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Adenosine A_1-receptor-mediated tonic inhibition of glutamate release at rat hippocampal CA3–CA1 synapses is primarily due to inhibition of N-type Ca^{2+} channels

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Abstract

The voltage-gated Ca^{2+} channels responsible for synaptic transmission at CA3–CA1 synapses are mainly P/Q- and N-types. It has been shown that tonic inhibition of transmission due to activation of adenosine A_1 receptors occurs at this synapse. We have recently developed a technique to monitor synaptically released glutamate which is based on synaptically induced glial depolarisation. Using this technique, we have examined the effects of different voltage-gated Ca^{2+} channel blockers on glutamate release. Under conditions in which the adenosine A_1 receptor was not blocked, ω-AgaIVA (a P/Q-type voltage-gated Ca^{2+} channel blocker) suppressed synaptically induced glial depolarisation to a greater extent than ω-CgTxGVIA (an N-type voltage-gated Ca^{2+} channel blocker) did. In contrast, in the presence of an adenosine A_1 receptor antagonist, ω-AgaIVA was less effective at suppressing synaptically induced glial depolarisation than ω-CgTxGVIA. These results indicate that, in the absence of adenosine A_1 receptor-mediated tonic inhibition, the contribution of N-type is much greater than that of P-type, and that N-types are the primary target of tonic inhibition in normal conditions in which adenosine A_1 receptor-mediated tonic inhibition is present.
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1. Introduction

Transmitter release at presynaptic terminals is one of the major targets for neuromodulation (Vizi, 2000). For example, it has been shown that activation of presynaptic receptors, such as metabotropic glutamate (Cartmell and Schoepp, 2000), adenosine A_1 (Dunwiddie and Masino, 2001), gamma-aminobutyric acid $(GABA)_B$ (Misgeld et al., 1995) and muscarinic and cannabinoid (Schlicker and Kathmann, 2001) receptors, modulates transmitter release at hippocampal CA3–CA1 synapses, and that inhibition of voltage-gated Ca^{2+} channels in the presynaptic terminals can be responsible for this modulation (Ambrosio et al., 1997; Mogul et al., 1993; Wu and Saggau, 1997).

Various studies have shown that N- and P/Q-type channels are responsible for glutamate release at CA3–CA1 synapses in the rat hippocampus (Luebke et al., 1993; Takahashi and Momiyama, 1993; Wheeler et al., 1994; Wu and Saggau, 1994b, 1997; Reuter, 1995; Reuter, 1996). Measuring Ca^{2+}-dependent [^{3}H]glutamate release from hippocampal synaptosomes using biochemical techniques, Luebke et al. (1993) reported that P-type channels play a more prominent role than N-type channels. Takahashi and Momiyama (1993) showed that excitatory postsynaptic currents in hippocampal CA1 neurons in slice preparations are suppressed to a greater extent by ω-AgaIVA, a P-type channel blocker, than by ω-CgTxGVIA, an N-type channel

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blocker (84% and 31% suppression, respectively). This finding was confirmed by Wheeler et al. (1994), who showed that the slope of the field excitatory post synaptic potential (fEPSP) at synapses from CA3 to CA1 pyramidal neurons in the hippocampus is reduced by ω-AgaIVA to a greater extent than by ω-CgTxGVIA (85% and 46%, respectively). Wu and Saggau (1994b) performed fast Ca^{2+} imaging of presynaptic terminals at rat CA3–CA1 synapses and reported that application of N- or P/Q-type channel blockers reduces presynaptic Ca^{2+} influxes by 21% or 35%, respectively. These studies showed that the contribution of P/Q-type channels is greater than that of N-type channels. In contrast, Reuter (1995) reported that, in rat hippocampal cell cultures, the exocytosis of the styryl dye, FM1-43, from synaptic vesicles during synaptic transmission following Ca^{2+} entry through Ca^{2+} channels is inhibited by ω-CgTxGVIA to a greater extent than by ω-AgaIVA.

However, most of the above studies were performed under conditions in which glutamate release from presynaptic terminals might have been suppressed by the action of various modulators. For example, Wu and Saggau (1994a) reported that the adenosine A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine (8-CPT), enhances both the presynaptic Ca^{2+} rise and the fEPSP, suggesting tonic inhibition by endogenous adenosine. It has been reported that adenosine suppress transmitter release by inhibiting voltage-gated Ca^{2+} channels (Dunwiddie and Masino, 2001). In order to determine which types of voltage-gated Ca^{2+} channels are responsible for transmitter release, it is necessary to compare contribution of voltage-gated Ca^{2+} channels in the absence of tonic inhibition.

Several different methods, such as the measurement of postsynaptic responses and presynaptic $[Ca^{2+}]_i$ changes, have been used to investigate the contribution of voltage-gated Ca^{2+} channels to transmitter release. However, these methods are rather indirect, as voltage-gated Ca^{2+} channels may be involved in determining postsynaptic responses and as there may be presynaptic $[Ca^{2+}]_i$ changes that do not contribute to transmitter release. We have recently developed a novel optical method to monitor synaptically induced glutamate release which involves detecting the depolarisation of glial cells caused by glutamate uptake using a voltage-sensitive dye and a fast optical imaging system (Kojima et al., 1999). In the present study, we monitored glutamate release at CA3–CA1 synapses in rat hippocampal slice preparations by using this method in the absence of adenosine-mediated tonic inhibition and examined the effects of specific blockers of N- and P/Q-type channels. We have confirmed that glutamate release is tonically inhibited by activation of adenosine A_1 receptors and demonstrated that, in the absence of inhibition, the contribution of N-type channels is greater than that of P/Q-type channels. Thus, N-type channels are the major target of the adenosine A_1 receptor-mediated inhibitory effect on transmission at CA3–CA1 synapses.

