AN ANTICONFLICT EFFECT OF GAMMA-1-GLUTAMYLTAURINE (LITORALON) IN RATS

Hisashi KURIBARA and Sakutaro TADOKORO
Division for Behavior Analysis, Behavior Research Institute, School of Medicine, Gunma University, 3-39-22 Showa-machi, Maebashi 371, Japan
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Abstract—Effects of gamma-1-glutamyltaurine (GT: Litoralon; Chinoin, Hungary) on rat's operant responses maintained under two types of negative reinforcement (avoidance) schedules: Sidman-type and discriminated avoidances and two types of positive reinforcement schedules and/or conflict schedules: differential water reinforcement of low rate (DRL 20 sec) and multiple fixed ratio 15/fixed ratio 15-punishment (MULT FR 15/FR 15-punishment) schedules were investigated to assess the central effects of GT. GT at 0.1–10 mg/kg i.p. induced no change in either the avoidance or DRL 20 sec responses. However, GT induced a prominent increase in the punished response (anticonflict effect) without eliciting a marked change in the non-punished response on the MULT FR 15/FR 15-punishment schedule. This behavioral change appeared not immediately, but gradually, when doses of more than 1 mg/kg were given; and a maximum level was achieved on days 3–6 and persisted for 7–14 days after the administration. The anticonflict effect of GT was synergistic with that of diazepam, a prototype antianxiety drug. No marked change in gross behavior was observed after GT. The present results suggest a possibility that GT has a central effect which correlates to the anticonflict effect.

Gamma-1-glutamyltaurine (GT: Litoralon; Chinoin, Hungary) (Fig. 1) is a dipeptide of glutamic acid and taurine, which was firstly discovered in bovine parathyroid gland by Feure and his coworkers (1, 2). They reported various effects of GT after chronic treatment (30–1500 μg/rat/day) such as a vitamin A-like effect and/or increase in serum vitamin A level (1–7), increase in serum cortisol level, increase in sensitivity of the adrenal gland to ACTH, increase in ACTH release from the pituitary gland (1, 3), and protection from X-ray irradiation (1) in rats. Clinically, GT showed cortisol-like effects (1). However, it has been unclear whether GT has central effects or not.

The purpose of this experiment was to assess the central effects of GT from the changes in the rat's operant responses maintained on both negative and positive reinforcement schedules.

MATERIALS AND METHODS

Animals: The experimental animals were 36 adult male rats of the Wistar strain provided at 4 weeks of age by the breeding colony at the Gunma University Medical School. Groups of 3 rats each were housed in stainless steel wire mesh cages of 25(W)×40(D)×20(H) cm with free access to a solid
diet (MF: Oriental Yeast Co., Tokyo) and tap water until start of the training under the operant situations. The breeding room was artificially illuminated by fluorescent lamps on a 12 hr light-dark schedule (light period: 6 a.m.–6 p.m.), and the room temperature was regulated to 23±2°C. Humidity was not controlled.

When the rats were 15 weeks of age and weighed 300–350 g, they were divided into 6 equivalent groups of 6 rats each. Two groups were subjected to the avoidance tests. The other 4 groups were subjected to the positive reinforcement and/or conflict tests. The animals used for the avoidance tests had been given access to food and water except during the period of the 2-hr avoidance sessions. The body weights of the animals used for the positive reinforcement and/or conflict tests were reduced to about 90% of those during free drinking by limiting the daily water intake to 10 ml/rat/day. However, the food was always available except during the period of the 1-hr experimental sessions.

Apparatus: The operant chambers used were made of aluminium and acrylfiber boards of 25(W)×20(D)×19(H) cm with a lever on a right side wall and a stainless steel floor grid. In addition, a water tray was attached to the chamber for the positive reinforcement schedules. The operant chamber and its attachments were contained in a wooden sound-attenuating box. The inside of the box was illuminated by a 10W fluorescent lamp and ventilated by a fan throughout the experimental sessions.

