Calcium-Stimulated Adenylyl Cyclases Required for Long-Term Potentiation in the Anterior Cingulate Cortex

Jason Liauw, Long-Jun Wu, and Min Zhuo
Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

Submitted 22 November 2004; accepted in final form 30 January 2005

Liauw, Jason, Long-Jun Wu, and Min Zhuo. Calcium-stimulated adenylyl cyclases required for long-term potentiation in the anterior cingulate cortex. J Neurophysiol 94: 878–882, 2005; doi:10.1152/jn.01205.2004. Activity-dependent long-term potentiation (LTP) in the CNS is thought to be important in learning, memory, development, and persistent pain. Here, we report that NMDA receptor-dependent LTP is the major form of long-term plasticity in the anterior cingulate cortex (ACC). In addition to N-methyl-D-aspartate (NMDA) receptors, L-type voltage-gated calcium channels are also required for inducing LTP. Activation of calcium-stimulated adenylyl cyclase subtype 1 (AC1) is essential for the induction of LTP in ACC neurons, while AC8 subunit partially contributes to forskolin-induced potentiation. Our results suggest that calcium-stimulated cAMP-dependent signaling pathways play a critical role in cingulate LTP.

INTRODUCTION

Neuronal activity in forebrain structures is thought to play a critical role in many brain functions including emotion, learning, and memory, and persistent pain (Doron and Ledoux 2000; Kandel 2001; Zhuo 2004). Synapses in these regions undergo long-term plastic changes in response to physiological stimuli or pathological insults (McKernan and Shinnick-Gallagher 1997; Rogan et al. 1997; Wei et al. 2001). Understanding the signaling pathways responsible for triggering plasticity can provide new insight into the mechanisms of forebrain function. Among many forms of synaptic plasticity, N-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) is the most widely studied and is thought to be important for learning and memory. In the hippocampal CA1 region, activation of NMDA receptors is critical for LTP induction (Collingridge and Bliss 1995; Nicoll and Malenka 1995). Subsequent activation of calcium-calmodulin (CaM) dependent adenylyl cyclases (AC), AC1 and AC8, by the calcium influx through the opened NMDA receptor channels contributes to synaptic potentiation in the hippocampus (Wang et al. 2003; Wong et al. 1999). In contrast, the roles of AC1 and AC8 in synaptic LTP of the anterior cingulate cortex (ACC) have not been reported. In this study, we investigated whether LTP in the ACC requires the activation of NMDA receptors and whether AC1 and AC8 activities are important for triggering synaptic potentiation in ACC neurons. Due to the lack of selective inhibitors for AC1 and AC8, we used a combination of genetic and pharmacological approaches to study the role of NMDA and AC1/AC8 in cingulate LTP.

METHODS

Animals

Adult wild-type and knockout (KO) mice (8–12 wk old) were anesthetized with 1–2% halothane. Transverse slices of the ACC (300 or 400 µM) were prepared using standard methods (Wei et al. 1999). Slices were transferred to a room temperature–submerged recovery chamber with oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) solution containing (in mM) 124 NaCl, 4.4 KCl, 25 NaHCO3, 1.0 NaH2PO4, 2.0 CaCl2, 2.0 MgSO4, and 10 glucose. After a 1-h recovery period, slices were transferred into recording chambers for either whole cell patch-clamp recordings or field potential recordings.

Whole cell patch-clamp recordings

Adult wild-type and knockout (KO) mice (8–12 wk old) were anesthetized with 1–2% halothane. Transverse slices of the ACC (300 or 400 µM) were prepared using standard methods (Wei et al. 1999). Slices were transferred to a room temperature–submerged recovery chamber with oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF) solution containing (in mM) 124 NaCl, 4.4 KCl, 25 NaHCO3, 1.0 NaH2PO4, 2.0 CaCl2, 2.0 MgSO4, and 10 glucose. After a 1-h recovery period, slices were transferred into recording chambers for either whole cell patch-clamp recordings or field potential recordings.

