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Metabotropic glutamate receptors mGluR4 and mGluR8 regulate transmission in the lateral olfactory tract-piriform cortex synapse

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Abstract

The piriform cortex (PC) is the primary terminal zone of projections from the olfactory bulb, termed the lateral olfactory tract (LOT). The PC plays a critical role in processing of olfactory stimuli and is also a highly seizure prone area thought to be involved in some forms of temporal lobe epilepsy. Pharmacological and immunohistochemical studies provide evidence for the localization of various metabotropic glutamate receptors (GluRs) in the PC. We employed whole cell patch clamp recordings from PC pyramidal cells to determine the roles of group III mGluRs in modulating synaptic transmission at the LOT–PC synapse. The group III mGluR agonist, L-AP4, induced a concentration-dependent inhibition of synaptic transmission at the LOT–PC synapse at concentrations that activate mGluR4 and mGluR8, but not mGluR7 or other mGluR subtypes ($EC_{50} = 473$ nM). In addition, the selective mGluR8 agonist, DCPG (300 nM), also suppressed synaptic transmission at the LOT synapse. Furthermore, the inhibitory actions of L-AP4 and Z-cyclopentyl-AP4, a selective mGluR4 agonist, were potentiated by the mGluR4 positive allosteric modulator, PHCCC (30 μ M). The high potency of L-AP4, combined with the observed effects of DCPG and PHCCC, suggests that both mGluR4 and mGluR8 play a role in the L-AP4-induced inhibition of synaptic transmission at the LOT–PC synapse.

Keywords

metabotropic glutamate receptors; group III mGluRs; L-AP4; DCPG; PHCCC; lateral olfactory tract; piriform cortex

1. Introduction

Metabotropic glutamate receptors (mGluRs) play an important role in modulating excitatory synaptic transmission at numerous synapses throughout the mammalian central nervous system (Conn & Pin 1997). The mGluRs are members of the family C G-protein-coupled receptors (GPCRs). To date, eight subtypes have been identified and are subdivided into three groups based on their sequence homology, pharmacological profiles, and downstream effector pathways. Group I includes mGluR1 and -5, which are coupled to G_q and activate PLC β ; group

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II includes mGluR2 and -3, which are coupled to $G_{i/o}$ and negatively modulate adenylate cyclase; group III consists of mGluR4, -6, -7, -8, which also couple to $G_{i/o}$ and negatively modulate adenylate cyclase activity. Both group II and III mGluRs are also known to regulate various ion channels, including voltage-gated calcium channels (Conn & Pin 1997).

A strong body of evidence now suggests that group III mGluRs function as presynaptic autoreceptors to reduce transmission at a variety of glutamatergic synapses (Abitbol et al., 2008; Ayala et al., 2008; Gereau & Conn 1995; Macek et al., 1996; Marino et al., 2003b; Marino et al., 2003a; Valenti et al., 2003; Valenti et al., 2005). However, until recently, there has been a lack of subtype selective ligands to precisely identify the role of individual group III mGluR subtypes in modulating excitatory neurotransmission at identified glutamatergic synapses.

Previous electrophysiological, pharmacological and immunohistochemical studies suggest that different group III mGluR subtypes are expressed in the piriform cortex (PC) where they can regulate excitatory synaptic transmission (Anson & Collins 1987; Benitez et al., 2000; Collins & Howlett 1988; Kinzie et al., 1997; Sugitani et al., 2004; Tan et al., 2006; Wada et al., 1998). The PC is the largest subdivision of the olfactory cortex and primarily responsible for processing olfactory information. It receives monosynaptic inputs from the lateral olfactory tract (LOT), which is formed from the axons of mitral and tufted cells located in the olfactory bulb. Anatomically, the PC is a relatively simple structure containing only three main layers. In the PC, lateral olfactory tract axon terminals form glutamatergic synaptic contacts onto the dendritic spines of pyramidal cells in both layers II and III (Haberly & Price 1978; Shipley & Ennis 1996). Layer II pyramidal cells receive the majority of the inputs from the olfactory bulb and recent findings have shown that this layer can be further subdivided into semilunar (SL, layer IIa) and superficial (SL, layer IIb) pyramidal cells based on their synaptic and firing properties (Suzuki & Bekkers 2006).

