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ORIGINAL ARTICLE

Glycine ingestion improves subjective sleep quality in human volunteers, correlating with polysomnographic changes

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Abstract

In human volunteers who have been continuously experiencing unsatisfactory sleep, effects of glycine ingestion (3 g) before bedtime on subjective sleep quality were investigated, and changes in polysomnography (PSG) during sleep were analyzed. Effects on daytime sleepiness and daytime cognitive function were also evaluated. Glycine improved subjective sleep quality and sleep efficacy (sleep time/in-bed time), and shortened PSG latency both to sleep onset and to slow wave sleep without changes in the sleep architecture. Glycine lessened daytime sleepiness and improved performance of memory recognition tasks. Thus, a bolus ingestion of glycine before bedtime seems to produce subjective and objective improvement of the sleep quality in a different way than traditional hypnotic drugs such as benzodiazepines.

Key words: amino acid, glycine, slow wave sleep latency, St Mary's Hospital Sleep Questionnaire, Stanford Sleepiness Scale.

INTRODUCTION

Glycine, a nonessential amino acid, is synthesized endogenously and plays an essential role in the peripheral and central nervous systems. We found in our previous study that glycine ingestion (3 g) before bedtime significantly improved subjective sleep quality in human volunteers who had been continuously experiencing unsatisfactory sleep.[1] Also, it was reported that 9 g of glycine, a threefold higher dose than that which has significant effect on subjective sleep, produced neither acute serious adverse events nor a daytime sleepiness carry-over effect.[2]

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In the present study, the effects of glycine on both sleep as subjectively observed and sleep as observed through polysomnography (PSG) were evaluated. Furthermore, the effects of glycine on daytime sleepiness and daytime cognitive function were also studied.

METHODS

Subjects

Eleven healthy volunteers (eight female and three male) aged 30–57 (mean ± SD = 40.5 ± 10.1) years who were engaged in work during the daytime participated in the present study. Women experiencing menstruation during the study period were excluded. The subjects were instructed beforehand to maintain their usual lifestyle and to avoid additional ingestion of alcohol and supplements containing such substances as caffeine or vitamin B_{12}. The subjects were asked before the start of the study to complete the Pittsburgh Sleep Quality Index form[3,4]

eliciting subjective evaluation of their sleep quality over the past month, together with a lifestyle checklist (questionnaires about their lifestyle and sleep characteristics such as sleep satisfaction).

Study design

The study design was a randomized single-blinded crossover trial. A set of examinations over two consecutive nights and days was repeated twice with an interval at least 1 week between them. The subjects entered the hospital each day of the trial, where PSG examination was carried out throughout the night. They took either granule glycine or a placebo (a reduced form of malt sugar with the same flavor as glycine) within 1 h before bedtime. The subjective quality of their sleep was evaluated the next morning using the St Mary's Hospital (SMH) Sleep Questionnaire.[5,6] After a clinical interview by a physician for assessment of possible acute adverse effects, each subject moved to another building (5 min walk) where daytime sleepiness and cognitive function were assessed at 08:00, 10:00 and 12:00 hours. Effects of the glycine on sleepiness were assessed again at 21:00 and 23:00 hours when the subjects came back to the hospital, which was followed by a second night of PSG and other examinations the next day.

Electroencephalograms (EEG, C3, C4, O1, and O2) were taken by PSG equipment (Sandman, Tyco Healthcare Japan, Tokyo, Japan) and the sleep stages were judged by visual inspection according to the Rechtschaffen and Kales criteria.[7] For the assessment of carryover effects related to daytime sleepiness, the Stanford Sleepiness Scale (SSS)[8] and a visual analog scale (VAS)[9] were used. These scales were translated into Japanese by the Department of Psychiatry, Jikei University School of Medicine. Daytime cognitive function was assessed through performance of a memory recognition task using a microcomputer (NoroPro Light System, Tokyo, Japan), where a target stimulus (a digit or letter) and a test stimulus (a digit or letter) were presented with an interval of 1500 ms between them, and the subject was requested to respond if the test stimulus was the same as the target stimulus.

All data during the first night were obtained exclusively for acclimatization of the subjects to the examination protocol, and thus were not incorporated into the results.

The study protocol was approved by the Institutional Review Boards of Ajinomoto Co. Inc. and of Ohta General Hospital. Written informed consent to participate in the study was obtained from all the subjects after they were given an explanation of the study and its potential risks. All of the procedures were carried out in accordance with Good Clinical Practice, the Helsinki Declaration, and related laws.