LTP in slices

For field potential recordings, transverse slices of cingulate cortex were rapidly prepared and maintained in an interface chamber at 28°C, where they were suffused with ACSF consisting of (in mM) 124 NaCl, 4.4 KCl, 2.0 CaCl2, 1.0 MgSO4, 25 NaHCO3, 1.0 NaH2PO4, and 10 glucose and bubbled with 95% O2-5% CO2. Slices were kept in the recording chamber for at least 2 h before the experiments. A bipolar stimulating electrode was placed in layer V, and extracellular field potentials were recorded with a glass microelectrode (3–12 MΩ, filled with ACSF) inserted into layer II/III. Based on preliminary

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
studies, five brief theta burst stimulations (TBSs) were used to induce LTP in ACC slices (Wei et al. 2002b). 

**Pharmacological inhibitors**

AP-5 and nifedipine were used to selectively block NMDA receptors and L-type voltage-gated calcium channels. SQ22536, an inhibitor of ACs, was used to inhibit the activity of ACs, whereas forskolin, a selective AC activator, was used to activate ACs. All chemicals and drugs were obtained from Sigma (St. Louis, MO), except for SQ22536, which was purchased from BIOMOL (Plymouth Meeting, PA). Drugs were applied to the perfusion solution.

**Data analysis**

Results were expressed as means ± SE. Statistical comparisons were performed with the Student t-test or one-way ANOVA. In all cases, P < 0.05 was considered statistically significant.

**RESULTS**

Glutamate is the major fast excitatory neurotransmitter in the cingulate cortex of rats (Sah and Nicoll 1991; Tanaka and North 1994; Wei et al. 1999). However, no direct study has been reported in adult mouse cingulate slices. Whole cell patch-clamp recordings from cingulate pyramidal cells of adult mice showed that fast excitatory postsynaptic currents (EPSCs) were elicited by delivering focal electrical stimulation (Fig. 1). When cells were held at −65 mV, EPSCs were slightly blocked by NMDA receptor antagonist AP-5 (100 μM; Fig. 1A). The remaining currents were completely blocked by the AMPA/kainate receptor antagonist; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); 10 μM; n = 12; Fig. 1A.

To determine if excitatory synaptic transmission in ACC slices undergoes LTP, we used TBS to induce synaptic potentiation (Frankland et al. 2001; Wei et al. 2002b). Preliminary experiments using one single TBS found that it did not cause
long-lasting potentiation \((n = 6, \text{ data not shown})\). Thus five bursts of TBS were used, and long-lasting synaptic potentiation was induced in ACC slices \((n = 15; \text{ mean, } 162.7 \pm 11.7\% \text{ of control}; P < 0.01 \text{ compared with baseline; Fig. 1B})\). Postsynaptic calcium-stimulated signaling pathways are important for synaptic potentiation in the CNS (Lisman and McIntyre 2001). NMDA receptors are important for the induction of LTP in the hippocampus and neocortex (Bear 1996; Collingridge and Bliss 1995; Nicoll and Malenka 1995). To test the role of NMDA receptors in ACC LTP, we carried out experiments in the presence of a selective NMDA receptor antagonist, AP-5. As shown in Fig. 1B, synaptic potentiation induced by TBS was completely abolished by bath application of 100 \(\mu M\) AP-5 \((n = 5; \text{ mean, } 96.8 \pm 5.7\% \text{ of control}; P < 0.01 \text{ compared with that in control solution})\). In addition to NMDA receptors, L-type voltage-gated calcium channels (L-VDCCs) are important for synaptic plasticity (Aniksztejn and Ben-Ari 1991; Grover and Teyler 1990; Huang and Malenka 1993; Wei et al. 1999). To determine the role of L-VDCCs, a selective L-VDCC antagonist nifedipine \((10 \mu M)\) was applied through the bath chamber. TBS produced significantly reduced or no synaptic potentiation in slices treated with nifedipine \((n = 10; \text{ mean, } 105.5 \pm 7.2\% \text{ of control}; P < 0.01 \text{ compared with control slices; Fig. 1, C and D})\). In contrast, basal synaptic responses were not significantly affected by nifedipine in the same slices.

