EFFECT OF TAURINE ON DEPOLARIZATIONS INDUCED BY
L-GLUTAMATE AND OTHER EXCITATORY AMINO ACIDS
IN THE ISOLATED SPINAL CORD OF THE FROG

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Abstract—The effect of taurine on depolarizing responses to L-glutamate (L-glu) was examined in isolated frog spinal cord using the sucrose gap method. Taurine (1 mM) reduced L-glu-induced depolarization in ventral and dorsal roots, even when calcium ion was deleted from or EGTA was added to the perfusate. GABA (1 mM) showed little or no effect on the L-glu response. A log dose-depolarization curve for L-glu was found to be shifted by taurine (1 mM) to the right in a non-parallel fashion. Strychnine blocked the taurine effect completely, while picrotoxin produced a partial reduction, and bicuculline exhibited no blockade at all. The depolarizations by 3-types of excitatory amino acids were also inhibited by taurine in the following order: N-methyl-D.L-aspartate > kainate > quisqualate. These results suggest a possibility that taurine but not GABA inhibits the depolarization mediated by excitatory amino acid receptors, and this may explain, in part, the inhibitory action of taurine in the central nervous system.

It has been suggested that taurine may function as a neuro-transmitter or neuromodulator in the central nervous system (CNS). Paying attention to a neuromodulatory role for taurine, especially its interaction with other transmitter candidates, presynaptically this amino acid has been shown to influence the release of transmitter candidates, L-glutamate (L-glu), γ-aminobutyric acid (GABA), noradrenaline and acetyl choline (1–3). On the other hand, many reports have provided evidence for the postsynaptic actions of taurine such as the increase of membrane conductance (4, 5), the decrease of the firing rate (6) and the reduction of synaptic potential induced by electrical stimulation (7). However, little is known about whether taurine modulates the postsynaptic action evoked by other transmitter candidates. Taurine also has pharmacologically strong anti-convulsant properties when applied intraventricularly, but not systemically (8, 9). The present study was designed to examine whether taurine affects postsynaptic neuronal excitations in the CNS. If taurine depresses the neuronal response to L-glu, a putative excitatory transmitter, the anti-convulsive action induced by this neutral amino acid may be, in part, interpreted. In order to investigate this interaction of taurine and L-glu, we used the isolated spinal cord of the frog for the following reasons: Recordings from ventral and dorsal roots of this preparation have been used in several laboratories to analyze pre- and postsynaptic actions of transmitter candidates (4, 6, 7); Amino acids tested on both frog and rat spinal neurons have shown parallel structure-activity relations (10–12); Test substances can be most easily applied to an isolated preparation in controlled concentrations.
Using the isolated spinal cord of the frog, a comparison was made of the taurine and GABA effects on L-glutamate-induced depolarization in ventral and dorsal roots.

**Materials and Methods**

All experiments were carried out with bullfrogs (*Rana catesbeiana*) weighing 100–200 g. The procedure for preparing the isolated perfused spinal cord was essentially the same as that described by Matsuura et al. (13) and Kudo et al. (14). The spinal cord with the 9th and 10th ventral and dorsal roots was carefully isolated, and this was perfused through a glass cannula, inserted into the anterior spinal artery, with oxygenated Ringer solution composed of 120 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 5.6 mM glucose and 1 mM Tris (hydroxymethyl) aminomethane (pH 7.3±0.1 adjusted by concentrated HCl, 16–18°C). All drug responses reported here were obtained with the medium to which 12 mM MgSO₄ was added and from which CaCl₂ was deleted, unless otherwise indicated.

The sucrose gap method was applied for recording DC-potential change in dorsal and ventral roots of the isolated spinal cord, using the same technique described previously (15). Potential differences between the spinal cord and the peripheral stumps of ventral or dorsal roots were detected by calomel electrodes.

Drugs used were: monosodium L-glutamate (Wako Pure Chemicals); taurine (Taisho Pharm.); γ-amino-n-butyric acid (GABA), glycol-etherdiamine tetraacetic acid (EGTA) and picrotoxin (Tokyo Kasei); bicuculline, N-methyl-D,L-aspartic acid, kainic acid and quisqualic acid (Sigma); and strychnine nitrate (Sanko).