Statistical analysis

For the PSG and the subjective sleep quality analysis, Wilcoxon's signed rank test was used, and for the daytime sleepiness and daytime cognitive function analysis, a two-way (treatment [glycine or placebo]/time of day) repeated measures ANOVA followed by Fischer's least significant difference (LSD) test was applied. Statistical significance was defined to be $P < 0.05$.

RESULTS

Subjects' background of daily sleep

The lifestyle questionnaire indicated that each subject had dissatisfaction with their sleep. The mean Pittsburgh Sleep Quality Index score was 8.07 ± 1.34 (range 7–11), indicating that the subjects had been continuously experiencing unsatisfactory sleep.

Effects of glycine on subjective quality of sleep

No serious adverse effects of the study protocol including glycine or placebo ingestion were observed during the study period.

Figures 1 and 2 show the effects of glycine on the subjective sleep quality assessed by the SMH Sleep

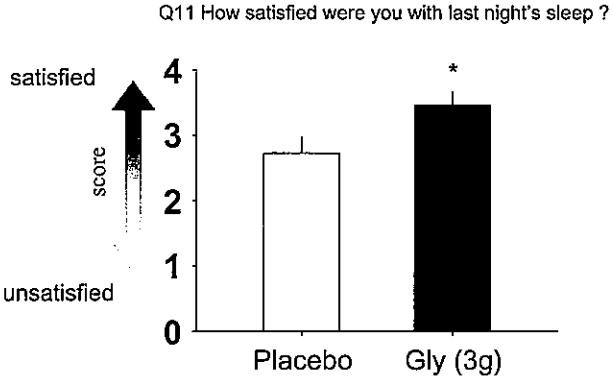

Figure 1 Effects of glycine (Gly) on responses to the St Mary's Hospital sleep questionnaire Question 11: "How satisfied were you with last night's sleep?" The higher the score, the more the individual is satisfied with their sleep. Data are expressed as mean ± SE. *$P < 0.05$.

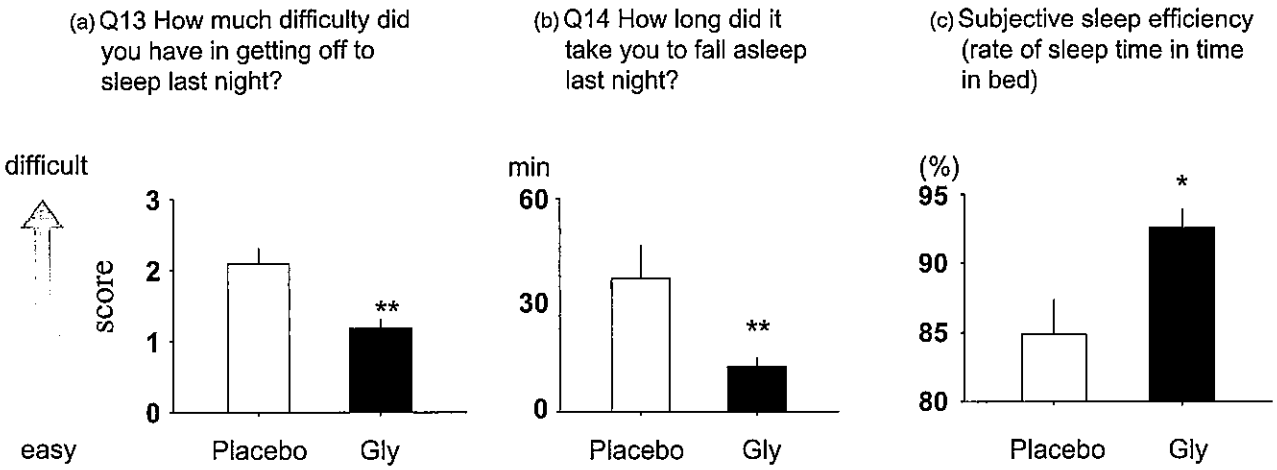

Figure 2 Effects of glycine (Gly) on subjective sleep quality. (a) Responses to St Mary's Hospital (SMH) sleep questionnaire Question 13: "How much difficulty did you have in getting to sleep last night?" The higher the score, the more difficult it was for the individual to get to sleep. (b) Responses to SMH sleep questionnaire Question 14: "How long did it take you to fall asleep last night?" (c) Subjective sleep efficacy measured as the ratio of sleep time to the whole time in bed. Data are expressed as mean ± SE. $*P < 0.05$; $**P < 0.01$.