2. Materials and methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Tokyo University of Pharmacy and Life Science, and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.1. Slice preparation

Hippocampal slices were prepared from 3-week-old male Wistar rats. The animals were decapitated under deep diethyl ether anaesthesia, and the brains removed and rapidly cooled in artificial cerebrospinal fluid [composition (in mM): 110 choline chloride for preparing slices or 124 NaCl for experiments, 2.5 KCl, 26 $NaHCO_3$, 10 glucose, 1.25 NaH_2PO_4, 2 $CaCl_2$ and 1 $MgCl_2$, pH 7.4] bubbled continuously with a gas mixture of 95% O_2 and 5% CO_2. Slices (400 μm thick) were prepared using a rotary slicer (Dosaka EM model DTY-7700, Japan).

2.2. Voltage-sensitive dye measurement

The method has been described in detail elsewhere (Kojima et al., 1999). Briefly, slices were maintained in a holding chamber for at least 30 min, then stained with the voltage-sensitive dye, RH155 (NK3041) (Molecular Probes, Eugene, OR, or Nippon Kankoh-Shikiso, Okayama, Japan) by submersion for 30 min in artificial cerebrospinal fluid containing 0.21 mg/ml of RH155. The stained slices were placed in an experimental chamber mounted on an inverted microscope (TMD-300, Nikon, Tokyo, Japan) and changes in dye absorption, which are related to the change in membrane potential, were measured at 700 nm using a high-speed (maximum frame rate 2 kHz) 16×16 photodiode array system (Agrus-50/PDA, Hamamatsu Photonics, Hamamatsu, Japan). The photo-currents generated at the photodiodes were converted to voltage using a current-to-voltage converter with a 50 MΩ feedback resistor and the data transferred to a sample-and-hold device, then DC-coupled to a 16 bit resolution analogue-to-digital converter. All optical signals were displayed as changes in light intensity divided by the total light intensity ($\Delta I/I$). In most experiments, a ×10 objective lens (NA 0.45) was used; in this case, each diode imaged an area of 52.5×52.5 μm. Experiments were performed at 24–28 °C. Synaptic responses were evoked by delivering a short current pulse of 200 μs duration using a bipolar tungsten electrode placed in the stratum radiatum to stimulate Schaffer collaterals. The stimulus was applied every 5 s and the optical signals averaged over 9–12 trials. To record extracellular field potentials, glass pipettes (1.5×90 mm, 1–5 MΩ) filled with artificial cerebrospinal fluid were inserted into the stratum radiatum. The potentials were amplified by a high-gain AC amplifier (MEZ-8201, Nihon-Koden, Tokyo, Japan), digi-

tised, and recorded on a computer (SD-512, EPSON, Japan). The field potential responses were averaged over five trials.

2.3. Drugs

6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; a non-*N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist), DL-2-amino-5-phosphonopentanoic acid (APV; an NMDA receptor antagonist), 8-cyclopentyl-1,3-dimethylxanthine (8-CPT; an adenosine A_1 receptor antagonist) and 2-chloro-N^6-cyclopentyladenosine (CCPA; an adenosine A_1 receptor agonist) were purchased from Sigma-Aldrich (Tokyo, Japan). 2-Carboxy-4-isopropyl-3-pyrrolidineacetic acid (DHK; a glial glutamate transporter antagonist) was purchased from Tocris Cookson (Bristol, UK). ω-AgaIVA and ω-CgTxGVIA were synthesized as described previously (Kim et al., 1994, 1995).

3. Results

3.1. Tonic inhibition of glutamate release at CA3–CA1 synapses by adenosine A_1 receptors

We have previously shown that glial glutamate transporter activity can be measured in the CA1 region of the hippocampus using an optical imaging technique (Kojima et al., 1999, Kawamura et al., 2004). Briefly, in normal artificial cerebrospinal fluid, the synaptically induced signals recorded from slices stained with the voltage-sensitive dye, RH155, consist of several components, including those due to presynaptic fibre volley, EPSPs and action potentials (Fig. 1A and B). In the presence of ionotropic glutamate receptor blockers, the signals due to EPSPs and action potentials disappear, leaving a slowly depolarising response that can be blocked by glutamate transporter blockers, such as DHK (Fig. 1B), and which is due to the depolarisation of astrocytes caused by the electrogenic activity of the GLT-1 glutamate transporter, which is stimulated by release of glutamate from the synapses. We therefore refer to this signal as synaptically induced glial depolarisation. The amplitude of the synaptically induced glial depolarisation signal is monotonically related to the intensity of stimulation (Fig. 1C–E).

In the present study, we examined whether application of an adenosine A_1 receptor antagonist substantially enhanced the synaptically induced glial depolarisation signal. Fig. 1C and D shows the effect of 0.5 μM 8-CPT on the synaptically induced glial depolarisation and field potential at three different stimulus intensities. In Fig. 1C and E1, the area of the synaptically induced glial depolarisation signal (0–120 ms) evoked by 0.1 or 0.3 mA was increased by 99.8% (n=5) and 150% (n=5), respectively. When the effect of 0.5 μM 8-CPT on the slope of the fEPSP was tested on a separate set of slice preparations (Fig. 1D), the slope of the fEPSPs evoked by 0.05, 0.1 and 0.3 mA was increased by 216% (n=5), 105% (n=5) and 75% (n=5), respectively (Fig. 1E2).

To confirm that the effect of 8-CPT was really due to inhibition of the adenosine A_1 receptor, we examined the effect of 8-CPT on the inhibition induced by an adenosine A_1 receptor agonist and on control transmission. Bath application of the specific adenosine A_1 receptor agonist, CCPA (0.5 μM), reduced both the amplitude of the synaptically induced glial depolarisation (Fig. 2A) and the slope of the fEPSP (Fig. 2B) to 60±9.2% (n=6) and 31±12% (n=4) of control levels, respectively. Using a combination of 0.5 μM 8-CPT and 0.5 μM CCPA, the amplitude of the synaptically induced glial depolarisation and the slope of fEPSP returned not to the basal level, but to a much higher level, the amplitude of the synaptically induced glial depolarisation reaching 226±32% (n=6) and the slope of the fEPSP 145±41% (n=4) of control levels (Fig. 2A and B). The values for the amplitude of the synaptically induced glial depolarisation and the slope of the fEPSP in the presence of 8-CPT alone were not significantly different (synaptically induced glial depolarisation p=0.34; fEPSP p=0.21 paired t-test) from those in the presence of both CCPA and 8-CPT (Fig. 2C1 and C2). Fig. 3 shows the dose dependency of the effect of 8-CPT on the amplitude of the synaptically induced glial depolarisation. The EC_{50} value was 0.9±1.1 μM.

