Korean J Physiol Pharmacol
Vol 7: 303–306, December, 2003

Modulation of Amygdala Synaptic Transmission by Metabotropic Glutamate Receptors

Jung Hyun Kim, Eun Jin Park, Duk Jin Chang, and Sukwoo Choi

School of Biological Sciences, Seoul National University, Seoul 151–742, Korea

Metabotropic glutamate receptors (mGluRs), classified into three groups (group I, II, III), play a critical role in modulation of synaptic transmission at central and peripheral synapses. In the present study, extracellular field potential recording techniques were used to investigate effects of mGluR agonists on excitatory synaptic transmission at thalamic input synapses onto the lateral amygdala. The non-selective mGluR agonist t-ACPD (100 μM) produced reversible, short-term depression, but the group III mGluR agonist L-AP4 (50 μM) did not have any significant effects on amygdala synaptic transmission, suggesting that group I and/or II mGluRs are involved in the modulation by t-ACPD. The group I mGluR agonist DHPG (100 μM) produced reversible inhibition as did t-ACPD. Unexpectedly, the group II mGluR agonist LCCG-1 (10 μM) induced long-term as well as short-term depression. Thus, our data suggest that activation of group I or II mGluRs produces short-term, reversible depression of excitatory synaptic transmission at thalamic input synapses onto the lateral amygdala. Considering the long-term effect upon activation of group II mGluRs, lack of long-term effects upon activation of group I and II mGluRs may indicate a possible cross-talk among different groups of mGluRs.

Key Words: Metabotropic glutamate receptors, Amygdala, Synaptic transmission

INTRODUCTION

The lateral amygdala is involved in the control of emotion, especially fear (Ledoux, 1995), and it appears that certain forms of learning and memory involve changes in amygdala function (Ledoux, 1995). The major excitatory input to the lateral amygdala arises from neurons in the thalamus that use glutamate as a neurotransmitter (Ledoux, 2000). Synaptic transmission at thalamic input synapses onto the lateral amygdala is mediated by AMPA receptors with very little involvement of NMDA receptors (Weisskopf et al, 1999).

mGluRs are G-protein-coupled receptors and are subdivided into three different groups (Pin & Acher, 2002). Group I mGluRs including mGluR 1 and 5 are coupled to PI hydrolysis, whereas Group II mGluRs including mGluR 2 and 3 are coupled to cAMP cascade. Group III mGluRs including mGluR 4, 6, 7, and 8 are coupled to cAMP cascade. Glutamate is a natural agonist for these receptors, and most of mGluRs exist around or at synapses. Activation of mGluRs has been shown to modulate synaptic transmission at various synapses such as hippocampal, striatal, cortical and thalamic synapses (Pin & Acher, 2002).

In the present study, we have examined the effect of the mGluR agonists on synaptic transmission at thalamic input synapses onto the lateral amygdala to define groups of mGluRs involved.

METHODS

Brain slices were prepared using previously described techniques (Choi & Lovinger, 1997; Choi & Tsien, 2000). Sprague Dawley rat (3 to 5 weeks old) were decapitated. The isolated whole brains were placed in an ice-cold (0 to 4°C) modified artificial cerebrospinal fluid (aCSF) solution. The composition of modified aCSF was as follows (in mM): 175 sucrose, 20 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1.3 $MgCl_2$, and 11 D-(+)-glucose. Coronal slices (400 μm thick) containing the amygdala were cut using a vibratome (Campden, UK), and were incubated in aCSF continuously bubbled at room temperature with 95% O_2/ 5% CO_2 for at least 3 hr before recordings. Just before transferring the slice to the recording chamber, the cortex overlying the amygdala was cut away with a scalpel so that, in the presence of picrotoxin, cortical epileptic burst discharges would not invade the amygdala (Huang & Kandel, 1998).

The recording chamber was continuously superfused with aCSF (30 to 32°C) at a flow rate of 1~2 ml/min. The aCSF contained (in mM): 120 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1.3 MgCl2, 2 $CaCl_2$, and 11 D-(+)-glucose. Picrotoxin (10 μM) was included in all experiments to minimize fast GABAergic transmission (Huang & Kandel, 1998). The slices were incubated in the recording chamber at least 30 min before the start of recordings.

