single-blind, placebo-controlled crossover-designed study was conducted to evaluate the effect of GABA on sleep. Sleep was evaluated by electroencephalography (EEG) after oral GABA administration. GABA significantly shortened sleep latency and increased the total non-rapid eye movement (non-REM) sleep time. Questionnaires showed that subjects receiving GABA realized its effects on sleep. In addition, the blood level of GABA after administration was investigated, and the absorption and metabolism rates of GABA were determined. GABA was quickly absorbed, and the blood level of GABA was the highest 30 min after oral administration, with a subsequent decrease in concentration. As GABA strongly affected the early stage of sleep, the effect of GABA on sleep may be connected to its levels in blood.

Keywords: γ-Aminobutyric acid, sleep, electroencephalogram, absorption

Introduction

γ-Aminobutyric acid (GABA) is an amino acid (Fig. 1) found in foods, such as vegetables, fruits, and fermented foods. In mammals, GABA is generated from glutamic acid by glutamic acid decarboxylase and functions as an inhibitory neurotransmitter in the central nervous system (1). It has been reported that low blood GABA levels are related to brain diseases (2-4); thus, it is recognized that GABA plays an important role in humans, particularly in the central nervous system. Since GABA is found in various foods, it can be obtained, to some extent, from a diet. However, the oral administration of high concentrations of GABA has shown distinct effects in humans. GABA can relieve anxiety (5), reduce psychological stress (6), and induce relaxation by increasing total and parasympathetic nerve activities (7). GABA can ameliorate psychological disorders, and because of this effect, many foods enriched in GABA, including drinks, candy, chocolates, and dietary supplements, have been released in the market in Japan and other countries.

It has been reported that over 20% of adults in Japan suffers from insomnia (8), and sleep is a serious social issue in Japan and other countries. Psychological disorders can greatly affect sleep quality and are regarded as a cause of insomnia (9,10). Because GABA can effectively relieve stress and anxiety, it is conceivable that GABA could improve sleep quality. Indeed, in a study that evaluated sleep in elderly people by using a questionnaire, it was shown that GABA improves sleep and reduces the frequency of urination at night (11).

However, there are few reports that objectively demonstrate the sleep-improving effects of GABA.

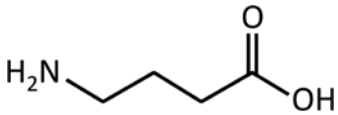

Fig. 1. The molecular structure of γ-aminobutyric acid (GABA)

In the present study, we aimed to investigate the effect of GABA on sleep by using electroencephalography (EEG), which made it possible to evaluate the sleep stage distribution precisely. Characteristics exhibiting subjects' quality such as sleep latency (i.e. the time taken for falling asleep) and non-rapid eye movement (non-REM) sleep (i.e. the deeper sleep) can be estimated from sleep stages. Furthermore, the rate of absorption and metabolism of GABA after oral administration was also investigated. GABA showed its effect mainly in the early stages of sleep, likely due to the rate of absorption and metabolism. Understanding the effect of GABA on sleep is essential, as it allows for sleep improvement by the ingestion of foods enriched with GABA.

Materials and Methods

Ethical considerations This study was approved by the Ethics Committee of Pharma Foods International Co., Ltd. (approval number: PF0078). The subjects consented to participate in this study after an explanation on the study procedures and potential risks. Written informed consent was obtained from all participants prior to

initiating the study procedures. All of the procedures were carried out in accordance with the code of the Declaration of Helsinki.

Study 1: Sleep evaluation by EEGs

Samples The test samples were composed of 112 mg GABA powder (100 mg GABA, 4.7 mg glutamic acid, 2.3 mg other amino acids, 3.4 mg minerals, and 1.6 mg water) or 112 mg of the placebo, dextrin, in a gelatin capsule. GABA powder was produced by natural fermentation using a specific strain of lactic acid bacteria (Pharma GABA®; 89% purity; Pharma Foods International Co., Ltd., Kyoto, Japan). From our preliminary examination, it has been found that the oral administration of GABA enabled subjects to feel the sure effect at a dose of 100 mg and therefore this study was conducted using 100 mg of GABA.