In central neurons, increases in postsynaptic calcium levels trigger activation of ACs and production of cAMP (Xia and Storm 1997). To examine the possible roles of ACs in synaptic potentiation, we used a selective AC inhibitor SQ22536 at three different doses \((1-100 \mu M)\). At a low dose of 1 \(\mu M\) SQ22536, synaptic potentiation induced by TBS was significantly reduced \((n = 5, 121.5 \pm 10.7\% \text{ of control}; P < 0.05 \text{ compared with control slices})\). However, at a higher dose of 10 \(\mu M\) \((n = 4)\) or 100 \(\mu M\) \((n = 8)\), synaptic potentiation was completely abolished \((10 \mu M, n = 4, \text{ mean, } 105.3 \pm 10.1\% \text{ of control}; 100 \mu M, n = 8, \text{ mean, } 84.3 \pm 6.0\% \text{ of control}; P < 0.01 \text{ compared with control slices in both cases; Fig. 2, A and B})\). Baseline synaptic responses were not affected by the same dose of SQ22536. If AC activity is required for LTP induction, we predicted that activation of ACs might cause LTP in ACC slices. To test this possibility, we bath-applied a selective AC activator forskolin \((10 \mu M)\). As expected, application of forskolin caused a prolonged enhancement of synaptic responses in ACC slices \((n = 8; \text{ mean, } 193.0 \pm 18.7\% \text{ of control}; P < 0.01 \text{ compared with baseline})\). Synaptic potentiation lasted for \(\geq 45\) min \((\text{Fig. 2C})\). Pretreatment slices with SQ22536 \((10 \mu M)\) blocked the potentiation induced by forskolin \((10 \mu M; n = 5; \text{ mean, } 103.3 \pm 10.2\% \text{ of control; Fig. 2C})\).

There are at least nine different isoforms of ACs that have been identified, each with a unique pattern of expression within the CNS at levels from the peripheral sensory nerves to the frontal cortex (Xia and Storm 1997). Among them, AC1 and AC8 are the two CaM-stimulated ACs in the CNS. They couple NMDA receptor–induced cytosolic Ca\(^{2+}\) increases to cAMP signaling pathways (Chetkovich and Sweatt 1993; Wong et al. 1999). To test if calcium-stimulated cAMP pathways contribute to synaptic enhancement within the ACC, we studied LTP induced by TBS in ACC slices of wild-type, AC1, and AC8 KO mice. In slices from wild-type mice, TBS induced significant potentiation of synaptic responses \((n = 9 \text{ slices/8 mice}; \text{ mean, } 168.6 \pm 16.7\% \text{ of control}; P < 0.01 \text{ compared with baseline; Fig. 3})\). However, in AC1 KO mice, synaptic potentiation was completely blocked \((n = 6 \text{ slices/5 mice}; \text{ mean, } 107.5 \pm 9.9\% \text{ of control}; P < 0.01 \text{ compared with wild-type mice; Fig. 3B})\). In contrast, synaptic potentiation in slices from AC8 KO mice was not significantly affected \((n = 6 \text{ slices/6 mice}; \text{ mean, } 145.4 \pm 14.6\% \text{ of control; no significant difference from wild-type})\). No obvious changes in basal synaptic responses were found between wild-type, AC1, and AC8 KO mice. Furthermore, we also measured synaptic potentiation in ACC slices of AC1 and AC8 double KO mice. We found that synaptic potentiation was abolished \((n = 9 \text{ slices/7 mice}; \text{ mean, } 85.2 \pm 8.6\% \text{ of control}; P < 0.01 \text{ compared with wild-type})\). These results indicate that calcium-stimulated AC1 plays an important role in ACC LTP.

We also wanted to see if AC1 and AC8 are required for LTP induced by activation of ACs. Forskolin \((10 \mu M)\) applied through bath solution caused significant enhancement of synaptic responses \((n = 6 \text{ slices/5 mice}; \text{ mean, } 199.3 \pm 20.3\% \text{ of control}; P < 0.01 \text{ compared with baseline; Fig. 3C})\). However,
in slices of AC1 and AC8 double KO mice, no synaptic potentiation was found after forskolin application at the same dose (n = 6 slices/5 mice; mean, 116.2 ± 16.3% of control; Fig. 3C). Synaptic potentiation observed in wild-type mice was significantly greater than that of AC1 and AC8 double KO mice (P < 0.05). To determine the possible different contribution of AC1 versus AC8 to forskolin-induced potentiation, we also performed similar experiments with forskolin in slices of AC1 or AC8 KO mice. As shown in Fig. 3D, we found that forskolin-induced potentiation was completely inhibited in slices of AC1 KO mice (n = 5 slices/5 mice; mean, 104.3 ± 9.1% of control) but not that of AC8 KO mice (n = 6 slices/5 mice; mean, 137.5 ± 8.5% of control). Compared with forskolin-induced potentiation in wild-type mice, the magnitude of potentiation was significantly reduced in slices from AC8 KO mice (P < 0.05), indicating the partial involvement of AC8 in forskolin-induced potentiation.