The synaptically induced glial depolarisation signals showed a paired-pulse facilitation for a paired pulse with a 50-ms inter-pulse interval, as did the field EPSPs (Figs. 1C and 2A). Interestingly, in contrast to the paired-pulse ratio for the slope of fEPSP, the paired-pulse ratio for the synaptically induced glial depolarisation was not significantly affected by the presence of CCPA or of 8-CPT (Fig. 2D).

These results confirm those of a previous report (Wu and Saggau, 1994a), which showed that synaptic transmission at CA3–CA1 synapses is tonically inhibited by activation of adenosine A_1 receptors. We also tested whether transmission was tonically inhibited by metabotropic glutamate receptors, but found that metabotropic glutamate receptor antagonists, such as (*RS*)-α-Methyl-4-phosphonophenylglycine (MPPG), had no significant effect on the amplitude of the synaptically induced glial depolarisation (data not shown).

3.2. Adenosine A_1 receptors control glutamate release by inhibiting N-type voltage-gated Ca^{2+} channels

To examine which voltage-gated Ca^{2+} channels were responsible for transmitter release and to understand the mechanisms by which adenosine A_1 receptors inhibit synaptic transmission, we compared the effects of blockers of P/Q- and N-type voltage-gated Ca^{2+} channels on the amplitude of the synaptically induced glial depolarisation in the presence and absence of 8-CPT. To block adenosine A_1 receptor-mediated inhibition, 10 μM 8-CPT was applied to

