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New Therapeutic Strategy for Amino Acid Medicine: Glycine Improves the Quality of Sleep

Makoto Bannai[1,*] and Nobuhiro Kawai[1]

[1]*Frontier Research Laboratories, Institute for Innovation, Ajinomoto Co., Inc., Kawasaki, Kanagawa 210-8681, Japan*

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Abstract. Glycine is a non-essential amino acid that has indispensable roles in both excitatory and inhibitory neurotransmission via *N*-methyl-D-aspartate type glutamate receptors and glycine receptors, respectively. We recently reported that glycine ingestion before bedtime significantly ameliorated subjective sleep quality in individuals with insomniac tendencies. Oral administration of glycine to rats was found to induce a significant increase in the plasma and cerebrospinal fluid glycine concentrations and a significant decrease in the core body temperature associated with an increase in cutaneous blood flow. The decline in the core body temperature might be a mechanism underlying glycine's effect on sleep, as the onset of sleep is known to involve a decrease in the core body temperature. Moreover, a low core body temperature is maintained during sleep in humans. Pharmacological studies investigating the mechanisms of glycine on sleep were also performed. In this review, we will describe both our recent findings regarding how and where orally administered glycine acts and findings from our rat study and human trials.

Keywords: non-essential amino acid, blood–brain barrier, vasodilatation, core body temperature, *N*-methyl-D-aspartate (NMDA) receptor

1. Introduction

The importance of sleep has recently been emphasized throughout the world. Nonetheless, a meta-analysis has revealed that approximately 30% of the general population present with insomnia symptoms (1). Insomnia is known to induce cognitive inefficiency, sleepiness, mood disruptions (2), impaired attention, and memory deficits (3). Thus, the amelioration of insomnia is very important for society.

We have recently found that glycine improves sleep quality in humans who have repeated complains about sleep (4, 5). This treatment is a novel approach to improve sleep via the administration of an amino acid. The objectives of this review are to elucidate the effects of glycine on sleep improvement and describe the effects of glycine in humans.

*Corresponding author. makoto_bannai@ajinomoto.com
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2. Glycine

Glycine is a non-essential amino acid and has the simplest molecular structure of all the amino acids. Animal cells obtain sufficient amounts of glycine by either *de novo* synthesis or through dietary proteins. Approximately 45 g of endogenous glycine is synthesized (6), and 3 – 5 g of glycine is taken up from the diet, daily in humans.

Endogenously synthesized glycine functions as an inhibitory neurotransmitter in the central nervous system via the glycine receptors (GlyRs) (7). In contrast, glycine also acts as a co-agonist for the channel opening of the *N*-methyl-D-aspartate subtype of ionotropic glutamate receptors (NMDARs) (8, 9). Glycine is also known as an anti-inflammatory agent that protects against disease states in animal models of ischemia–reperfusion, injury, and transplantation (10). Moreover, glycine has been reported to attenuate the increase in free fatty acids and the accumulation of abdominal fat in sucrose-fed rats (11).

Given that glycine is often considered to be a biologically neutral molecule, it is used as an isonitrogenous control in amino acid supplementation studies. In this

context, we used glycine as a placebo in a human trial to investigate the functions of other amino acids. The unexpected results of this human trial instigated the study of the effects of glycine on sleep.

3. The pharmacokinetics of glycine

To investigate the effects of glycine on improving sleep, we first examined the permeability of the blood–brain barrier to externally administered glycine in rats (12). The concentration–time curve showed that the oral administration of 2 g/kg glycine increased the plasma glycine concentration to 5371 ± 507 μM (C_{max}), which was 13-fold higher than that of control animals given vehicle alone (vehicle controls) at 30 min (T_{max}) after administration. In the cerebrospinal fluid, the glycine levels reached 52.7 ± 4.4 μM (C_{max}), which was 6-fold higher than that of the control animals at 30 min (T_{max}). The cerebrospinal fluid (CSF) concentration of glycine has been shown to be higher than the ED_{50} of NMDARs (100 – 300 nM) and lower than that of GlyRs (90 – 100 μM) (13), suggesting that orally administered glycine directly acts on NMDARs. In our study, the cortical levels of glycine reached 1376 ± 30 pmol/mg wet tissue (C_{max}), which was 2-fold higher than that observed in the vehicle controls after 4 h (T_{max}). For a detailed analysis, we investigated the glycine uptake into the brain using the brain uptake index (BUI) method (14, 15). The brain uptake of glycine varied with respect to the injected quantities of glycine, ranging from 0.67 to 2.5 mmol/kg. The calculated BUIs in the range were between 2.6% and 9.0%, and these values were almost stable. The BUIs of the essential amino acids, which are transported by a specific transporter (16), have been shown to decrease according to their injected quantities (17). A high dose of the essential amino acids would saturate their specific transporters, resulting in a lower BUI. Taken together, these observations indicate that glycine passively diffuses across the blood–brain barrier by nonspecific transportation.

