Enhancement of NMDA receptor-mediated synaptic potential evoked in rat medial-amygdala neuron following olfactory bulbectomy

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Synaptic potentials evoked in the medial amygdala (m-AMG) neurons were studied in vitro slice preparations obtained from normal and olfactory bulbectomized rats. Local stimulation induced a sequence of responses: a fast EPSP, a fast IPSP and a slow EPSP. The fast EPSP was suppressed by kynurenic acid (KYN) at a concentration of 1 mM but not by 3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP) at concentrations up to 20 μM. The slow EPSP was reversibly blocked by both KYN (1 mM) and CPP (5–10 μM). Addition of bicuculline methiodide (50 μM) to the bath suppressed the fast IPSP and augmented both the fast and slow EPSPs leading to burst discharges. In a small population of m-AMG neurons, the slow EPSP was followed by a slow IPSP. The slow IPSP was suppressed by phaclofen (500 μM) but not by bicuculline methiodide (up to 100 μM). In slice preparations obtained from olfactory bulbectomized rats, local stimulation evoked burst discharges, which were similar to those observed when bicuculline methiodide was applied to slice preparations obtained from normal rats. These results suggest that GABA_A receptors mediating fast IPSP and N-methyl-D-aspartate (NMDA) receptors mediating slow EPSP regulate activities of m-AMG neurons and that the enhancement of NMDA receptor mediating slow EPSP is responsible for the hyperexcitability of m-AMG neurons following olfactory bulbectomy.

INTRODUCTION

The amygdala (AMG) is a subcortical formation of the limbic system and has been implicated to be important in epileptogenesis. Experimental evidence indicates that the AMG has the lowest seizure threshold when stimulated electrically and requires the fewest number of stimulations to induce kindling, a generalized convulsive response. Recently, agents which antagonize the action of N-methyl-D-aspartate (NMDA) have been shown to suppress kindling and kindled seizures in the AMG. Contrary to this, agents which antagonize GABA receptors have been reported to facilitate kindling and kindled seizures in the AMG. These facts indicate that synaptic activation of NMDA and GABA receptors may play an important role in regulating neuronal excitability in the AMG.

We previously reported that the threshold for after-discharges decreased significantly and kindling was facilitated following olfactory bulbectomy in the medial (m)-AMG. The present study was undertaken to clarify synaptic potentials evoked by local stimulation in the m-AMG and to elucidate the mechanism for changes in neuronal excitability following olfactory bulbectomy by using in vitro slice preparations.

MATERIALS AND METHODS

Male Wistar rats weighing 150–250 g were decapitated under ether anesthesia and the brains were rapidly removed. In some of the early experiments, rats were anesthetized with ketamine (20 mg/kg, i.p.) prior to decapitation. Each brain was trimmed with a razor blade to a block containing the region of the AMG. Parasagittal slices with a thickness of 400 μm were cut from the block with a vibratome and placed in an interface-type recording chamber with the bath temperature maintained at 36 °C. Local stimulation (intensity 5–40 V, duration 200 μs, frequency 0.6 Hz) was applied to the m-AMG through a bipolar electrode gently placed on the surface of the slice. The stimulating electrode was made by twisting a pair of 80 μm diameter nichrome wires insulated except at the tip (impedance 10–50 kΩ). The recording electrode was positioned visually at a distance of 0.5–1.0 mm from the stimulating electrode. Intracellular recordings were obtained through a glass micro-electrode filled with 2 M K-citrate and a high input impedance amplifier (Neurodata IR183). Electrical responses were stored in a videocassette recorder through a PCM converting system (Sony PCM-501 ES) with a build-in low pass filter and plotted on an X-Y plotter. The Krebs solution for superfusion of the slices was composed of (in mM): NaCl 124, KCl 5.0, KH₂PO₄ 1.24,

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NaHCO<sub>3</sub> 26, CaCl<sub>2</sub> 2.4, MgSO<sub>4</sub> 1.3 and glucose 10. Olfactory bulbectomy was carried out bilaterally by suctioning through a hole made in the skull just above the olfactory bulbs under sodium pentobarbital (40 mg/kg i.p.) anesthesia approximately 10 days before the experiment. The extent of olfactory bulbectomy was verified histologically by making 50-μm sagittal sections stained with cresyl violet.