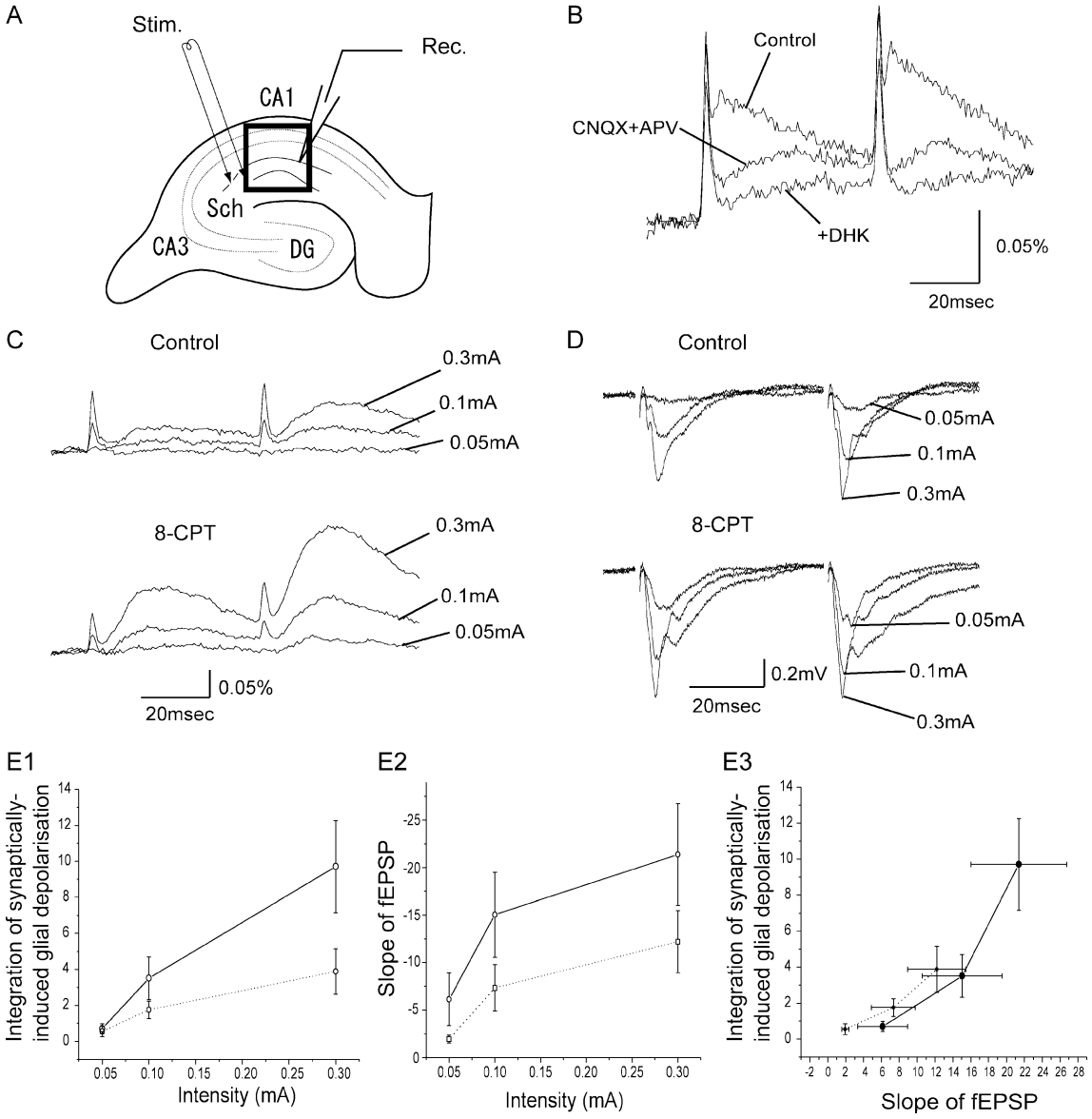

Fig. 1. Synaptically induced glial depolarisation monitored optically in the hippocampal CA1 area. (A) A slice was stained with a voltage-sensitive dye RH155. The slice was electrically stimulated by a bipolar electrode (Stim.) positioned in the stratum radiatum. An extracellular recording electrode (Rec.) was placed in the stratum radiatum to record the fEPSP. The changes in absorption were measured from the area indicated by a rectangle. (B) Sample traces of the change in absorption in response to a pair of stimuli recorded from the stratum radiatum. In control artificial cerebrospinal fluid, the response consists of three main components: a very fast spike-like component due to activity of presynaptic fiber volley, a fast component, and a slow component. Blockage of ionotropic glutamate receptors by applying CNQX (10 μM) and APV (50 μM) abolished the fast responses, leaving slow depolarising responses. Bath application of DHK (1 mM), a selective GLT-1 glutamate transporter antagonist, suppressed the synaptically induced glial depolarisation. (C, D) Synaptically induced glial depolarisation and fEPSP evoked by stimulus at various intensities (0.05–0.3 mA) in control conditions (top) and in the presence of 8-CPT (0.5 μM), a specific adenosine A_1 receptor blocker (bottom). (E1, E2) Dependence of the initial slope of the fEPSP (n=6) and the amplitude of the synaptically induced glial depolarisation (n=6) on stimulus intensity in control conditions (dashed line) and in the presence of 0.5 μM 8-CPT. (E3) The relationship between the fEPSP (n=6) and the synaptically induced glial depolarisation (n=6) at different stimulus intensities in control conditions (dashed line) and in the presence of 0.5 μM 8-CPT.

the bathing medium, then synaptically induced glial depolarisation was elicited by applying five repetitive stimuli at 10 ms intervals in order to increase the signal to noise ratio, a stimulation pattern which itself does not cause a change in the amplitude of the synaptically induced glial depolarisation (Kawamura et al., 2004). ω-AgaIVA was used to block P/Q-type channels and ω-CgTxGVIA to block N-type channels; on the basis of the results of Wu and Saggau (1994b), to obtain a maximal effect, both blockers were applied at a concentration of 1 μM for 20 min before testing for suppression of synaptically induced glial depolarisation in the presence of the voltage-gated Ca^{2+} channel blockers.

Fig. 4 shows representative responses (Fig. 4A) and the summarised results (Fig. 4B) of the effects of Ca^{2+} channel

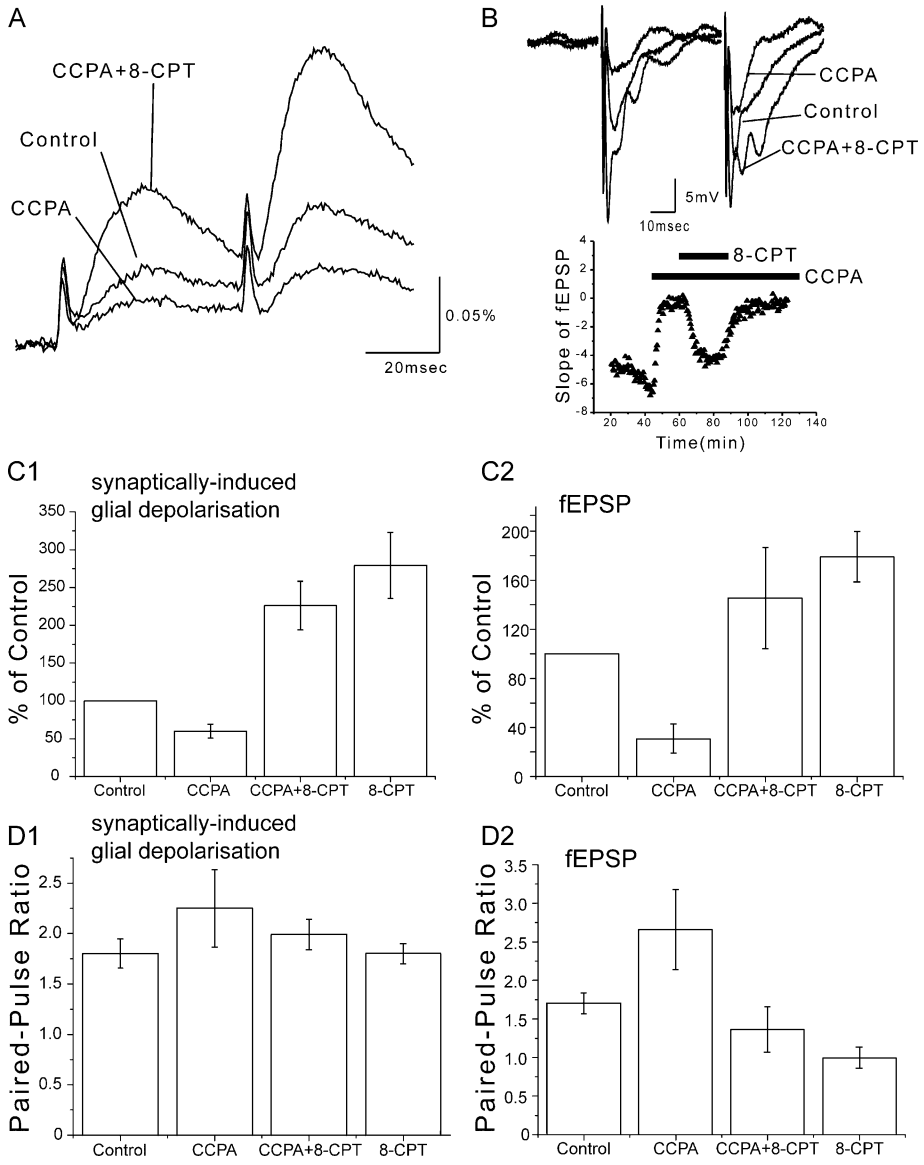

Fig. 2. Tonic inhibition of glutamate release by adenosine A_1 receptors. (A) Typical experiment showing the effects of application of CCPA (0.5 μM) and co-application of CCPA and 8-CPT (both 0.5 μM) on the synaptically induced glial depolarisation. CNQX(10 μM) and APV(50 μM) were present in the bath in all experiments. (B) Effects of application of CCPA (0.5 μM) and co-application of CCPA and 8-CPT (both 0.5 μM) on the fEPSP. Top: A typical experiment showing the field EPSPs. Bottom: Time-course of changes in the initial slope of the fEPSP during application of CCPA and co-application of CCPA and 8-CPT. Pooled data for the effects of CCPA alone and CCPA plus 8-CPT on the synaptically induced glial depolarisation (C1, n=4) and the fEPSP (C2, n=4). The signals were normalised to those before application of CCPA or 8-CPT. Effects of CCPA and co-application of CCPA and 8-CPT on the paired-pulse ratio of the synaptically induced glial depolarisation (D1) and the fEPSP (D2). Each column and bar indicates the mean and S.E.M.

