Paired-pulse depression of the N-methyl-D-aspartate receptor-mediated synaptic potentials in the amygdala

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- 1 An *in vitro* slice preparation of rat amygdala was used to study the paired-pulse depression of the N-methyl-D-aspartate (NMDA) receptor-mediated synaptic potential e.p.s.p._{NMDA}.
- 2 The e.p.s.p._{NMDA} was isolated pharmacologically by applying a solution containing the non-NMDA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and the γ -aminobutyric acid_A (GABA_A) blocker picrotoxin and increasing the stimulus intensity.
- 3 When two stimuli of identical strength were applied in close succession, the second e.p.s.p._{NMDA} was depressed. This paired-pulse depression was seen with interstimulus intervals of between 100 ms and 2000 ms; the maximal depression was observed at interval of 200 ms.
- 4 Superfusion of phaclofen or 2-hydroxy-saclofen inhibited the paired-pulse depression indicating the involvement of GABA_B receptors.
- 5 Bath applications of Ba²⁺ or intracellular injection of Cs⁺ to block post- but not presynaptic GABA_B receptors failed to inhibit the paired-pulse depression (PPD).
- 6 Incubation of slices with pertussis toxin prevented the postsynaptic hyperpolarization induced by baclofen. The PPD of e.p.s.p._{NMDA}, however, was not affected by pertussis toxin treatment.
- 7 These results suggest that GABA released by the first stimulus acts on GABA_B receptors to suppress the second e.p.s.p._{NMDA} via mechanisms other than activation of a postsynaptic GABA_B receptor-coupled K⁺ conductance.

Keywords: N-methyl-D-aspartate receptor; GABA_B receptor; paired-pulse depression

Introduction

Electrophysiological and immunohistochemical studies have established that γ -aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the amygdala (Le Gal La Salle, 1976; Takagi & Yamamoto, 1981; McDonald, 1985). Synaptic release of GABA from inhibitory interneurons produced a dual component inhibitory postsynaptic potential (i.p.s.p.) in the basolateral amygdala (BLA): a fast, bicuculline-sensitive increase in Cl⁻ conductance mediated by GABA_A receptors (f-i.p.s.p.), and a late, phaclofen-sensitive increase in K⁺ conductance mediated by GABA_B receptors (l-i.p.s.p.) (Rainnie *et al.*, 1991; Gean & Chang, 1992; Washburn & Moises, 1992). The functional role of f-i.p.s.p. is thought to maintain normal levels of amygdalar excitability; blockade of f-i.p.s.p. with bicuculline or picrotoxin invariably leads to the development of epileptiform activity (Gean & Shinnick-Gallagher, 1987). The role of GABA_B receptors is, however, not well understood in this area of the brain.

Recently, it has been demonstrated that the sensitivity of presynaptic GABA_B receptors is reduced in amygdala kindled animals (Asproдини *et al.*, 1992). This suggests that the reduction in GABA_B-mediated presynaptic inhibition at excitatory terminals may contribute to the enhancement of glutamatergic transmission (Gean *et al.*, 1989; Rainnie *et al.*, 1992). On the other hand, Hosford *et al.* (1992) have shown that the receptor number but not the equilibrium dissociation constant of GABA_B is increased in neocortical membranes of lethargic mice, a mutant strain with spontaneous seizures. In addition, both proepileptic and antiepileptic effects of baclofen, a GABA_B receptor agonist, have been reported in various *in vivo* and *in vitro* models (Ault & Nadler, 1983; Swartzwelder *et al.*, 1986; Mott *et al.*, 1989; Karlsson *et al.*, 1992). Therefore, it is likely that GABA_B receptors may play both inhibitory and disinhibitory roles in the expression of epileptiform activity, depending on the sites of action.

In the present study, we isolated the N-methyl-D-aspartate (NMDA) receptor-mediated synaptic potential (e.p.s.p._{NMDA}) pharmacologically and found that its amplitude was markedly decreased for a period following the conditioning stimulation. This paired-pulse depression (PPD) of e.p.s.p._{NMDA} could be reversed by phaclofen or 2-hydroxy-saclofen indicating the involvement of GABA_B receptors. GABA_B receptors are present on the pre- as well as postsynaptic neurones and activation of both sites of GABA_B receptors could be responsible for the paired-pulse inhibition of e.p.s.p._{NMDA}. To determine the site of action, we applied various pharmacological agents known to block the postsynaptic GABA_B receptor-activated K⁺ conductance. We found that external Ba²⁺ or internal Cs⁺ did not block the PPD. Furthermore, the PPD was unaffected by pertussis toxin pretreatment. These results suggest that the PPD of e.p.s.p._{NMDA} in the amygdala is produced by activating GABA_B receptors that are not coupled to a Ba²⁺-sensitive K⁺ conductance.