The following drugs were used: 3-[(±)-2-carboxypiperezin-4-yl]propyl-1-phosphonic acid (CPP; Tocris Neuramin), kynurenic acid (KYN; Sigma), bicuculline methiodide (Sigma), phaclofen (Tocris Neuramin) and (±)-baclofen (Ciba-Geigy). Numerical data are presented as mean ± S.D.

RESULTS

Intracellular recordings were obtained from 63 m-AMG neurons which had a resting membrane potential of −63.0 ± 5.8 mV and an input resistance of 73.1 ± 9.2 MΩ. Local stimulation evoked a sequence of responses: a fast depolarization (peak amplitude 5.3 ± 2.2 mV, duration 7.7 ± 3.6 ms), a fast hyperpolarization (peak amplitude 2.5 ± 1.3 mV, duration 21.5 ± 7.7 ms) and a slow depolarization (peak amplitude 3.5 ± 1.2 mV, duration 103 ± 43 ms) in the majority of the m-AMG neurons (n = 51). These values were measured at the resting membrane potential with stimulus intensity at just subspike threshold (e.g. 5–15 V). The amplitudes of both fast and slow depolarization were increased enough to trigger an action potential with increasing stimulus intensity. Both the amplitude and duration of the fast depolarization decreased with an injection of a continuous depolarizing current and increased with hyperpolarizing currents (Fig. 1A). The injection of a depolarizing current increased the amplitude of the fast hyperpolarization and hyperpolarizing currents decreased it. The polarity of the fast hyperpolarization could be easily reversed to the depolarizing direction with a hyperpolarizing current injection (Fig. 1A). These results would indicate that fast depolarization and fast hyperpolarization are excitatory postsynaptic potential (EPSP) and an inhibitory postsynaptic potential (IPSP), respectively.

The slow depolarization, however, displayed unconventional change upon intracellular current injections.

![Fig. 1. Postsynaptic potentials evoked in an m-AMG neuron. A: changes in the responses by continuous application of depolarizing and hyperpolarizing intracellular currents. The current intensity is indicated on the left of each trace. B: effects of bath applied CPP (10 μM) and KYN (1 mM) on the responses evoked by local stimulation in an m-AMG neuron. Upward arrows in this and other figures indicate the onset of each local stimulation. Records are averages of four successive responses. Calibrations in A also apply to B.](image-url)
Fig. 2. Postsynaptic potential evoked in an m-AMG neuron by a high frequency train. A: increase in the number of trains applied. High-frequency stimulation (multiple dots) consisted of 1, 3, 6 and 12 trains with a 5-ms interval. B: effects of CPP (10 μM) on postsynaptic potential evoked by a high frequency train. Calibrations in A also apply to B.

The depolarizing current injection increased the duration of the slow depolarization and triggered repetitive action potentials. On the other hand, a decrease in amplitude and duration of the response was observed when a relatively large hyperpolarizing current (e.g. -0.2 nA) was injected (Fig. 1A). Because the blockade of the slow

Fig. 3. Postsynaptic potentials evoked in an m-AMG neuron which have a slow hyperpolarization. A: changes in responses by continuous application of depolarizing and hyperpolarizing intracellular currents. The current intensity is indicated on the left of each trace. B: effects of phaclofen (500 μM) and bicuculline methiodide (50 μM) on the responses evoked by local stimulation in an m-AMG neuron. Records are averages of 4 successive responses. Calibrations in A also apply to B.