Both mGluR4 and mGluR8 have been shown to be expressed in cells of the olfactory bulb and are uniformly distributed on presynaptic membranes in the LOT synaptic terminals in layer I of the PC (Benitez et al., 2000; Wada et al., 1998). In addition, immunohistochemical studies also confirm the localization of mGluR7 in the presynaptic terminals of layer Ia in the PC (Wada et al., 1998). Consistent with these data, application of the group III mGluR agonist, L-AP4, has been shown to reduce excitatory synaptic transmission at the LOT-PC synapse in several electrophysiology studies (Anson & Collins 1987; Collins & Howlett 1988; Tan et al., 2006). However, in previous studies, subtype-selective ligands for individual group III mGluR subtypes have not been available, making it impossible to rigorously determine the roles of specific group III mGluRs in regulating transmission at this synapse. We have used novel subtype selective pharmacological ligands that act at specific group III mGluR subtypes to test the hypothesis that L-AP4-induced inhibition of excitatory transmission at LOT-PC synapse is mediated by both mGluR4 and mGluR8.

2. Materials and Methods

2.1. Compounds

L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4), (S)-3,4-Dicarboxyphenylglycine ((S)-DCPG), N-Phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide (PHCCC), and 2S-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-xanth-9-yl propanoic acid (LY341495) were purchased from Tocris Bioscience (Ellisville, MO). Z-Cyclopentyl AP4 ((Z)-1(RS)-amino-3(RS)-phosphonocyclopentanecarboxylic acid) (Crooks et al., 1986) was supplied by Dr. Rodney Johnson (University of Minnesota).

2.2. Animals

All animals used in these studies were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental protocols were in accordance with all applicable guidelines regarding the care and use of animals. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) approved facility with free access to food and water. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Brain Slice Electrophysiology

Sagittal brain slices (290–300 μm) were prepared from 15 to 25-day-old Sprague-Dawley rats (Charles River, Wilmington, MA). Animals were anesthetized with isoflurane, decapitated, and brains were rapidly removed and submerged in an ice-cold choline chloride replacement solution containing (in mM): 126 Choline Chloride, 26 NaHCO_3 , 1.2 NaH_2PO_4 , 2.5 KCl, 8 MgSO_4 , 1.3 MgCl_2 , 10 glucose, oxygenated with 95% O_2 and 5% CO_2 . Slices were prepared using a Vibratome (Vibratome 3000, St. Louis, MO), transferred to a chamber containing oxygenated artificial cerebrospinal fluid (ACSF; in mM: 130 NaCl, 3.5 KCl, 1.25 NaH_2PO_4 , 24 NaHCO_3 , 10 glucose, 1.5 CaCl_2 , 1.5 MgCl_2), incubated at 37 °C for 30 min, and then equilibrated at room temperature for at least 45 minutes before recording. In all experiments, 5 μM glutathione and 500 μM pyruvate were included in the choline chloride buffer and in the holding chamber ACSF.

Excitatory postsynaptic currents (EPSCs) were recorded from layer IIb of the PC in the whole-cell voltage clamp mode using a Warner 501A amplifier (Warner Instruments, Hamden, CT). During recordings, slices were maintained fully submerged on the stage of a brain slice chamber perfused with heated (32 °C) and oxygenated ACSF at 2 ml/min. Superficial (SP) pyramidal neurons (layer IIb) were distinguished from semilunar (SL) granule neurons within in layer II of the PC according to cell morphology, somatic location (SP, deep layer II; SL, superficial layer II), and electrical properties (input resistance, firing properties, and membrane potential) (Suzuki & Bekkers 2006). SP pyramidal neurons were visualized with an Olympus BX51WI upright microscope (Olympus, Lake Success, NY) coupled with a 40 $\times$ water immersion objective and Hoffman optics. Borosilicate glass pipettes were pulled using a Flaming/Brown micropipette puller (model P-97; Sutter Instruments, Novato, CA) to produce patch electrode resistances of 2.0 to 4.0 M Ω when filled with an intracellular solution containing (in mM): 140 K-gluconate, 7 NaCl, 4 Mg-ATP, 0.3 $\text{Na}_3\text{-GTP}$, 10 HEPES (pH adjusted to 7.3 with KOH and osmolality of ~295 mOsm). Concentric bipolar tungsten electrodes were placed in the lateral olfactory tract (LOT) to the more caudal section of the PC in slices corresponding to Watson and Paxinos rat brain atlas (figures 10–15) (Paxinos & Watson 1998). Stimulation of the LOT (2–20 V, 0.20 ms) evoked EPSCs recorded from layer IIb pyramidal cells of the PC. EPSCs were recorded at a holding potential of –60 mV, and bicuculline (10 μM) was added to the bath solution to block GABA_A-mediated inhibitory currents. In some experiments, the bath solution contained a low concentration of DNQX (6,7-dinitroquinoxaline-2,3-dione) (1 μM), to minimize polysynaptic activity as previously reported by (Suzuki & Bekkers 2006). The voltage-clamp signal was low-pass-filtered at 2 kHz, digitized at 10 kHz, and acquired using a Clampex9.2/DigiData 1332 system (Molecular Devices, Sunnyvale, CA). Compounds were made in a 1000 $\times$ stock and diluted into ACSF immediately before use. After a stable baseline was recorded for 5 to 10 min, the effect of each compound on baseline EPSC amplitude was examined.