**DISCUSSION**

In this study, we characterized the synaptic mechanisms of LTP in the ACC of adult mice. We showed that both NMDA receptors and L-VDCCs are required for the induction of ACC LTP by TBS. Considering the important roles of both NMDA receptors and L-VDCCs in calcium-dependent signaling pathways in neurons (Bito et al. 1996; Dolmetsch et al. 2001; West et al. 2001), we propose that LTP is a key mechanism in plastic changes in ACC neurons. Supporting the involvement of calcium-stimulated pathways in ACC LTP, we showed that genetic deletion of AC1 and AC8 blocked the induction of TBS-induced LTP, providing strong evidence for a role of calcium-stimulated AC1 and AC8 in ACC plasticity. Among two different isoforms of ACs that both are expressed in the ACC neurons (Wei et al. 2002a), we found that AC1 is essential for LTP induced by TBS or forskolin. This may be in part due to the fact that AC1 is four to five times more sensitive to calcium than AC8 (Cali et al. 1996). In case of forskolin application, we found that deletion of AC8 significantly reduced the potentiation. We believe that this may be due to the nonselective activation of ACs by forskolin. Consistent with previous studies on adult rats (Chetkovich and Sweatt 1993; Sah and Nicoll 1991; Tanaka and North 1994; Wei et al. 1999), we showed, using whole cell patch-clamp recordings, that fast EPSCs recorded from ACC neurons were completely blocked by a selective NMDA receptor blocker, AP-5, plus a selective AMPA/kainate receptor antagonist CNQX. These results support that glutamate is the fast excitatory transmitter in the ACC of adult mice.

Two forms of LTP have been reported in the hippocampus in regard to their sensitivity to NMDA receptor and/or L-type VDCC blockade. In the hippocampus, LTP induced by moderate tetanic stimulation, including late-phase LTP, are completely blocked by NMDA receptor antagonist AP-5 (100 μM) (Wei et al. 2000). Very strong tetanic stimulation induced NMDA receptor–insensitive potentiation that is sensitive to the blockade of L-VDCCs (Aniksztejn and Ben-Ari 1991; Grover and Teyler 1990). In the ACC, we showed that the blockade of either NMDA receptors or L-VDCCs completely blocked ACC LTP in slices from adult wild-type mice, suggesting that both are required for the induction of LTP in the ACC. One obvious consequence of the activation of NMDA receptors and L-VDCCs is the increase in postsynaptic calcium concentration. In this study, we provided pharmacological and genetic evidence that calcium-stimulated ACs are important for synaptic potentiation in the ACC. Both AC1 and AC8 likely contribute to synaptic potentiation, and AC1 plays a more important role in the induction of LTP. Considering that AC1 is more sensitive than AC8 to calcium, we propose that AC1 is likely the
major calcium sensor for NMDA receptors and L-VDCCs in ACC neurons.

These results show that the cAMP signaling pathway contributes to early synaptic potentiation in the ACC. This finding is consistent with reports from many other regions of the brain (Moss et al. 1992; Rosenmund et al. 1994; Trudeau et al. 1996). cAMP clearly contributes to the synaptic potentiation observed 5–40 min after induction. Our results suggest that the enhancement of synaptic responses within the ACC require synaptic activation of AC1 and less or no AC8. These results provide strong evidence for the different roles of AC1 and AC8 in ACC LTP. We cannot rule out the possibility of the involvement of AC8 in other forms of synaptic potentiation in the ACC and other related areas. Understanding synaptic plasticity within the ACC will help us dissect the cortical processing and formation of long-term memory under both physiological and pathological conditions.

Acknowledgments

We thank Drs. Louis Muglia and Dan Storm for providing the AC1 and AC8 knockout mice and S. Ko for the critical reading of the manuscript.

Grants

This work was supported by grants from the EJLB-Canadian Institutes of Health Research Michael Smith Chair in Neurosciences and Mental Health, Canadian Research Chair, Canadian Institute for Health Research, and National Institute of Neurological Disorders and Stroke Grant NS-42722 to M. Zhuo.

References


Collingridge GL and Bliss TV. Memories of NMDA receptors and LTP. Trends Neurosci 18: 54–56, 1995.