Fig. 4. Burst discharges evoked in m-AMG neurons during perfusion with bicuculline methiodide (50 μM). A: effects of CPP (10 μM) on a burst discharge and responses evoked by a depolarizing current pulse injection. The line drawings below the traces indicate the duration and intensity of the injected current. B: effects of baclofen (100 μM) on a burst discharge and response evoked by a depolarizing current pulse injection. In the middle trace, the membrane potential was returned to the original level by injecting a depolarizing current. Calibrations in A also apply to B.
depolarization with strong membrane hyperpolarization suggested an involvement of NMDA receptor activation, the effects of CPP, an antagonist of NMDA receptor, on the slow depolarization were examined. Addition of CPP to the bath at a concentration of 5-10 μM markedly and reversibly suppressed the slow depolarization by 87 ± 4% (n = 4) without affecting the fast EPSP (Fig. 1B). The duration of the fast IPSP increased slightly during the application of CPP. CPP did not cause any noticeable changes in the membrane potential or the input resistance. KYN, a non-selective antagonist of excitatory amino acid receptor, at a concentration of 1-2 mM completely suppressed both the fast EPSP and the slow EPSP (n = 5) (Fig. 1B). KYN also increased the amplitude and duration of the fast IPSP (Fig. 1B). The effect of high frequency stimulation was studied in 14 m-AMG neurons. Both the amplitude and duration of the slow EPSP increased as the number of trains was increased (Fig. 2A). On the other hand, the amplitude of the fast EPSP and the fast IPSP progressively declined during high frequency stimulation (Fig. 2B). Bath application of CPP at a concentration of 10 μM completely suppressed the slow EPSP evoked by high frequency stimulation (n = 3) (Fig. 2B). The fast IPSP reappeared during the application of CPP.

In the remaining 12 neurons, the sequence of responses described above was accompanied by slow hyperpolarization (peak amplitude 3.3 ± 0.9 mV, duration 540 ± 83 ms) (Fig. 3). In contrast to the fast IPSP, the amplitude of the slow hyperpolarizing potential was first increased (e.g. 0.1 nA) and then decreased (e.g. 0.2 nA) with injecting depolarizing current (Fig. 3A). An injection of a hyperpolarizing current also decreased the amplitude of the slow hyperpolarizing potential. The polarity of the slow hyperpolarizing potential was not reversed by the hyperpolarizing current (e.g. -0.1 nA) which was enough to reverse the polarity of the fast IPSP (Fig. 3A). Because the nature of this hyperpolarizing response was similar to that of GABA receptor-mediated synaptic potential described in other regions of the central nervous system, the effects of phaclofen, an antagonist of GABA receptor, on this hyperpolarization was examined. Phaclofen at a concentration of 500 μM markedly and reversibly depressed the slow hyperpolarization by 90 ± 5% (n = 3) without affecting the fast
Excitatory potentials evoked in the m-AMG when the ablation was extended to the brain areas caudal to the anterior olfactory nucleus were left fully intact \( (n = 5) \) (Fig. 5B). When ablation was limited to the main olfactory bulbs and the accessory olfactory bulbs and the anterior olfactory nucleus were left fully intact \( (n = 5) \) (Fig. 5C), burst discharges were not induced. The burst discharges were dependent on the membrane potential (Fig. 6B) and were markedly depressed by CPP \( (10 \mu M) \) (Fig. 6C).

**DISCUSSION**

Excitatory potentials evoked in the m-AMG

Local stimulation evoked a dual component of EPSPs, a fast EPSP followed by a slow EPSP, in the m-AMG. Both the amplitude and duration of the slow EPSP increased (up to 40%) during the application of phaclofen. Bicuculline methiodide at a concentration of 5–10 \( \mu M \), on the other hand, completely suppressed the fast IPSP without affecting the slow hyperpolarization. At the same time, both the fast and slow EPSPs were augmented, leading to burst discharges. The latter component of the burst discharge induced during perfusion with a bicuculline methiodide-containing solution was reversibly depressed by an application of CPP at a concentration of 10 \( \mu M \) without affecting response generated by an injection of a depolarizing current pulse \( (n = 4) \) (Fig. 4A). Baclofen at a concentration of 100 \( \mu M \) resulted in the following changes in the m-AMG neurons \( (n = 5) \): (1) a hyperpolarization of 6.1 ± 1.1 mV, (2) a 14.3 ± 3.5% decrease in the input resistance, (3) a marked depression of synaptically induced responses and (4) a slight decrease in the number of action potentials generated by depolarizing current pulses. During the application of baclofen, the burst discharges were markedly suppressed even when the membrane potential was returned to the control level after injecting a depolarizing current (Fig. 4B).