To record field potentials at thalamic input synapses to the lateral amygdala, we placed a bipolar stimulating

Corresponding to: Sukwoo Choi, School of Biological Sciences, Seoul National University, Gwanak-gu, Sillim-dong, Seoul 151-742, Korea. (Tel) 82-2-880-6700, (Fax) 82-2-872-1993, (E-mail) sukwoo12@snu.ac.kr

ABBREVIATIONS: mGluR, metabotropic glutamate receptors; aCSF, artificial cerebrospinal fluid; NMDA, N-methyl D-aspartic acid; AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DHPG, 3,5-Dihydroxyphenylglycine.

electrode in the thalamic afferent fibers innervating the lateral amygdala, which is located in the ventral part of the striatum, just above the central nucleus of the amygdala, just medial to the lateral amygdala. A trunk of the thalamic afferent fibers appeared to be well isolated from other structures, and it could easily be visualized under our microscope. A stimulating electrode was located specifically on the trunk to elicit the field potential. The recording electrode (>1.0 MΩ) was filled with 0.9% NaCl and placed in the dorsal subregion of the lateral amygdala.

Synaptic responses were elicited at 0.017 Hz. For the baseline field potential recording, 50% of the maximum amplitude was used. The range of stimulus intensity and duration for each pulse was 0.1~0.3 mA and 0.1~0.2 ms, respectively.

Extracellular field potentials were amplified using a DP-301 amplifier (Warner Instrument Co., CT), and the output was digitized with a DIGIDATA 1322A interface (Axon instruments Inc., Foster City, CA). The digitized signals were stored and analyzed with a PC computer using pClamp 8 (Axon Instruments Inc., Foster City, CA).

Drugs used were D-AP5, picrotoxin and kynurenic acid from Sigma-Aldrich (St. Louis, MO). DHPG, LCCG-I, L-AP4 and CPCCOEt were from Tocris Cookson (Ballwin, MO). Drugs were made up in stock solutions and diluted more than 1000 times into aCSF. Picrotoxin and CPCCOEt were made up in DMSO.

RESULTS

The field potential in our experimental condition had a constant and short latency of about 5 ms, followed high frequency (50 Hz) stimulation reliably and without failure, and it could be blocked by kynurenic acid (5 mM), a non-selective glutamate receptor antagonist (Fig. 1A and B). These findings suggest that the field potential measured in the present study reflects glutamatergic, monosynaptic responses at thalamic input synapses to the lateral amygdala.

The non-selective mGluR agonist t-ACPD (100 μM) produced a depression, which was fully reversed upon washout. In 4 of the 4 slices examined, we observed a decrease in PS amplitude (Fig. 2A, 56.5±2.7% of baseline PS). This finding suggests the involvement of an mGluR in the modulation of amygdala synaptic transmission, but did not clarify which mGluRs was involved.

To determine the mGluR subtype(s) involved in the modulation of amygdala synaptic transmission, we examined the effect of group I, II and III selective mGluR agonists on synaptic transmission at thalamic input synapses onto the lateral amygdala. The effects of the group III mGluR agonist L-AP4 was examined first. Treatment with the group III mGluR agonist L-AP4 (50 μM) produced no significant changes in amygdala synaptic transmission (Fig. 2B, 3.2±11.1% decrease in PS amplitude relative to baseline, P>0.6 paired t-test, n=8). This suggests that the group III mGluRs are not involved in the mGluR modulation of amygdala synaptic transmsission.

We also examined the effect of the group I mGluR selective agonist DHPG on amygdala synaptic transmission. Treatment with the group I mGluR agonist DHPG (100 μM) produced a short-term depression (Fig. 3A, 52.5± 1.8% of baseline PS, P<0.01 paired t-test, n=5). Furthermore, the DHPG effect was blocked by the group I mGluR selective antagonist CPCCOEt, suggesting that the DHPG exerts its effect by activating group I mGluRs (data not shown).

Next, we examined the effect of the group II mGluR selective agonist LCCG-1 on amygdala synaptic trans-