2.4. Statistical Analysis

All electrophysiology data analysis was performed using Clampfit software (v9.2, Molecular Devices, Sunnyvale, DA), Origin (v6, Microcal Origin, Northampton, MA), GraphPad Prism (GraphPad Software Inc, San Diego, CA), and Excel (Microsoft Corp., Redmond, WA).

Statistical analysis were performed using either the student's unpaired *t*-test or a standard one way ANOVA with Bonferroni's multiple comparison test, or Dunnett's post hoc test to compare multiple sets of data in the GraphPad Prism software. Averaged data are presented as mean $\pm$ standard error of the mean (S.E.M.). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Activation of group III mGluRs modulates synaptic transmission at the LOT-PC synapse

To test the hypothesis that activation of group III mGluRs modulates synaptic transmission at the LOT-PC synapse, we determined the effects of multiple concentrations of a selective group III mGluR agonist, L-AP4, on evoked EPSCs. Consistent with previous electrophysiology studies using field recordings (Tan et al., 2006), application of L-AP4 (300 nM) caused a significant reduction in evoked EPSC amplitudes that was reversible upon washout (70.6 ± 4.8 % of baseline, $P < 0.05$, $n = 5$; Fig. 1A and B). The inhibition of EPSCs by L-AP4 was concentration dependent with a calculated EC_{50} value of 473 nM (Fig. 1C). In both recombinant and brain slice systems, it has been shown that nanomolar concentrations of L-AP4 are sufficient to activate both mGluR4 and mGluR8, while much higher millimolar concentrations of L-AP4 are required for activation of mGluR7 (Ayala et al., 2008; Schoepp et al., 1999). Given that mGluR6 is not localized within the CNS (Nakajima et al., 1993), the high potency of L-AP4 at the LOT-PC synapse suggests that its effects are mediated by mGluR4 or mGluR8.

A decrease in synaptic transmission induced by L-AP4 could result from a pre- or postsynaptic depression of neurotransmitter release at the LOT-PC synapse. However, several studies have shown that the group III mGluRs are localized presynaptically within the PC region (Benitez et al., 2000; Kinzie et al., 1997; Wada et al., 1998) and primarily function as presynaptic autoreceptors throughout many glutamatergic synapses in the CNS (Conn & Pin 1997). For example, previous electrophysiology studies using paired pulse facilitation protocols demonstrated that group III mGluRs agonists, including L-AP4, mediated their effects by a presynaptic mechanism at the LOT-PC synapse (Anson & Collins 1987; Tan et al., 2006). In this present study, L-AP4 had no effect on input resistance at all concentrations tested (10 μ M L-AP4, 107.8 ± 3.8 % of baseline, $P = 0.17$, $n = 5$; data not shown). Thus, the inhibitory actions of L-AP4 are likely mediated by a presynaptic decrease in transmitter release, rather than postsynaptic actions.

