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Identification of two taurine receptor subtypes on the primary afferent terminal of frog spinal cord

[1]Yoshihisa Kudo, Eri Akiyoshi & Hiroyuki Akagi

Laboratory of Neuropharmacology, Department of Neuroscience, Mitsubishi-Kasei Institute of Life Sciences, Minamiooya 11, Machida, Tokyo 194, Japan

1 Effects of taurine on primary afferent terminals in the frog spinal cord were examined by a sucrose-gap method applied to a dorsal root (9th or 10th segment).

2 In a normal Ringer solution, taurine (1 mM, applied for 5 s at a rate of 0.04 ml s^{-1}, 0.2 μmol) caused a hyperpolarization, but a higher concentration (10 mM, applied at the same rate, 2.0 μmol) caused a biphasic response consisting of a hyperpolarization followed by a slow onset depolarization. A similar biphasic response could also be observed in tetrodotoxin-treated preparations.

3 When the concentration of extracellular Mg^{2+} was increased up to 9.0 mM, the depolarizing response to taurine was augmented. The rate of the augmentation was dependent upon the extracellular Mg^{2+} concentration.

4 The depolarizing effect was selectively antagonized by bicuculline in concentrations (10–30 μM) that had no significant antagonizing action on γ-aminobutyric acid (GABA)-induced depolarization. On the other hand the hyperpolarizing effect of taurine was selectively reduced by strychnine (0.1 μM) which had no antagonizing effect on responses to glycine.

5 These results suggest that in the frog spinal cord there are at least two subtypes of taurine receptor whose pharmacological profiles resemble GABA and glycine receptors in the mammalian central nervous system, and whose sensitivity may be modulated by extracellular Mg^{2+}.

Introduction

Taurine, an amino acid containing sulphoxyl group, has been postulated as an inhibitory neurotransmitter in the vertebrate central nervous system (Haas & Hosli, 1973; Kaczmarek & Adey, 1974; McBride and Frederickson, 1980; Okamato & Sakai, 1980). Previous studies revealed that strychnine, a glycine antagonist, greatly reduced or abolished the depolarization in the dorsal root induced by taurine and by antidromic stimulation of the ventral root in the frog spinal cord (Barker *et al.*, 1975a; Nicoll *et al.*, 1976; Kudo *et al.*, 1983). Moreover we found that dendrobine, an alkaloid which was reported to block the inhibitory effect of glycine on the cat spinal cord (Curtis *et al.*, 1973), blocked the effect of taurine and the presynaptic inhibition in the frog spinal cord (Kudo *et al.*, 1983). Although in the mammalian central nervous system bicuculline is known to be a potent competitive antagonist of the effect of γ-aminobutyric acid (GABA) (Curtis *et al.*, 1971), we could not observe a clear antagonistic action of bicuculline on the depolarization of the dorsal root induced by GABA in the frog spinal cord (Kudo *et al.*, 1981). These results support the suggestion that taurine rather than GABA may participate in the presynaptic inhibition induced by antidromic stimulation of the ventral root in the frog spinal cord (Barker *et al.*, 1975b). In this paper we report a further study of the effect of taurine on the primary afferent terminal of the frog spinal cord.

[1] Author for correspondence.

Methods

Isolated, intra-arterially perfused spinal cords of the bullfrog (*Rana catesbiana*, 100–150 g) were used. The technique for preparing the cords was the same as that described elsewhere (Kudo *et al.*, 1975; Kudo, 1978). An arterial cannula (about 200 μm in diameter) was inserted into the ventral spinal artery and the spinal cord was perfused with Ringer solution having the following composition (mM): NaCl 117, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.1, glucose 5.5,