Methods

Brain slices (500 μ m in thickness) of the amygdala were prepared from male Sprague-Dawley rats weighing 125–200 g. The rats were decapitated and the brains were quickly removed and submerged in chilled (0–4°C) artificial cerebral spinal fluid (ACSF) of the following composition (in mM): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, NaH₂PO₄ 1.2 and glucose 11. The brain was subsequently hemisected and cut transversely. A block of tissue containing the amygdala was dissected from the right hemisphere. Two or three transverse amygdalar slices were obtained with a Vibroslice (Campden Inst.) and placed in a beaker of ACSF oxygenated with 95% O₂:5% CO₂.

The slices were kept at room temperature for at least 1 h before recording. A single slice was then transferred to the

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recording chamber where it was held submerged between two nylon nets and maintained at $32 \pm 1^\circ\text{C}$. The chamber consisted of a circular well of a low volume (1–2 ml) and was constantly perfused at a rate of $2\text{--}3\text{ ml min}^{-1}$. A bipolar stimulating electrode was placed in the ventral endopyriform nucleus and stimulated with monophasic constant voltage pulses. The NMDA receptor-mediated synaptic component (e.p.s.p._{NMDA}) was pharmacologically isolated with 6-cyano-7-nitroquinoline-2,3-dione (CNQX, $10\text{ }\mu\text{M}$) and picrotoxin ($50\text{ }\mu\text{M}$). In experiments involving pertussis toxin treatment, slices were incubated in ACSF solution containing pertussis toxin ($5\text{ }\mu\text{g ml}^{-1}$) for at least 7 h before recording. Control preparations were treated with the same protocol in a pertussis toxin-free solution. Previous study on septal neurones has shown that adenosine- and baclofen-induced hyperpolarizing effects were blocked in slices pretreated with pertussis toxin *in vitro* (Hasuo *et al.*, 1992).

Intracellular recordings were made from basolateral amygdala (BAL) neurones by use of glass microelectrodes filled with 3 M KCl ($60\text{--}120\text{ M}\Omega$). In some experiments, the microelectrodes were filled with 3 M CsCl in order to block postsynaptic GABA_B receptor-mediated K^+ conductance. Electrical signals were amplified using an Axoclamp-2A amplifier and connected to a Data-6100 digital oscilloscope (Data Precision Co.) for data analysis and subsequent plotting of fast transient potentials. All data were expressed as mean $\pm$ s.e. Statistical analysis was performed using Student's *t* test, and a *P* value of less than 0.05 was considered to be statistically significant.

All drugs were freshly prepared from stock solutions, dissolved in ACSF and applied by superfusion. DL-2-amino-5-phosphonovaleate (DL-APV), picrotoxin and pertussis toxin were obtained from Sigma Chemicals; 6-cyano-7-nitroquinoline-2,3-dione, phaclofen and 2-hydroxy-saclofen from Research Biochemicals Inc.

Results

Stable intracellular recordings were obtained from 63 neurones of control slices and 11 neurones of pertussis toxin-pretreated slices. Twenty out of 63 neurones from control slices were recorded with CsCl-filled microelectrodes. Cells have resting membrane potentials more negative than -55 mV and action potential amplitude greater than 70 mV . The resting membrane potential and neuronal input resistance were $-65.7 \pm 0.6\text{ mV}$ and $43.5 \pm 1.7\text{ M}\Omega$ ($n = 43$), respectively. The NMDA receptor-mediated synaptic potential (e.p.s.p._{NMDA}) was isolated pharmacologically by the method of Bashir *et al.* (1991), in which afferent fibres were stimulated in the presence of CNQX ($10\text{ }\mu\text{M}$) and picrotoxin ($50\text{ }\mu\text{M}$) and increasing the stimulus intensity (Bashir *et al.*, 1991). In this study, the e.p.s.p._{NMDA} has a time to peak of $44.5 \pm 1.6\text{ ms}$ with $10\text{--}90\%$ duration of $96.7 \pm 2.2\text{ ms}$ ($n = 43$).

