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Activation of adenosine A_2 receptors enhances high K^+-evoked taurine release from rat hippocampus: a microdialysis study

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Summary. The present study was designed to examine which type of adenosine receptors was involved in enhancement of high K^+-evoked taurine release from *in vivo* rat hippocampus using microdialysis. Perfusion with 0.5 or 5.0 mM adenosine enhanced high K^+-evoked taurine release. Perfusion with 2 μM R(−)-N^6-2-phenylisopropyladenosine (PIA), a selective adenosine A_1 receptor agonist, did not modulate taurine release. Perfusion with 1 μM 1,3-dipropy1-8-cyclopentylxanthine (DPCPX), a selective adenosine A_1 receptor antagonist, increased taurine release. On the other hand, perfusion with 20 μM 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethyl-carboxamide-adenosine (CGS21680), a selective adenosine A_{2A} receptor agonist, enhanced taurine release, while perfusion with 1 mM 3,7-dimethyl-propagylxanthine (DMPX), an adenosine A_2 receptor antagonist, did not affect taurine release. These results demonstrate that adenosine enhances high K^+-evoked taurine release via activation of adenosine A_{2A} receptors from both neurons and glial cells of *in vivo* rat hippocampus.

Keywords: Amino acids – Adenosine – Taurine – Hippocampus – Microdialysis – Spreading depression

Introduction

Adenosine, which is released during cerebral ischemia, increases cerebral blood flow and decreases neuronal excitability. It has been suggested that adenosine acts as a neuroprotective agent, preventing damage from ischemia and excitotoxicity (for recent reviews, see Fredholm et al., 1994; Von Lubitz et al., 1995; Brundege and Dunwiddie, 1997). Numerous studies have shown that taurine, which is released during ischemia and high K^+ stimulation, has many biological functions including osmoregulation and neuromodulation in the central nervous system (for reviews see Huxtable, 1992; Saransaari and Oja, 1992).

Madelian et al. (1988) first reported that adenosine, a neuromodulator, stimulated cAMP-mediated taurine release from *in vitro* cultured astrocytes. We have recently reported that an increase in endogenous adenosine by adenosine transport inhibitors enhances high K^+-evoked taurine release from rat hippocampus (Kaku et al., 1994; Hada et al., 1996). Moreover, Miyamoto and Miyamoto (1996) have also reported that exogenous adenosine increases taurine release from *in vivo* rabbit brain regions including hippocampus. However, the receptor subtype involved has not been specified in *in vivo* preparations. Therefore, we have carried out the present study to determine which type of adenosine receptor is associated with adenosine-induced release of taurine from *in vivo* rat hippocampus using microdialysis.

Materials and methods

The experimental procedures have been described in detail in our previous papers (Kaku et al., 1994; Hada et al., 1996). In brief, main points are described here. Male Wister rats were anesthetized with urethane (1.5 g/kg, i.p.) during experiments. We stereotaxically implanted a microdialysis probe (CMA/10, CMA/Microdialysis) into the dorsal hippocampus in order to apply drugs and to collect dialysates. The basal perfusion medium was an artificial cerebrospinal fluid (ACSF) (composition in mM: NaCl 132.8; KCl 3.0; $CaCl_2$ 2.0; $MgCl_2$ 0.7; $NaHCO_3$ 24.6; urea 6.7; glucose 3.7). The perfusion flow rate was 2.0 μl/min and controlled by a microinjection pump (CMA/100, CMA/Microdialysis).

We performed three series of experiments. In the first series of experiments, three groups of rats were dialysed through probes with 2 mm active membrane length. Five rats served as a control group and received 100 mM K^+ alone for 30 min (high K^+ alone group). The other 5 rats received 0.5 mM adenosine plus 100 mM K^+ for 30 min (0.5 mM adenosine group). Moreover, 6 rats received 5.0 mM adenosine plus 100 mM K^+ for 30 min (5.0 mM adenosine group). In both groups, adenosine was applied 30 min before perfusion with 100 mM K^+ for 30 min and thus the total perfusion time was 60 min.

In the second series of experiments, three groups of rats were dialysed through probes with 1 mm active membrane length. Nine rats served as a control group and received 75 mM K^+ alone for 40 min (high K^+ alone group). The other 5 rats received 2 μM R(−)-N^6-2-phenylisopropyladenosine (PIA), an adenosine A_1 receptor agonist, plus 75 mM K^+ for 40 min (PIA group). Five rats received 20 μM 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethyl-carboxamide-adenosine (CGS21680), a selective adenosine A_{2A} receptor agonist, plus 75 mM K^+ for 40 min (CGS21680 group). PIA or CGS21680 was applied 20 min before perfusion with 75 mM K^+ for 40 min and thus the total perfusion time was 60 min.