NaH_2PO_4 0.25, with the pH adjusted to 7.6 ± 0.1 by addition of $NaHCO_3$. In some experiments concentrations of Ca^{2+} and Mg^{2+} were changed as indicated in the text. The perfusion rate was approx. 0.3 ml min^{-1}. The temperature of the recording chamber was kept at 20°C ± 1°C with a thermomodule temperature control unit (Daia medical system, DTC-100). The potential difference between the spinal cord and the peripheral dorsal root stump (9th dorsal root) was assessed by means of a sucrose-gap method (Kudo *et al.*, 1975; Kudo, 1978). The 10th dorsal root was stimulated to evoke the dorsal root potential. Taurine (Wako Pure Chem.) or GABA (Wako Pure Chem.) was dissolved in Ringer solution in a concentration of 1–10 mM and perfused for 5 s at a rate of 0.04 ml s^{-1} (0.2–2 μmol) through fine polyethylene tubing placed in a glass cannula for perfusion using a microtube pump (LKB 2155) and timer-operated three-way electric valves (General valve, USCC1T-3C-12D). Bicuculline (Sigma) was dissolved in a small amount of 0.1 N HCl and diluted to make a stock solution (1 mM), which was further diluted with Ringer solution to 10–30 μM before use (pH 7.6 ± 0.1). Strychnine (Sigma) was also prepared as a stock solution (0.1 mM) and diluted with Ringer solution to 0.1 μM (pH 7.6 ± 0.1). These antagonists were applied by replacing the perfusing medium with drug-containing Ringer solution.

Results

Biphasic effect of taurine on the primary afferent terminal

Taurine in a lower concentration (1 mM applied for 5 s, 0.2 μmol) caused a marked hyperpolarization of the primary afferent terminal but had no substantial effect on the dorsal root potentials induced by the stimulation of the adjacent dorsal root (DR-DRP) ($n = 13$ Figure 1a). However, a higher concentration of taurine (10 mM applied for 5 s, 2.0 μmol) caused a fast-onset hyperpolarization which was followed by a slow-onset depolarization, while DR-DRPs were greatly reduced ($n = 16$, Figure 1b). Since a biphasic action of taurine could also be observed in preparations treated with tetrodotoxin (0.3 μM), the effects of the amino acid could be ascribed to a direct action on the primary afferent terminal ($n = 3$, Figure 1c). On the other hand GABA (1 mM applied for 5 s at the same rate as above, 0.2 μmol) caused a marked depolarization which was never preceded by a hyperpolarization (Figure 1d).

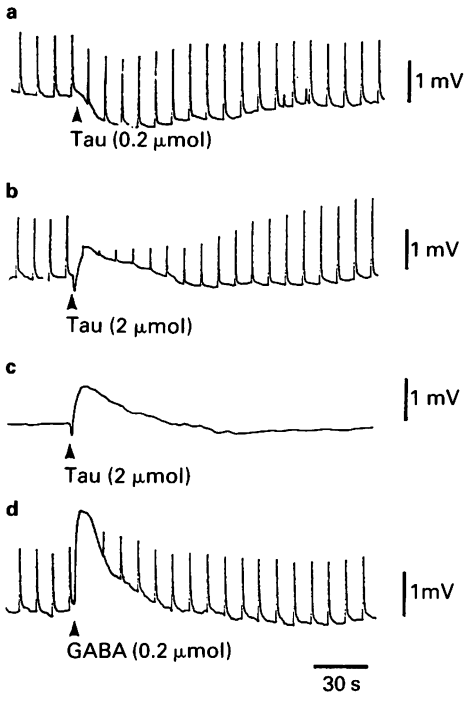

Figure 1 Effects of taurine and γ-aminobutyric acid (GABA) on the primary afferent terminal of the frog spinal cord. (a) Taurine (Tau) (1 mM applied for 5 s at a rate of 0.04 ml s^{-1}, 0.2 μmol) cause a hyperpolarization. (b) A higher concentration of taurine (10 mM applied for 5 s at the same rate as above, 2.0 μmol) induced a hyperpolarization followed by a slow-onset depolarization. (c) The biphasic response to taurine could also be observed in a preparation treated with tetrodotoxin (0.3 μM). (d) GABA (1 mM applied for 5 s at the same rate as above, 0.2 μmol) caused a fast-onset depolarization which was not preceded by a hyperpolarization.