When two stimuli of identical strength were applied in close succession, the second e.p.s.p._{NMDA} was depressed in every neurone tested. This paired-pulse depression (PPD) was seen with interstimulus intervals of between 100 ms and 2000 ms ; the maximal depression was observed at interval of 200 ms . A representative experiment is illustrated in Figure 1a and the pooled data from 14 cells are shown in Figure 1b. The extent of PPD was dependent on the stimulus strength used; greater depression occurred when higher stimulus intensities were used ($n = 6$). However, higher stimulus intensities appeared to increase the extent of PPD not by reducing the amplitude of second e.p.s.p._{NMDA}, but largely by increasing the amplitude of first e.p.s.p._{NMDA}. Therefore, PPD is not due to a depletion of neurotransmitter stores.

We examined whether GABA_B receptors are involved in PPD by application of GABA_B antagonists. Figure 2a showed that in control medium the amplitude of the second e.p.s.p._{NMDA} was depressed relative to that of the first.

Figure 1 Time course of the paired-pulse depression of e.p.s.p._{NMDA}. (a) Pairs of e.p.s.p._{NMDA} evoked in the presence of CNQX ($10\text{ }\mu\text{M}$) and picrotoxin ($50\text{ }\mu\text{M}$) by two identical stimuli delivered at intervals of between 100 ms and 2000 ms . The resting membrane potential was -67 mV . (b) Graph of the peak amplitude of the second e.p.s.p._{NMDA} as a percentage of the first, against interstimulus interval for 14 cells. For abbreviations in this and subsequent legends, see text.

Superfusion with 1 mM phaclofen caused a reduction in e.p.s.p._{NMDA} ($68.0 \pm 3.9\%$, $n = 8$). To quantify the effect, the amplitude of the first e.p.s.p._{NMDA} in the presence of 1 mM phaclofen was matched to its control level by increasing the stimulus intensity. As illustrated in Figure 2b, phaclofen reduced the percentage depression in 9 of 9 slices. The percentage depressions, at an interstimulus interval of 200 ms , were $84.0 \pm 3.3\%$ for control, $23.8 \pm 7.5\%$ in the presence of 1 mM phaclofen ($P < 0.001$, $n = 9$) and $86.9 \pm 2.4\%$ after washout of phaclofen. We also tested the more potent analogue 2-hydroxy-saclofen (2-OH-saclofen , $200\text{ }\mu\text{M}$) (Kerr *et al.*, 1988; Bowery, 1989). This agent reversibly reduced the paired-pulse depression in a similar manner. The percentage depressions, at an interstimulus interval of 200 ms , were $88.4 \pm 2.4\%$ for control, $35.7 \pm 4.1\%$ in the presence of $200\text{ }\mu\text{M}$ 2-hydroxy-saclofen ($P < 0.001$, $n = 7$) and $82.1 \pm 4.5\%$ following washout of the 2-hydroxy-saclofen (Figure 3).

Phaclofen (1 mM) and 2-OH-saclofen ($200\text{ }\mu\text{M}$) both depressed the amplitude of the first e.p.s.p._{NMDA} by $68.0 \pm 3.9\%$ ($n = 8$) and $69.7 \pm 4.0\%$ ($n = 7$) respectively. However, in the presence of baclofen ($5\text{ }\mu\text{M}$), 2-OH-saclofen did not cause a further suppression of e.p.s.p._{NMDA}. On the

Figure 2 Phaclofen blocks the paired pulse depression (PPD). (a) Intracellular recordings of paired e.p.s.p.-NMDAs with interstimulus interval of 200 ms. Top tracing: PPD of e.p.s.p.-NMDA. Second tracing shows that phaclofen (1 mM) blocked the PPD. The amplitude of e.p.s.p.-NMDA was substantially depressed by phaclofen. To compensate for this the size of the first e.p.s.p.-NMDA of the pairs in the presence of phaclofen was matched to the control by increasing the stimulus intensity. Fourth tracing demonstrates that the e.p.s.p.-NMDA could be blocked by DL-APV (50 μM). Resting membrane potential = -63 mV. (b) Mean percentage depressions in control, during perfusion of phaclofen (1 mM) and after washout of this drug. $n = 9$. * $P < 0.001$ vs. control.

other hand, 2-OH-saclofen reversed the baclofen-induced presynaptic inhibition (Figure 5d). Similar results were obtained with phaclofen (1 mM, $n = 3$, data not shown). The reduction of the e.p.s.p.-NMDA by phaclofen and 2-OH-saclofen and the occluding effect of baclofen on 2-OH-saclofen response are consistent with a partial agonist action of these compounds at presynaptic GABA_B receptors (Kerr *et al.*, 1990).