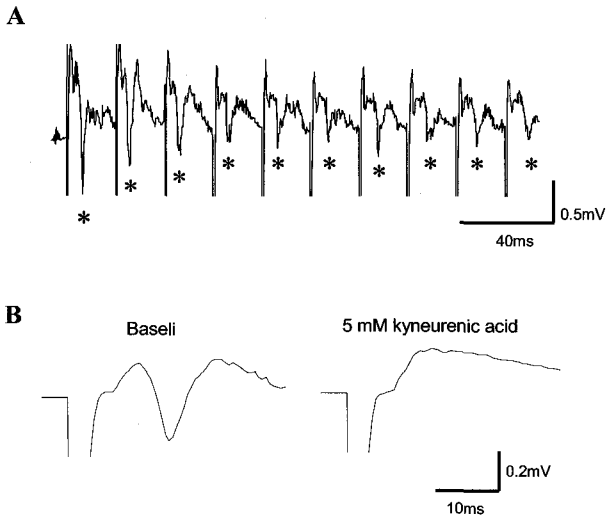

Fig. 1. Monosynaptic field potentials recorded in the thalamo-amygdala pathway. A, The synaptic potential recorded during a 50 Hz tetanus. The synaptic response followed the tetanus in a one-for-one manner without failure. B, The synaptic potential was blocked by kynurenic acid (5 mM). Asterisks indicate the synaptically-driven field potential component after each stimulus.

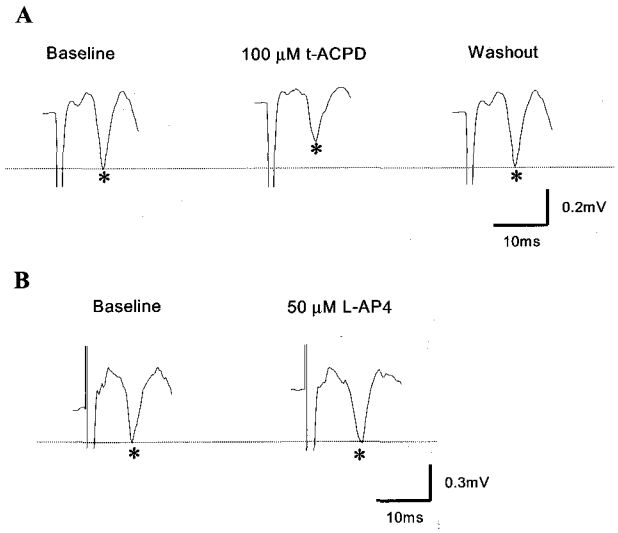

Fig. 2. Short-term inhibition of excitatory synaptic transmission by mGluRs at thalamic input synapses onto the lateral amygdala. A, the average normalized population spikes (PS) before, in the presence of and after the washout of 100 μM t-ACPD. B, the average normalized population spikes (PS) before, in the presence of 50 μM L-AP4. Please note that saturating concentration of L-AP4 did not have any significant effects on PS. Asterisks indicate the synaptically-driven field potential component after each stimulus. Averaged PSs for 5~10 episodes are shown in A and B.

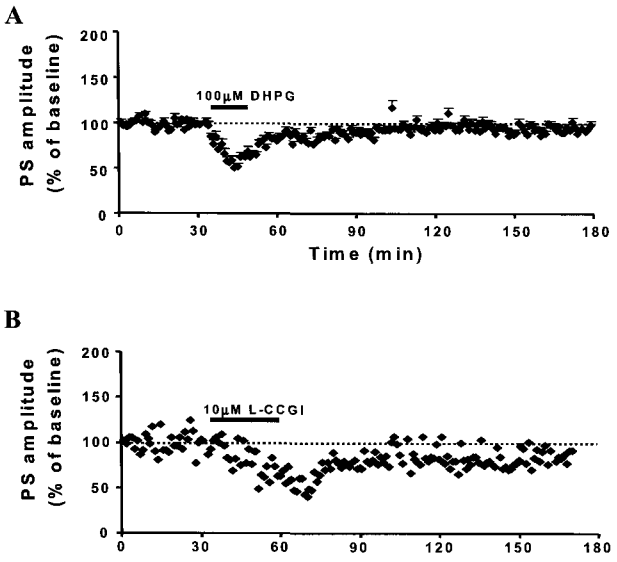

Fig. 3. Modulation of amygdala synaptic transmission by Group I and II mGluRs. A, Averaged PS amplitude plots showing that treatment of the Group I mGluR agonist DHPG reversibly reduced evoked PS amplitude. B, PS amplitude plots from a representative experiment showing that treatment of Group II mGluR agonist LCCG-I produced long-term as well as short-term depression. Bar indicates the duration of application of the mGluR agonists.

mission. Treatment with the group II mGluR agonist LCCG-I (10 μM) produced a depression, which was not completely reversed even with extensive washout (Fig. 3B). This long-term depression by LCCG-I has already been observed in the previous study by Heinbockel & Pipe (2000). These authors concluded that LCCG-I produced a presynaptic form of long-term depression. However, the result with LCCG-I was quite puzzling to us because the non-selective mGluR agonist t-ACPD, which should activate group II mGluRs, produced only short-term depression. Although we do not understand mechanisms underlying this phenomenon, we speculate that interactions among different mGluRs prohibit the long-term effect by group II mGluRs.