Subjects Subjects were selected from 32 Japanese volunteers recruited for this study. They usually worked at offices and had no serious health problems. For the screening of subjects, the Pittsburgh Sleep Quality Index (PSQI) questionnaire (12) was used. The PSQI questionnaire consists of 18 questions about the state of sleep and the sleep quality of each subject, and the data obtained are expressed as a score out of 21 points. A higher PSQI score indicates lower sleep quality. Volunteers with a PSQI score greater than or equal to six qualified for this study, because they were suspected to have a sleep disorder and were classified as poor sleepers (13). However, volunteers who had serious sleep disorders such as sleep apneas, nocturnal myoclonus, restless legs syndrome, and nocturnal frontal lobe epilepsy were excluded. Finally, ten subjects (average age: 37.7±11.5, age range: 24-57, 6 male subjects and 4 female subjects), who were suspected to have small sleep disorders but were not patients, participated in this study. No subjects dropped out throughout the study.

EEG measurement EEG measurements were performed using a portable, one-channel EEG device (Sleep Scope; SleepWell Co., Ltd., Osaka, Japan) and disposable electrodes, in accordance with a method described previously (14). Sleep was classified into four stages, wakefulness, REM sleep, light non-REM sleep (stage N1 and N2), and deep non-REM sleep (stage N3), according to the criteria of the American Academy of Sleep Medicine, 2007 (15). REM and non-REM sleep time were expressed as the percentage of each sleep time in the total sleep time. Total non-REM sleep time was defined as the summation of the light non-REM sleep time and the deep non-REM sleep time. Sleep latency and non-REM sleep latency were defined as the time from going to bed to the first non-REM sleep and the time from going to bed to the first deep non-REM sleep, respectively. Sleep efficiency was defined as the percentage of sleep time versus the total time in bed.

Visual analogue scale (VAS) VAS questionnaire comprised three scales of evaluation for test subjects; ease of falling asleep, feelings upon awakening, and satisfaction with sleep. Each scale represented a 100 mm horizontal line and points marked on the line indicated subjects' perception of state. Points marked on the left end of line suggested no feelings and points marked on the right end of line suggested much feelings, respectively. The length from the left end of the line to the marked point was measured and VAS score was determined from the length in millimetres.

Study design and test procedure A randomized, single-blind, placebo-controlled, crossover-designed study was performed. The ten subjects were randomly divided into two groups of five people each (group 1 and group 2). The study consisted of two intake periods (1 week each) and a wash out period (1 week) between the intake periods. During the first intake period, subjects in groups 1 and 2 took GABA and the placebo, respectively, 30 min before going to bed everyday for a week. During the second intake period, the treatments were exchanged between groups (i.e. subjects in group 1 and group 2 took the placebo and GABA, respectively). For two consecutive nights three days prior to each intake period, the subjects went to bed wearing the EEG instrument for adaptation. On the night prior to each intake period, EEG recordings were obtained while the subject was sleeping to serve as the baseline data. EEGs were also collected on the last night of the intake periods. On days that EEGs were recorded, alcohol and drugs, such as cold medicine and hypnotics, were prohibited. In addition, food and drinks, such as coffee, tea, and other items that might affect natural sleep, were also prohibited 2 h before going to bed. Subjects were requested to maintain constant lifestyle patterns regarding their eating habits, exercise, daily work, and so on throughout the study. On the day following the EEG measurement, subjects evaluated their sleep with respect to ease of falling asleep, feelings upon awakening, and satisfaction with sleep by using VAS. Subjects gave high scores if they felt positive effects from the test samples. In addition to VAS, PSQI was used to evaluate the sleep quality of each subject.

Statistical analysis Data were expressed as the mean±SD of the change in values before and after administration. The EEG measurements and questionnaires were analysed using the paired *t*-test and Wilcoxon signed-rank test, respectively. Significant differences between the GABA group and the placebo group were evaluated, and a probability value of less than 5% was considered significant.

Study 2: Blood GABA levels after administration

Samples Participants were given 100 mL of water with 200 mg of GABA. The same GABA sample as that used in study 1 was used.

Subjects Ten healthy Japanese subjects (average age: 32.4±4.8, age range: 23-37, 4 male subjects and 6 female subjects) were recruited and all of them participated in this study. They usually worked at offices but they were not the same as those in study 1. They had no serious health problems as well as sleep disorders.