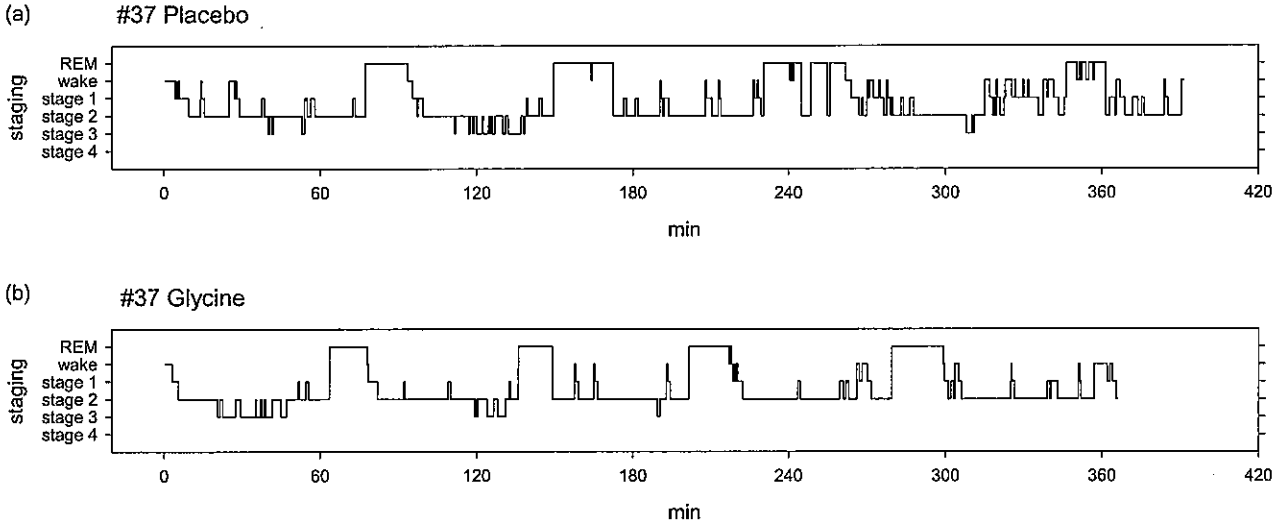

Figure 3 Representative hypnograms following administration of (a) placebo and (b) glycine. Transition of sleep phases over time after getting into bed is shown for subject no. 37.

Questionnaire. Significant beneficial effects of glycine were revealed in Question 11 "*How satisfied were you with last night's sleep?*" ($P = 0.046$, Fig. 1), Question 13 "*How much difficulty did you have in getting to sleep last night?*" ($P = 0.008$, Fig. 2a), and Question 14 "*How long did it take you to fall asleep last night?*" ($P = 0.002$, Fig. 2b). Further, glycine significantly increased the subjective sleep efficacy (ratio of sleep time to the whole time in bed, $P = 0.018$, Fig. 2c).

Effects of glycine on PSG parameters

Figure 3 shows one typical hypnogram where either more frequent appearance of deep sleep during an early sleep period, or a decrease in wake up after sleep onset (WASO) were observed following glycine ingestion.

Figure 4 shows that glycine ingestion significantly shortened the latency to sleep onset (latency to the first appearance of stage 2 sleep, $P = 0.01$) and to slow wave

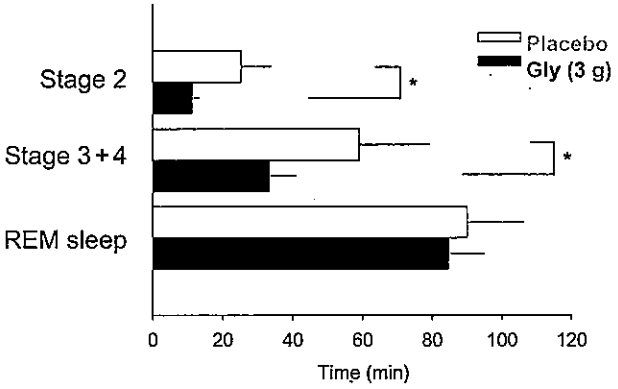

Figure 4 Effects of glycine (Gly) on the latency to sleep stages. Data are expressed as mean ± SE. *$P < 0.05$.