In the third series of experiments, three groups of rats were dialysed through probes with 2 mm active membrane length. Five rats served as a control group and received 100 mM K^+ alone for 30 min. Five rats received 1 μM 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), an adenosine A_1 receptor antagonist, plus 100 mM K^+ for 30 min (DPCPX group). The other 5 rats received 1 mM 3,7-dimethyl-propagylxanthine (DMPX), an adenosine A_2 receptor antagonist, plus 100 mM K^+ for 30 min (DMPX group). DPCPX or DMPX was applied 30 min before perfusion with 100 mM K^+ for 30 min and thus the total perfusion time was 60 min.

Dialysates were collected every 10 min. Taurine content was determined after precolumn derivatization with *o*-phthaldialdehyde by high performance liquid chromatography using a fluorescence detector. A capillary column (BAS, C-18, 5 μm, monomeric 1.0 ϕ × 100 mm) was used for taurine analysis. The mobile phase was phosphate buffer

(pH 6.0) with 0.1 mM EDTA-2Na, 10% acetonitrile and 3% tetrahydrofuran and was pumped at a flow rate of 60μl/min.

The data are expressed as means ± S.E.M. Taurine release is expressed as a percentage, taking the mean basal amount released during ACSF perfusion before application of high K^+ or any drug as 100%. Total evoked taurine release is the cumulative amount of taurine released during a 60-min period following the onset of high K^+ perfusion. This was obtained by subtracting the amount of taurine released before any drug perfusion from that released during the 60-min period following the onset of high K^+ perfusion. Taurine release is expressed as a percentage, taking the mean total net amount of taurine released by high K^+ alone as 100%.

To determine the difference between experimental groups and time course of taurine release, statistical analysis was performed by a mixed type analysis of variance (ANOVA) with repeated measures and post-hoc tests (Fisher's protected least significant difference). For single comparisons, the significance of differences between means was determined by Student's two-tailed *t*-tests. P values < 0.05 were considered to be significant.

Results

Effect of adenosine on high K^+-evoked release of taurine

The basal levels of taurine from hippocampal dialysates collected through the microdialysis probes with 1 and 2mm long active membrane were 0.718 ± 0.038μM (n = 56) and 1.538 ± 0.045μM (n = 92), respectively. Figure 1A shows the time course of high (100mM) K^+-evoked release of taurine in high K^+ alone (control), 0.5 and 5.0mM adenosine groups. In each group, high K^+ increased taurine release. The taurine levels gradually increased and then reached maximal values 10min after the onset of reperfusion with ACSF. In the control group, perfusion with high K^+ alone increased taurine release to a maximal value of 320.27 ± 36.24% (n = 5). Perfusion with 0.5 and 5.0mM adenosine dramatically enhanced taurine release to a maximal value of 596.43 ± 82.02% (n = 5) and of 500.80 ± 57.36% (n = 6), respectively.

To assess the degree of the effect of adenosine on net taurine release, we estimated the total net amount of taurine released during the 60-min period following the onset of high K^+ perfusion in each group. When compared with the control (100.00 ± 13.26%; n = 5), perfusion with 0.5 and 5.0mM adenosine significantly increased the total net amount of taurine release to 249.69 ± 55.78% (n = 5) and 207.68 ± 33.16% (n = 6), respectively ($P < 0.05$) (Fig. 1B).

Effect of adenosine receptor agonists on high K^+-evoked release of taurine

In order to determine which type of adenosine receptor is associated with the enhancement of taurine release by adenosine, we examined effects of the selective adenosine A_1 and A_2 receptor agonists and antagonists on high K^+-evoked taurine release. Figure 2 shows the effects of PIA, the selective adenosine A_1 receptor agonist and CGS21680, the selective adenosine A_{2A} receptor agonist, on high (75mM) K^+-evoked taurine release. Perfusion with high K^+ alone dramatically released taurine to a maximal value of 494.51 ± 54.19% (n = 9). Perfusion with 2μM PIA did not change taurine release. In