The passive membrane properties and synaptic responses evoked by local stimulation were examined in 18 m-AMG neurons from 7 rats that received olfactory bulbectomy. The resting membrane potential \( (-64.5 \pm 6.0 \text{ mV}) \) and input resistance \( (74.5 \pm 8.2 \text{ M} \Omega) \) were not significantly altered by olfactory bulbectomy. Local stimulation with intensity at just subspike threshold induced a similar sequence of responses as described above including fast EPSP, fast IPSP and slow EPSP. While the stimulus intensity was increased, the slow EPSP markedly increased leading to burst discharges, which were not seen in slices prepared from normal rats at the same range of stimulus intensity (e.g. 25–40 V). These changes in synaptic potentials were observed only when the ablation was extended to the brain areas caudal to the main olfactory bulbs including the accessory olfactory bulbs and the anterior olfactory nucleus \( (n = 13) \) (Fig. 5B). When ablation was limited to the main olfactory bulbs and the accessory olfactory bulbs and the anterior olfactory nucleus were left fully intact \( (n = 5) \) (Fig. 5C), burst discharges were not induced. The burst discharges were dependent on the membrane potential (Fig. 6B) and were markedly depressed by CPP \( (10 \mu M) \) (Fig. 6C).

Inhibitory potentials evoked in the m-AMG

Local stimulation also evoked a dual component of IPSPs, a fast IPSP and a slow IPSP. The fast IPSP, which was induced near the inflection between the fast and slow EPSPs, was depressed by high-frequency stimulation and bicuculline. The reduction of the fast EPSP during high frequency stimulation may be due to a depletion of Ca\(^{2+}\) in presynaptic terminals. The dual component of IPSPs was similar to the responses recorded in many regions of the central nervous system including the spinal cord, the cerebral cortex, the hippocampus, the dentate gyrus, the subthalamus, the entorhinal cortex and the septum. In the m-AMG, however, a fast IPSP could be observed near the inflection between the fast and slow EPSPs when low-frequency stimulation was applied with a relatively low intensity.

The m-AMG receives afferent projections from its vicinity such as the cortical AMG, the anterior amygdaloid area and the substantia innominata. It also receives afferent projections from the main and accessory olfactory bulbs, the bed nucleus of the stria terminalis, the hypothalamus, the thalamus and the lower brain stem. The results obtained from the olfactory bulbectomy experiments suggest that both fast and slow EPSPs are mediated through ascending fibers originating in the brain areas caudal to the anterior olfactory nucleus and projecting collaterals into the m-AMG.

**DISCUSSION**

Excitatory potentials evoked in the m-AMG

Local stimulation evoked a dual component of EPSPs, a fast EPSP followed by a slow EPSP, in the m-AMG neurons in in vitro preparations. The slow EPSP was voltage-dependent, potentiated by high frequency stimulation and blocked by CPP, an antagonist of NMDA receptor. These results indicate that activation of NMDA receptors is involved in the mediation of this potential. On the other hand, the fast EPSP was blocked by KYN, a non-selective antagonist of excitatory amino acid receptor, but not by CPP. KYN acts as an antagonist at both NMDA and non-NMDA receptors but did not significantly interact with other neurotransmitters (e.g. acetylcholine). These facts suggest that the fast EPSP is mediated by activation of non-NMDA receptors. The reduction of the fast EPSP during high frequency stimulation may be due to a depletion of Ca\(^{2+}\) in presynaptic terminals. The dual component of EPSPs was similar to the responses recorded in many regions of the central nervous system including the spinal cord, the cerebral cortex, the hippocampus, the dentate gyrus, the subthalamus, the entorhinal cortex and the septum. In the m-AMG, however, a fast IPSP could be observed near the inflection between the fast and slow EPSPs when low-frequency stimulation was applied with a relatively low intensity.

