Contrary roles of kainate receptors in transmitter release at corticothalamic synapses onto thalamic relay and reticular neurons

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Corticothalamic fibres, which originate from layer VI pyramidal neurons in the cerebral cortex, provide excitatory synaptic inputs to both thalamic relay neurons and reticular neurons; reticular neurons in turn supply inhibitory inputs to thalamic relay neurons. Pyramidal cells in layer VI in the mouse somatosensory cortex highly express mRNA encoding kainate receptors, which facilitate or depress transmitter release at several synapses in the central nervous system. We report here that contrary modulation of transmitter release from corticothalamic fibres onto thalamic relay and reticular neurons is mediated by activation of kainate receptors in mouse thalamic ventrobasal complex and thalamic reticular nucleus. Exogenous kainate presynaptically depresses the synaptic transmission at corticothalamic synapses onto thalamic relay neurons, but facilitates it at corticothalamic synapses onto reticular neurons. Meanwhile, the lemniscal synaptic transmission, which sends primary somatosensory inputs to relay neurons, is not affected by kainate. In addition, GluR5-containing kainate receptors are involved in the depression of corticothalamic synaptic transmission onto relay neurons, but not onto reticular neurons. Furthermore, synaptically activated kainate receptors mimic these effects; high-frequency stimulation of corticothalamic fibres depresses synaptic transmission onto relay neurons, but facilitates it onto reticular neurons. Our results suggest that the opposite sensitivity of kainate receptors at the two corticothalamic synapses is governed by cortical activity and regulates the balance of excitatory and inhibitory inputs to thalamic relay neurons and therefore their excitability.

Corticothalamic (CT) fibres originating from layer VI pyramidal neurons in the cerebral cortex supply glutamatergic synaptic inputs to both thalamic relay neurons (thalamocortical (TC) neurons) and reticular (RT) neurons; RT neurons in turn supply inhibitory inputs to TC neurons (Baughman & Gilbert, 1981; Jones, 1985; Ohara & Lieberman, 1985; Bourassa *et al.* 1995; Eaton & Salt, 1996; Kao & Coulter, 1997; Cox *et al.* 1997; Kim & McCormick, 1998; Golshani *et al.* 1998). Anatomically, a single CT axon originating from a layer VI pyramidal neuron innervates TC neurons and its axonal collaterals also innervate RT neurons (Deschenes *et al.* 1998). The balance of excitatory and inhibitory inputs is

considered to be critical for the control of TC neurons and therefore the processing of sensory information and modification of sensory receptive field properties (Sillito *et al.* 1983; Holdefer *et al.* 1989; Norton & Godwin, 1992; Zhu & Lo, 1998). The CT inputs, interacting with the RT neuron-mediated inhibitory inputs, are also implicated in the synchronization of intrathalamic oscillatory activities, which is associated with the arousal state (Steriade *et al.* 1993; von Krosigk *et al.* 1993; Destexhe *et al.* 1998) and certain neurological disorders such as absence epilepsy (Avoli & Kostopoulos, 1982; Bal *et al.* 2000; Blumenfeld & McCormick, 2000; Bessaih *et al.* 2006; Zhu *et al.* 2006; Alexander & Godwin, 2006).

It has been reported that activation of groups II and III metabotropic glutamate receptors (mGluRs) decreases the release of transmitter from CT synapse to TC neurons

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in the dorsal lateral geniculate nucleus (dLGN) (Turner & Salt, 1999; Alexander & Godwin, 2005). Furthermore, endogenous glutamate released by high frequency stimulation of CT fibres activates group II mGluRs to attenuate CT synaptic transmission (Alexander & Godwin, 2005). Despite the previous studies on mGluRs, however, little is known regarding the role that ionotropic glutamate receptors play in the presynaptic modulation of CT synapses onto TC neurons (CT–TC synapses). As for the CT synapses on RT neurons (CT–RT synapses), it is unknown even whether there is presynaptic modulation mediated by ionotropic glutamate receptors.

Activation of presynaptic kainate receptors (KARs), one of the ionotropic glutamate receptors, is known to regulate transmitter release from excitatory and inhibitory nerve terminals at various synapses, although there is limited anatomical evidence that KARs are localized at presynaptic terminals (Darstein *et al.* 2003). Endogenous glutamate released by synaptic activation induces KAR-mediated presynaptic modulation, suggesting that presynaptic KARs function as autoreceptors at the same synapse from which transmitter release occurs (Lauri *et al.* 2001*a*,*b*; Schmitz *et al.* 2001*b*; Delaney & Jahr, 2002; Kidd *et al.* 2002).

There are five KAR subunits (GluR5, GluR6, GluR7, KA1 and KA2) that can coassemble to form heteromeric KARs (Cui & Mayer, 1999; Paternain *et al.* 2000). Layer VI of cerebral cortex, as compared with the supra-granular layer, expresses a high level of mRNAs of KARs in the rodent brain in *in situ* hybridization studies. mRNAs of all five KAR subunits exist in layer VI cortical neurons. In particular, GluR7 and KA2 mRNAs are highly expressed in layer VI (Wisden & Seeburg, 1993; Bischoff *et al.* 1997). Immunohistochemical studies demonstrate that cortical pyramidal neurons in layer VI are labelled by anti-GluR6/7 and/or KA2 antibodies (Petralia *et al.* 1994). And, anti-body of anti-GluR5, 6 and 7 subunits is labelled at CT–TC and CT–RT synapses, although the exact location of KARs at CT synapses onto TC and RT neurons remains to be identified (Bolea *et al.* 2001).

In the present study, we showed contrary effects of KAR activation on the transmitter release at CT–TC and CT–RT synapses in the thalamic ventrobasal complex (VB) and thalamic reticular nucleus of mouse. Exogenous kainate presynaptically depresses CT synaptic transmission onto TC neurons, whereas it potentiates the synaptic transmission onto RT neurons. In addition, we also found that GluR5-containing KARs are involved in the depression of CT–TC synaptic transmission, but not CT–RT synaptic transmission. Finally, we showed that synaptic release of endogenous glutamate, by stimulation of high-frequency trains to CT fibres, mimics the KAR-mediated presynaptic modulation. Taken together, these results suggest that the excitability of TC neurons is regulated by cortical activity. Under the condition that CT

fibres were stimulated at high frequency, depressed CT–TC EPSCs along with enhanced RT-TC IPSCs that result from the facilitation of CT–RT EPSCs lead to the decrease in the excitability of TC neurons.

Methods

All experiments were carried out on mice in accordance with the institutional guidelines for animal experimentation, and approved by the animal research committee of the National Institute for Physiological Sciences. C57BL/6 Cr mice of both sexes (postnatal day p14–23, 106 mice) were deeply anaesthetized with halothane and decapitated, and brains were dissected rapidly. Horizontal slices $300\ \mu m$ thick (Castro-Alamancos, 2002) were cut in ice-cold, continuously oxygenated (95% O_2–5% CO_2) sucrose Ringer solution using a vibrating microtome (VT1000S; Leica, Nussloch, Germany) and kept in a submerged chamber for more than 1 h with 95% O_2–5% CO_2-saturated ACSF at room temperature. The sucrose Ringer solution contained (in mM): 234 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgCl_2$, 0.5 $CaCl_2$, 25 $NaHCO_3$ and 10 glucose. ACSF contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 20 glucose. ACSF was equilibrated with 95% O_2–5% CO_2 (pH 7.3) and infused at a rate of 3.0 ml min^{-1}. During recordings, ACSF containing 10 μM (−)-bicuculline methobromide (Tocris Cookson, Avonmouth, UK) and 5 μM CGP 55348 (Tocris Cookson) was used for blocking GABA$_A$ and GABA$_B$ receptors, respectively. All experiments were performed at 30–32°C.