blockers in the absence of 8-CPT. Both Ca^{2+} channel blockers suppressed the amplitude of the synaptically induced glial depolarisation without affecting the time-course of the effect. To estimate the relative contribution of different types of Ca^{2+} channels to transmitter release, the degree of suppression caused by a blocker of a specific Ca^{2+} channel type should be normalised to that caused by a potent non-specific Ca^{2+} channel blocker. In the presence of DHK, synaptically induced glial depolarisation was suppressed to such an extent that the addition of 300 μM Cd^{2+} to the bathing medium did not suppress the signal further (data not shown). To normalise the amplitude of the synaptically induced glial depolarisation, we therefore subtracted the amplitude of the signal obtained in the presence of DHK from that obtained in its absence.

When the adenosine A_1 receptor was not blocked, application of ω-AgaIVA (1 μM) alone, ω-CgTxGVIA (1 μM) alone or co-application of ω-AgaIVA and ω-CgTxGVIA reduced the synaptically induced glial depolarisation to 44.4±6.5% (n=10), 67.4±7.3% (n=10) or 30.3±3.6% (n=10) of control levels, respectively (Fig. 4B). In contrast, in the presence of 8-CPT (10 μM), the corresponding values were 47.9±5.9% (n=6), 32.0±5.7% (n=4) or 22.7±7.7% (n=5) of control levels (Fig. 5B).

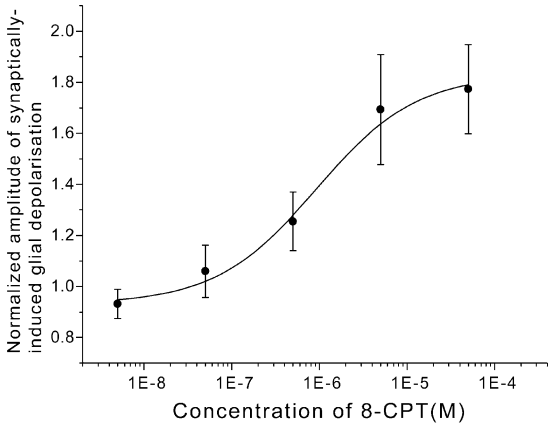

Fig. 3. Dose dependency of the effects of 8-CPT on the amplitude of the synaptically induced glial depolarisation. The amplitude of the synaptically induced glial depolarisation in the presence of 8-CPT was normalised to the value before applying 8-CPT. The bars show the S.E.M. (n=6). Data points were fitted to a sigmoidal curve (Y=[−0.9/{1+(x/EC^{50})$^{0.8}$}+1.8]) using the χ^2 method. The EC_{50} value was 0.9±1.1 μM.

These results showed that, in the presence of adenosine A_1 receptor-mediated suppression, the contribution of the P/Q-type channel was greater than that of the N-type channel, but, when adenosine A_1 receptor-mediated suppression was removed, the contribution of the N-type Ca^{2+} channel was greater than that of the P/Q-type channel.

4. Discussion

In this study, we monitored glutamate transmitter release by optically recording glial glutamate transporter activity, and found that the contribution of N-type voltage-gated Ca^{2+} channels to glutamate release was greater than that of P/Q-type voltage-gated Ca^{2+} channels when adenosine A_1 receptors were inhibited. This indicates that glutamate release at hippocampal CA3–CA1 synapse is tonically suppressed by activation of adenosine A_1 receptors and that the suppression is mainly due to inhibition of N-type voltage-gated Ca^{2+} channel.

The method we used in this study to measure glutamate release involved the monitoring of glutamate transporter activity in glial cells. We have shown in a previous report (Kojima et al., 1999) that the signal is due to glial transport on the basis that the signal can be recorded in a preparation in which postsynaptic cells are absent and that the signal was almost absent in slice preparations made from GLT-1 knock out mice. Still we cannot rule out the possibility that a