The schedule-controlling and data-recording apparatus consisting of relays, timers and electro-magnetic counters were located in an adjacent room.

Operant schedules: Two types of negative reinforcement (avoidance) schedules, namely, the Sidman-type avoidance schedule (response-shock interval=30 sec, and shock-shock interval=5 sec) (8) and the discriminated avoidance schedule without an escape contingency (intertrial interval=25 sec, and warning stimulus (WS) presentation period=5 sec) (9, 10) were used. The shock was an electric current of 150 V, 1 mA, 50 Hz AC applied for 0.3 sec to the rat by passing it though the floor grid. The WS was a 1,000 Hz tone sounded from a speaker along with the lighting of a small lamp. The 2-hr experimental sessions for the avoidance tasks were held every other day during the training period and at 1–3 day intervals during the drug testing period. The indices of the rat's avoidance responses were response rate (lever-presses/min) and shock rate (number of shocks delivered/min) for the Sidman-type avoidance response and response rate and avoidance rate (number of avoidance responses/number of WS presentations ×100) for the discriminated avoidance response. One of the two groups of the nonwater deprived rats was subjected to the Sidman-type avoidance task, and the other group of rats was subjected to the discriminated avoidance task.

Differential water reinforcement of low rate (DRL 20 sec) and a multiple fixed ratio 15/fixed ratio 15-punishment (MULT FR 15/FR 15-punishment) of water reinforcement schedules were used for the positive reinforcement and/or conflict schedules. Each reinforcement consisted of 0.05 ml of water. On the DRL schedule, responses spaced for more than 20 sec apart were reinforced. On the MULT FR 15/FR 15-punishment schedule, every 15th response was reinforced throughout the session. However, during the periods of WS presentation (punished period), each reinforcement was followed by an electric shock of 100 V, 0.5 mA, 50 Hz AC for 0.3 sec as the punishment for the response. In addition, an unavoidable shock of the same intensity was delivered at the end of each warning period following Estes and Skinner's conditioned suppression
procedure (11). The advantage of this modified Geller-type conflict procedure (12) for assessment of antianxiety drugs had been discussed in our previous report (13). The punished periods were inserted at intervals of 15 min, each of which persisted for 5 min. The 1-hr experimental sessions for the positive reinforcement tests were held every day during the training periods and at 1–3 day intervals during the drug testing periods. The indices of the rat’s behaviors were response rate and reinforcement rate (number of water reinforcement/min) for the DRL 20 sec response and response rates during both the punished and non-punished periods for the MULT FR 15/FR 15-punishment response. A group of 6 rats was subjected to the DRL 20 sec task. The conflict behavior was less stable than the avoidance and DRL behaviors, and the baseline performance tended to drift after a long term experiment. Three groups of 6 rats each were subjected to the MULT FR 15/FR 15-punishment task in order to reconfirm the anticonflict effect of GT in the different rats and to assess a combined effect with diazepam.

When the rat’s operant behaviors achieved stable levels for 5–6 consecutive sessions, the drug administration tests were started.

Drugs: The drugs used and the doses administered were GT, 0.1–10 mg/kg i.p.; taurine (Sigma), 0.1–10 mg/kg i.p.; and diazepam (Cercin Inj., Takeda), 0.5 mg/kg s.c. GT and taurine were dissolved in physiological saline, and the diazepam-preparation was diluted by 20% propylene glycol. Each injection volume was fixed to 1 ml/kg.

In the assessment of the effects of single administration of GT and taurine, these drugs were administered immediately before the start of the session at intervals of 7–20 days. The doses given were changed generally from the lower to the higher. However, one group of rats in the MULT FR 15 FR 15 punishment test was administered GT in the order of 1, 0.3, 0.1 and 3 mg/kg. In the combined experiment of GT with an anti-anxiety drug, diazepam was given 3 days after the administration of GT. The experiments were carried out between 9 a.m. and 6 p.m.

