The Effect of Subacute Supplementation of Taurine on Spatial Learning and Memory

Koichi ITO*, Matevž ARKO*, Tomohiro KAWAGUCHI, Masayoshi KUWAHARA, and Hirokazu TSUBONE

Department of Comparative Pathophysiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Abstract: Although the effect of taurine on the heart and liver is well studied, there has been no direct observation concerning the effect of taurine on spatial learning and memory at the behavior level. In this study, we tested the effect of subacute taurine supplementation with evaluation by the Morris water maze method. Although swim distance to find the platform of taurine-supplemented rats was significantly longer than that of control rats due to increase of swimming velocity, escape latency and the efficacy of learning and memory was comparable in both groups. These results suggest that taurine supplemented orally does not affect the learning and memory function.

Key words: learning and memory, taurine, water maze

Taurine, 2-aminoethylsulfonic acid, is widely distributed throughout the body including the liver and heart. The roles of endogenous taurine in the liver and heart are to increase bile acid secretion by forming bile acid conjugate [2] and regulate Ca^{2+} kinetics to protect and improve the heart function [17], respectively. In the brain, taurine is also abundant, especially in the hippocampus, and modulates synaptic transmission as an inhibitory neuromodulator interacting with γ-aminobutyric acid type A (GABA_A) or glycine receptors [9, 22]. Recent electrophysiological studies using rat brain preparations, however, have shown that taurine application induced long-lasting synaptic potentiation [3, 6]. The features of this taurine-induced synaptic potentiation are similar to those of long-term potentiation (LTP) [4, 19], which is a typical example of synaptic plasticity, and is thought to be a basic model for learning and memory [8], indicating that taurine is likely to be involved in learning and memory.

Despite accumulation of findings on the relationship between taurine and synaptic plasticity by electrophysiological studies, the effect of taurine on learning and memory at the behavior level is complicated. Studies of acute administration of taurine indicate that it has no effect on humans [1, 20] and mice [15], whereas studies investigating the subacute or chronic effect of taurine, often taken exogenously in food supplements on a daily basis, are still controversial. Sanberg and Fibiger reported that taurine impaired memory functions [16]. On the other hand, El Idrissi reported that taurine improved learning and retention in aged mice [5]. In these conflicting studies, the passive avoidance test was adopted to...
evaluate the effect of taurine. Because taurine was reported to change pain sensitivity [11, 18], not only the passive avoidance test but also another kind of behavioral test needs to be applied.

The Morris water maze test is well known and is the most widely used behavioral test for learning and memory dependent on the hippocampus [14]. Nevertheless, no study has investigated the subacute effect of taurine using this test. The purpose of this study was to elucidate the subacute potency of taurine on spatial learning and memory by the Morris water maze method.

Animal care and treatment complied with the guidelines of the University of Tokyo, Japan, which are consistent with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. In this study, young-adult male Ila:Wistar rats derived from Ishikawa Laboratory Animals (12 weeks, Saitama Experimental Animals Supply Co., Ltd., Saitama, Japan), weighing 313–397 g, were used. They were housed three to five animals per cage under 12-h light/dark cycles (lights on at 8:00 am) in a temperature-controlled room (24°C). The rats were randomly assigned to either a control group (n=5) or a 1% taurine (Wako Pure Chemical Industries, Osaka, Japan) supplementation group (n=6). They were given free access to food and water (or water including 1% taurine for taurine-supplemented rats) for seven days, starting on the day before the first day of the experiments. All experiments were carried out between 10:00 and 16:00. A circular swimming pool made of grey painted plastic, 150 cm in diameter, 45 cm in height, was filled with tap water. The water temperature was maintained at 22–23°C. A round disk platform made of transparent clear Plexiglas (12 cm in diameter, 30 cm in height) was submerged 1.0–1.5 cm below the water surface, at the center of one of the quadrants of the pool that were made by two imaginary perpendicular lines crossing at the center of the tank. It was maintained there during all experiments. Four visual cues were posted on the walls of the experimental room. The experiments were recorded on video and the scores for latency to escape to the platform, distance traveled from the starting point to the platform, and the swimming speed of the rats were later measured and analyzed with the EthoVision 3.1 video tracking system (Noldus Information Technology, The Netherlands). The rats were handled 5 min per day for a week before the training session. The procedure of the water maze test was the same as previously reported by Kikusui et al. [10]. Briefly, one session consisting of six consecutive trials was given for four consecutive days. A trial started with leaving a rat in the tank facing the wall, and the rat was allowed to swim freely to the escape platform. If the rat did not find the platform within 90 s, it was gently guided to it. The rat was allowed to remain on the platform for 60 s after finding it and was then removed from the tank to be put at the next starting point in the tank. The starting points were varied in a pseudo-randomized manner on the circumference of the semicircle opposite the platform. The location of the platform was unchanged throughout the experiments. On the day after the last session, the control and taurine-supplemented rats were forced to swim to find the platform which had been placed in a different position, and we evaluated how the rats tried to find it. This session had only one trial. All values are represented as mean ± SE. Statistical analysis was carried out using two-way repeated analysis of variance (ANOVA) and the significant level for all statistical tests was set at 0.005 (***) or 0.05 (*).