Influences of extracellular Mg^{2+} on the effects of taurine

The taurine-induced biphasic effects were markedly influenced by the concentration of extracellular Mg^{2+}. The hyperpolarization induced by taurine (1 mM applied for 5 s) changed to a biphasic response consisting of an initial fast-onset hyperpolarization followed by a marked depolarization when the preparation was perfused with low Ca^{2+} (0.1 mM) and high Mg^{2+} (9.0 mM) medium to abolish synaptic transmission. On the other hand the amplitude of GABA-induced depolarization was not influenced substantially after treatment with high Mg^{2+} Ringer solution. When the extracellular Mg^{2+} concentration was varied from 0.1 to 9.0 mM (Ca^{2+} concentration was fixed at 1.8 mM), the amplitude of the

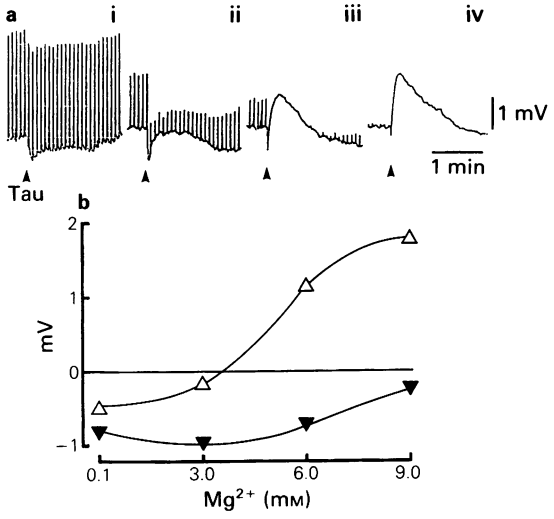

Figure 2 Change in the shape of the primary afferent response to taurine in medium including various concentrations of Mg^{2+}. (a) Representative data for the changes in the shape of taurine (Tau) (10 mM applied for 5 s, 2 μmol)-induced potential in the primary afferent terminal. Mg^{2+} concentration was increased from (i) 0.1 to (ii) 3.0, (iii) 6.0 and (iv) 9.0 mM, the Ca^{2+} concentration being maintained at 1.8 mM. (b) The size (in mV) of hyperpolarization (▼) and depolarization (△) induced by taurine plotted against the concentration of Mg^{2+}.

depolarization induced by taurine (10 mM applied for 5 s) increased and that of the hyperpolarization apparently decreased as the Mg^{2+} concentration increased (Figure 2a and b). The effects of Mg^{2+} were shown to be reproducible in five separate preparations. In two preparations perfused with media in which the Mg^{2+} concentration was fixed at 0.1 mM and the concentration of Ca^{2+} was increased to 3–10 mM, we could not observe augmentation of the amplitude of the depolarization as was observed in media containing high Mg^{2+} concentrations. When Ni^{2+} or Mn^{2+} was used as a substitute for Mg^{2+}, there was no augmentation of the taurine-induced slow onset depolarization ($n = 2$).

Table 1 Hyperpolarization and depolarization of the primary afferent terminal induced by taurine and effects of strychnine and bicuculline on them

Antagonists	n	*% response to taurine* *Hyperpolarization*	*Depolarization*
Strychnine (0.1 μM)	4	0.0 ± 0.0	85.4 ± 6.8
Recovery (Wash 30 min)		37.4 ± 16.5	96.5 ± 6.4
Bicuculline (30 μM)	4	180.7 ± 10.5	38.2 ± 8.5
Recovery (Wash 30 min)		118.2 ± 20.4	110.3 ± 10.6

The % responses were calculated dividing the amplitude of the response to taurine in the presence of the antagonist (for 20 min) by that of the control response (before the application of antagonist). Each value is the mean ± s.e.mean. Depolarization-dominant effect of taurine (10 mM applied for 5 s at rate of 0.04 ml s^{-1}, 2.0 μmol) obtained in a medium containing Mg^{2+} 9.0 mM and Ca^{2+} 0.1 mM.

Effects of bicuculline and strychnine on taurine-induced biphasic responses

Since a marked antagonizing effect of strychnine on the taurine-induced depolarization on the primary afferent terminal of the frog spinal cord has been reported (Barker *et al.*, 1975a; Nistri & Morelli, 1978; Kudo *et al.*, 1983), we used strychnine as an antagonist for taurine to examine the profiles of the biphasic effects. When a higher concentration of strychnine (10 mM) which was used in the previous studies was applied, the effects of taurine on the primary afferent terminal was greatly reduced (Table 2). However, a much lower concentration of strychnine (0.1 μM) selectively antagonized the fast onset hyperpolarization (Figure 3a upper trace, Table 1) which was induced by a higher concentration of taurine (10 mM applied for 5 s, 2.0 μmol) in a high Mg^{2+} (9.0 mM) and low Ca^{2+} (0.1 mM) medium. In this condition, the hyperpolarization was apparently abolished, while the depolarizing response was minimally affected. The recovery of the depolarization after 30 min washing was almost complete, but that of the hyperpolarization was at most 40% (Table 1). In preparations perfused with a medium containing Mg^{2+} (3.0 mM) and Ca^{2+} (0.1 mM), a lower concentration of taurine (1 mM applied for 5 s, 0.2 μmol) caused a 'hyperpolarization-dominant' effect. Strychnine (0.1 μM) markedly reduced the hyperpolarization in these preparations (Figure 3a, lower trace). Strychnine in concentrations of 0.1 and 10 μM markedly augmented the depolarizing effect of glycine on the primary afferent terminal (Table 2) as has been reported for the motoneurone of frog spinal cord (Evans *et al.*, 1976).