RESULTS

Effects on the avoidance responses: After establishment of the behavioral baselines, the mean response and shock rates ± S.E. of the 6 rats on the Sidman-type avoidance schedule were 6.4 ± 0.6/min and 0.30 ± 0.04/min, respectively, and the mean response and avoidance rates ± S.E. on the discriminated avoidance schedule were 3.1 ± 0.2/min and 95 ± 1%, respectively.

Figure 2 shows the daily changes in the Sidman-type avoidance response by means of the response and shock rates. Figure 3 shows the daily changes in the discriminated avoidance response by means of the response and avoidance rates. The data after the administration of 0.1 mg/kg GT were excluded from the figure presentation. GT at 0.1–10 mg/kg produced no change in either the
Sidman-type or discriminated avoidance response.

Taurine at 0.1–10 mg/kg also did not induce a marked change in either the Sidman-type or discriminated avoidance response (not shown in Fig.).

Effects on DRL 20 sec response: After establishment of the behavioral baseline on the DRL 20 sec schedule, the mean response and reinforcement rates±S.C. were 3.2±0.1/ min and 1.0±0.06/min, respectively.

GT and taurine 0.1–10 mg/kg did not induce marked change in the DRL 20 sec response (not shown in Fig.).

Effects on MULT FR 15/FR 15-punishment response: After the achievement of baseline performance under the MULT FR 15/FR 15-punishment schedule, the mean response rates during the non-punished and punished periods for the 3 groups of 6 rats each were 58±6/min and 2.0±0.6/min, 64±8/min and 3.7±1.2/min, and 50±5/min and 3.0±1.3/min, respectively. There were no statistically significant differences in the response rates among the groups.

Figure 4 shows the daily changes in the MULT FR 15/FR 15-punishment response by means of the non-punished and punished response rates.

![Figure 3](image)

Fig. 3. Daily changes in the response on the discriminated avoidance schedule (intertrial interval=25 sec, and warning stimulus presentation period=5 sec) without an escape contingency by means of the average response rate (closed circles with solid lines) and the average avoidance rate (open circles with broken lines) after single administration of gamma-1-glutamyltaurine at 0.3, 1, 3 and 10 mg/kg i.p. The rat's avoidance response was observed for 2 hr per day at intervals of 1–3 days. N=6.

![Figure 4](image)

Fig. 4. Daily changes in the response on multiple fixed ratio 15/fixed ratio 15-punishment water reinforcement schedule by means of the average non-punished response rate±S.E. (closed circles with solid lines) and the average punished response rate±S.E. (open circles with broken lines) after single administration of gamma-1-glutamyltaurine at 0.1, 0.3, 1 and 3 mg/kg i.p. for the first group of 6 rats. The rat's behavior was observed for 1 hr per day at intervals of 1–3 days.
Fig. 5. Daily changes in the response on multiple fixed ratio 15/fixed ratio 15-punishment water reinforcement schedule by means of the average non-punished response rate±S.E. and the average punished response rate±S.E. after single administration of gamma-1-glutamytaurine at 0.3, 1, 3 and 10 mg/kg i.p. for the second group of 6 rats. The data are expressed in the same way as in Fig. 4.

Single administration of 0.1–3 mg/kg GT to the 1st group of 6 rats. The doses administered to this group were changes in the order of 1, 0.3, 0.1 and 3 mg/kg. Figure 5 shows the changes in the non-punished and punished response rates after the administration of 0.3–10 mg/kg GT to the 2nd group of 6 rats. The doses administered to this group were changed in the order of lower to higher. More than 1 mg/kg of GT induced an increase in the punished response without a marked change in the non-punished response. The increase in the punished response appeared at days 1–2, achieved the maximum level during the 3–6th days, and disappeared during the 7–14th days after administration. The persistence of the behavioral change tended to be dose-dependent. However, the maximum levels of behavioral change achieved were not dose-dependent.