3.2. PHCCC potentiates L-AP4-induced inhibition of synaptic transmission at the LOT-PC synapse

PHCCC has recently been characterized as a highly selective positive allosteric modulator of mGluR4 (Maj et al., 2003; Marino et al., 2003b). Most notably, PHCCC alone does not activate mGluR4, but potentiates the response to activation of mGluR4 by orthosteric agonists. In addition to effects in recombinant systems, PHCCC significantly potentiates the effects of L-AP4 in activating mGluR4 at synapses in the globus pallidus (Marino et al., 2003b; Marino et al., 2003a), substantia nigra (Valenti et al., 2005), and cerebellar cortex (parallel fiber-purkinje cell synapse) (Abitbol et al., 2008), where mGluR4 is the primary mGluR subtype regulating synaptic transmission. To further differentiate the role of mGluR4 and mGluR8 in regulating synaptic transmission at the LOT-PC synapse, we tested the ability of PHCCC to potentiate the inhibitory effects of L-AP4. At a concentration of 300 nM, L-AP4 induced a small but discernable effect on transmission at the LOT-PC synapse (Fig. 1A). Thus, we chose this submaximal concentration of L-AP4 and a concentration of PHCCC (30 μ M) previously shown to maximally potentiate mGluR4 and to enhance L-AP4 responses at other synapses (Ayala et al., 2008; Marino et al., 2003b; Valenti et al., 2005). PHCCC significantly potentiated L-AP4-induced inhibition of synaptic transmission at the LOT-PC synapse (300 nM L-AP4, 70.6 ± 4.8 % of baseline; 300 nM L-AP4 + 30 μ M PHCCC, 50.5 ± 4.9 % of baseline, $P < 0.05$, $n =$

5; Fig 2A, B and C). The synergistic effects of L-AP4 and PHCCC suggest that L-AP4 action in the LOT-PC is mediated at least in part by activation of mGluR4.

3.3. Selective activation of mGluR8 by DCPG modulates synaptic transmission at the LOT-PC synapse

The finding that mGluR4 plays a role in regulating transmission at the LOT-PC synapse does not rule out the possibility that mGluR8 may also contribute to L-AP4-induced inhibition of transmission at this synapse. Because the effects of L-AP4 are consistent with activation of both mGluR4 and mGluR8, we used the selective agonist of mGluR8, DCPG (Thomas et al., 2001). Consistent with selectivity of this compound for mGluR8 in cell lines, we have previously shown that DCPG has no effect on transmission at synapses in the rat globus pallidus, and substantia nigra, where mGluR4 plays a predominant role in regulating synaptic transmission (Valenti et al., 2003; Valenti et al., 2005). Application of 300 nM DCPG significantly reduced synaptic transmission at the LOT-PC synapse ($52.1 \pm 4.2\%$ of baseline, $P < 0.001$, $n = 4$; Fig 3 A and B). These data suggest that mGluR8 also plays a role in the L-AP4-induced inhibition at this synapse and contributes to the regulation of excitatory transmission in the PC.

It has previously been shown that the effects of L-AP4 are mediated by a presynaptic depression of neurotransmitter release at the LOT-PC synapse (Anson & Collins 1987; Tan et al., 2006). However, DCPG-induced reduction of transmission at the LOT-PC could be mediated by a pre- or postsynaptic mechanism. To address this question, we performed paired-pulse facilitation experiments in the absence or presence of 300 nM DCPG. Application of 300 nM DCPG significantly increased the paired pulse facilitation ratio in the LOT-PC synapse (Control, 2.00 ± 0.30 versus 300 nM DCPG, 3.46 ± 0.52 , $P < 0.05$, $n = 5$; Fig 3C). In addition, 300 nM DCPG was without effect on input resistance values ($96.6 \pm 3.8\%$ of baseline, $P = 0.32$, $n = 4$; data not shown). These data suggest that the effects of DCPG at the LOT-PC synapse are mediated by a presynaptic mode of action.

3.4. Z-Cyclopentyl-AP4 requires PHCCC to reduce synaptic transmission at the LOT-PC synapse

The studies above suggest that mGluR4 plays a role in modulating synaptic transmission in the PC. To further test this hypothesis, we determined whether selective activation of mGluR4 with a selective agonist would result in similar inhibitory effects observed for L-AP4 (Fig. 1 and Fig. 2). We recently reported that a conformationally restrained analogue of L-AP4, Z-Cyclopentyl-AP4, acts as an agonist with a higher potency for mGluR4 than mGluR8 and is without effect on mGluR7. Furthermore, Z-cyclopentyl-AP4, in the presence of PHCCC, has been shown to have high selectivity for activating mGluR4 with minimal effects on both mGluR7 and mGluR8 (Ayala et al., 2008).