TC and RT neurons in VB and thalamic reticular nucleus were visualized under a microscope (BX50WI; Olympus, Tokyo, Japan) equipped with an infrared differential inter-ference contrast video system (C2400–79H; Hamamatsu Photonics, Hamamatsu, Japan). Whole-cell recordings were carried out using 2–4.5 MΩ recording pipettes containing (in mM): 120 caesium methane-sulphonate, 10 Hepes, 1 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 20 NaCl, 5 QX-314, 2 ATP-Na_2, and 0.5 GTP-Na (pH 7.3 with CsOH, 298–310 mosmol l^{-1}) for voltage-clamp recordings.

An EPC9 double patch-clamp amplifier (HEKA, Lambrecht, Germany) was used for voltage-clamp whole-cell recordings. EPSCs were recorded with filtering at 3–10 kHz and digitized at 50 kHz. Series resistances were monitored online and the uncompensated series resistance was typically less than 15 MΩ. A series resistance compensation of 70% was used during recordings from the two types of neurons. Most of the synaptic responses were recorded as NMDAR-mediated EPSCs, which were measured at +40 mV of holding potential in the presence of a selective AMPAR antagonist, GYKI 53655

(50 μM), to block only AMPARs, but not KARs. Synaptic responses were evoked using a concentric electrode (tip diameter, 25 μm; Inter Medical, Nagoya, Japan) placed on the internal capsule for recording CT responses, because all CT fibres pass though the internal capsule. We first made whole-cell patch recording from a RT neuron in the thalamic reticular nucleus, and confirmed that CT–RT EPSCs were elicited by stimulating CT fibres. We then searched for a TC neuron (with a second recording electrode) in the VB, from which CT–TC EPSCs were evoked by the same CT fibre stimulation. Typically, the two neurons were aligned along a straight line which intersected at almost a right angle with an imagery curved line running through the anteromedial and posterolateral extremes of the thalamic reticular nucleus in our horizontal slice orientation. For recording lemniscal responses, the electrode was placed on the medial lemniscus, as reported previously (Miyata & Imoto, 2006). The stimulus consisted of a 100 μs bipolar pulse of constant current steps ($< 100 \mu$A) delivered using a biphasic isolator (BAK electronics, Oxford, UK). Lemniscal synaptic responses were recorded as non-NMDAR-mediated EPSCs, which were measured at -70 mV of holding potential. The stimulus was delivered at 0.05 Hz. Data were acquired using the PULSE program (HEKA, version 8.54). Pulse Fit (HEKA, version 8.54) and Igor Pro (Wavemetrics, Lake Oswego, OR, USA) were used to analyse the obtained data. The statistical significance was determined using Student's t test for unpaired or paired data depending on the experimental design; the paired t test was used unless stated otherwise. Data shown are means $\pm$ S.D. unless otherwise stated.

Drugs

DL-2-Amino-5-phosphonopentanoic acid (DL-AP5), [R-(R^*, S^*)]-5-(6,8-dihydro-8-oxofuro[3,4-e]-1,3-benzo-dioxol-6-yl)-5, 6, 7, 8-tetrahydro-6,6-dimethyl-1, 3-dioxolo[4,5-g]isoquinolinium bromide ((−)-bicuculline methobromide), (2S)-3-[[(1S)-1-(3, 4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid (CGP55845), 2, 3-dioxo-6-nitro-1, 2, 3, 4-tetra hydrobenzo[f]quinoxaline-7-sulphonamide disodium salt (NBQX disodium salt), (RS)-a-cyclopropyl-4-phosphonophenylglycine (CPPG), (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate (MK-801), (RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl) propanoic acid (ATPA), and (RS)-α-methyl-4-carboxyphenylglycine (MCPG) were purchased from Tocris Cookson (Avonmouth, UK). GYKI 53655 was purchased from Sigma-Aldrich (St Louis, MO, USA). LY382884 was a gift from Eli Lilly and Company.

Results

CT–TC and CT–RT EPSCs are differently altered by applied kainate

We first recorded AMPA receptor (AMPAR)-mediated CT-EPSCs simultaneously from both TC and RT neurons in the VB and thalamic reticular nucleus. CT–TC EPSCs and CT–RT EPSCs showed similar synaptic properties to those reported previously (de Curtis *et al.* 1994; Turner & Salt, 1998; von Krosigk *et al.* 1999; Granseth *et al.* 2002; Alexander *et al.* 2006; Miyata & Imoto, 2006). First, these EPSCs increase monotonically with stimulus intensity, indicating that they are composed of a large number of small unitary events. Second, the synaptic responses display paired-pulse facilitation when the same stimulus is delivered twice at a short interval (Fig. 1*A*). We then examined whether KAR activation alters CT-EPSCs in TC and RT neurons. In TC neurons, the peak amplitude of EPSCs decreased to $76.7 \pm 20.0\%$ ($P < 0.01$, $n = 10$) 5 min after the bath-application of 500 nM kainic acid (KA: kainate). In contrast, even in the case of stimulation of the same root of CT fibres, KA increased the peak amplitude of EPSCs to $136.8 \pm 27.0\%$ ($P < 0.01$, $n = 10$) in RT neurons. However, KA at these concentrations had no effect on the holding currents ($99.2 \pm 2.9\%$ of that in control, $P > 0.05$) of the two types of neurons. Thus, these changes of EPSC amplitudes are not likely to result from the direct postsynaptic effect of KA. Because NMDARs are insensitive to KA, we examined the effect of KA on synaptic transmission by monitoring NMDAR-mediated EPSCs. NMDAR-mediated currents were pharmacologically isolated by bath-application of GYKI53655 (50 μM), a specific antagonist of AMPAR, and observed at $+40$ mV of holding potential. The application of GYKI53655 at 50 μM, a concentration commonly used in other studies (Vignes *et al.* 1998; Bureau *et al.* 1999; Lauri *et al.* 2001*a*; Li *et al.* 2001; Rebola *et al.* 2007), obviously abolished AMPAR-mediated EPSCs in CT–TC and CT–RT synapses (see Supplementary Fig. 1). KA at several concentrations was bath-applied during stimulation (Fig. 4). A low concentration of KA (50 nM) had no effect on the amplitude of NMDAR-mediated EPSPs in CT–TC synapses, whereas a high concentration of KA (500 nM) evidently decreased NMDAR-mediated EPSCs in CT–TC synapses (Fig. 2*A* and *B*, $85.0 \pm 13.0\%$, $P < 0.01$, $n = 20$). KA at both concentrations, however, increased the CT–RT EPSCs (Fig. 3*A* and *B*, $123.5 \pm 14.0\%$, $P < 0.01$, $n = 20$ at 50 nM; $155.2 \pm 17.0\%$, $P < 0.01$, $n = 20$ at 500 nM). These changes in amplitude of EPSCs were associated with clear changes in paired pulse ratio, which is classically considered to inversely correlate with release probability at synaptic terminals (Dobrunz & Stevens, 1997; Zucker & Regehr, 2002). The paired pulse ratio was increased from $216.1 \pm 21.7\%$ to $268.3 \pm 15.3\%$ ($P < 0.01$) by
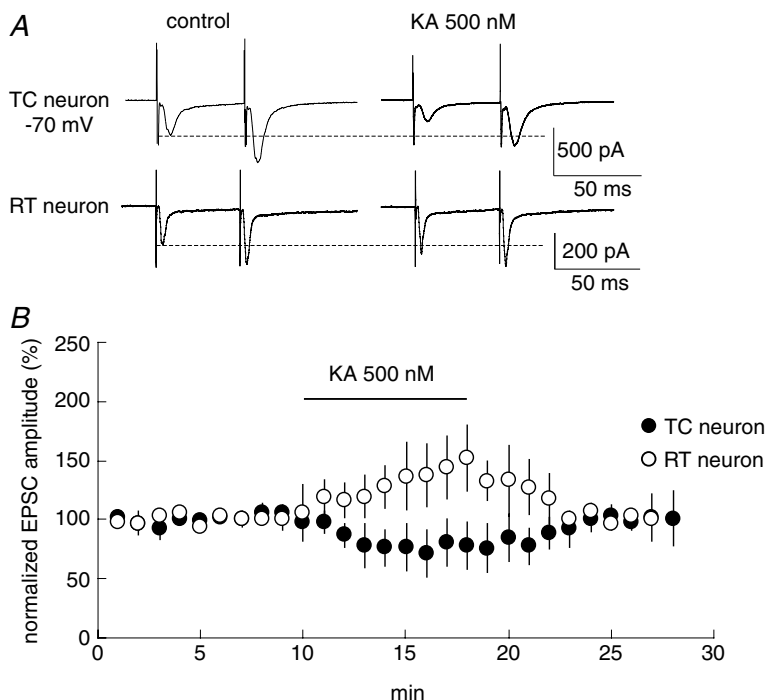
A