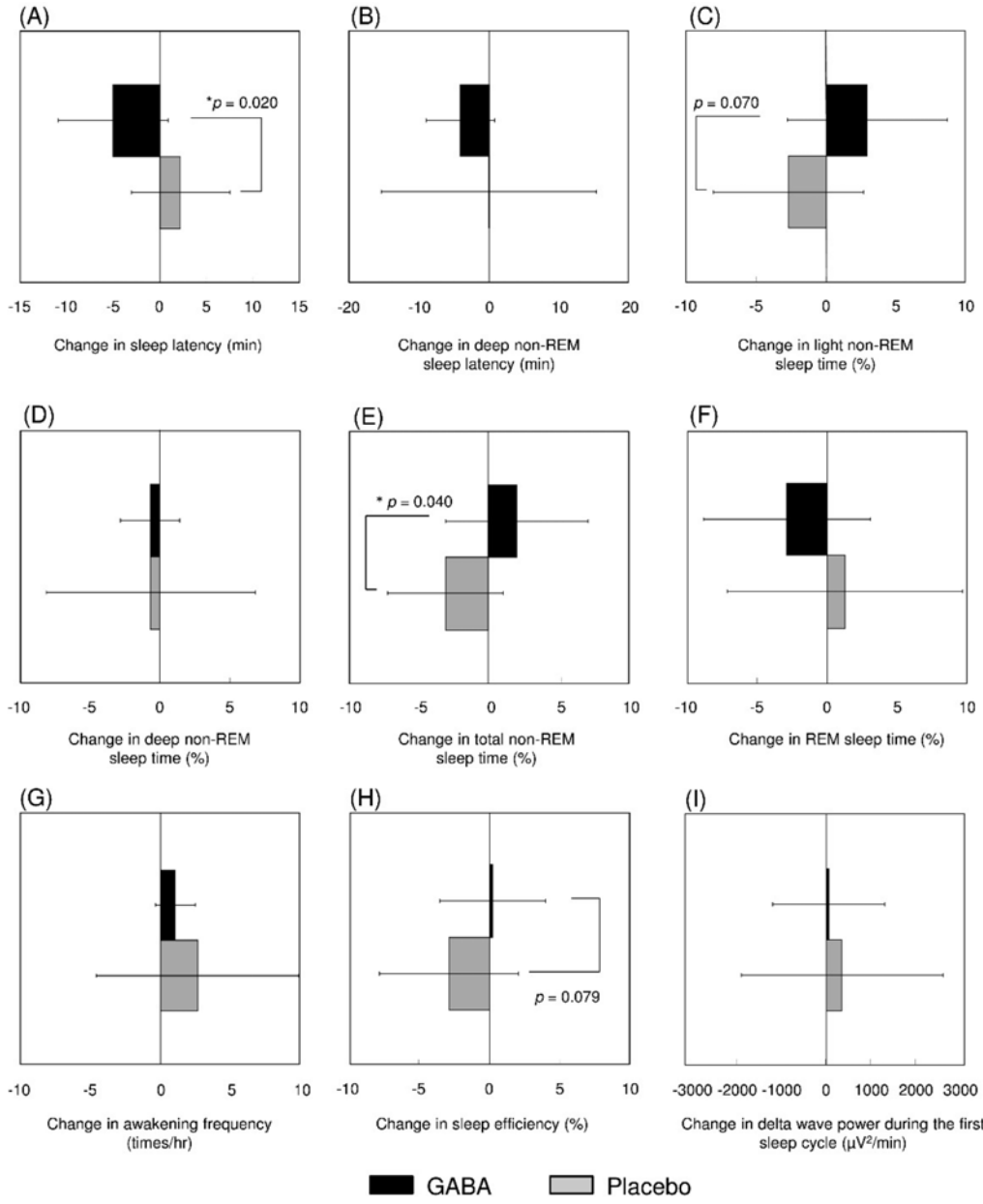

Fig. 2. EEG test results. Values are the mean±SD of the changes in sleep latency (A), deep non-REM sleep latency (B), light non-REM sleep time (C), deep non-REM sleep time (D), total non-REM sleep time (E), REM sleep time (F), awakening frequency (G), sleep efficiency (H), and delta wave power during the first sleep cycle (I) before and after sample administration. * indicates a significant difference compared with the placebo (p<0.05).