**Inhibitory potentials evoked in the m-AMG**

Local stimulation also evoked a dual component of IPSPs, a fast IPSP and a slow IPSP. The fast IPSP, which was induced near the inflection between the fast and slow EPSPs, was depressed by high-frequency stimulation and bicuculline. The reduction of the fast IPSP during high frequency stimulation may be produced by activation of NMDA receptors because the fast IPSP reappeared with an application of CPP. The fast IPSP does not seem to be mediated disynaptically activated GABAergic interneurons since blockade of preceding fast EPSP by KYN augmented this response. Although the origin of GABAergic afferents is not known, the present results suggest that blockade of GABA\(_A\) receptor-mediated fast IPSP is closely related to the development of NMDA receptor-mediated slow EPSP leading to burst discharges. The fast IPSP seems to be sufficient to suppress the slow NMDA receptor-mediated EPSP because it could hyperpolarize...
postsynaptic membranes into the range at that NMDA receptor-linked channels can be inactivated by extracellular Mg\(^{2+}\) (refs. 22, 26). Thus, the fast IPSP appears to play an important role in regulating neuronal excitability in the m-AMG.

In a small population of the m-AMG neurons tested, a slow IPSP which followed the slow EPSP was observed. The slow IPSP was long-lasting (up to 1 s) and blocked by phaclofen, a presumed antagonist of GABA\(_B\) receptor\(^{19}\), indicating the involvement of activation of GABA\(_B\) receptors in the mediation of this potential. Phaclofen at the same concentration as used in this study has been shown to block long-lasting IPSPs in the hippocampus\(^7,30,31\) the neocortex\(^{18}\), the thalamus\(^{31}\) and the septum\(^{12}\). This long-lasting inhibition does not seem to play a role in regulating neuronal excitability since the blockade of this response by phaclofen did not induce burst discharges. In the present experiment, however, baclofen, an agonist of GABA\(_A\) receptor\(^2\), had a potent suppressive effect on bicuculline-induced epileptiform discharges. It is likely that this effect resulted from the well-known presynaptic action by which baclofen decreases the release of excitatory neurotransmitters\(^1,21,27\).

**Effects of olfactory bulbectomy on synaptic potentials in the m-AMG**

The burst discharges were recorded from slices prepared from the olfactory bulbectomized rats and were markedly suppressed by CPP. In slice preparations from normal rats, a similar burst activity could be induced during an application of bicuculline. These results strongly suggest that the NMDA receptor-mediated component of synaptic response is augmented following olfactory bulbectomy. This can also account for our previous findings that the threshold for afterdischarges decreased significantly and kindling was facilitated following olfactory bulbectomy in the m-AMG\(^{26}\).

Reduction of GABAergic inhibition is one possible mechanism for these changes in synaptic responses of the m-AMG following olfactory bulbectomy. In fact, it has been reported that the GABA content in the m-AMG is significantly reduced following olfactory bulbectomy\(^{15}\). Although the precise mechanisms for the reduction of GABAergic IPSP are unclear, it is considered that the brain areas which project GABAergic efferents to the m-AMG are damaged by olfactory bulbectomy. One of the presumable regions is the anterior olfactory nucleus since such phenomena could be observed only when the ablation was extended to this area. The reduction of GABAergic inhibition has also been demonstrated contributing to the enhancement of the excitability by activating NMDA receptors in the development of kindling. Stelzer et al.\(^{32}\) have observed a reduction of GABAergic inhibition and an enhancement of NMDA receptor-mediated responses in hippocampal neurons using in vitro model of kindling produced by massive stimulation of afferent pathway. Furthermore, Gean et al.\(^{10}\) have suggested that a long-lasting loss of GABAergic IPSPs is a cause of bursting activityactivating NMDA receptors in the AMG from their in vitro studies using animals kindled in vivo.

In addition to the GABAergic system, the catecholaminergic system may play an important role in the regulation of neuronal excitability in the AMG. It has been reported that the depletion of norepinephrine in the AMG caused by injection of 6-hydroxydopamine into the AMG facilitated the development of kindling\(^5,23\). Tonner et al.\(^{34}\) found that the turnover rate of norepinephrine in the m-AMG markedly decreased by olfactory bulbectomy in the rat. Thus, the changes in the function of the norepinephrinergic system may be another possible mechanism for neuronal hyperexcitability observed in the m-AMG following olfactory bulbectomy.

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**REFERENCES**