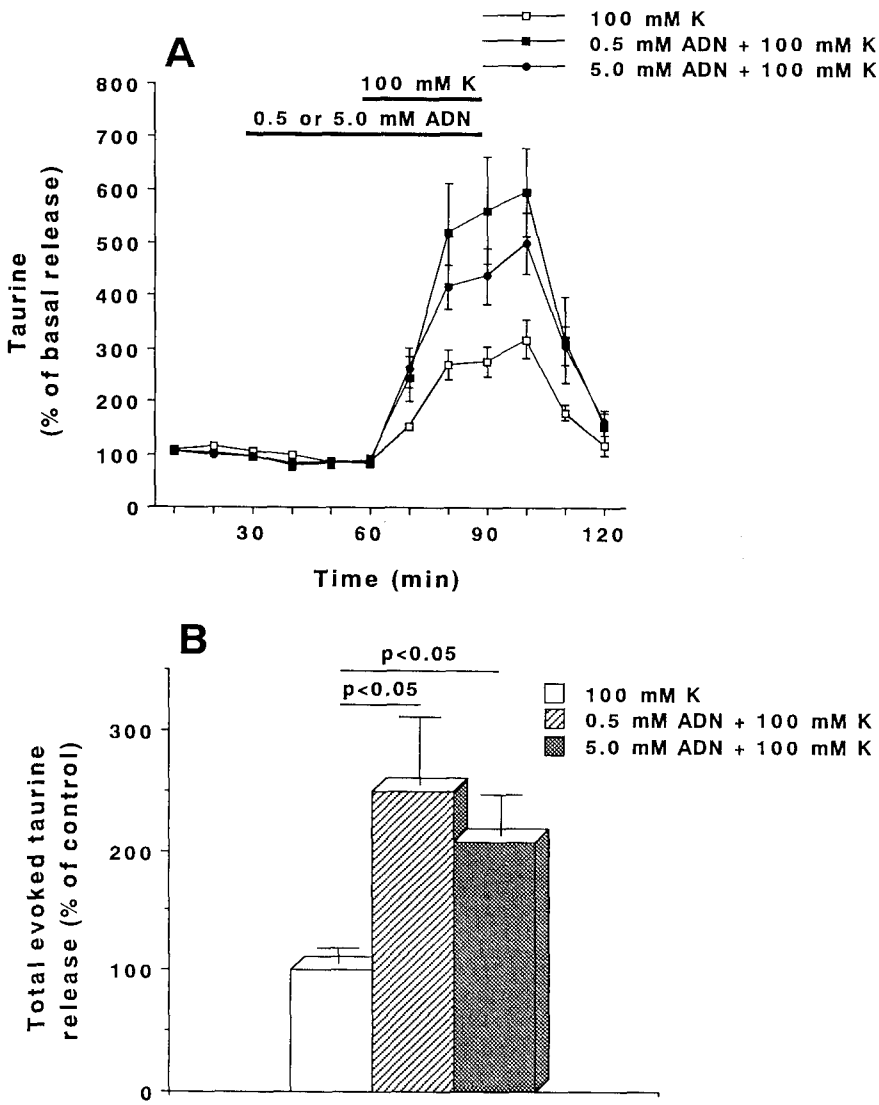

Fig. 1. **A** Time course of taurine release evoked by perfusion with high K^+ alone, 0.5 or 5.0 mM adenosine (ADN) + 100 mM K^+. Each point represents the mean ± SEM of taurine released for 5 or 6 experiments as a percentage of the basal level. Symbols: 100 mM K^+ alone (control) (□); 0.5 mM adenosine (ADN) + 100 mM K^+ (■); 5.0 mM adenosine (ADN) + 100 mM K^+ (●). A mixed type ANOVA with repeated measures design was carried out to analyse the time course of taurine release. The main effect of group was not significant [$F(2/13) = 3.326$, $P > 0.05$] but that of time was significant [$F(11/143) = 76.730$, $P < 0.0001$]. The interaction between group and time was also significant [$F(22/143) = 4.246$, $P < 0.0001$]. Fisher's protected least significant difference post-hoc test showed a significant group difference ($P < 0.05$) between the high K^+ alone group and the 0.5 mM adenosine group. **B** The effect of adenosine on the total net amount of high K^+-evoked taurine release. The total net amount of taurine release was obtained by subtracting the basal amount of taurine released before adenosine application from that released during the 60-min period following the start of high K^+ perfusion. Each column with a vertical bar represents the mean ± SEM of 6 samples obtained from 5 or 6 animals and is expressed as a percentage, taking the mean total net amount of high K^+-evoked taurine release in the high K^+ alone group as 100%. Note that perfusion with 0.5 mM adenosine significantly enhanced high K^+-evoked taurine release, when compared with the high K^+ alone group ($P < 0.05$)