B

Figure 1. The bath-application of KA induces contrarily alternations of the amplitude of CT-EPSCs onto TC and RT neurons
A, simultaneous recordings of CT-EPSCs ($V_h = -70$ mV, $n = 10$) from TC and RT neurons in 500 nM kainate treatments. *B*, effects of 500 nM KA on amplitudes of CT–TC (●) and CT–RT EPSCs (○) and average of five recordings normalized to average control period amplitude. Values are means ± s.d.

KA (500 nM) in CT–TC synapses (Fig. 2*C*). In contrast, the ratio of CT–RT EPSCs was significantly decreased from $217.9 \pm 45.5\%$ to $187.8 \pm 36.2\%$ ($P < 0.01$) and to $153.4 \pm 20.6\%$ ($P < 0.01$) by KA at 50 nM and 500 nM, respectively (Fig. 3*C*). We further examined the changes in the coefficient of variation (CV) of EPSCs, which is also generally used to monitor changes in release

probability (Del Castillo & Katz, 1954), following the bath-application of KA in CT–TC and CT–RT synapses. The bath-application of 500 nM KA increased the CV of CT–TC EPSCs from 0.10 ± 0.02 to 0.17 ± 0.06 ($P < 0.01$), but decreased the CV of CT–RT EPSCs from 0.17 ± 0.04 to 0.13 ± 0.05 ($P < 0.01$, Figs 2*D* and 3*D*).
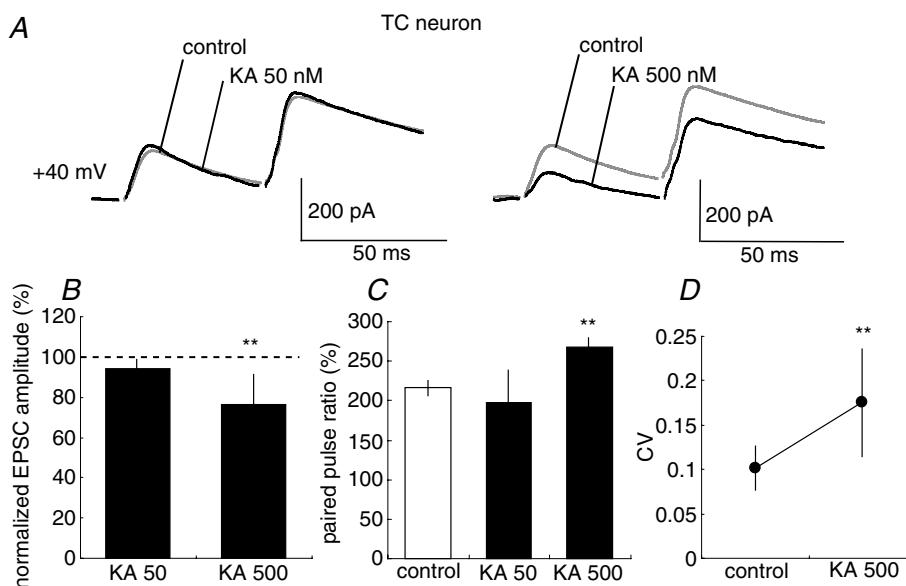
A

B *C* *D*

Figure 2. CT–TC EPSCs are decreased by KA application
A, traces show NMDAR-EPSCs ($V_h = +40$ mV) in CT–TC synapses with the paired pulse stimulation at 50 ms interval in control and KA treatments (50 nM, left traces; 500 nM, right traces). Each trace is the average of traces evoked by five stimuli at 0.05 Hz. *B*, effects of 50 (KA 50) and 500 nM KA (KA 500) on amplitudes of CT–TC EPSCs (**$P < 0.01$, $n = 20$ and 20, respectively). *C*, summary of effects of 50 and 500 nM KA on paired-pulse ratio. *D*, summary of effects of 50 and 500 nM KA on coefficient of variation of EPSCs. Values are means ± s.d.
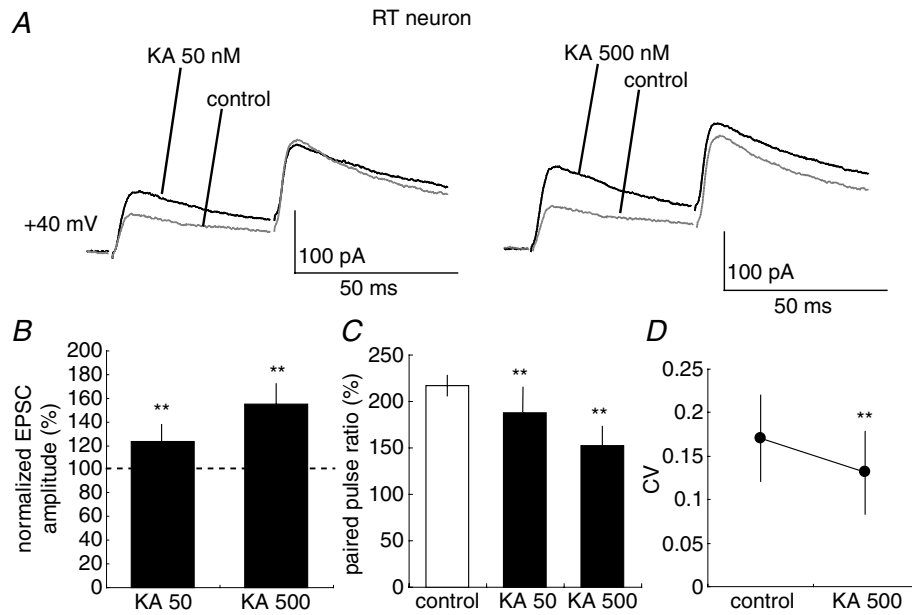
Figure 3. CT–RT EPSCs are increased by KA application