Since bicuculline was reported to reduce the depolarizing effect of β-alanine whose structure and pharmacological properties are quite similar to those of taurine (Barker *et al.*, 1975a), we tested the effect of bicuculline as an antagonist of the depolarizing action induced by taurine. As shown in the upper trace in Figure 3b and Tables 1 and 2, bicuculline (10–30 μM) selectively and reversibly reduced the depolarization induced by taurine (10 mM applied for 5 s, 2.0 μmol), while the hyperpolarization was apparently augmented. In the preparation where a 'hyperpolarization-dominant' response to taurine

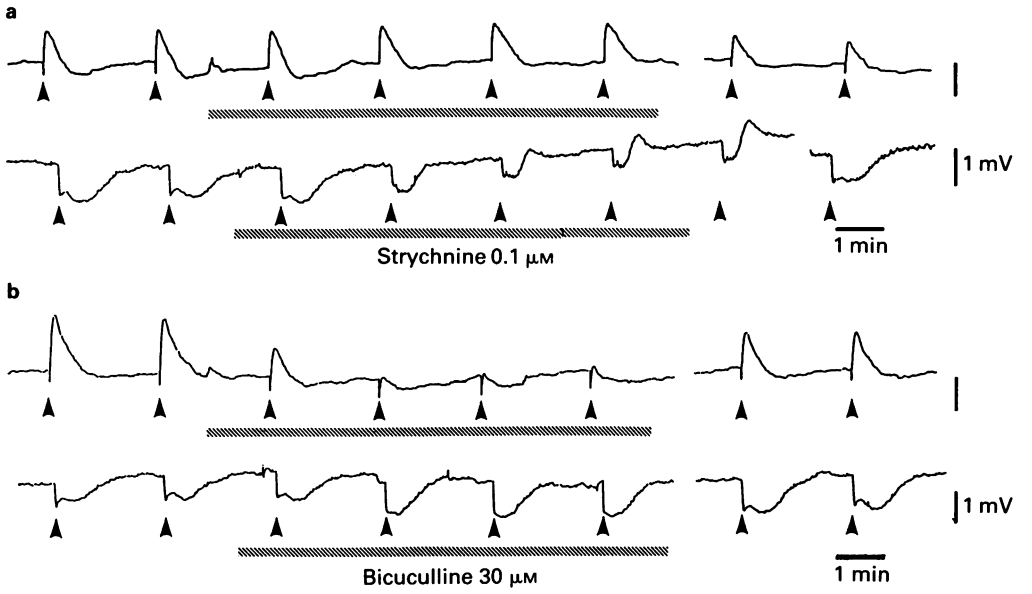

Figure 3 Effects of strychnine and bicuculline on the taurine-induced potentials in the primary afferent terminal. The effects of bicuculline (30 μM) and strychnine (0.1 μM) on the potentials induced by taurine were tested in preparations perfused with a medium containing high Mg^{2+} (9.0 mM) and low Ca^{2+} (0.1 mM) (upper traces in a and b) and perfused with a medium containing lower Mg^{2+} (3.0 mM) and 0.1 mM Ca^{2+} (lower traces in a and b). In the former medium a higher concentration of taurine (10 mM applied for 5 s at the same rate as described in Figure 2) caused depolarization-dominant effects. In the latter medium the amino acid in a lower concentration (1 mM applied for 5 s at the same rate as above) caused hyperpolarization-dominant effects. At arrow heads taurine was infused once every 5 min. Strychnine or bicuculline was applied during the time indicated by hatched bars. Breaks in the recording after the withdrawal of antagonists indicate 30 min pause in recording.

was observed as described above, bicuculline (30 μM) apparently augmented the hyperpolarization, which may be attributable to the unmasking effect of the agent by the selective antagonizing action on taurine-induced depolarization (Figure 3b, lower trace). Interestingly bicuculline (10 and 30 μM) showed no antagonizing action on the depolarization of the primary afferent terminal induced by GABA (1 mM applied 5 s, 0.2 μmol) (Table 2).

