INHIBITORY ACTION OF TAURINE ON MOTONEURON OF FROG SPINAL CORD

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(Received January 17, 1985)

Using the sucrose gap method, the effect of taurine on the spinal cord of the bullfrog was investigated. Taurine inhibited the spontaneous potential and the ventral root reflex potential elicited by the stimulation of the dorsal root. The excitability of motoneuron did not necessarily diminish following taurine application. Taurine inhibited the responses induced by excitatory neurotransmitter candidates such as, glutamate and substance P, in a concentration dependent manner in the preparation, where the neurotransmission was abolished by Ca²⁺-deprivation and/or Mg²⁺-supplement. From these results, the depressant action of taurine on the electrical activities in the frog spinal cord may be interpreted, in part, by the inhibition of the response evoked by excitatory transmitter at postsynaptic site.

Keywords — taurine; glutamate; substance P; spinal cord; reflex; motoneuron

INTRODUCTION

A report that taurine level in the central nervous system (CNS) is high has provided the basis for the suggestion that taurine plays an important role in the physiology of the CNS, namely neurotransmitter or neuromodulator.¹ Neuropharmacologically, taurine has been shown to exhibit a strong inhibitory action on neurons,² when applied directly to the CNS. In the present study, we investigated the effect of taurine on neurons using the isolated frog spinal cord, which can provide apparently normal biological responses for a long time and has been used to analyze the actions of chemical substances.³ Moreover, this preparation will serve for analyzing the actions of the drugs which do not penetrate the blood-brain barrier. We now report that taurine exerts the strong inhibitory effect on the spinal cord of the frog and blocks the depolarization induced by substance P as well as glutamate.

MATERIALS AND METHODS

Experiments were conducted on the isolated and perfused spinal cord of bullfrogs (Rana catesbeiana), weighing 100—150 g. The technique for preparing the isolated spinal cord was essentially the same as that described by Kudo.⁴ The spinal cord was carefully isolated with the 9th and 10th ventral and dorsal roots. Ringer solution was perfused through a glass cannula inserted into the anterior spinal artery as soon as possible. The perfusing rate was 0.5 ml/min and temperature was maintained at 16—18 °C. Ringer solution consisted 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 1 N HCl) and bubbled with 100% oxygen. In some experiments, all or part of Ca²⁺ ion was removed and Mg²⁺ ion (3—12 mM as MgSO₄) was added to Ringer solution. The membrane potential of the ventral root was recorded using the sucrose gap method.⁵ Calomel electrodes were used to measure the difference in potential between a spinal cord and a peripheral stump of the ventral root. Reflex potential in the ventral root was elicited by the stimulation of the dorsal root which was put on bipolar platinum electrodes. The dorsal root was stimulated with supramaximal rectangular pulses (0.05 ms duration and a frequency of 0.2 Hz). The excitability of motoneuron was also
measured by Wall's technique. A tungsten microelectrode was inserted into the motoneuron pool, which was stimulated by a submaximum current pulse (0.2 Hz, 0.05 ms), and the evoked potential was measured at the ventral root by a sucrose gap method. Drugs used were: L-glutamate monosodium salt (Wako Pure Chem.); taurine (Tokyo Kasei); substance P (Protein Research Foundation).

RESULTS
1) Effect of Taurine on the Ventral Root Potential
In normal Ringer solution, the spontaneous fluctuating potential was recorded in the ventral root of the frog spinal cord. As shown in Fig. 1, the spontaneous potential faded out when 1 mM taurine was applied to the spinal cord. The membrane potential also changed variously. Namely, taurine caused a depolarization, hyperpolarization or no change in membrane potential. The spontaneous potential reappeared after the cessation of the taurine perfusion.

The ventral root potential evoked by the stimulation of the dorsal root diminished following the application of 1 mM taurine (Fig. 2). The reflex potential restored to the control level by perfusing with the medium without taurine.

2) Effect of Taurine on the Excitability of the Ventral Root
Fig. 3 shows the effect of taurine on the excitability of the motoneuron detected at the ventral root. Since taurine variously shifted the membrane potential of the motoneuron, the excitability of the motoneuron was diversely modified by the amino acid. When the membrane potential was depolarized by taurine, the excitability usually rose (Fig. 3). Contrary, the excitability reduced following the hyperpolarization induced by the amino acid.

FIG. 1. Effect of Taurine (TAU) on the Ventral Root Potential
Direct current potential in the ventral root was measured by sucrose gap method. Note that the spontaneous potential disappeared following 1 mM taurine application. The horizontal bar indicates duration of exposure of taurine.

A control  B TAU 5 min
C TAU 10 min  D wash

FIG. 2. Change of Ventral Root Potential Elicited by the Stimulation of the Dorsal Root Following 1 mM Taurine Application
D shows the ventral root potential 30 min after washing taurine.