A, traces show NMDAR EPSCs ($V_h = +40$ mV) in CT–RT synapses with paired pulse stimulation at 50 ms intervals in control and KA treatments (50 nM, left traces; 500 nM, right traces). Each trace is average of traces evoked by five stimuli at 0.05 Hz. *B*, effects of 50 and 500 nM KA on amplitudes of CT–RT EPSCs (**$P < 0.01$, $n = 20$ and 20, respectively). Values are means ± s.d. *C*, summary of effects of 50 and 500 nM KA on paired-pulse ratio. Values are means ± s.d. *D*, summary of effects of 50 and 500 nM KA on coefficient of variation of EPSCs. Values are means ± s.d.

Comparison of the dose–response relationships for the effect of KA on the amplitudes of CT–TC and CT–RT EPSCs showed that KA distinctly affects CT–TC and CT–RT synaptic transmission (Fig. 4). We did not observe the dose-dependent crossover from facilitation to depression of EPSCs in the two types of neuron as previously observed in the cerebellum and hippocampus (Schmitz *et al.* 2001*a*; Delaney & Jahr, 2002). KA at concentrations lower than 1 μM had no marked effect on the holding currents of the two types of neurons at +40 mV (less than ∼30 pA). However, KA at 2 μM increased the holding current to ∼110 pA at +40 mV. An antagonist of AMPAR/KAR, NBQX (5 μM), blocked the effects of KA in both types of synaptic transmission. In CT–TC synapses, amplitude of the EPSCs was not affected by KA in the presence of NBQX (100.0 ± 6.0% at 50 nM and 98.8 ± 4.2% of control at 500 nM, $P > 0.05$, $n = 7$). Moreover, CT–RT EPSCs were not altered either (98.6 ± 12.0% at 50 nM and 101.0 ± 4.2% of control at 500 nM, $P > 0.05$, $n = 8$) by KA with NBQX. Taken together, these results indicated that KA contrarily modulated CT–TC and CT–RT synaptic transmissions, which were accompanied by clear changes in presynaptic parameters, such as paired pulse ratio and CV.

Presynaptic modulation by KARs is not observed at lemniscal synapses

We then examined whether the presynaptic modulation of KARs exists at lemniscal synapses, which convey somatosensory inputs to TC neurons. We distinguished lemniscal–TC EPSCs from CT–TC EPSCs on the basis of their characteristics. First, lemniscal EPSCs in the vast majority of the neurons exhibited all-or-none responses
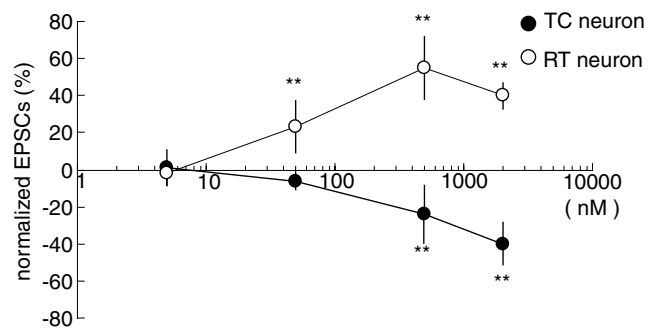

Figure 4. Dose-dependent modulation of CT–TC and CT–RT EPSCs by KA

Effects of 5, 50 and 500 nM, and 2 μM KA on NMDAR-EPSCs in CT–TC and CT–RT synapses ($n = 7$, 20, 20, 5 for CT–TC EPSCs, $n = 5$, 20, 20, 5 for CT–RT EPSCs, respectively; **$P < 0.01$). Values are means ± s.d.

and the threshold stimulation resulted in a unitary response that always had the same amplitude. Second, all lemniscal EPSCs led to paired-pulse depression (Fig. 5*A*). These synaptic properties have been previously reported in detail (Miyata & Imoto, 2006). In contrast to CT–TC synapses, lemniscal EPSPs were unaffected by applied KA. The amplitude of lemniscal EPSPs was not changed by KA ($101.2 \pm 7.4\%$ of that in control at 50 nM, $P > 0.05$, $n = 7$; $101.3 \pm 8.7\%$ at 500 nM, $P > 0.05$, $n = 7$, Fig. 5*A* and *B*). Furthermore, the paired pulse ratio did not significantly change following KA bath-application (paired pulse ratio was $53.9 \pm 11.2\%$ for control; $53.9 \pm 8.2\%$ at 50 nM, $P > 0.05$; $55.2 \pm 8.1\%$ at 500 nM, $P > 0.05$, Fig. 5*C*). CV was also not affected by KA ($P > 0.05$, Fig. 5*D*). These results are consistent with the previous observation in field EPSPs (Binns *et al.* 2003).

It was previously reported that postsynaptic AMPARs at retinogeniculate synapses, which are the primary sensory synapses in LGN and have a similar structure to lemniscal synapses, are desensitized under normal synaptic transmission (Chen *et al.* 2002). To exclude the possibility that the desensitized AMPARs may mask the effect of KARs at lemniscal synapses, the same experiment was also performed in the presence of bath-applied cyclothiazide (CTZ), an inhibitor of AMPAR desensitization. CTZ (75 μM) increased the peak amplitude of AMPAR-mediated EPSCs ($111.0 \pm 8.5\%$ of control; $n = 4$) and paired pulse depression ratio ($53.9 \pm 11.2\%$ for control, $76.2 \pm 2.0\%$ with CTZ). Under

this condition, the subsequent application of KA (500 nM) did not alter the peak amplitude of EPSCs ($102.0 \pm 2.3\%$ of that without KA, $P > 0.05$, $n = 4$) and paired pulse ratio ($101.8 \pm 2.0\%$ of that without KA, $P > 0.05$), indicating that KARs did not contribute to transmitter release in lemniscal synapses.