4. The pharmacological effects of glycine on sleep in rats

Sleep and core body temperature (CBT) are significantly correlated. CBT has a circadian oscillation; i.e., it drops at the onset of sleep (18, 19), continues to decrease during sleep (20) and gradually rises as a person wakes (21). We investigated the effects of glycine on CBT in rats (22). A total of 2 g/kg of orally administered glycine significantly decreased CBT in addition to causing vasodilatation. Furthermore, intracerebroventricular administration of glycine (130 nmol) also induced vasodilatation. We then performed a pharmacological study to identify the primary glycine binding receptor in the brain. Pretreatment with the NMDAR antagonists AP5 and CGP78608 into the lateral ventricle attenuated the vasodilatation induced by glycine, whereas pretreatment with the GlyR antagonist strychnine did not. In addition, a single treatment of the NMDAR glycine site agonist D-serine resulted in a significant increase in subcutaneous blood flow. Taken together, these results suggest that administered glycine primarily acts on NMDARs.

Next, we investigated the effect of glycine on several hypothalamic nuclei to identify the primary action site, as the hypothalamus is the center for both sleep and body temperature regulation. After unilateral or bilateral microinjection into the medial preoptic area (mPOA, the center of thermoregulation), dorsal subparaventricular zone (dSPZ, a regulator of thermogenesis and sleep), or the suprachiasmatic nucleus (SCN, the center of the circadian rhythm), vasodilatation was only present when glycine was bilaterally injected into the SCN, suggesting that the bilateral action of the SCN is necessary for inducing vasodilatation. Together, our findings indicate that orally administered glycine passes into the brain passively and acts on NMDARs in the SCN, resulting in vasodilatation and, subsequently, a decreased CBT.

The effect of glycine on sleep in rats was also investigated using electroencephalogram/electromyogram (EEG/EMG) recordings. Glycine (2 g/kg) significantly increased non-REM (NREM) sleep and reduced wake state in sleep-disturbed rats after 2 h of oral administration. These studies in rats revealed that glycine can improve sleep by decreasing CBT.

Finally, we have previously reported that the oral administration of glycine increases extracellular serotonin release in the rat prefrontal cortex (23). This release of serotonin should indirectly support the glycine-mediated improvement in sleep quality.

5. Glycine and sleep in humans

We have previously reported three human volunteer studies to subjectively assess the effect of glycine on sleep. Individuals with continuous complaints about the quality of their sleep were recruited and given either 3 g of glycine or a placebo before bedtime. In the first study (4), a randomized double-blinded crossover trial, 19 female volunteers (24 – 53 years of age; average, 31.1 years) participated. All of the subjects had complained about their sleep quality. Their Pittsburgh Sleep Quality Index (PSQI) scores were 6 or greater, indicating that the subjects had continuously experienced unsatisfactory sleep. The subjective quality of sleep was evaluated using the St. Mary's Hospital (SMH) Sleep Questionnaire

(24) and the Space-Aeromedicine (SAM) Fatigue Checklist (25). Glycine significantly improved the feeling of fatigue the next morning, indicating that glycine helps improve sleep quality.

The second study (5), a randomized single-blinded crossover trial, included 11 volunteers (8 females and 3 males; 30 – 57 years of age; average, 40.5 years), whose mean PSQI score was 8.07, indicating repeated unsatisfactory sleep. Polysomnographic (PSG) examinations were performed throughout the night, and the subjective quality of sleep was evaluated using the SMH Sleep Questionnaire. Furthermore, daytime sleepiness was assessed at 08:00, 10:00, 12:00, 21:00, and 23:00 on the day following the examination. The PSG examinations revealed a stabilized sleep state and a shortened latency to both the sleep onset and slow-wave sleep, with no alterations in the sleep architecture. Glycine also subjectively improved the volunteers' satisfaction with their sleep, the difficulty of sleep onset, and sleep efficiency. Furthermore, daytime sleepiness was significantly improved in the morning, as measured by the Visual Analogue Scale (26). Taken together, these findings indicate that glycine improves sleep quality both subjectively and objectively.

No serious side effects have been observed with the administration of 31 g/day of glycine (27). The third trial (28) investigated any acute adverse events and daytime sleepiness after the administration of 9 g of glycine. This study was an open trial because any safety problems needed to be addressed quickly if they occurred. A total of 12 volunteers (6 females and 6 males; 25 – 39 years of age; average, 34.0 years) participated in the study. Their mean PSQI score was 4.41, indicating no particular problems regarding sleep. Glycine (9 g) administered during the day did not induce sleepiness and had no adverse effects. Together, the results of these three aforementioned human trials indicate that glycine improves sleep quality in a subjective and objective manner and has no serious adverse effects.

6. Conclusion

We found that orally administered glycine acts on NMDARs in the SCN and decreases CBT, resulting in an improvement in sleep quality. However, given that neuronal projections from the SCN are abundant, the change in CBT is not simply a glycine-induced phenotype that supports sleep quality. The unknown functions of the projections from the SCN should be clarified in future studies.

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