Phaclofen and 2-OH-saclofen could act as partial agonists, rather than as antagonists, at the presynaptic GABA_B receptors to reduce transmitter release and thereby diminish the PPD observed. We therefore tested if baclofen produced a similar effect on the PPD. We found that application of baclofen (10 nM) mainly decreased the amplitude of first e.p.s.p.-NMDA with little effect on the second e.p.s.p.-NMDA; consequently the extent of PPD was reduced. However, when the amplitude of first e.p.s.p.-NMDA was increased by increasing the stimulus intensity to obtain the comparable amplitude in the pre-baclofen condition, the degree of PPD was little affected ($n = 6$, data not shown).

The block of paired-pulse inhibition by phaclofen and 2-OH-saclofen suggests that GABA_B receptors are respon-

Figure 3 2-Hydroxy-saclofen blocks the paired pulse depression (PPD). (a) PPD of e.p.s.p.-NMDA evoked at interstimulus interval of 200 ms under control conditions (top tracing), in the presence of 2-OH-saclofen (200 μM, middle tracing) and after washout of the drug (lower tracing). The amplitude of the first e.p.s.p.-NMDA in the presence of 2-OH-saclofen was depressed and was subsequently matched to its initial level by increasing the stimulus intensity. Resting membrane potential = -68 mV. (b) Mean percentage depressions in control, during perfusion of 2-OH-saclofen (0.2 mM) and after washout of the drug; $n = 7$. * $P < 0.001$ vs. control.

sible for suppression of second e.p.s.p.-NMDA. Since an increase in postsynaptic K⁺ conductance induced by the GABA_B receptor activation is known to be blocked by Ba²⁺ (Thomson & Gähwiler, 1992), we therefore tested the effects of Ba²⁺ on the paired-pulse depression. Superfusion with Ba²⁺ (0.5 mM) depolarized the membrane potential by 3–8 mV and increased neuronal input resistance ($85 \pm 12\%$, $n = 7$). Figure 4a shows that, in the presence of Ba²⁺ (0.5 mM), baclofen (10 μM) induced little, if any, hyperpolarizing response. In 6 cells, superfusion with 10 μM caused a -7.3 ± 0.5 mV membrane hyperpolarization. In the presence of Ba²⁺ (0.5 mM), baclofen produced little hyperpolarization (-0.4 ± 0.3 mV, $n = 6$). The difference is significant at $P < 0.001$ (unpaired *t* test).

In 7 out of 7 cells examined, Ba²⁺ produced a large depolarizing wave with several action potentials atop it. The amplitude of the depolarizing wave was then reduced by partially blocking NMDA receptors with 10 μM DL-APV to match the control levels. The e.p.s.ps remaining under these conditions were apparently mediated by NMDA receptors because they could be blocked completely by further increasing the concentration of DL-APV to 50 μM. Under these conditions, the e.p.s.p.-NMDA still exhibited paired-pulse depression (Figure 4b) which was not significantly different from the corresponding controls (percentage depressions at interstimulus interval of 200 ms, control: $76.7 \pm 3.3\%$, in the

Figure 4 Barium fails to block the paired pulse depression (PPD). (a) Chart record illustrating the effect of baclofen ($10 \mu\text{M}$) on a basolateral amygdala neurone in the presence of Ba^{2+} (0.5 mM). During the experiment, -0.1 nA hyperpolarizing current pulses (upper trace) were injected intracellularly to monitor the neuronal input resistance. (b) (1) PPD of e.p.s.p.-NMDA at interstimulus intervals of 200 ms and 400 ms . (b) (2) Application of Ba^{2+} (0.5 mM) produces a large depolarizing wave with several action potentials atop it. The amplitude of the depolarizing wave was subsequently reduced by partially blocking NMDA receptors with $10 \mu\text{M}$ DL-APV to match the control level. (b) (3) Thirty minutes after washout of Ba^{2+} . (c) Graphic analysis of the effect of barium on the PPD at interstimulus intervals of 200 ms ($n = 7$) and 400 ms ($n = 8$).

presence of Ba^{2+} : $69.7 \pm 2.5\%$, $P = 0.12$; at interstimulus interval of 400 ms , control: $73.4 \pm 4.2\%$, in the presence of Ba^{2+} : $66.1 \pm 4.2\%$, $P = 0.24$ (Figure 4c) and application of phaclofen (1 mM) reduced the extent of depression (data not shown).