Contrary roles of KA in release probability measured by MK-801

To confirm the presynaptic action of KARs, we examined release probability more directly using a standard assay; the rate of blockade of NMDAR-EPSCs was measured using an irreversible, open-channel blocker of NMDAR, MK-801 (Hessler *et al.* 1993; Rosenmund *et al.* 1993). We compared the rate of progressive NMDAR blockade in the absence and presence of 500 nM KA. The NMDAR-EPSC amplitude was set at about 200 pA in all experiments. After obtaining a stable baseline, MK-801 (40 μM) was added to the bath, and stimulation was paused for 10 min to allow MK-801 to equilibrate in the slice. Then, the resumed stimulation (at 15 s intervals) progressively blocked NMDAR-EPSCs. The rate of blockade of NMDAR-EPSCs by MK-801 was significantly decreased by 500 nM KA in CT–TC synapses (decay time constant (τ) of stimulus number; $\tau = 23.8 \pm 5.9$ *versus* 32.3 ± 7.9; $n = 10$, $P < 0.05$, unpaired *t* test, Fig. 6*A* and *B*). In contrast, the rate of blockade was increased in CT–RT synapses ($\tau = 15.0 \pm 9.6$
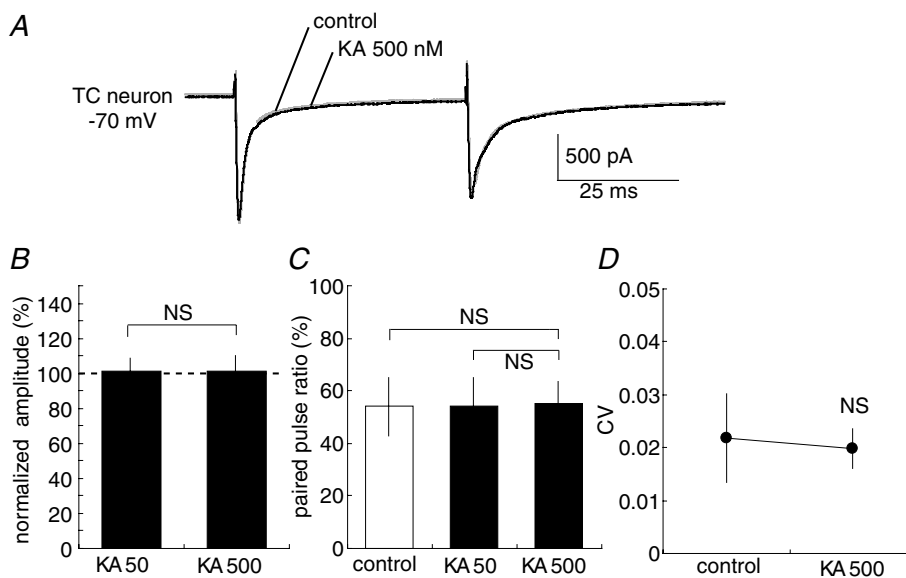

Figure 5. Medial lemniscal fibre–TC neuron EPSCs are not affected by KA application
A, lemniscal EPSCs ($V_h = -70$ mV) with the paired pulse stimulation at 50 ms intervals for control and 500 nM KA treatments on TC neurons. Each trace is average of traces evoked by five stimuli at 0.05 Hz. *B*, effects of 50 and 500 nM KA on amplitudes of lemniscal EPSCs ($n = 7$; NS, not significant). Values are means ± S.D. *C*, summary of effects of 50 and 500 nM KA on paired-pulse ratio. Values are means ± S.D. *D*, summary of effects of 50 and 500 nM KA on coefficient of variation of EPSCs. Values are means ± S.D.

versus 5.23 ± 2.9; $n = 7$, $P < 0.05$, Fig. 6*B* and *D*). Because NMDARs are insensitive to KA (Hollmann & Heinemann, 1994), these results showed directly that the activation of KARs causes contrasting changes in the probability of glutamate release in CT–TC and CT–RT synapses. The MK-801 blocking effects in the presence of NBQX (10 μM) were not significantly different from those measured in the presence of GYKI53655 ($\tau = 23.8 \pm 5.9$ *versus* 23.4 ± 7.0 in CT–TC synapses, $n = 7$; $\tau = 15.0 \pm 9.6$ *versus* 14.5 ± 10.2 in CT–RT synapses, $n = 7$, $P > 0.05$), indicating that presynaptic KARs were not tonically activated by ambient glutamate level at CT–TC and CT–RT synapses.

GluR5-containg KARs are involved in presynaptic modulation in CT–TC synapses, but not in CT–RT synapses

The target neuron-specific difference in presynaptic modulation by KA in CT synapses might originate from different KAR subunit compositions at the two presynaptic terminals onto TC and RT neurons. To examine this hypothesis, we bath-applied ATPA, a GluR5 agonist (Clarke *et al.* 1997), and then observed NMDAR-mediated EPSCs in CT–TC and CT–RT synapses. We applied ATPA at 1 and 10 μM, which are commonly used concentrations in hippocampal slices (Lauri *et al.* 2001*b*; Clarke & Collingridge, 2002). ATPA application mimicked the effect of KA in CT–TC

synapses, but not in CT–RT synapses. ATPA significantly decreased NMDAR-mediated EPSCs ($69.4 \pm 11.5\%$ and $54.9 \pm 10.6\%$ at 1 and 10 μM, respectively, $P < 0.01$ at both concentrations, $n = 7$ at each concentration, Fig. 7*A* and *B*) with increasing paired pulse ratio in CT–TC synapses (from $282.2 \pm 40.5\%$ to $320.2 \pm 38.0\%$ and $340.2 \pm 43.5\%$ at 1 and 10 μM, respectively, $P < 0.05$, Fig. 7*C*). In contrast, ATPA did not affect amplitudes of NMDAR-mediated EPSCs in CT–RT synapses (changed to $105 \pm 11.3\%$ and $98 \pm 4.5\%$ at 1 and 10 μM, respectively, $n = 7$, Fig. 7*D* and *E*) and paired pulse ratio ($225.1 \pm 6.5\%$ for control, $212.1 \pm 6.9\%$ and $222.3 \pm 9.6\%$ at 1 and 10 μM, respectively, $P > 0.05$ at the both concentrations, Fig. 7*F*). These results suggest that GluR5-containing KARs contributed to the presynaptic modulation in CT–TC synapses, but not in CT–RT synapses. We therefore examined whether the KAR-mediated effect is indeed sensitive to LY382884, an antagonist of GluR5 in CT–TC synapses (Fig. 8). The bath-application of LY382884 (10 μM) alone did not affect CT–TC EPSCs, suggesting that GluR5 was not tonically activated by normal synaptic transmission (Fig. 8*A*). However, the preincubation of neurons with LY382884 completely blocked the inhibitory effect of KA on CT–TC synaptic transmission. In the case of application of LY382884 with KA (500 nM), the amplitude and paired pulse ratio of CT–TC EPSCs did not significantly change ($101.5 \pm 8.34\%$ and $98.5 \pm 6.2\%$ of the control, percentage change of EPSC amplitude and paired pulse
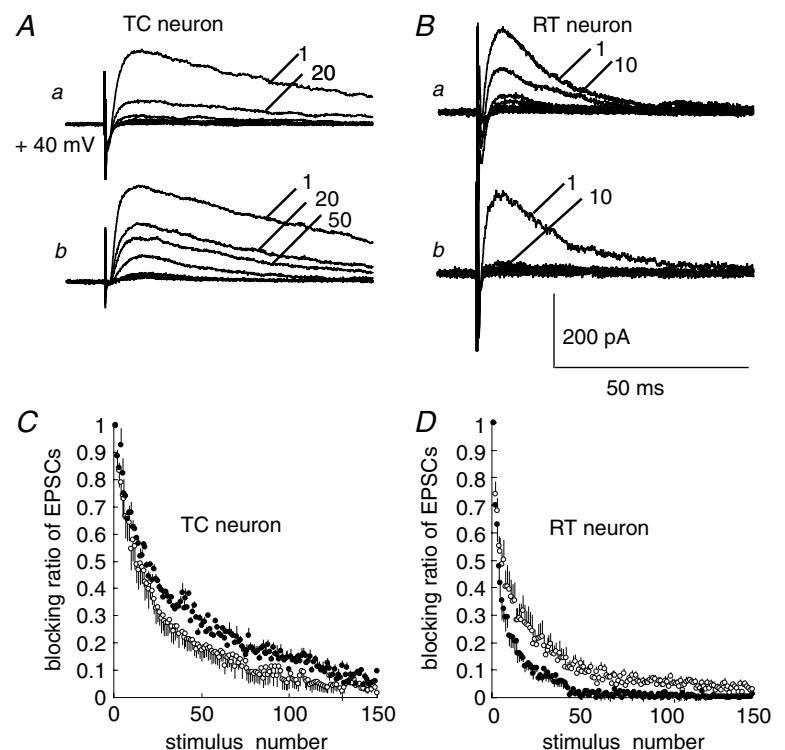