**Results**

**Effects of taurine on L-glutamate-induced depolarizations:** All responses to amino acids were obtained in the Ca²⁺-free and 12 mM MgSO₄-added medium, with CaCl₂ deleted, unless otherwise indicated.

|   | Depolarization (mV) | Control (%) | N |
|---|---------------------|-------------|---|
| DR | 5 mM                | 0.61±0.32   | 97.3±6.8 | 5 |
|   | 7.5 mM              | 2.39±0.35   | 85.3±9.2 | 6 |
|   | 10 mM               | 4.53±0.84   | 65.3±5.3 | 7 |
|   | 12.5 mM             | 6.66±0.94   | 43.1±3.8 | 7 |
|   | Tau. 0.1 mM         | 2.45±0.40   | 70.3±13.2 | 6 |
|   | 1 mM                | 2.45±0.40   | 40.2±4.3 | 8 |
|   | GABA 1 mM           | 2.62±0.53   | 71.9±5.8 | 7 |
|   | 10 mM               | 6.29±0.69   | 36.2±2.8 | 4 |
| VR | 5 mM                | 0.75±0.27   | 100.7±3.1 | 7 |
|   | 7.5 mM              | 2.32±0.56   | 91.3±8.5 | 7 |
|   | 10 mM               | 5.15±0.83   | 65.4±6.5 | 7 |
|   | 12.5 mM             | 5.58±1.29   | 57.4±8.1 | 7 |
|   | Tau. 0.1 mM         | 1.74±0.43   | 64.7±10.5 | 6 |
|   | 1 mM                | 1.74±0.43   | 33.3±3.8 | 8 |
|   | GABA 10 mM          | 3.24±0.80   | 60.5±5.7 | 5 |

* a) The mean amplitude (±S.E.) of depolarizations evoked by high-K⁺, taurine or GABA alone. b) The mean relative amplitude (%±S.E.) of L-glutamate-induced depolarizations during application of high-K⁺, taurine or GABA, the value before K⁺, taurine or GABA application being taken as 100%. c) Number of experiments with different spinal cords. d) Little or no change in the membrane potential following 0.1 mM taurine application. DR: dorsal root, VR: ventral root.
Mg\(^{2+}\)-containing Ringer solution, the condition capable of effectively blocking chemical synaptic transmission (15). As shown in Table 1, the addition of 0.1 mM taurine to the medium scarcely altered the potential in either the ventral or the dorsal root. One mM taurine, however, evidently depolarized both ventral and dorsal roots, the dorsal root being somewhat more strongly depolarized than the ventral root (2.45 mV and 1.74 mV, Table 1). The application of L-glu to the cord preparation for a period of 30 sec produced an apparent depolarization with rapid onset and offset in both ventral and dorsal roots (Fig. 1-A). L-Glu was then tested after the preparation was exposed to 1 mM taurine for 4 min. As illustrated in Fig. 1, taurine application remarkably and reversibly inhibited the depolarization induced by L-glu infusion in both ventral and dorsal roots. In addition, L-glu-induced depolarization was markedly prolonged in the presence of 1 mM taurine (Fig. 1-A). Similar effects were confirmed to occur even in the presence of calcium ion, namely, in 1.8 mM Ca\(^{2+}\)-and 20 mM Mg\(^{2+}\)-containing Ringer solution (results not illustrated). With Ringer solution containing EGTA (1 mM), the application of taurine still reduced the depolarization induced by L-glu in ventral and dorsal roots (Fig. 2-A).

As shown in Fig. 3, 1 mM taurine shifted the log dose-response curve for L-glu in a non-parallel manner to the right.