Figure 6 shows the changes in the non-punished and punished responses in the 3rd group of 6 rats after the combined administration of 1–10 mg/kg GT i.p. with 0.5 mg/kg diazepam s.c. Diazepam was administered 3 days after the administration of GT, and the data at the diazepam session are shown in this Fig. The results after the administration of saline+propylene glycol, saline+diazepam, and GT+propylene glycol are also shown in this Fig. Diazepam at 0.5 mg/kg moderately but significantly increased the punished response when compared with the saline+propylene glycol control value (P<0.05, Student’s t-test). GT at 1–10 mg/kg+propylene glycol also induced a significant increase in the punished response as compared with that after the saline+propylene glycol value (P<0.05 or 0.01). A prominent increase in the punished response was observed when diazepam was administered 3 days after the administration of GT. Here, the punished response rate after the combined administration of 1 and 3 mg/kg GT with 0.5 mg/kg diazepam was signifi-
cantly higher (P<0.05 or 0.01) than those after the administration of the corresponding single doses of GT or diazepam alone. No marked change in non-punished response was observed after the combined administration of GT and diazepam.

**Effects on gross behaviors:** After the administration of 0.1–10 mg/kg GT and 0.1–10 mg/kg taurine, no marked change in gross behavior such as ataxia and acceleration or depression of motor activity and drinking was observed. However, 0.5 mg/kg diazepam induced a slight ataxia.

**DISCUSSION**

The present experiment demonstrated that GT did not induce any change in either the avoidance responses or the DRL 20 sec water reinforcement response. However, GT induced a prominent increase in the punished response without eliciting a marked change in the non-punished response under the MULT FR 15/FR 15-punishment water reinforcement schedule. The increase in the punished response may not have been due to a drug-induced thirst because there was no marked change in the gross behaviors after GT. Furthermore, GT did not produce a marked change in either the daily pattern of drinking and the daily total water intake in the non-water deprived rats (unpublished data) or in the responses under the DRL 20 sec water reinforcement schedule and FR 15 non-punished response during the non-punished period under the MULT FR 15/FR 15-punishment schedule. Moreover, the increase in the punished response was also unlikely to be due to glutamic acid and/or taurine since we have previously reported (14) that both glutamic acid (over 1,000 mg/kg p.o.) and glutamate (over 500 mg/kg p.o. and s.c.) did not induce an increase in the punished response, but induced a transient decrease in the FR response. Taurine alone also did not elicit any change in the operant responses tested in the present schedules. These results suggest that the increase in the punished response was due to GT. Unfortunately, no study on the metabolic process of GT has been carried out after the systemic administration of GT. However, Török et al. (15) reported that GT might be synthesized in the rat brain. According to our experience,
intraventricular administration of 0.2–0.4 mg/kg GT induced no marked change in the gross behavior except for a slight sedation in mice (unpublished data).

Generally, it has been considered that anti-anxiety drugs specifically increase punished response (anticontlict effect) (12), while antipsychotic drugs suppress avoidance responses (16, 17). Therefore, it is considered that GT has a central action which correlates to the anticonflict effect. The effect of GT on the punished response was synergistic with that of diazepam, a prototype antianxiety drug. This result also supports the possibility that GT has an anticonflict effect. However, benzodiazepine derivatives, meprobamate and barbiturates sometimes elicit an anticonflict effect with ataxia (12, 13). In contrast, GT did not induce ataxia even though it produced a marked increase in the punished response. A clear dose-effect relationship could not be observed in the maximum level of increase in the punished response. Moreover, the behavioral change after GT persisted for a long time. These results suggest that the profile of the central action of GT is somewhat different from those of benzodiazepine derivatives, meprobamate, and barbiturates. However, the mechanism of the anticonflict effect of GT could not be explained only by the results of the present experiment. No peptide which has an anticonflict effect has been found yet. A further study must be carried out to investigate the roles of peptides including GT on animal behaviors, in particular on conflict behavior.

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