We recorded neurones using CsCl-filled microelectrodes since the postsynaptic GABA_A receptor-activated K^+ current was blocked by internal Cs^+ (Gahwiler & Brown, 1985). A progressive increase in spike duration was observed over $15\text{--}40 \text{ min}$ after penetration of neurones with a Cs^+ -containing electrode (Figure 5b). In addition, as depicted in Figure 5a, baclofen failed to affect the membrane poten-

Figure 5 Lack of effect of intracellular Cs^+ on the paired pulse depression (PPD). (a) Chart record showing that baclofen failed to affect the membrane potential and input resistance in a basolateral amygdala neurone impaled with a 3 M CsCl-filled microelectrode. (b) Action potentials elicited by intracellular current injection ($+0.3 \text{ nA}$, 100 ms) in the same neurone as in (a). (c) Graphic analysis of the effect of intracellular Cs^+ on the PPD. The percentage depressions, at an interstimulus interval of 200 ms , were $15.7 \pm 2.5\%$ for control ($n = 14$) and $17.6 \pm 2.1\%$ ($n = 13$, $P = 0.55$) for neurones recorded with CsCl-filled microelectrodes. (d) 2-OH-saclofen ($200 \mu\text{M}$) reversed the baclofen ($5 \mu\text{M}$)-induced presynaptic inhibition of e.p.s.p.-NMDA. (e) (1) e.p.s.p.-NMDAs evoked at interval of 400 ms in the presence of baclofen ($5 \mu\text{M}$). Baclofen reduced the amplitude of e.p.s.p.-NMDAs. The amplitude of first e.p.s.p.-NMDA was increased by increasing the stimulus intensity; however, the e.p.s.p.-NMDAs still exhibited PPD in the presence of baclofen. (e) (2) Application of 2-OH-saclofen ($200 \mu\text{M}$) reversed the PPD.

tial or neuronal input resistance in the same neurone. However, paired stimulation at an interval of 200 ms evoked e.p.s.p.-NMDAs that displayed PPD. The second e.p.s.p.-NMDA was depressed by an average of $82.4 \pm 2.1\%$ ($n = 13$), which was not significantly different from the control PPD ($84.3 \pm 2.5\%$, $n = 14$, $P = 0.55$) (Figure 5c). Moreover, superfusion of 2-OH-saclofen reversed the PPD in neurones recorded with CsCl (Figure 5e).

Previous study in the amygdala has shown that pre- and

Figure 6 Pertussis toxin treatment blocks the postsynaptic action of baclofen but does not affect the paired pulse depression (PPD). (a) (1) Chart record illustrating the effect of baclofen ($10 \mu\text{M}$) on a basolateral amygdala neurone which was exposed to PTX $5 \mu\text{g ml}^{-1}$ for 7 h. During application of baclofen, -0.16 nA hyperpolarizing current pulses were injected to monitor membrane input resistance. (a) (2) Sample traces from (a) (1) were expanded on the oscilloscope to illustrate that baclofen does not affect the input resistance in neurones pretreated with PTX. (b) PPD of e.p.s.p.-NMDA evoked with different stimulus intensities. (a) and (b) were taken from the same cell. Graphic analysis of the effect of PTX on the PPD. The percentage depressions, at interstimulus interval of 200 ms, were $15.7 \pm 2.5\%$ for control ($n = 14$) and $15.7 \pm 1.8\%$ ($n = 11$, $P = 0.97$) in neurones pretreated with PTX.

postsynaptic GABA_B receptors can be distinguished on the basis of their sensitivity to pertussis toxin treatment (Asprodini *et al.*, 1992). We incubated the slices in pertussis toxin-containing ACSF ($5 \mu\text{g ml}^{-1}$) for 7 h and examined its effect on the PPD. As shown in Figure 6a, both membrane hyperpolarizing and resistance decreasing effects normally produced by baclofen were blocked in basolateral amygdala neurones of pertussis toxin-pretreated slices. Superfusion of $10 \mu\text{M}$ baclofen which caused a $-7.3 \pm 0.5 \text{ mV}$ ($n = 6$) hyperpolarization in neurones from untreated slices produced no hyperpolarization in neurones from PTX-treated slices ($-1.0 \pm 0.4 \text{ mV}$, $n = 6$, $P < 0.005$). In contrast, the PPD of

e.p.s.p.-NMDA was not significantly different between neurones obtained from control and PTX-treated slices (Figure 6b). The percentage depressions, at an interstimulus interval of 200 ms, were $84.3 \pm 2.5\%$ for control ($n = 14$) and $84.2 \pm 1.8\%$ ($n = 11$, $P = 0.97$) in neurones pretreated with PTX (Figure 6c).