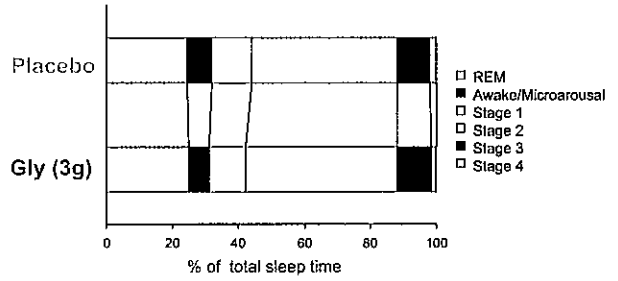

Figure 5 Effects of glycine (Gly) on sleep architecture. REM, rapid eye movement; WASO, wake-up after sleep onset.

sleep (SWS) (latency to the first appearance of stage 3, $P = 0.019$). The latency to rapid eye movement (REM) sleep, however, was not changed. The sleep architecture, that is, the ratios of each sleep stage length in the whole sleep period, was not altered by glycine ingestion (Fig. 5).

Effects of glycine on daytime sleepiness and daytime cognitive function

Figure 6 shows the effect of glycine ingestion on the daytime sleepiness in terms of the total score on the SSS. A tendency for glycine ingestion to improve this score ($F_{1,40} = 4.568$, $P = 0.058$) and a significant effect of time of day on this score ($F_{4,40} = 5.984$, $P < 0.001$) were observed. The interaction between treatment and time of day was not significant ($F_{4,40} = 1.435$, $P = 0.240$).

Figure 7 shows the effect of glycine ingestion on daytime sleepiness as seen in replies to item 2 of the VAS, "*How sleepy are you?*". Only the interaction between treatment and time of day was close to being significant

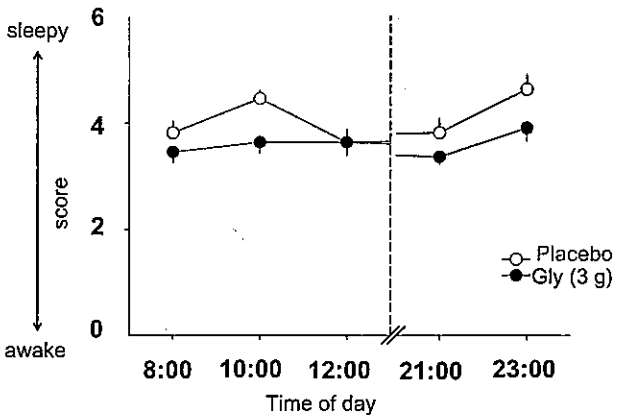

Figure 6 Effects of glycine (Gly) on daytime sleepiness expressed through Stanford Sleepiness Scale. Data are expressed as mean ± SE. A tendency for glycine ingestion to improve this score ($P = 0.058$) was observed.

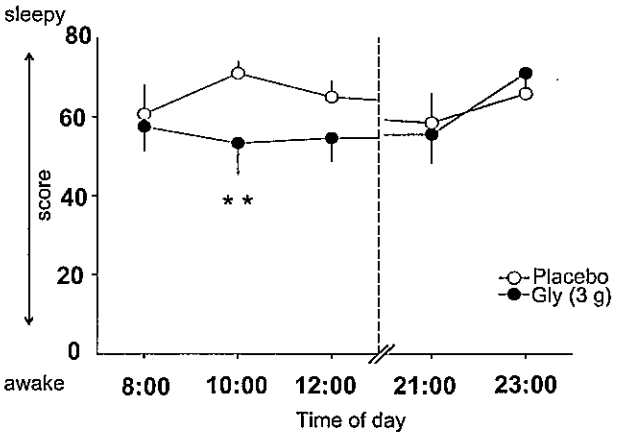

Figure 7 Effects of glycine (Gly) on daytime sleepiness expressed through Visual Analog Scale. Data are expressed as mean ± SE. **$P < 0.01$.

($F_{4,40} = 2.518$, $P = 0.056$). Fischer's LSD test revealed that at 10:00 hours, glycine reduced daytime sleepiness ($P = 0.009$).

These observations suggested that daytime sleepiness was lessened by glycine ingestion.

With regard to daytime cognitive function, only the interaction between the effect of the glycine ingestion on the correct response ratio of the memory recognition task and time of day was significant ($F_{2,20} = 6.303$, $P = 0.008$). Fischer's LSD test revealed that at 12:00 hours, glycine ingestion the previous night significantly improved the performance of the memory recognition task ($P = 0.004$, Fig. 8a). No significant