Plasma GABA level measurement Subjects were prohibited from eating and drinking from the night before the measurement until the measurement was completed. Blood was collected 10 min before, and 30 and 60 min after GABA administration. Heparin was added to each blood sample, and the samples were centrifuged to separate the plasma. Plasma GABA concentration was analysed by high performance liquid chromatography (HPLC) by Japan Clinical Laboratories, Inc. (Kyoto, Japan).

Statistical analysis Data were expressed as the mean±SD. The statistical significance of the time-course data was analysed using the paired t-test with Bonferroni correction. A probability value of less than 5% was considered significant.

Results and Discussion

The results of sleep evaluation by EEG are shown in Fig. 2. Oral administration of GABA significantly shortened sleep latency by 5.0 min (p=0.020, Fig. 2A). The mean value of sleep latency of subjects before GABA administration was approximately 10 min, and their sleep latency after GABA administration was reduced by half.

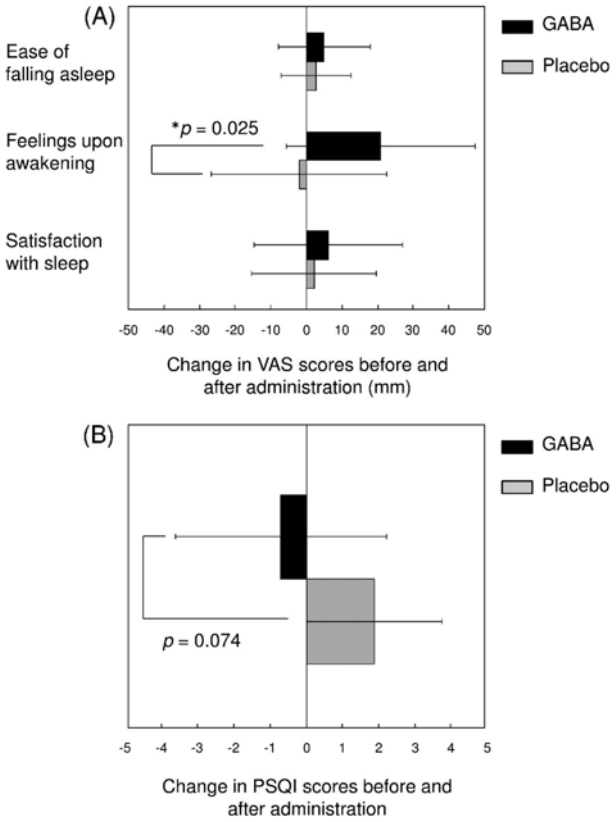

Fig. 3. The VAS questionnaire and PSQI results. Values are the mean±SD of the changes in VAS value with respect to sleep satisfaction, feelings upon awakening, ease of falling asleep (A), and changes in PSQI score (B) before and after sample administration. * indicates a significant difference compared with the placebo (p<0.05).

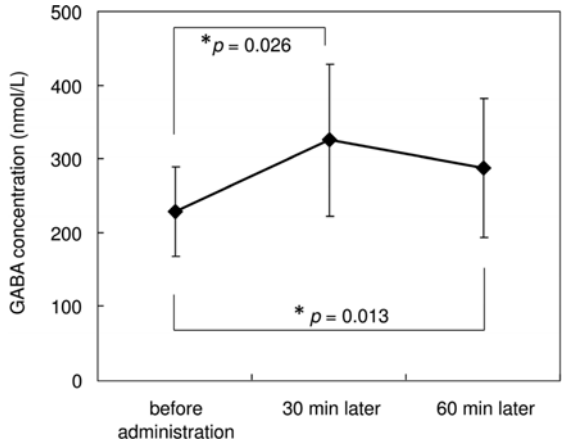

Fig. 4. Plasma GABA concentration before, 30 min after, and 60 min after GABA administration. Values are the mean±SD. * indicates a significant difference compared with the value before administration (p<0.05).

Although there was no significant difference, deep non-REM sleep latency was also shortened in GABA group by 4.1 min (Fig. 2B). These results indicate that GABA may help people fall asleep quickly and easily. In other words, GABA exhibited its effect during the early stage of sleep. Besides shortening sleep latency, GABA significantly increased total non-REM sleep time by 2.2% (p=0.040, Fig. 2E). This is favorable because non-REM sleep is a deeper sleep and is thought to rest both the brain and the body. In particular, the time from falling asleep to the first REM sleep, which was in the early stage of sleep and consisted of light and deep non-REM sleep, was longer in the GABA group compared to the placebo (data not shown). This also indicated that GABA was effective, especially during the early stage of sleep.