Effect of GABA on L-glu-induced depolarization: In Ca\(^{2+}\)-free and 12 mM Mg\(^{2+}\)-...
containing Ringer solution, 1 mM GABA itself always caused a depolarizing response in the dorsal root (Table 1), while in the ventral root, a variable responses such as small hyperpolarizations, biphasic responses or very little voltage change were observed. After exposing the tissue preparation to 1 mM GABA for 4 min, the L-glu response was recorded. As illustrated in Fig. 4-A, 1 mM GABA application resulted in little or no reduction in the amplitude of depolarization evoked by L-glu in both ventral and dorsal roots. The L-glu response, however, was found to be greatly diminished by a higher concentration of GABA (10 mM), especially in the dorsal root (Fig. 4-B). At such a high concentration, GABA itself caused a large depolarization, especially in the dorsal root (6.29±0.69 mV, Table 1). We therefore examined whether the L-glu response was modulated by the high-K⁺ (5–12.5 mM) application which caused intensive depolarizations in both ventral and dorsal roots. As indicated in Table 1, the magnitudes of L-glutamate-evoked depolarizations in ventral and dorsal roots were diminished by the presence of high-K⁺ in a dose-dependent fashion. In the dorsal root, the amplitude of the depolarization induced by 7.5 mM K⁺ was similar to that by 1 mM GABA, 2.39 mV and 2.62 mV (Table 1); and moreover, the effect on the L-glu response of 7.5 mM K⁺ was also similar to that by 1 mM GABA (85.3% and 71.9%). Such a similarity was also observed between 12.5 mM K⁺ and 10 mM GABA.
(6.56 mV and 6.29 mV, 43.1% and 36.2%) (Table 1). In the ventral root, the depolarization produced by GABA itself was not so marked as that in dorsal root, namely, 3.24 mV at 10 mM GABA. The suppressive effect of 10 mM GABA on the L-glu response was similar to that of 0.1 mM taurine, the values being 60.5% and 64.7% (Table 1). This indicates that taurine depressed the responses to L-glu with a potency approx. 100 times that of GABA.

As shown in Fig. 5, L-glu responses at concentrations of 0.1–10 mM were only slightly affected by GABA (1 mM) in both ventral and dorsal roots.

Effects of strychnine, bicuculline and picrotoxin on the inhibitory action of taurine: In order to obtain further information about taurine action, inhibitory amino acid blockers, strychnine, bicuculline and picrotoxin, were tested for their antagonistic actions. As shown in Table 2, the inhibitory effect of taurine on L-glu-induced depolarization was completely antagonized by strychnine (0.1 mM) in both ventral and dorsal roots. Bicuculline (0.01 mM) did not affect the taurine action at all, while picrotoxin (0.1 mM) showed a moderate antagonism (Table 2). In addition, the taurine effect on the dorsal root was somewhat more sensitive to picrotoxin than that on the ventral root (Table 2).

Effect of taurine on three excitatory amino acids: It has been indicated that excitatory amino acid receptors may be divided into three types: kainate, N-methyl D-aspartate and quisqualate preferring receptors (16–18). The effects of taurine on the depolarizations induced by these 3 amino acids were then examined. In the dorsal root, taurine almost equally inhibited all depolarizations induced by kainate (0.01 mM), N-methyl D,L-aspartate (NMDL-A, 1 mM), quisqualate

![Fig. 5. Effect of GABA on the log dose-response curve for L-glutamate recorded from the ventral (VR) or the dorsal root (DR). Abscissa: L-glutamate concentration in log (mM). Ordinate: L-glutamate-induced depolarization (AVN), normalized with respect to the response to 1 mM L-glutamate. Each point is the mean±S.E. for 5 experiments. ○: VR response, control. △: DR response, control. ●: VR response, during GABA (1 mM). ▲: DR response, during GABA (1 mM).](image)

### Table 2. Effects of bicuculline, picrotoxin and strychnine on taurine action

|          | % Depolarization | VR       | DR       |
|----------|------------------|----------|----------|
| Control  |                  | 38.1±3.2 (14) | 44.4±4.0 (13) |
| Bicuculline (0.01 mM) |          | 39.4±4.1 (3) | 33.6±7.1 (3) |
| Picrotoxin (0.1 mM) |                  | 63.6±6.8 (5)** | 83.3±8.3 (5)** |
| Strychnine (0.1 mM) |                  | 102.7±3.4 (4)** | 109.5±3.2 (4)** |

% Depolarization: the mean relative amplitude (% ±S.E.) of L-glutamate-induced depolarizations during taurine application, the value before taurine application being taken as 100%, with and without (control) amino acid antagonists, the number of experiments being indicated in parentheses. For statistical analysis, Student's t-test was used. **P<0.01, ***P<0.001 as compared to the control.
Table 3. Effect of taurine on the depolarization evoked by L-glutamate and three excitants

|                | % Depolarization |
|----------------|------------------|
|                | VR (mM)          | DR (mM)          |
| L-glutamate 1 mM | 38.1±3.2 (14)    | 44.4±4.0 (13)    |
| NMDL-A 1 mM    | 23.6±2.0 (5)*    | 33.9±4.8 (5)     |
| Kainate 0.01 mM | 40.0±6.4 (6)     | 41.3±3.6 (6)     |
| Quisqualate 0.005 mM | 53.9±6.5 (6)*   | 44.3±5.4 (4)     |

% Depolarization: the mean relative amplitude (% ±S.E.) of the depolarizations elicited by L-glutamate or three excitants, the value before 1 mM taurine application being taken as 100%. The number of experiments are indicated in parentheses. *P<0.05 as compared to L-glutamate (38.1±3.2%).