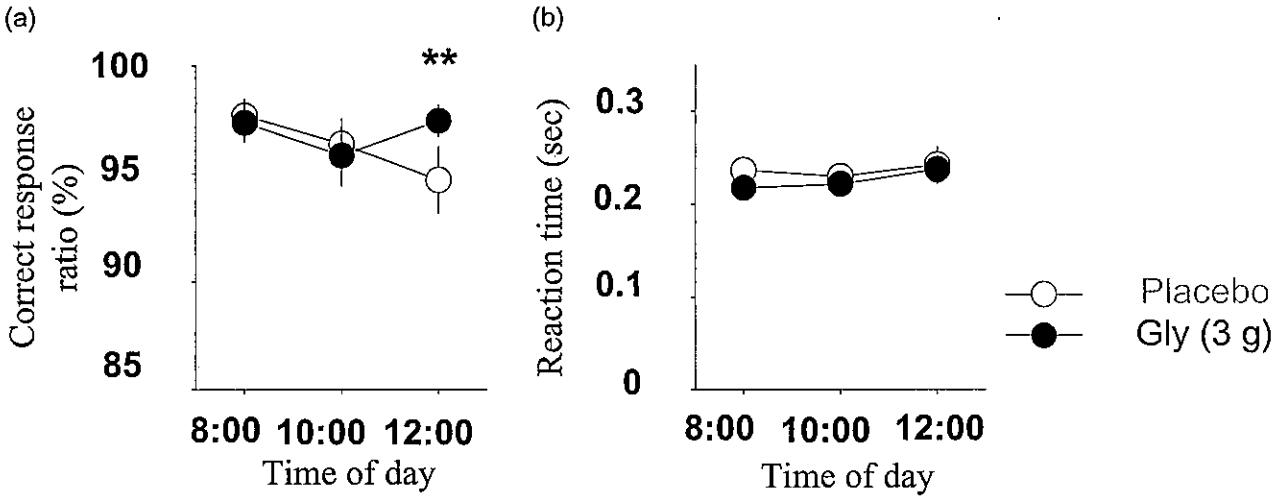

Figure 8 Effects of glycine (Gly) on daytime cognitive function: (a) cognitive function via memory recognition task; and (b) reaction time. Data are expressed as mean ±SE. $^{**}P < 0.01$.

changes in the reaction time were observed (Fig. 8b). These observations indicate that glycine may have effects on the cognitive function but not on motor function.

DISCUSSION

The present PSG results from subjects who had been continuously experiencing unsatisfactory sleep demonstrated that ingestion of 3 g of glycine before bedtime significantly shortened the latency to sleep onset and latency to SWS emergence without changes in the sleep architecture. These objective PSG data correlated with the subjectively observed improved sleep qualities such as satisfaction with sleep, a shortened latency to falling asleep, and sleep efficacy.

Traditional hypnotic drugs such as benzodiazepines enhance the γ-aminobutylic acid-A receptor and increase stage 2 sleep but alter the sleep architecture by reducing relative lengths of SWS and REM sleep.[10,11] The induction of daytime sleepiness and reduction of daytime cognitive function by benzodiazepines[12–14] would be consequences of such alteration of the sleep architecture. However, the proportions of each sleep stage of subjects in this study were unchanged, showing that glycine does not modify the sleep architecture itself. Their hypnograms traced a typical stable nocturnal sleep cycle, from deeper to shallower sleep with very few sleep interruptions. Furthermore, this study suggested reduction of daytime sleepiness and showed significant improvement of cognitive function following glycine ingestion.

A bolus ingestion of glycine appears to accelerate the onset of sleep and the first appearance of SWS in persons having chronically low quality of sleep. Furthermore, we have previously reported that glycine ingestion during daytime does not produce acute sleepiness.[2] Traditional hypnotics induce acute sleepiness, irrespective of either time of day or the recipients' background of sleep quality.

Our autoradiographic study in rats revealed the specific accumulation of intravenously administered glycine in the pineal body.[15] Furthermore, we found that concentration of glycine in the pineal body was much higher than in either the plasma or the cerebrospinal fluid and that glycine transporter (GlyT1) was highly expressed in the pineal body.[15] These observations suggest that glycine transporter is responsible for active transport of glycine into pinealocytes.

Judging from these observations, the mode of action of glycine is unique, and very different from hypnotics, although the neural mechanism of action, including the releasing mechanism of glycine into the cerebrospinal fluid of the third ventricle, is still to be elucidated.

The importance of a "good night's sleep" is not in doubt. The use of chemical hypnotics is widespread, although they have well-known problems. Therefore there is a need for a safe and reliable sleep regulator for those occasions when sleep is disturbed. Our present results .suggest that glycine produces subjective and

objective improvement of sleep quality and leads to a natural sleep pattern, including reduction of WASO, an early appearance of SWS and maintenance of REM sleep. This would provide the individual experiencing unsatisfactory sleep the means to restore a good night's sleep.

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