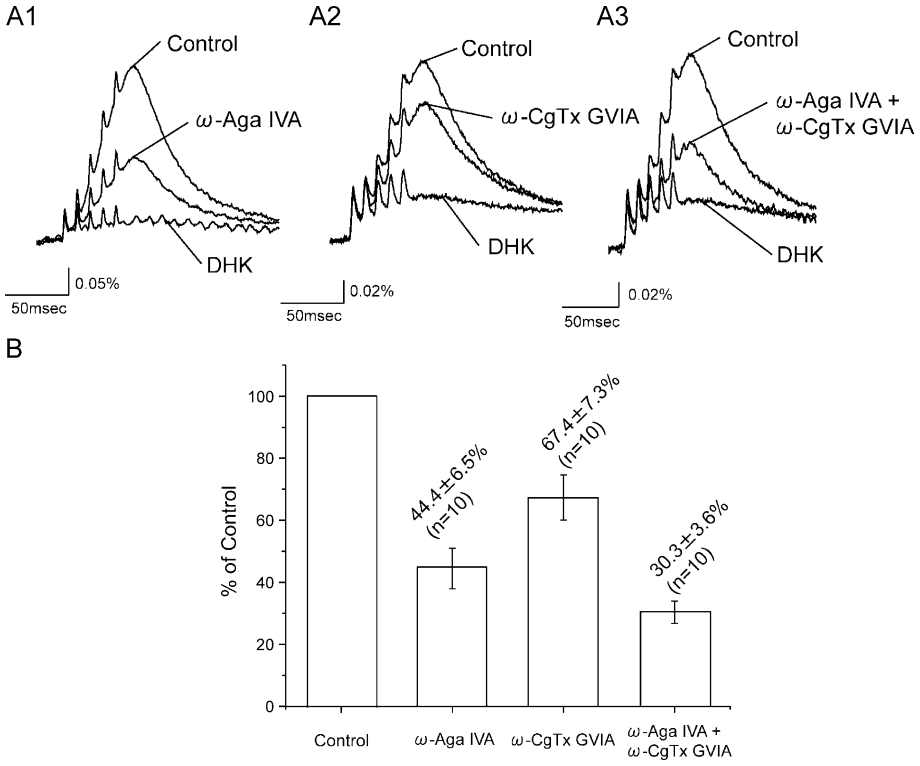

Fig. 4. Effects of Ca^{2+} channel blockers on the amplitude of the synaptically induced glial depolarisation in the absence of an adenosine A_1 receptor blocker. (A) The synaptically induced glial depolarisation was measured in normal artificial cerebrospinal fluid, and the blockers for the P/Q-type channel, ω-AgaIVA (1 μM, A1), and/or the N-type channel, ω-CgTxGVIA (1 μM, A2) were applied. Responses were evoked by delivering 5 stimuli with a 10-ms interval to Schaffer collaterals. The effect of application of ω-CgTxGVIA was smaller than that of ω-AgaIVA. Application of DHK (1 mM) suppresses the component due to glutamate uptake, leaving a component which is not related to glutamate release. CNQX (10 μM) and APV (50 μM) were present in the bath in all experiments. (B) Statistical comparison of the effects of Ca^{2+} channel blockers on the synaptically induced glial depolarisation in the absence of 8-CPT (10 μM). After subtracting the DHK-insensitive component, the signal was integrated over the first 200 ms and normalised to the value in control conditions. Each column and bar indicates the mean and S.E.M.

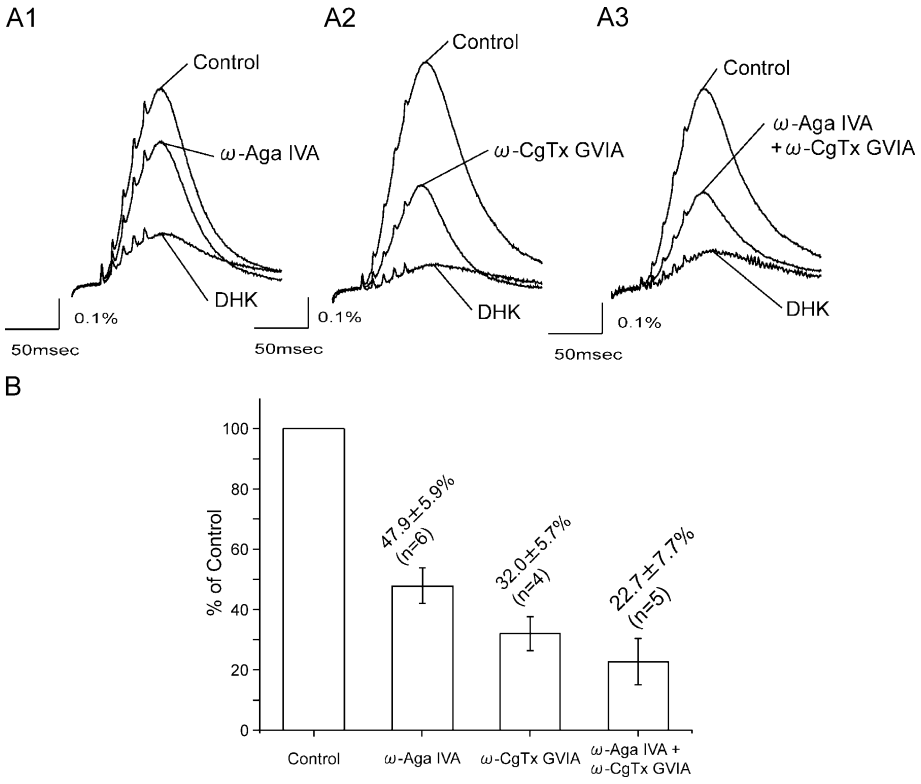

Fig. 5. Effects of Ca^{2+} channel blockers on the amplitude of the synaptically induced glial depolarisation in the presence of an adenosine A_1 receptor blocker. (A) The synaptically induced glial depolarisation was measured in the presence of 8-CPT (10μM), and the blockers for the P/Q-type channel, ω-AgaIVA (1 μM, A1), and/or the N-type channel, ω-CgTxGVIA (1 μM, A2) were applied. Responses were evoked by delivering five stimuli with a 10 ms interval to Schaffer collaterals. The effect of application of ω-CgTxGVIA was greater than that of ω-AgaIVA. Application of DHK (1 mM) suppresses the component due to glutamate uptake, leaving a component which is not related to glutamate release. CNQX(10 μM) and APV(50 μM) were present in the bath in all experiments. (B) Statistical comparison of the effects of Ca^{2+} channel blockers on the synaptically induced glial depolarisation in the presence of 8-CPT (10 μM). After subtracting the DHK-insensitive component, the signal was integrated over the first 200 ms and normalised to the value in control conditions. Each column and bar indicates the mean and S.E.M.

part of the synaptically induced depolarizing signal in the presence of glutamate receptor antagonists could be due to activities of neuronal glutamate transporters because the voltage-sensitive dye we used in this study preferentially stains glial cells but stains neuronal cells as well (Momose-Sato et al., 1999). If it was the case, however, it would not confound our results, because, either way we can monitor amount of glutamate release by measuring the signal that we refer to as synaptically induced glial depolarisation.