Figure 6. KA contrarily modulates release probability of CT synapses onto the two types of neurons

A, traces show MK-801-mediated blockade of synaptic transmission at CT–TC synapses. *a*, normal ACSF; *b*, in the presence of 500 nM KA. Added numbers on traces indicate the stimulus number during MK-801 application. *B*, traces show MK-801-mediated blockade of synaptic transmission at CT–RT synapses. *a*, control; *b*, in the presence of 500 nM KA. *C*, average blocking ratio in EPSC amplitude (mean ± s.e.m.) in normal ACSF (○) and in the presence of 500 nM KA (●) at CT–TC synapses. *D*, average blocking ratio in EPSCs amplitude in normal ACSF (○) and in the presence of 500 nM KA (●) at CT–RT synapses.
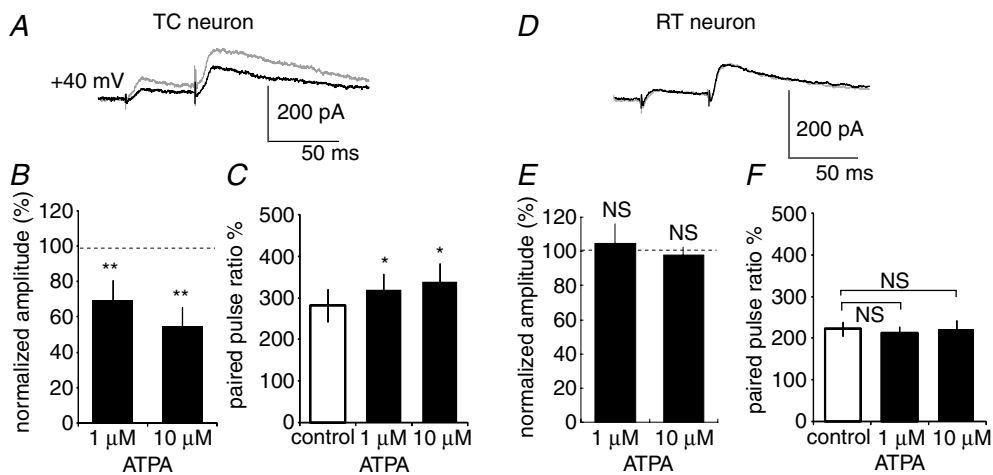
Figure 7. ATPA, an agonist of GluR5-containing KARs, mimics the effect of KA in CT–TC but not in CT–RT synapses
A, CT–TC EPSCs with paired pulse stimulation at 50 ms interval in control (grey) and ATPA application (black line). Each trace is the average of traces evoked by five stimuli at 0.05 Hz. *B*, effects of ATPA on amplitudes of CT–TC EPSCs (mean ± s.d.; **$P < 0.01$, $n = 10$). *C*, summary of effects of ATPA on paired-pulse ratio in CT–TC EPSCs (mean ± s.d.; *$P < 0.05$, $n = 7$). *D*, CT–RT EPSCs with paired pulse stimulation at 50 ms intervals in control (grey) and ATPA application (black line). *E*, effects of ATPA on amplitudes of CT–RT EPSCs (NS, not significant; $n = 10$). *F*, summary of effects of ATPA on paired-pulse ratio in CT–RT EPSCs (NS, not significant; $n = 7$).
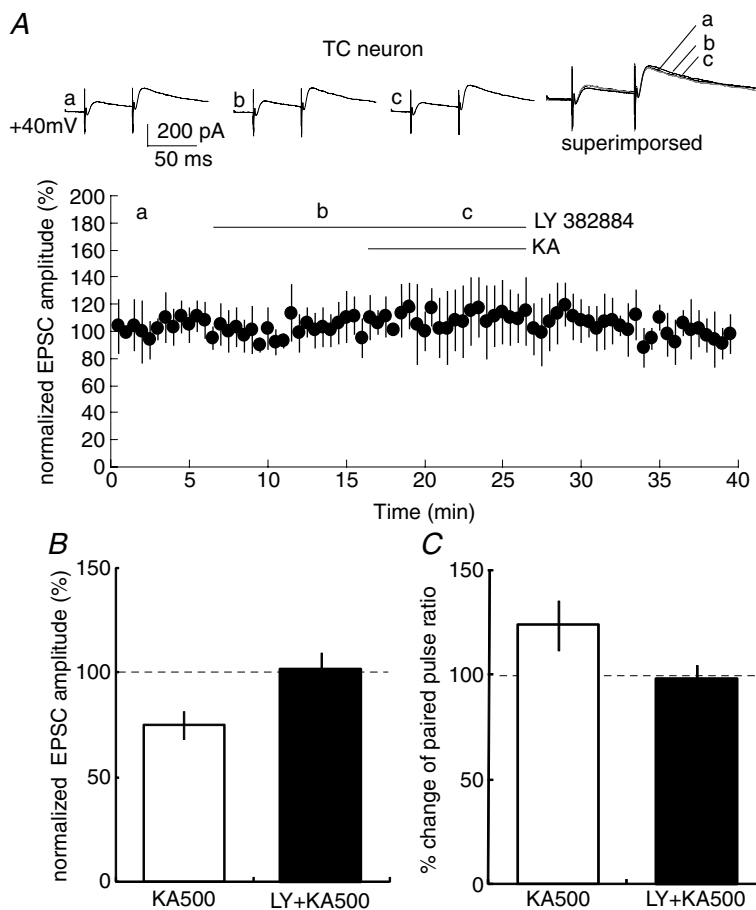

Figure 8. Pretreatment with GluR5 antagonist abolishes the effect of KA in CT–TC synapses
A, upper traces show EPSCs for control (*a*), in the presence of LY382884 (10 μM) (*b*), and in the presence of LY 382884 with KA (500 nM) (*c*). Graph shows that pretreatment with LY382884 (10 μM) abolishes the depression of EPSCs induced by KA in CT–TC synapses. *B*, effects of LY 382884 on KA-induced depression of amplitudes of CT–TC EPSCs (mean ± s.d.; *$P < 0.05$, $n = 20$ and 6, respectively). *C*, comparison of paired-pulse ratio of CT–TC EPSCs in the presence of KA with and without LY 382884 (*$P < 0.05$, $n = 20$ and 6, respectively). KA500 and LY + KA500 indicate the application of KA in the absence and presence of LY 382884, respectively.

ratio, respectively, $n = 6$, $P > 0.05$, Fig. 8*A–C*). These results demonstrated that GluR5-containing KARs are involved in the inhibition of transmitter release at CT–TC synapses.