A control  B TAU 10 min
C TAU 20 min  D TAU 30 min

FIG. 3. Effect of 1 mM Taurine on Excitability of Motoneurons
An example showing little change of the motoneuron excitability by taurine.
TABLE I. Change by Mg\(^{2+}\) Concentrations of Taurine Effect on Glutamate Response

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>No. of preparations</th>
<th>Glutamate response during taurine (%)</th>
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</thead>
<tbody>
<tr>
<td>Ca(^{2+}) (mM)</td>
<td>Mg(^{2+}) (mM)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>1.8</td>
<td>20</td>
<td>5</td>
</tr>
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</table>
| a) Values represent the mean±S.E.M. of the relative glutamate responses during taurine application, the value before taurine application being taken as 100%

3) Inhibitory Action of Taurine on the Depolarization Induced by Glutamate

In order to prevent the interneuronal effect, 20 mM Mg\(^{2+}\) ion was added to the perfusate. In this condition, the ventral root potential induced by the stimulation of the dorsal root disappeared. The depolarization of the motoneuron induced by 1 mM glutamate was reduced by 1 mM taurine (Table I). Other treatment was employed to block the synaptic transmission. Ca\(^{2+}\) ion was omitted and Mg\(^{2+}\) ion was added to the perfusate. As shown in Table I, the inhibitory action of taurine reduced as Mg\(^{2+}\) ion added to the perfusate was increased.

4) Effect of Taurine on the Depolarization Induced by Substance P

In a normal Ringer, substance P depolarized the motoneuron but the depolarization was not reproducible. In Ca\(^{2+}\) free and 12 mM Mg\(^{2+}\)-Ringer, the response was also not reproducible. Then, the perfusate was selected that Ca\(^{2+}\) ion was diminished to one tenth (0.18 mM) and 6 mM of Mg\(^{2+}\) ion was added. In this condition, the reflex potential evoked by the stimulation of the dorsal root disappeared. The repetitive applications of substance P (0.05 mM) induced the same depolarization of the motoneuron. Fig. 4 shows the effect of taurine on the substance P response. The substance P response was reduced by taurine (0.1 mM) and reappeared following the wash of taurine. The substance P response disappeared with the application of taurine (1 mM), being shown in Fig. 4 and Table II.

5) Effect of Taurine on the Glutamate Response in the Low Ca\(^{2+}\) and Mg\(^{2+}\) Added Perfusate

The glutamate (0.5 mM) response was stable on the repetitive application in the same perfusate.

![Graph showing reduction of Substance P (SP) response following taurine application in the ventral root. SP (0.05 mM) was applied for 30 s, a period indicated by the bar. The horizontal bar indicates duration of exposure of taurine. Recovery from taurine effect on SP response was slow following the washout of a higher concentration (1 mM) of taurine.]

![Graph showing effect of taurine on glutamate (Glu) response in the ventral root. Glutamate (0.5 mM) was applied for 30 s, a period indicated by the bar. Glutamate response still appeared after 1 mM taurine application.]

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ate as that used in the substance P experiment. The application of taurine to the spinal cord reduced the glutamate response (Fig. 5 and Table II).

DISCUSSION
Taurine inhibited the spontaneous potential in the motoneuron and the ventral root potential evoked by the stimulation of the dorsal root in the frog spinal cord preparation. But the excitability of the motoneuron was not necessarily depressed by the application of taurine. Probably, the excitability of the motoneuron depends on the membrane potential produced by taurine. From these results, the inhibitory action of taurine on the motoneuron is unlikely to be interpreted by the reduction of the motoneuron excitability.

Then, we examined the effect of taurine on the excitatory transmitter candidates, glutamate and substance P. Taurine inhibited the glutamate response. The inhibitory action of taurine was augmented by reducing the external concentration of Mg$^{2+}$ ion. One possible explanation for this result is that Mg$^{2+}$ ion blocks the inhibitory action of taurine. However, this is unlikely since there is no difference between the effects of taurine in the perfusates containing 12 and 20 mM Mg$^{2+}$ ion (Table I). Another explanation is possible. Evans et al. proposed that there are two types of receptors for excitatory amino acids which are sensitive and non-sensitive to Mg$^{2+}$ ion. Accordingly, taurine may strongly inhibit the glutamate response sensitive to Mg$^{2+}$ ion. At present, however, we have no data to resolve this problem.

Substance P caused depolarizations in the motoneuron, which was reproducible in 0.18 mM Ca$^{2+}$ and 6 mM Mg$^{2+}$ perfusate. Taurine inhibited the substance P response in a dose-dependent manner. A high concentration of taurine (1 mM) abolished the depolarization induced by substance P. Recently we reported that taurine inhibited the depolarization induced by various excitatory amino acids. These responses never disappeared with the application of taurine (1 mM). In the present experiment, we confirmed that 1 mM taurine also did not abolish the glutamate response in 0.18 mM Ca$^{2+}$ and 6 mM Mg$^{2+}$ perfusate. These results suggest that substance P response is very sensitive to taurine in the frog spinal cord.

In conclusion, the inhibitory effect of taurine on the transynaptic activity in the frog spinal cord can be interpreted, in part, by the inhibition of the responses evoked by some excitatory transmitters, such as glutamate and substance P.

REFERENCES

<table>
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<tr>
<th>Table II. Inhibitory Action of Taurine on Substance P and Glutamate Responses in 0.18 mM Ca$^{2+}$ and 6 mM Mg$^{2+}$ Ringer</th>
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<tbody>
<tr>
<td>Taurine (mM)</td>
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<tr>
<td>--------------</td>
</tr>
<tr>
<td>0.01</td>
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<tr>
<td>0.1</td>
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<td>1</td>
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$^{a)}$ Values represent the mean S.E.M. of the relative substance P or glutamate responses during taurine application, the value before taurine application being taken as 100%. A number in parentheses indicates the number of preparations.