Discussion

The present results suggest the existence of two discrete subtypes of the taurine receptor on the primary afferent terminal of the frog spinal cord. Taurine has previously been reported to have mainly a depolarizing effect on the primary afferent terminal of the frog spinal cord (Barker *et al.*, 1975a; Nicoll *et al.*, 1976; Nistri & Constanti, 1976), although a hyperpo-

Table 2 Effects of strychnine and bicuculline on the inhibitory amino acid-induced depolarization in the primary afferent terminal of the frog spinal cord

Agents	*Dose* (μM)	% *depolarizing response to*		
		GABA (n)	*Glycine* (n)	*Taurine* (n)
Strychnine	0.1	100.6 ± 0.8 (4)	144.7 ± 17.8 (4)	85.4 ± 6.8 (4)
	10	97.2 ± 7.9 (5)	180.7 ± 12.1 (3)	7.7 ± 4.8 (5)
Bicuculline	10	92.2 ± 6.6 (4)	98.6 ± 2.5 (4)	50.4 ± 8.4 (4)
	30	90.6 ± 5.2 (3)	102.5 ± 4.5 (3)	38.2 ± 8.5 (4)

The % responses were calculated in the same way as described in Table 1. Each value is the mean ± s.e.mean. γ-Aminobutyric acid (GABA, 0.2 μmol), glycine (2.0 μmol) and taurine (2.0 μmol) were applied in the same way as described in Table 1.

larizing effect has also been observed (Ono *et al.*, 1983). Differences in Mg^{2+} concentration may account for this discrepancy. Studies where taurine caused depolarization generally employed high concentrations of Mg^{2+} to block neurotransmission (e.g. Barker *et al.*, 1975a, used 20 mM Mg^{2+}). In contrast, studies in which the amino acid caused hyperpolarization used Mg^{2+}-free Ringer solution (Ono *et al.*, 1983). In the present study the effect of taurine on the primary afferent terminal was found to be dependent upon the extracellular Mg^{2+} concentration. Although we do not know the mechanism of action of Mg^{2+} on the taurine-induced effect, one possibility is a direct modulatory effect of extracellular Mg^{2+} on the taurine receptor or some Mg^{2+}-dependent endogenous modulatory mechanism. Such a modulatory effect of Mg^{2+} is reminiscent of the antagonizing effect of the ion on the excitatory amino acid receptor (Ault *et al.*, 1980) and the modulation of the sensitivity of the N-methyl-D-aspartic acid receptor by the ion (Nowak *et al.*, 1984).

One of the important findings in the present study is the characteristic antagonizing actions of strychnine and bicuculline on the biphasic effect of taurine. The selective antagonizing action of strychnine on the hyperpolarizing action of taurine was obvious only at lower concentrations (less than 0.1 μM). The agent never antagonized the depolarization induced by glycine even at a concentration of 10 μM. Unexpectedly, the selectivity of bicuculline as an antagonist of taurine-induced depolarization was quite high. As we have already reported (Kudo *et al.*, 1981) and have confirmed in the present study, bicuculline (10 μM) did not antagonize GABA-induced depolarization of the primary afferent terminal of the bullfrog spinal cord, whereas the agent antagonized the taurine-induced depolarization. The apparent augmentation of the hyperpolarization is presumably due to the selective inhibition of the depolarizing response.

The present results support the suggestion that taurine may participate as an inhibitory neurotransmitter in the characteristic presynaptic inhibition induced by antidromic stimulation of the ventral root (Barker *et al.*, 1975b). Furthermore our results suggest that taurine has two receptor subtypes that are distinct from GABA and glycine receptors but have selective sensitivity to bicuculline and strychnine in the primary afferent terminal of the frog spinal cord. Frog spinal cord may provide some important clues to reveal the evolutionary changes in the inhibitory system in the vertebrate central nervous system.

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