The results of sleep evaluation by VAS questionnaires and PSQI are shown in Fig. 3. Every item of VAS and PSQI was improved in GABA group. It was possible to say that subjects in the present study felt certain effects after use of GABA. In particular, feelings upon awakening were significantly improved (p=0.025, Fig. 3A). Some drugs used for sleep treatments are too strong and drowsiness can remain in next morning. For example, some benzodiazepines, the most often-used drug for sleep, have strong efficacy with several days-half-life in human body (16). Of course, there are numbers of benzodiazepines with sedative effects and short half-life; however, remaining drowsiness after using strong drugs is the point at issue. In the present study, it was shown that GABA had no such influence, and that subjects felt fine upon waking up. As is often the case with poor sleepers, their own evaluation of their sleep quality does not correspond to their actual sleep. Therefore, poor sleepers sometimes cannot be satisfied, even if their sleep is improved. On the other hand, in the present study, subjects felt that their sleep was improved, which could be a beneficial effect of GABA on sleep.

In order to investigate the reason why GABA can exhibit its effect during the early stage of sleep, blood GABA levels after GABA administration was studied. The result of blood level test is shown in Fig. 4. The blood GABA level elevated 30 min after administration from 244 to 329 nmol/L (p=0.026) and it was decreased to 290 nmol/L 60 min after administration. Above result suggested that GABA was quickly absorbed and metabolized and this may explain why GABA was effective in the early stage of sleep. It can be thought that GABA exerts its effect in a short time after oral administration because of quick absorption. Thus, the effects of GABA may be connected to the level of GABA in the blood. However, GABA is never ineffective after falling asleep. Total non-REM sleep time significantly increased (Fig. 2E), and light non-REM time (Fig. 2C) and sleep efficiency (Fig. 2H) increased with a slight trend toward significance (p=0.070 and p=0.079, respectively). In addition, sixty minutes after GABA administration, the blood GABA level was decreased to 290 nmol/L; however, levels remained higher than before administration (Fig. 4). These results support that GABA is effective throughout sleeping.

Various factors cause sleep disorders. People suffering from insomnia often have psychological problems, such as stress, thereby causing insomnia (9,10). As GABA can relieve stress and anxiety (5,6), the sleep-improving effect of GABA may be associated with this function. In contrast, we previously confirmed that oral administration

of GABA lowered core body temperature in humans (unpublished data). It is known that reduced core body temperature can affect sleep onset (17). Given that oral GABA administration can activate parasympathetic nerves (7), which control body thermoregulation, there is a high probability that GABA improves sleep through the activation of parasympathetic nerves and the reduction of core body temperature. Although GABA is one of the major inhibitory neuro transmitters in the central nervous system, it has been reported that ordinary dose of GABA by oral ingestion do not permeate the blood-brain barrier (18). Therefore, it has been considered that GABA may act on the peripheral nervous system of the digestive organs and not the central nervous system (6). The mechanism of GABA-induced stress relief and body temperature reduction and its effects on sleep remain unclear and require further study.

In the present study, several sleep components, including sleep latency, non-REM sleep time, and sleep efficiency, deteriorated in the placebo group. This phenomenon was probably caused by changes in environmental factors, such as air temperature and humidity on the day of measurement. The quality of sleep can be worsen if the weather is sultry and unpleasant on the night of the EEG measurement. Therefore, it is appropriate to evaluate the results of GABA by comparing them to those of the placebo as a reference.

This study showed that the blood GABA level was increased after GABA administration but the increase was small. There is a question of whether the blood GABA level is enough to exhibit the activity. However, there has been no report that evaluates the bioavailability of orally administrated GABA or the physiological blood GABA level to exert the beneficial effect on sleep disorder or other symptoms. This study is the first report that discusses the relation between the rate of absorption and metabolism of orally administered GABA and its effect on sleep. It is hoped that further studies will be conducted and discussion will become active with this study as a point of departure for the discussion about the above question.

In Japan and other countries, GABA is regarded as a beneficial food ingredient and is used in functional foods which are frequently consumed to obtain various GABA-induced effects. The present study provides important results and suggests that functional foods containing GABA can be useful in improving sleep.

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Disclosure The authors declare no conflict of interest.

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