We measured the body weights of the rats and the amounts of liquid they drank for the seven consecutive days including the four consecutive experiment days. The amount of liquid consumed by the taurine-supplemented rats was significantly higher than the control group (P<0.01, Student’s t-test). Each rat in the taurine-supplemented group drank 34.4 ± 1.1 g water per day, while each rat in the control group drank 30.6 ± 0.5 g water per day, and approximately the same amount of liquid was consumed each day in both groups. The taurine-supplemented group therefore consumed a mean dose of 0.34 g taurine per day, which was approximately 0.94 g taurine/kg per day. In addition, the body weights of taurine-supplemented rats were slightly higher than controls although the difference was not statistically significant. Sympathetic nerve activity suppresses deprivation of salt and water. This response might be suppressed by taurine, resulting in urination increase [7]. It is likely that this effect of taurine induced a significant increase in intake of water, resulting in a slight increase of body weight. Moreover, the increase
of liquid consumption implied that ingested taurine was effective in the body.

In escape latency analysis, the taurine-supplemented rats’ time required to find the platform was comparable with that of the control rats (Fig. 1A). To discover the learning effect, we calculated the ratios of all values to the first trial of day 1. There were no significant differences between the control and taurine-supplemented groups (Fig. 1B). Moreover, we analyzed the day-by-day changes by adopting the data of the first trials from day 1 to day 4, and the ratio of each trial to the escape latency of the first trial of day 1 to examine the memory effect. Neither of these analyses showed any significant differences between the two groups (Figs. 1C and 1D). The swim distances of taurine-supplemented rats were significantly longer than those of control rats on day 1 (F value: 10.5, \( P < 0.005 \), interactions between treatment and day: \( P < 0.0001 \)), although there were no significant differences after day 2 (Fig 2A). We note that there seemed to be a difference in the first trial of day 1, although it did not involve learning and memory. We regard this difference as meaningless, because swimming velocity of taurine-supplemented rats was significantly faster only on the first trial of day 1 (\( P < 0.005 \), Student’s \( t \)-test) and rats on the first trial on day 1 were eager to swim along the wall of the pool (Fig. 4), indicating that the increase in swim distance was unhelpful in finding the platform. Therefore, swim distance which was calculated by multiplying the escape latency with swimming velocity was longer in taurine-supplemented rats in spite of no ties to learning and memory. To discover the learning effect in swim distance, we also calculated the ratio of all values to the first trial of day 1. The learning effect showed no significant differences between the control and taurine-supplemented groups (Fig. 2B). We also analyzed day-by-day changes by adopting the data of the first trials from day 1 to day 4. Although taurine-supplemented rats showed significantly longer swim distances than control rats, due to increase of swimming velocity (F value: 14.2, \( P < 0.05 \), interactions between treatment and day: \( P < 0.0001 \)).
The ratio of each trial to the swim distance of the first trial of day 1 did not show any significant differences (Fig. 2D), indicating absence of memory effect. Analysis using distance to zone, the integral of the distance between the rats and the platform for every 0.16 s, showed that the taurine-supplemented group tended to swim longer distances than the control group only on day 1 ($P=0.051$, Fig. 3A). However, the learning effect in distance to zone showed no significant differences between the two groups (Fig. 3B). Although day-by-day changes of distance to zone showed that taurine-supplemented rats tended to swim longer distances than the control group only on day 1 ($P=0.051$, Fig. 3A), the ratio of each trial to the distance to zone of the first trial of day 1 showed no significant differences (Fig. 3D), indicating absence of memory effect. As the results of the Morris water maze test often include confounders due to physical parameters such as swimming ability, the results might depend on the physical abilities of the rats in part.