Synaptically released glutamate induces KARs-mediated presynaptic modulation

Is KAR-mediated presynaptic modulation of CT synaptic transmission induced by endogenous glutamate released from CT synaptic terminals? To answer this question, we stimulated CT fibres at a high frequency to raise the ambient glutamate concentration at synaptic clefts. It has been reported that the high-frequency stimulation of CT synapses activates several metabotropic glutamate receptors (mGluRs). For example, mGluR1, a member of Group I mGluRs, is activated by high-frequency stimulation of CT fibres and produces slow EPSPs (Godwin *et al.* 1996; Lee & McCormick, 1997; Golshani *et al.* 1998; Turner & Salt, 1998; von Krosigk *et al.* 1999). Moreover, high frequency stimulation of CT fibres activates presynaptic mGluR2, a member of Group II mGluRs, which inhibits

CT–TC synaptic transmission (Alexander & Godwin, 2005). Thus, to exclude the effects of mGluRs on transmitter release during high-frequency stimulation, we observed the EPSCs in the presence of a group I/II mGluR antagonist, (*RS*)-MCPG (0.5 mM), and a group III mGluR antagonist, CPPG (0.5 mM). NMDAR-mediated EPSCs evoked with a single stimulus of CT fibres were not affected by blocking KARs with NBQX (100.2 ± 2.3% of that in the control at 10 μM, $n = 7$), indicating that the ambient concentration of glutamate under a single stimulus was not sufficiently high to activate KARs. However, both CT–TC and CT–RT synapses were affected by NBQX depending on the stimulus frequency of CT fibres train stimulation. Train stimulation of five pulses was carried out from 20 to 100 Hz. CT–TC EPSCs (NMDAR-mediated EPSCs at 50 μM GYKI53655) were facilitated by NBQX when EPSCs were stimulated at a stimulus frequency higher than 20 Hz. The average change in peak amplitude of the fifth EPSC in the presence of NBQX significantly increased to 120 ± 5.4% (mean ± S.E.M., $P < 0.05$, $n = 7$), 133 ± 6.6% ($P < 0.05$) and 135 ± 11.9% at 20 Hz, 50 Hz and 100 Hz, respectively (Fig. 9*A* and *C*). In contrast to the facilitatory effect on
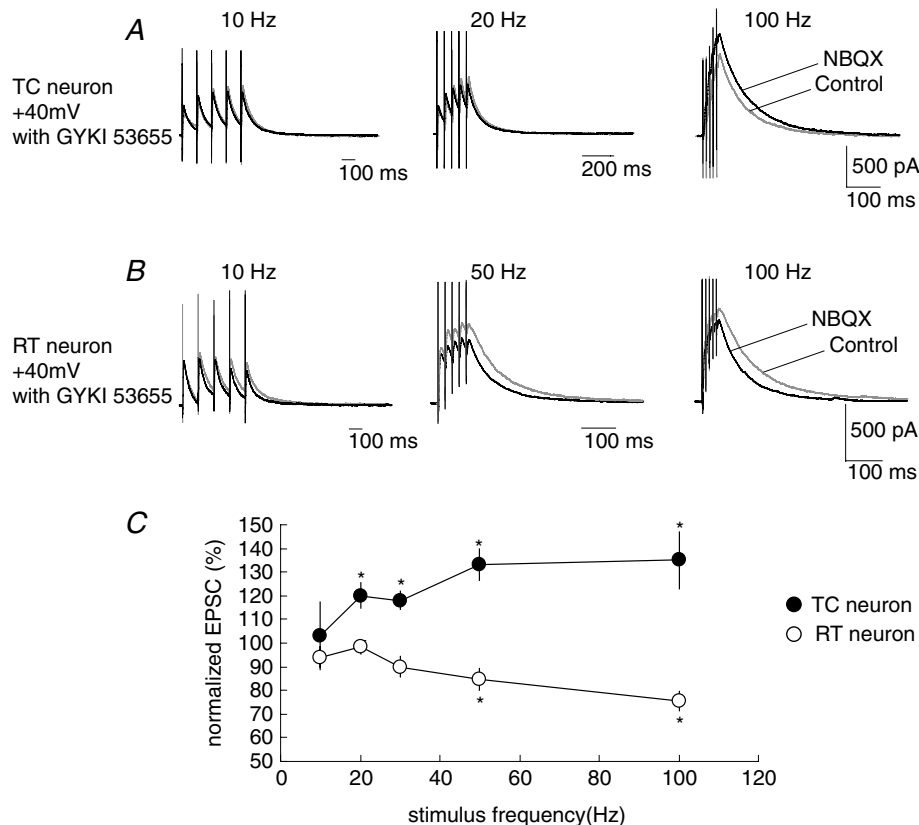

Figure 9. Synaptically released glutamate induces KAR-mediated presynaptic modulation
A, CT–TC EPSCs stimulated at 20 s intervals with trains of five stimuli at 10–100 Hz for control and with the addition of 10 μM NBQX. *B*, CT–RT EPSCs with trains of five stimuli at 10–100 Hz for control and with the addition of 10 μM NBQX. *C*, average alternation of peak amplitude (mean ± S.E.M.) after fifth stimulus by NBQX at stimulus frequencies in CT–TC ($n = 10$) and CT–RT synapses ($n = 7$; *$P < 0.05$).

CT–TC EPSCs, the amplitudes of CT–RT EPSCs were decreased by NBQX at stimulus frequencies higher than 30 Hz. NBQX decreased the peak amplitudes of the fifth EPSC to $84.6 \pm 4.5\%$ and $75.4 \pm 4.5\%$ at 50 Hz ($P < 0.05$, $n = 7$) and 100 Hz ($P < 0.05$), respectively (Fig. 9*B* and *C*).

Discussion

We report here that the activation of presynaptic KARs can contrarily alter the strength of glutamatergic synaptic transmission at the two types of CT synapses onto TC and RT neurons in mouse VB and thalamic reticular nucleus. Our major findings are as follows. (1) Activation of KARs presynaptically depresses CT–TC synaptic transmission, but facilitates CT–RT synaptic transmission. (2) In contrast to CT synapses, lemniscal synaptic transmission is not influenced by KA. (3) GluR5-containing KARs contribute to the KAR-mediated presynaptic modulation in CT–TC synapses, but not in CT–RT synapses. (4) High-frequency train stimulation of CT fibres mimics the KAR-mediated modulation of CT synaptic transmissions. A presynaptic locus of KAR regulation of CT synaptic transmission is explainable on the basis of three things. First, accompanying changes in EPSC amplitude induced by the bath-application of KA, the paired pulse facilitation ratio increases in CT–TC synapses, but decreases in CT–RT synapses. Second, the CV of EPSCs, another parameter of presynaptic release probability, increases in CT–TC synapses, but decreases in CT–RT synapses. Third, the rate of blockade of NMDAR-EPSCs using MK801 is decreased in CT–TC synapses, but increased in CT–RT synapses in the presence of KA.

Bolea and colleagues have reported that an exogenous application of kainate has no effect on frequency of miniature EPSCs in TC and RT neurons, suggesting the lack of presynaptic effect of kainate at CT synapses (Bolea *et al.* 2001). The discrepancy between their study and ours may be attributed to the differences in experimental conditions; miniature EPSCs contain synaptic inputs from all excitatory synapses onto TC and RT neurons, which may mask the component of CT synaptic input. In addition, they recorded EPSCs at a lower temperature (22–25°C), which is known to reduce kainate receptor-mediated currents (Kidd & Isaac, 2001).