Discussion

The present study demonstrates that the e.p.s.p.-NMDA isolated with CNQX/picrotoxin exhibits PPD at interstimulus intervals of 100–2000 ms. The mechanism underlying PPD appeared to involve the GABA_B receptors because phaclofen and 2-hydroxy-saclofen reduced or blocked the depression. However, the lack of a significant difference in the external Ba^{2+} , internal Cs^{+} or PTX-treated compared to control neurones suggests that GABA released by the first stimulus reduced the second e.p.s.p.-NMDA by an action other than that of opening a postsynaptic GABA_B receptor-mediated K^{+} conductance.

The involvement of GABA_B receptors in activity-dependent decrease of synaptic potentials evoked by repetitive activation of afferent pathways has been demonstrated recently (Deisz & Prince, 1989; Davies *et al.*, 1990; Calabresi *et al.*, 1991). In the hippocampus, the mechanism underlying paired-pulse depression of i.p.s.ps can be explained by a negative feedback phenomenon which involves GABA acting on presynaptic GABA_B receptors to inhibit its own release (Davies *et al.*, 1990; Otis *et al.*, 1993; Davies & Collingridge, 1993). On the contrary, activation of postsynaptic GABA_B receptors resulting in an increase in K^{+} conductance which hyperpolarizes neurones in a region where NMDA receptor-operated channels are blocked by Mg^{2+} is responsible for paired-pulse inhibition of e.p.s.p.-NMDA (Morrisett *et al.*, 1991). Baclofen-induced postsynaptic hyperpolarizing responses are known to be blocked by 0.5 mM Ba^{2+} (Washburn & Moises, 1992 and the present study) or internal Cs^{+} (Gahwiler & Brown, 1985 and the present study). The inability of external Ba^{2+} or internal Cs^{+} to reverse PPD in the present study suggests that GABA_B-mediated postsynaptic activation of K^{+} conductance is unlikely to be the underlying mechanism. Since the stimulus intensity was increased in order to evoke the e.p.s.p.-NMDA in the presence of CNQX, we speculate that the released GABA by the first stimulus spills out from GABAergic synapses to act on glutamatergic fibre terminals and reduces the transmitter release. A recent demonstration that a brief tetanus but not a single conditioning stimulus releases enough GABA to diffuse to the presynaptic receptors on excitatory nerve terminals (Isaacson *et al.*, 1993) is consistent with this hypothesis.

The observation that phaclofen or 2-OH-saclofen suppressed the amplitude of e.p.s.p.-NMDA is in accord with previous reports in the amygdala and hippocampus (Asprodini *et al.*, 1992; Hosford *et al.*, 1992) and reflects that these acidic analogues of GABA are in fact partial agonists rather than true antagonists (Kerr *et al.*, 1990). In the present study, we also demonstrate that the degree of PPD is reduced under conditions where transmitter release is decreased by baclofen. Furthermore, 2-OH-saclofen and phaclofen, but not baclofen, are able to reverse the PPD. These results suggest that presynaptic mechanism involving GABA_B receptors is the major contributor to the PPD.

In the basolateral amygdala, it has previously been shown that 2-OH-saclofen and phaclofen block the late i.p.s.p. and reduce the hyperpolarizing effect of baclofen, both of which are mediated by postsynaptic GABA_B receptors. In the present study, we observed that 2-OH-saclofen and phaclofen also reversed the baclofen-induced presynaptic inhibition of e.p.s.p.-NMDA and paired-pulse depression. These data suggest that pre- and postsynaptic GABA_B receptors share a similar pharmacological profile on the basis of antagonist sensitivity in the amygdala. However, the lack of an antagonistic effect

of Ba^{2+} and Cs^{+} on the PPD suggests that pre- and post-synaptic receptors are coupled to different effectors.