To check if taurine induced loss of motility, we analyzed swimming velocity at the same time. Taurine-supplemented rats swam faster than control rats on the first trial of day 1 ($P<0.005$, Student’s $t$-test), although the mean swimming velocity of taurine-supplemented rats on day 1 was not significantly different from that of control rats (Fig. 4A), indicating that the athletic performance of taurine-supplemented rats tended to improve. Therefore, the swim distances and distance to zone in taurine-supplemented rats might be overestimated.

We also investigated whether either of the groups utilized special strategies to find the platform. We tested two parameters: the time spent in the quadrant where the platform was and the time spent in the inner circle area. We separated the surface area of the pool into two areas, a small concentric circular area (100 cm in diameter) and the surrounding torus-shaped area, and measured the amount of time the rats spent in the small concentric circular area. No significant difference was observed between the control and taurine-supplemented rats (Figs. 4B and 4C), suggesting that neither group

Fig. 2. Swim distance comparison. (A) Swim distance of taurine-supplemented rats was significantly longer than controls only on day 1 of the experimental period ($P<0.05$). (B) All values were normalized to the value of the first trial of day 1 to examine the learning effect. There were no significant differences between the two groups. (C) Day-by-day analysis adopting the data of the first trials from day 1 to day 4 showed significant differences between the two groups ($P<0.005$). (D) Data of Fig. 2C were normalized to the value of the first trial of day 1 to examine the memory effect. There were no significant differences between the two groups.
adopted a special pattern of movement to find the platform (e.g. swimming along the wall or looking at a particular direction when swimming). These results suggest that taurine-supplemented rats swam longer distances compared to control rats due to physical motility such as swimming velocity, but the abilities of learning and memory were not affected by subacute taurine supplementation.

An autoradiographic study suggested that exogenous taurine is found in the brain as in the heart and liver, but that the amount of taurine in the brain is small and the uptake is slow since it took more than 24 h for $^{35}$S-taurine to diffuse into the brain [13]. A study using taurine transporter-deficient mice showed decrease of taurine level in the brain and functional derangement [21], and Malcangio and colleagues reported that intracerebroventricular administration of taurine inhibited mice in the passive avoidance test [12]. Thus, our results in which taurine supplementation did not affect the learning and memory function suggest that exogenous taurine, even at the high concentration used in this study (0.94 g taurine/kg of body mass), has difficulty passing the blood-brain barrier which is consistent with the autoradiographic study.

In summary, we found that taurine supplemented orally to rats did not affect spatial learning and memory as tested by the Morris water maze system, although it did improve the physical ability of swimming. The Morris water maze test is a representative test of spatial learning and memory related to the hippocampus, and a combination of this behavioral test with other tests such as the eight-arm radial maze test or the object special test would be more useful for understanding the effect of taurine on learning and memory. In addition, the direct effect of taurine on the brain at the behavior level remains unknown, so further studies will be needed.

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Fig. 4. (A) Physical effect of taurine. Mean velocity of swimming of taurine-supplemented rats was significantly faster than controls on the first trial of day 1 (P<0.005, arrow), although the mean swimming velocity of taurine-supplemented rats on day 1 was not significantly different from that of controls. The time spent in the quadrant, in which the platform was placed (B), and the time spent in the inner concentric circle, which had a diameter of 100 cm (C) were not significantly different between the taurine-supplemented rats and controls. On day 5, we changed the position of the platform and tested the rats’ way of finding it but no significant difference was observed between the two groups.