We first determined the ability of Z-cyclopentyl-AP4 to reduce transmission at the LOT-PC synapse in the absence of PHCCC. Consistent with our previous findings (Ayala et al., 2008), Z-cyclopentyl-AP4 induced a small reduction of transmission at the LOT-PC synapse ($100 \mu\text{M}$ Z-cyclopentyl-AP4, $87.4 \pm 2.7\%$ of baseline, $P < 0.05$, $n = 5$; Fig. 4A and B). Z-cyclopentyl-AP4 is a structural analogue of L-AP4 and did not significantly change input resistance values ($101.7 \pm 1.4\%$ of baseline, $P = 0.83$, $n = 5$; data not shown), suggesting that its inhibitory actions are also mediated by a presynaptic mechanism. Furthermore, the effect of Z-cyclopentyl-AP4 was significantly potentiated in the presence of PHCCC ($100 \mu\text{M}$ Z-cyclopentyl-AP4 + $30 \mu\text{M}$ PHCCC, $50.3 \pm 7.8\%$ of baseline, $P < 0.05$, $n = 5$; Fig. 4A and B), providing further evidence in support of an mGluR4 regulation of transmission in the LOT-PC synapse.

3.5. LY341495 antagonizes the L-AP4-induced inhibition of synaptic transmission at the LOT-PC synapse

LY341495 is a general mGluR antagonist with nanomolar potency at group II mGluRs while micromolar concentrations are required to antagonize group I and III mGluR subtypes (Fitzjohn et al., 1998; Kingston et al., 1998; Niswender et al., 2008; Schoepp et al., 1999). To verify that the actions of L-AP4 were mediated by activation of a group III mGluR function and not by any off target mechanisms, we determined the response to L-AP4 in the presence of LY341495. Application of 100 μ M LY341495, a concentration at least 4-fold higher than the IC_{50} of this compound at recombinant group III mGluRs, inhibited the response to L-AP4 (300 nM L-AP4, 70.6 ± 4.8 % of baseline versus 300 nM L-AP4 + 100 μ M LY341495, 94.4 ± 1.7 % of baseline; $P < 0.001$, $n = 5$; Fig. 5A and B). We found no evidence for an effect of LY341495 alone on transmission at the LOT-PC synapse (100 μ M LY341495, 94.6 ± 5.3 % of baseline, $P > 0.05$, $n = 5$; Fig. 5A and B). These data, taken together with the high potency of L-AP4 and an observed reduction of transmission in the presence of DCPG, suggest that the L-AP4 modulation of synaptic transmission at this synapse is most likely attributed to actions at both mGluR4 and mGluR8.

4. Discussion

In this study we employed a combination of novel subtype selective pharmacological ligands and whole-cell patch clamp techniques to determine the role of specific subtypes of group III mGluRs in regulating synaptic transmission at the LOT-PC synapse. Previous immunohistochemical studies revealed the presence of group III mGluRs in the LOT-PC synapse (Benitez et al., 2000; Wada et al., 1998). Furthermore, the group III mGluR agonist L-AP4 reduces excitatory transmission in the PC (Anson & Collins 1987; Collins & Howlett 1988; Tan et al., 2006). However, these previous studies did not identify the individual group III mGluR subtypes responsible for regulating transmission in the PC due to the previous lack of subtype selective ligands for group III mGluRs. Here, we report that mGluR4 and mGluR8 are the primary group III mGluRs mediating the L-AP4-induced reduction of synaptic transmission at the LOT-PC synapse.

Using novel ligands, we have established a role for both mGluR4 and mGluR8 to reduce synaptic transmission. Specifically, low concentrations of L-AP4 reduce evoked EPSC amplitude, consistent with an effect on the high affinity group III mGluRs, mGluR4 and mGluR8. In addition, the selective allosteric potentiator of mGluR4, PHCCC, can enhance the effects of both L-AP4 and the mGluR4 selective ligand Z-Cyclopentyl-AP4. Confirming a role for mGluR8 in mediating this effect, DCPG also reduced transmission with a potency consistent with selective activation of mGluR8. The effect of L-AP4 was deemed selective for mGluR activation as opposed to an off target effect by blockade with the mGluR selective antagonist LY341495. Furthermore, ours and previous studies confirm that the actions of these compounds are through a presynaptic mechanism.