There is a possibility that the applied adenosine A_1 receptor agonist and/or antagonist acted on the glial glutamate transporter, rather than on transmitter release. However, application of the adenosine A_1 receptor blocker also facilitated the fEPSPs, implying that the blocker acted on release, not on uptake. To support presynaptic site of drug actions, an immunohistochemical study by Ochiishi et al. (1999) reported that adenosine A_1 receptor immunoreactivity was not detected on glial cells. In addition, it has been reported that glial transporters can be regulated by activation of adenosine A_2 receptors, but not adenosine A_1 receptors (Nishizaki et al., 2002). Thus, it is unlikely that the adenosine A_1 receptor agonist and/or antagonist acted on glial glutamate transporters.

Immunohistochemical studies reported that not only presynaptic but also post synaptic reactivities of adenosine A_1 receptors were detected (Ochiishi et al., 1999, Rebola et al., 2003), and that adenosine A_1 receptors co-localize with postsynaptic NMDA glutamate receptors and N- or P/Q-type voltage-gated Ca^{2+} channels (Rebola et al., 2003). Therefore adenosine A_1 receptor agonist and/or antagonist might have acted on postsynaptic sites. In our experimental condition, however, responses of postsynaptic neurons were blocked by antagonists against ionotropic glutamate receptors. Therefore, signals due to activities of postsynaptic membranes or due to uptake of glutamate from the recurrent terminals of post synaptic neurons were unlikely to be involved in synaptically induced glial depolarisation signal in this study.

It has been shown in previous reports that activation of presynaptic metabotropic glutamate receptors would suppress glutamate release from presynaptic terminals (Cartmell and Schoepp, 2000). To test this possibility, we tested the effects of agonists and antagonists for mGluRs on synaptically induced glial depolarisation signals in normal conditions in which the adenosine A_1 receptor agonist was absent. We found that mGluR agonists suppressed synap-

tically induced glial depolarisation significantly, whereas the antagonists did not significantly enhance synaptically induced glial depolarisation. Although N-type voltage-gated Ca^{2+} channel are the most common voltage-gated Ca^{2+} channels shown to be inhibited by mGluR agonists in literatures (Anwyl, 1999), it is possible that presynaptic mGluRs be more effectively activated in the presence of adenosine A_1 receptor antagonist and the activated mGluRs target P/Q voltage-gated Ca^{2+} channels. We have not tested the effects of mGluR agonists/antagonists in the presence of adenosine A_1 receptor antagonists. Still, we should be able to claim N-type voltage-gated Ca^{2+} channel as the main regulator in normal conditions.

Both in the presence and absence of 8-CPT, the degree of blockade after co-application of ω-AgaIVA and ω-CgTxGVIA was less than the sum of the blockades produced by ω-AgaIVA alone or ω-CgTxGVIA alone. This non-linearity is not surprising, since it has been shown by Reuter (Reuter, 1995) that ω-AgaIVA-sensitive voltage-gated Ca^{2+} channels and ω-CgTxGVIA-sensitive voltage-gated Ca^{2+} channels coexist in more than half of the synaptic terminals of hippocampal cell cultures, and, since the amplitude of the postsynaptic response is likely to be related to the power of the presynaptic Ca^{2+} current (Augustine et al., 1985), the non-linearity may be due to the power relationship between the presynaptic Ca^{2+} current and transmitter release at those terminals in which N- and P/Q-type voltage-gated Ca^{2+} channels coexist. If the tonic suppression of glutamate release mediated by adenosine A_1 receptors is mainly due to inhibition of voltage-gated Ca^{2+} channels, we should be able to estimate the degree of tonic inhibition of N- and P/Q-type voltage-gated Ca^{2+} channels by activation of adenosine A_1 receptors at CA3–CA1 synapses and also the relative contributions of these channels in the absence of inhibition. We assumed that the relationship between the amplitude of the synaptically induced glial depolarisation and the Ca^{2+} rise due to entry through voltage-gated Ca^{2+} channels to be as follows.

Amplitude of the synaptically induced glial depolarisation

$$= constant \times \left(\Delta\left[Ca^{2+}\right]_{\mathrm{pre}}\right)^{m}$$

Wu and Saggau (1994b) obtained a value of m=3.5 by analysing their Ca^{2+} imaging data recorded from presynaptic terminals at CA3–CA1 synapses, simultaneously per-