(0.005 mM) and L-glutamate (1 mM, Table 3). In the ventral root, the depolarization evoked by 3 types of excitatory amino acids as well as L-glutamate were also inhibited by taurine in the following order: NMDL-A>L-glutamate>kainate>quisqualate (Table 3).

Discussion

The purpose of this study was to explore the ability of neuroinhibitory amino acids, taurine and GABA, to antagonize L-glutamate responses in the ventral and dorsal roots, probably in motoneurones and primary afferent terminals, respectively. Taurine at 0.1 mM, which caused little or no change in the memran potential (Table 1), significantly reduced the L-glutamate response (Figs. 1 and 2). In addition, 1 mM taurine, which itself depolarized the ventral and dorsal roots (Table 1), significantly suppressed the response to L-glutamate (Figs. 1 and 2). Thus, taurine was found to antagonize the L-glutamate response. It was also shown that the taurine action is little altered by the presence and absence of calcium ion in the perfusion solution (Figs. 1 and 2), suggesting that the effect is independent of external calcium ion, although possible interactions between taurine and calcium ion have been described by others (1, 19–21). This discrepancy might arise from the concentration of taurine used and also from the difference in experimental animals or organs, but the reason remains obscure at present.

The log dose-depolarization curve for L-glutamate was shifted by taurine (1 mM) to the right in an apparently non-parallel fashion (Fig. 3); thus taurine seems to be a non-competitive antagonist of L-glutamate in the frog spinal cord.

This taurine effect probably does not occur at the level of the L-glutamate receptor sites since this neutral amino acid does not appear to interfere with the binding of L-glutamate to neuronal membrane fractions (22). In view of the well-known Na dependency of the L-glutamate response, the results obtained here might indicate that taurine influences Na channel to be activated by L-glutamate (22).

The suppressive action of taurine on the L-glutamate response was completely antagonized by strychnine, moderately by picrotoxin, but not by bicuculline (Table 2). These findings may suggest that taurine acts on either the receptor or linked ionophores for glycine-like amino acids, but not GABA.

The depolarization of frog motoneurones produced by L-glutamate was often followed by a hyperpolarization (Fig. 1-A). Although the precise mechanism underlying this hyperpolarization is not known, it is reasonable to assume that it is due to the activation of a membrane pump which extrudes the intracellular Na accumulated during neuronal depolarization (23). If this is the case, it might be possible to attribute the prolongation of L-glutamate-induced depolarization by
taurine application (Fig. 1-A) to the suppression of the membrane pump by taurine.

High dose of GABA (10 mM) significantly inhibited the L-glu response, but low doses (0.1 and 1 mM) did not (Fig. 1). The reduction of the L-glu response was also observed during the depolarization caused by high-K+ (Table 1). The extent of this reduction depended on the magnitude of the K+-induced depolarization. The interpretation for this finding could be that high-K+ solution shifts the membrane potential closer to the equilibrium potential of the ion involved in the L-glu response, probably Na+ ion, thereby decreasing the electrochemical gradient for this ion across the membrane. In addition, high concentration of GABA (10 mM) is known to increase extracellular K+ in the frog spinal cord (24) and also to produce a reproducible depolarization in the nerve fiber which had been severed from the spinal cord (4). It is therefore possible that the suppression of the L-glu response by high dose of GABA (10 mM) is ascribed to the elevation of extracellular K+ and/or the huge depolarization induced by this neutral amino acid.

In view of the concept that excitatory amino acid receptors in the vertebrate CNS may be classified into three different types (16-18), the effects of taurine on neuronal depolarizations induced by 4 different excitatory amino acids (Table 3) were also studied. The presence of such receptor populations in these preparations, however, could not be detected using the taurine action as an index since the responses elicited by kainate, quisqualate and NMDL-A as well as L-glu were all significantly suppressed by taurine (1 mM, Table 3).

It has been suggested that L-glu could function as an excitatory neurotransmitter in a variety of CNS neurones. In addition, this amino acid is known to produce convulsions when administered either systemically or intracerebrally (25). Our results that the L-glu response is markedly suppressed by taurine may partly explain the anti-convulsive effect of taurine.

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