DISCUSSION

We have observed that agonism of group I mGluRs produced short-term depression of synaptic transmission at thalamic input synapses onto the lateral amygdala. In contrast, agonists of group II mGluRs produced long-term depression as well as short-term depression of synaptic transmission. Suprisingly, the non-selective mGluR agonist t-ACPD induced only short-term depression. Thus, activation of group I or II appears to produce short-term depression, whereas activation of group II alone has additional long-term effects. Furthermore, co-activation of different groups of mGluRs seems to mask the long-term effects by group II mGluRs.

Currently, we do not know much about the expression pattern of mGluRs identified with immunocytochemical tools. mGluR5, one member of group I mGluRs, has been shown to exist in postsynaptic spines of pyramidal neurons of the lateral amygdala (Rodriguez et al, 2002). From our preliminary experiment, mGluR1, another member of Group I mGluRs, appeared to exist at synaptic sites in the lateral amygdala (unpublished observation by J. Y. Kim, E. J. Kim, S. Choi). These anatomical data further support group I mGluR modulation of synaptic transmission at thalamic input synapses onto the lateral amygdala, although we do not know whether the group I mGluR agonists act on pre- or postsynaptic mGluRs. Group II mGluRs appear to be expressed at presynaptic sites, since the group II mGluR agonists have been shown to act presynaptically (Heinbockel & Pipe, 2000). These anatomical and functional data support the idea that mGluRs have an important role in synaptic transmission through distinct receptor subtypes in the lateral amygdala.

It should be noted that non-selective activation of mGluRs produced only short-term depression. This particular result implies some kind of interaction between different groups of mGluRs since activation of group II mGluRs alone produced a form of long-term depression. Possibly activation of mGluRs other than group II mGluRs inhibits long-term effects by group II mGluRs. In fact, interactions between different mGluRs have been reported in the previous studies (Rodriguez-Moreno et al, 1998).

It is well known that short-term modulation of synaptic transmission by mGluRs often involves direct modulation of ion channels by G-proteins (Pin & Acher, 2002). On the contrary, intracellular messengers such as PI and cAMP have been known to produce rather irreversible, long-term effects such as long-term potentiation and long-term depression. Therefore, group I mGluRs are likely to exert their effects on synaptic transmission through ion channels, whereas modulation by group II mGluRs may involve intracellular signal molecules as well as ion channels.

One of the most important questions related to this study would be whether these mGluRs can be stimulated by endogenous glutamate in physiological conditions. There are several factors which determine degree of mGluR activation in vivo; 1) distance between mGluRs and release sites, 2) amount of glutamate release, 3) diffusion barriers between mGluRs and release sites. Among these factors, amount of glutamate release can be easily altered by neuronal activities such as frequency of presynaptic activation. The higher the frequency is, the more the glutamate release would be. Therefore, it will be very important to find out a firing pattern of presynaptic neurons optimal for activation of each mGluR, and to determine if such patterns exist in vivo.

ACKNOWLEDGEMENTS

This work was supported by Korea Research Foundation Grant (KRF-2001-003-D00086).

REFERENCES

Choi S, Lovinger DM. Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. *Proc Natl Acad Sci USA* 94: 2665–2670, 1997

Choi S, Klingauf J, Tsien RW. Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses', *Nat Neurosci* 3: 330–336, 2000

Huang YY, Kandel ER. Postsynaptic induction and PKA-depen-

dent expression of LTP in the lateral amygdala, *Neuron* 21: 169–178, 1998

LeDoux JE. Emotion: clues from the brain. *Annu Rev Psychol* 46: 209–235, 1995

Ledoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 23: 155–184, 2000

Pin JP, Acher F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr Drug Target CNS Neurol Disord* 2002 1: 297–317, 2002

Rodrigues SM, Bauer EP, Farb CR, Schafe GE, LeDoux JE. The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. *J Neurosci* 22: 5219–5229, 2002

Rodriguez-Moreno A, Sistiaga A, Lerma J, Sanchez-Prieto J. Switch from facilitation to inhibition of excitatory synaptic transmission by group I mGluR desensitization. *Neuron* 21: 1477–1486, 1998

Weisskopf MG, Bauer EP, LeDoux JE. L-type voltage-gated calcium channels mediate NMDA-independent associative long-term potentiation at thalamic input synapses to the amygdala. *J Neurosci* 19: 10512–10519, 1999