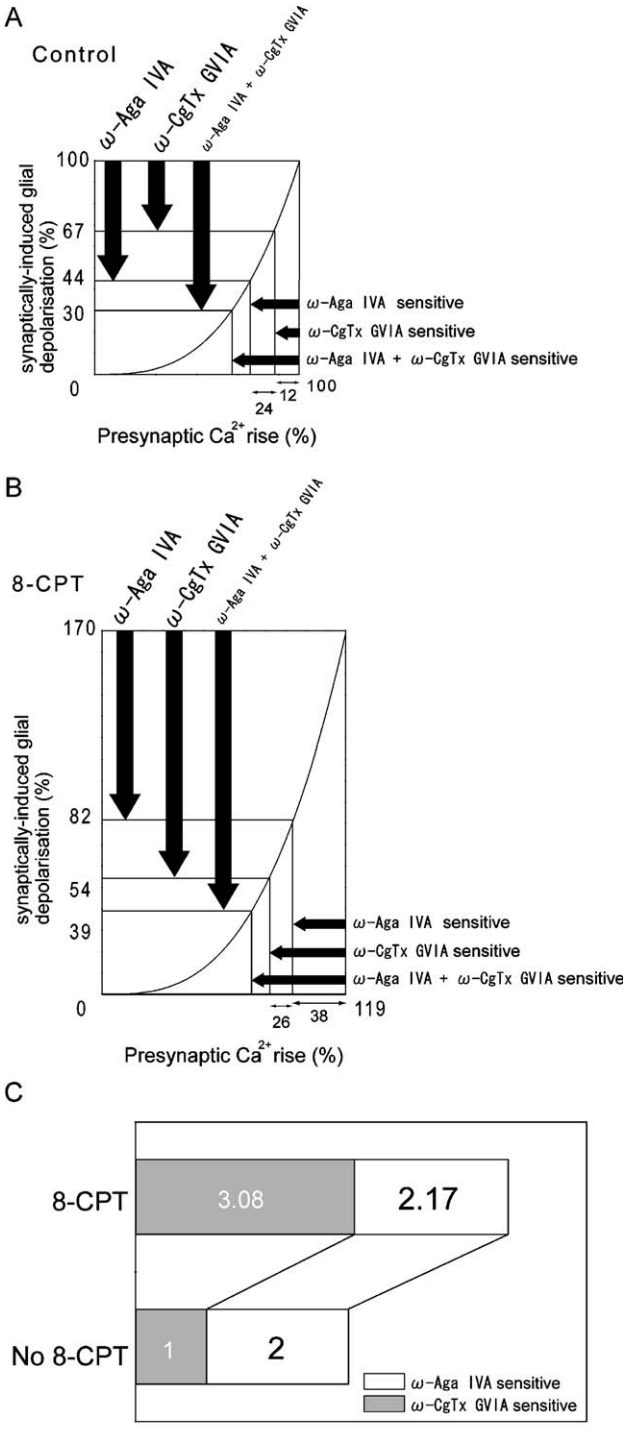

Fig. 6. An analysis of the contribution of voltage-gated Ca^{2+} channels to glutamate release. (A) The relationship between the synaptically induced glial depolarisation and the calculated presynaptic Ca^{2+} influx in the absence of an adenosine A_1 receptor blocker. In this calculation, the amplitude of the synaptically induced glial depolarisation was assumed to be proportional to the third power of the change in the $[Ca^{2+}]_{pre}$; synaptically induced glial depolarisation=constant×$(\Delta[Ca^{2+}]_{pre})^3$. The ordinate shows the relative amplitude of synaptically induced glial depolarisation normalized to the value in the control condition in which 8-CPT was absent. The vertical arrows show the extent of the suppression by blockers for voltage-gated Ca^{2+} channels. The abscissa shows the relative change in calculated presynapitc Ca^{2+} concentration normalized to the value in the control condition. The horizontal arrows show the extent of suppression. (B) The relationship between the synaptically induced glial depolarisation and the calculated presynaptic Ca^{2+} influx in the presence 8-CPT, an adenosine A_1 receptor blocker. The ordinate and the abscissa are normalized to the value obtained in the control condition in which 8-CPT was absent. (C) Comparison of the calculated contribution of voltage-gated Ca^{2+} channels to the change in the $[Ca^{2+}]_{pre}$. The values are normalised to the contribution of the ω-CgTxGVIA-sensitive component in the absence of adenosine A_1 receptor-mediated inhibition.

formed with field EPSP measurements. Reid et al. (1998) estimated m=3.1 or 3.3 at hippocampal autapses. Reid et al. (1998) showed that the values for N- and P/Q-types were not significantly different. Applying this formula, we calculated the relative contribution of different voltage-gated Ca^{2+} channels to the Ca^{2+} rise, based on the effects of the different channel blockers on the synaptically induced glial depolarisation in the absence and in the presence of 8-CPT, the adenosine A_1 receptor blocker. In the absence of 8-CPT, the amplitude of synaptically induced glial depolarisation was reduced by ω-CgTxGVIA alone and ω-AgaIVA alone to 67.4±7.3% and 44.4±6.5% of the control respectively. For m=3, these values correspond to 12% and 24% blockade of the total Ca^{2+} rise (Fig. 6A). In the presence of 10 μM 8-CPT, the amplitude of the synaptically induced glial depolarisation increased to 170% (Fig. 3), corresponding to an increase in the Ca^{2+} rise of 119%. In this condition, the amplitude of synaptically induced glial depolarisation by ω-CgTxGVIA alone and ω-AgaIVA alone was 32.0±5.7% and 47.9±5.9% of control respectively, corresponding to 38% and 26% blockade of the total Ca^{2+} rise in the control conditions with no 8-CPT (Fig. 6B). Thus, the relative contribution of N- and P/Q-type voltage-gated Ca^{2+} channels was 1:2 in the absence of 8-CPT and 3.08:2.17 in the presence of 8-CPT (Fig. 6C). A similar conclusion was reached when we assume m=2, 4 or 5. We can therefore conclude that the N-type voltage-gated Ca^{2+} channel is suppressed by tonic activation of adenosine A_1 receptors, whereas the P-type voltage-gated Ca^{2+} channel is not significantly affected.

Although it has been shown that N- and P/Q-type voltage-gated Ca^{2+} channels are responsible for transmitter release at many synapses in the central nervous systems, the distribution and the contribution of these voltage-gated Ca^{2+} channels seem to vary from synapses to synapses (Reid et al., 1997, 2003; Reuter, 1996). Scholz and Miller (1995) and Iwasaki et al. (2000) also have shown that the relative contribution of N-type versus P/Q-type changes during development. The results of our study suggest a possibility that the extent of adenosine A_1 receptor-mediated tonic inhibition can be one of the reasons for the heterogeneity of voltage-gated Ca^{2+} channels responsible for transmitter release.

The source of adenosine responsible for tonic regulation of synaptic transmission is largely unknown, and we did not make attempts to analyze the source of adenosine in our study. Two possible sources have been suggested: dephosphorylation of ATP released either from presynaptic terminals or glial cells (Newman, 2003; Cunha and Ribeiro, 2000; Dunwiddie and Masino, 2001; Yawo and Chuhma, 1993). Release of adenosine via passive or active transporters is also suggested (Dunwiddie and Masino, 2001). The basal extracellular level of adenosine is reported to be in the range of 25–250 nM. Since the affinity of adenosine A_1 receptors to adenosine is reported to be around 70 nM (Dunwiddie and Masino, 2001), the basal level of adenosine is high enough to provide tonic activation of adenosine A_1 receptors.

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