Collectively, our findings are consistent with past studies examining the localization of group III mGluRs in the PC (Benitez et al., 2000; Wada et al., 1998). In particular, both mGluR4 and mGluR8 have been shown to be expressed in the olfactory bulb and are uniformly distributed on presynaptic membranes in the LOT synaptic terminals in layer I of the PC (Benitez et al., 2000; Wada et al., 1998). However, these studies also report the localization of mGluR7 in the presynaptic terminals of layer Ia in the PC (Wada et al., 1998). Indeed, our results do not exclude the possibility that mGluR7 may also contribute to the regulation of synaptic transmission at the LOT-PC synapse. However, a limited number of pharmacological tools currently exists to reliably delineate the role of mGluR7 in overexpression and native systems, and moreover in this present study. AMN082 has been reported to be an mGluR7 selective agonist (Mitsukawa et al., 2005; Suzuki et al., 2007). However, we recently found that AMN082 does not inhibit

synaptic transmission in the hippocampus at the Schaffer collateral-CA1 synapse, where mGluR7 is the dominant presynaptic autoreceptor. AMN082 was also without effect in cell-based assays of mGluR7-induced activation of the GIRK potassium channel (Ayala et al., 2008). Because AMN082 does not have verified effects on mGluR7 in multiple systems, this compound has not proven useful for determining the roles of mGluR7 in native systems such as the PC. Based on the low affinity of glutamate at mGluR7 and its high affinity at activating mGluR4 and mGluR8 it is likely that these receptors regulate different modes of transmission. Along the same lines, we have previously shown at the Schaffer collateral-CA1 synapse that mGluR7 becomes the primary group III mGluR regulating transmission at this synapse in adult aged rats. With this in mind, our current studies used juvenile aged animals which does not rule out the possibility that mGluR7 could play a role in adult animals at the LOT-PC synapse with a minimal role in juvenile animals; indeed, previous immunohistochemical data using adult aged rats report the localization of mGluR7 in layer Ia of the PC.

It is interesting to note that the data presented suggest that the effects of activation of both mGluR4 and mGluR8 are not strictly additive. Interestingly, DCPG or Z-cyclopentyl-AP4 plus PHCCC induce about a 50% inhibition of EPSCs. Thus, if the effects of these compounds were additive, a maximally effective concentration of L-AP4 should induce a complete inhibition of EPSCs. However, the maximal response to L-AP4 is somewhat comparable to the depression achieved by the more selective compounds (i.e. DCPG or Z-cyclopentyl-AP4 plus PHCCC) and L-AP4 clearly does not fully suppress EPSCs. This suggests that the combined effects of activating both mGluR4 and mGluR8 are at least partially redundant and that maximal activation of both receptor subtypes does not induce a completely additive depression of synaptic transmission.

The reduction of synaptic transmission at the LOT-PC cortex by group III mGluR activation could have several functional implications. First, it could have a significant effect on olfactory perception and processing, given that the PC receives direct inputs from the olfactory bulb, which in turn receives direct inputs from the primary odorant receptors in the olfactory epithelium (Haberly & Price 1978; Wilson 2001). In line with this idea, it has been recently reported that group III mGluR activation in the PC is necessary for rat habituation to odor mediated-behaviors (Best et al., 2005; Yadon & Wilson 2005). Second, the PC has also been implicated as a critical region for the generation and propagation of temporal lobe seizures (Doherty et al., 2000; Hoffman & Haberly 1991; Loscher & Ebert 1996; Shimosaka et al., 1994). In fact, recent studies reveal that unilateral low frequency stimulation (LFS) of the central piriform reduces amygdaloid-kindled induced seizures (Zhu-Ge et al., 2007). In light of these data, it would be interesting to test the hypothesis that a reduction in transmission mediated by mGluR4 and mGluR8 could affect the initiation and propagation of seizures activity in the PC (Loscher & Ebert 1996; Zhu-Ge et al., 2007). Such *in vivo* studies would also contribute to a growing body of literature identifying the potential role of subtype selective mGluR ligands to treat a variety of epileptic seizures (Doherty & Dingledine 2002).

In summary, we have identified mGluR4 and mGluR8 as the primary group III mGluR subtypes responsible for reducing synaptic transmission at the LOT-PC synapse. This conclusion is based on the high potency of L-AP4 at this synapse, combined with the observed inhibitory effects of Z-Cyclopentyl-AP4, DCPG, and potentiation of agonist activity in the presence of PHCCC. However, these data do not rule out the possibility that mGluR7 may also play an important role in regulating transmission at this synapse as well. Taken together, these data provide insight into the functional role of specific subtypes of group III mGluRs in the PC.