It has been previously established that the GABA_B receptor directly activates postsynaptic K^{+} channels via a pertussis toxin-sensitive G-protein (Dutar & Nicoll, 1988). In agreement with previous reports, the hyperpolarizing action of baclofen was abolished in the PTX-treated slices. However, paired-pulse stimulation caused a $84.2 \pm 1.8\%$ depression of the second e.p.s.p._{NMDA}. This result suggests hyperpolarizing membrane potential through the activation of postsynaptic GABA_B receptors could not account for the PPD. Stimulation of Ca^{2+} -phospholipid-dependent protein kinase C (PKC) (Ben-Ari *et al.*, 1992; Chen & Huang, 1991) and cyclic AMP-dependent protein kinase A (PKA) (McVaugh & Waxham, 1992; Huang *et al.*, 1993; Cerne *et al.*, 1993) has recently been shown to modulate positively NMDA responses. Postsynaptic GABA_B receptors may depress e.p.s.p._{NMDA} by activating G-proteins that interfere with the functions of these kinases. However, in the present study, PTX treatment did not block the PPD, suggesting it is unlikely that GABA_B receptors act through postsynaptic G-proteins to suppress e.p.s.p._{NMDA}.

The mechanism(s) by which activation of GABA_B receptors on excitatory axon terminals inhibits transmitter release has not yet been established. An activation of Ba^{2+} -sensitive K^{+} current resulting in hyperpolarization of the presynaptic nerve terminals could lead to reduced transmitter release. However, our data indicate the PPD could not be explained by such a presynaptic hyperpolarization since Ba^{2+} failed to affect it. Considering the long duration (>2 s) of PPD, it is nevertheless possible that a Ba^{2+} -insensitive K^{+} current (Wagner & Dekin, 1993) with slow inactivation time constant might underlie the presynaptic hyperpolarization. Other possibilities include a direct inhibition of presynaptic Ca^{2+} current by GABA_B receptors (Dolphin & Scott, 1986; Scholz & Miller, 1991; Pfrieger *et al.*, 1994) or an inhibition of transmitter release subsequent to Ca^{2+} influx (Scanziani *et al.*, 1992).

Interestingly, we observed that there is no stimulus strength-response relationship i.e. irrespective of the stimulus intensities applied, the amplitude of second e.p.s.p._{NMDA} was constant. In addition, baclofen mainly decreased the amplitude of first e.p.s.p._{NMDA} with little effect on the second one,

suggesting presynaptic GABA_B receptors may be maximally activated at low stimulus intensities. These results are consistent with a previous report that the EC_{50} of baclofen for the presynaptic action was 5 nM representing a 200 fold difference for its postsynaptic action ($\text{EC}_{50} = 1 \mu\text{M}$) in the amygdala.

In the mammalian brain, the NMDA subtype of glutamate receptor is thought to play an important role in many forms of neuronal plasticity such as epilepsy, learning and memory (Collingridge & Singer, 1990; Meldrum & Garthwaite, 1990). In the amygdala, an enhancement in NMDA receptor-mediated responses has been demonstrated in both acute and chronic models of epilepsy (Geaun *et al.*, 1989; 1990; Rainnie *et al.*, 1992). Furthermore, activation of NMDA receptors is required for the induction of long-term potentiation (LTP) in this region of the brain (Geaun *et al.*, 1993). The present study shows that GABA_B receptor antagonists reverse the PPD of e.p.s.p._{NMDA} suggesting that GABA_B receptors play an important role in the regulation of amygdala neuronal excitability. Indeed, a recent study by Asprodini *et al.* (1992) indicated that a reduced sensitivity of GABA_B receptors located on the nerve terminal of glutamate afferents underlies the kindling-induced epileptogenesis.

In summary, we have shown that GABA_B receptor activation plays an important role in paired-pulse depression of e.p.s.p._{NMDA} in the amygdala. We have also demonstrated that PPD is not blocked by external Ba^{2+} or internal Cs^{+} . Furthermore, pretreating the slices with pertussis toxin, which blocked the hyperpolarizing action of baclofen, did not alter the PPD. Taken together, these results suggest that GABA released by the first stimulus acting on GABA_B receptors to suppress the second e.p.s.p._{NMDA} is via mechanisms other than activation of a postsynaptic GABA_B receptor-coupled K^{+} conductance. The present study is therefore in contrast to a previous report where the reduction of the second e.p.s.p._{NMDA} in the hippocampus is thought to result from activation of postsynaptic K^{+} conductance (Morrisett *et al.*, 1991).

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