Target neuron-specific presynaptic modulation of KARs

Our results demonstrated that KARs display a target neuron-specific modulation of transmitter release at CT synapses. To our knowledge, this is the first report of the existence of such a modulation in the cortico-thalamic system. The target neuron-specific modulation of KARs has also been observed in the cerebellum and hippocampus. KAR activation presynaptically facilitates synaptic transmission of parallel fibre synapses onto Purkinje cells, but depresses transmission of parallel fibre synapses onto stellate cells (Delaney & Jahr, 2002). In the hippocampus, presynaptic KARs facilitate glutamate release at Schaffer collateral synapses onto somatostatin-positive (SOM) interneurons, but not onto non-SOM interneurons (Sun & Dobrunz, 2006). The anatomical evidence that a single CT fibre from a pyramidal neuron in layer VI of the barrel cortex innervating both types of TC and RT neurons in VB and thalamic reticular nucleus (Deschenes *et al.* 1998) raises the possibility that distinct subunits of KARs may exist at the two types of terminals innervating TC and RT neurons, respectively. It is also possible that this difference stems from the types of CT fibres targeting TC and RT neurons. CT fibres are anatomically distinct and classified into at least two types. One type originates from pyramidal neurons located in the upper part of layer VI in a barrel column of the cortex and restrictedly terminates at a barreloid in the VB. The other type originates from the lower part of layer VI pyramidal neurons in a barrel column and broadly projects over the thalamic ventroposterial medial nucleus and the thalamic posterior nucleus (Deschenes *et al.* 1998). Both types of CT fibres have collaterals terminating RT neurons. However, the patterns of their projections to the thalamic reticular nucleus are not homogeneous (Deschenes, 1998). In addition, pyramidal neurons in layer VI, a source of CT fibres, are classified into several subtypes on the basis of their dendritic morphology and excitatory inputs (Zarrinpar & Callaway, 2006). Thus, the difference in KA sensitivity between TC and RT neurons may be attributed to the predominance of different types of CT fibres. However, we cannot distinguish these two possibilities because in our study we stimulated a bundle of CT fibres, instead of a single CT fibre.

Role of KAR subunits in CT synapses

Our results shown in Fig. 7 suggest strongly that GluR5-containing KARs contribute to presynaptic modulation in CT–TC synapses. The property of GluR5-containing KARs to depress transmitter release has also been observed in the hippocampus (Chittajallu *et al.* 1996; Vignes *et al.* 1998; Clarke & Collingridge, 2002) and cortex (Campbell *et al.* 2007). Two mechanisms underlying this depression have been considered: the inhibition of presynaptic Ca^{2+} influx induced by depolarization block of presynaptic fibres (Kamiya & Ozawa, 1998) and depolarization-independent metabotropic action (Frerking *et al.* 2001; Lauri *et al.* 2006; Vesikansa *et al.* 2007). It should be noted that GluR6 containing KARs may also contribute to depress the

transmitter release in hippocampal CA1 region (Kamiya & Ozawa, 1998). Our results do not rule out the possible role for GluR6 subunit in the depression of transmitter release at CT–TC synapses, as GluR5 and GluR6 can form functional heteromeric receptors (Paternain *et al.* 2000). As for the facilitation effect at CT–RT synapses, our results indicate that other subunits of KARs, except GluR5, are involved. The presynaptic role for the facilitation effect has been well studied in the hippocampal CA3 region using subunit-specific knockout mice of GluR6, GluR7 and KA2 (Contractor *et al.* 2003; Breustedt & Schmitz, 2004; Pinheiro & Mulle, 2006; Pinheiro *et al.* 2007). Homometric or heterometoric KARs of these subunits may be involved in the facilitation of transmitter release at CT–RT synapses. Bolea and colleague have reported that anti-GluR5, 6, and 7 subunit antibody is labelled at CT–TC and CT–RT synapses by post-embedding immunogold labelling (Bolea *et al.* 2001). Although 20% of gold particles are located in postsynaptic site in this study, more than 60% of gold particles are located in the synaptic cleft, making it difficult to ascertain the exact location (i.e. at the presynaptic site or postsynaptic site). The native receptor composition and their specific localization as well as density of the receptor on axon terminals of CT fibres remain to be identified. Further studies are needed to precisely determine their subunit composition at the two types of terminals of CT fibres.

Relation to developmental changes of KARs

It has been reported that the expression pattern of mRNAs of KAR subunits changes during development. In layer VI of rat cerebral cortex, mRNA of GluR5 is detectable during postnatal day P12, but only weakly detectable in adult (Bahn *et al.* 1994; Binns *et al.* 2003). In the thalamus, mRNA of GluR5 is highly expressed around birth with a strong decline after postnatal day P12 (Bahn *et al.* 1994; Binns *et al.* 2003). In physiological studies, presynaptic KARs that mediate synaptic depression at TC synapses are lost by the end of postnatal day P7 in layer IV of cerebral cortex (Kidd *et al.* 2002). It has also been reported that presynaptic KARs tonically inhibit glutamate release onto CA1 pyramidal cells during postnatal day P7 (Lauri *et al.* 2006). Our results were obtained from juvenile animals (P14–23). It is unknown at the moment if the effect observed in our study is maintained in the adult.

Functional implication of KAR-mediated presynaptic modulation in thalamic synapses

KAR-mediated presynaptic modulation is dependent on stimulus frequency of CT fibre train stimulation, suggesting that KARs are activated by augmenting the accumulation of glutamate at CT synapses. The difference in stimulus frequency-dependent KAR modulation between CT–TC and CT–RT synapses provides a mechanism for manipulating the output of TC neurons, depending on cortical activity. That is to say, KAR-mediated presynaptic modulation would finally suppress the excitability of TC neurons when CT fibres are activated at high frequency; along with the depression of CT–TC EPSCs, GABAergic inhibition of TC neurons as a result of the facilitation of CT–RT EPSCs would be enhanced.

CT feedback activities involving the thalamic reticular nucleus underlie thalamic synchrony and slow oscillation as in the case of absence epilepsy. Experimental observations reveal that spindle oscillations can undergo transition to a hyper-synchronous rhythm in the thalamus through high-frequency stimulation of CT fibres (Deschenes *et al.* 1998; Destexhe, 1998; Bal *et al.* 2000; Blumenfeld & McCormick, 2000). In addition, it has been reported that genetic juvenile absence epilepsy is due to the linkage of GluR5 gene mutation in humans (Sander *et al.* 1997; Izzi *et al.* 2002). Thus, frequency-dependent KAR-mediated modulation of CT synaptic transmissions may prevent the abnormal thalamic synchrony and/or hyperexcitability in absence epilepsy. Unlike CT synapses, lemniscal synaptic transmission is not influenced by activation of KARs, mGluRs and acetylcholine receptors (Castro-Alamancos, 2002; Alexander & Godwin, 2005), thereby ensuring primary sensory information is precisely preserved.

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Supplemental material

Online supplemental material for this paper can be accessed at:
http://jp.physoc.org/cgi/content/full/jphysiol.2008.164996/DC1