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Figure 1. L-AP4 reduces synaptic transmission at the LOT-PC synapse in rat brain slices

(A) Representative excitatory postsynaptic currents (EPSCs) recorded from superficial (SP) pyramidal neurons in layer IIb of the PC using whole-cell patch clamp techniques. EPSCs were elicited by stimulating the lateral olfactory tract (LOT). Traces show before, during, and after a 5 min application of group III mGluR agonist, L-AP4 (300 nM). (B) Averaged time course illustrating rapid and reversible inhibitory effects of L-AP4 (300 nM) ($n = 5$, $P < 0.05$). (C) Concentration–response curve for L-AP4 showing a reduction of EPSC amplitude induced by increasing concentrations of L-AP4. The concentration producing half-maximal reduction in EPSC amplitude is 473 nM ($n = 4 - 6$ cells for each concentration). Data are expressed as a percentage of baseline EPSC amplitude and represent the mean $\pm$ S.E.M.

Figure 2. PHCCC potentiates the effects of L-AP4 at the LOT-PC synapse in rat brain slices
 (A) Representative traces depicting the effects of 300 nM L-AP4 on EPSC amplitude in the presence and absence of, a selective positive allosteric modulator at mGluR4, PHCCC (300 μM L-AP4, $70.6 \pm 4.3\%$ of baseline; 300 μM L-AP4 + 30 μM PHCCC, $50.5 \pm 4.9\%$ of baseline; $P > 0.05$). (B) Averaged time course illustrating a rapid and reversible potentiation of L-AP4-induced inhibition of transmission in the presence of PHCCC (30 μM). (C) Bar graph depicting that the inhibitory effect of 300 nM L-AP4 in the presence of PHCCC (30 μM) is significantly increased ($P > 0.05$). Data are expressed as percentage of baseline EPSC amplitude and represent the mean $\pm$ S.E.M. ($n = 5$ for each experiment).

Figure 3. DCPG decreases synaptic transmission at the LOT-PC synapse in rat brain slices
 (A) Representative traces depicting the effects of a selective mGluR8 agonist, DCPG (300 nM) on EPSC amplitude in the PC. (B) Averaged time course before, during, and after a 5 min bath application of DCPG (300 nM). DCPG induced a rapid and reversible reduction of synaptic transmission (52.1 ± 4.2 % of baseline, $n = 4$, $P < 0.001$). (C) Bar graph illustrating the effect of 300 nM DCPG on paired pulse facilitation. DCPG significantly increased the paired pulse facilitation ratio in the LOT-PC synapse (Control, 2.00 ± 0.30 versus 300 nM DCPG, 3.46 ± 0.52 , $P < 0.05$, $n = 5$). EPSCs were elicited with a half-maximal voltage (0.20 ms duration) with an inter-pulse-interval equal to 50 ms. Data are expressed as a percentage of baseline EPSC amplitude and represent the mean $\pm$ S.E.M.

Figure 4. PHCCC dramatically potentiates the effects of Z-cyclopentyl-AP4 at the LOT-PC synapse in rat brain slices

Top panels (A) and (B) Representative traces displaying the effects of Z-cyclopentyl-AP4 (100 μM) in the absence and presence of PHCCC (30 μM). Bottom panels (A) and (B) Averaged time courses depicting the effects of Z-cyclopentyl-AP4 in the absence and presence of PHCCC (100 μM Z-cyclopentyl-AP4 $87.4 \pm 2.7\%$ of baseline; 100 μM Z-cyclopentyl-AP4 + 30 μM PHCCC, $50.3 \pm 7.8\%$ of baseline; $P > 0.05$). Data are expressed as a percentage of EPSC baseline amplitude and represent the mean $\pm$ S.E.M. ($n = 5$ for each experiment).

Figure 5. LY341495 antagonizes the effect of L-AP4 at the LOT-PC synapse in rat brain slices (A) Representative EPSCs and (B) bar graph illustrating the effect of 300 nM L-AP4 on EPSC amplitude in the absence and presence of 100 μ M LY341495 in the PC (300 nM L-AP4, 70.6 ± 4.8 % of baseline; 300 nM L-AP4 + 100 μ M LY341495, 94.4 ± 1.7 % of baseline; $P < 0.001$). LY341495 (100 μ M) was without effect on synaptic transmission alone ($P > 0.05$). Data are expressed as a percentage of baseline EPSC amplitude and represent mean $\pm$ S.E.M. ($n = 5$ cells for each experiment).