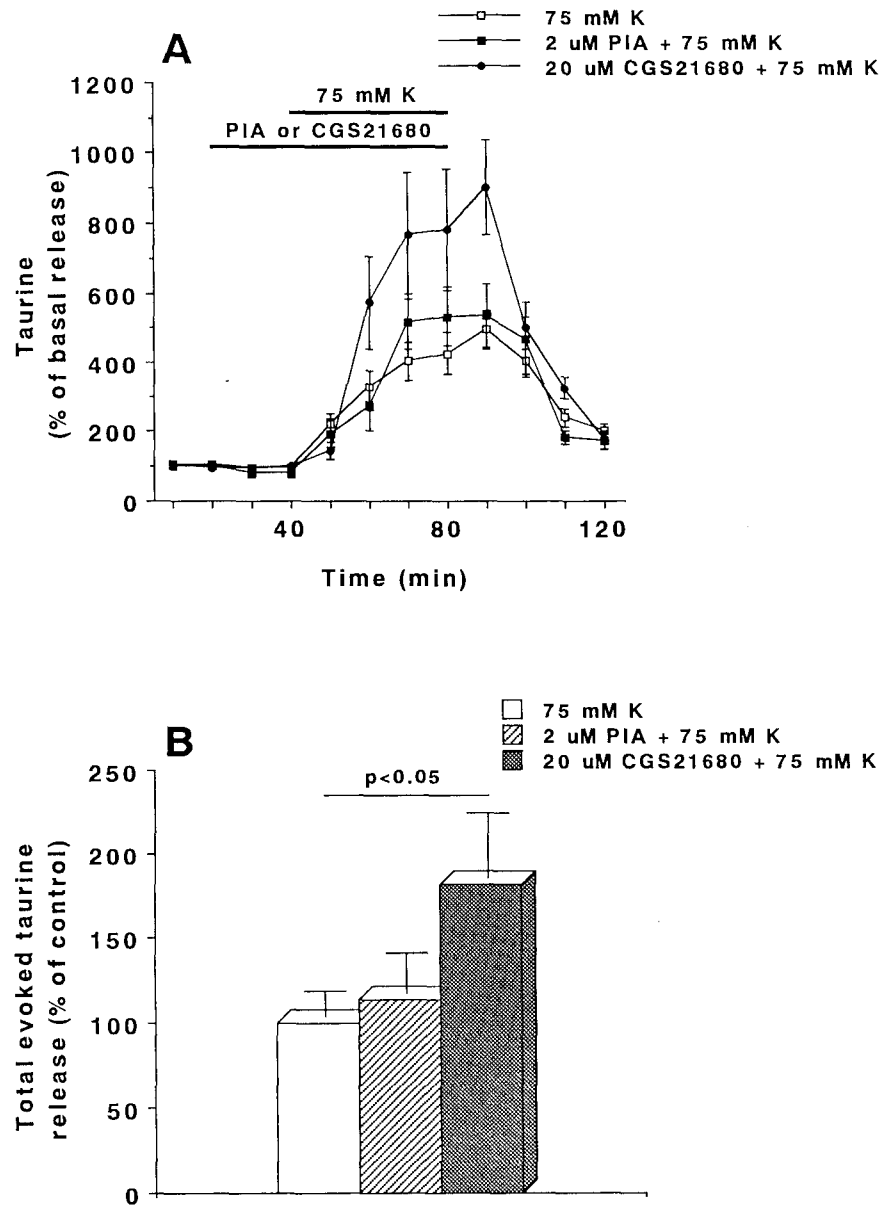

Fig. 2. A Time course of taurine release evoked by perfusion with 75 mM K^+ alone (control) (□), 2 μM PIA + 75 mM K^+ (■) or 20 μM CGS21680 + 75 mM K^+ (●). A mixed type ANOVA with repeated measures design was carried out to analyse the time course of taurine release. The main effect of group was not significant [$F(2/16) = 3.554$, $P > 0.05$] but that of time was significant [$F(11/176) = 51.765$, $P < 0.0001$]. The interaction between group and time was also significant [$F(22/176) = 3.753$, $P < 0.0001$]. Fisher's protected least significant difference post-hoc test showed a significant group difference ($P < 0.05$) between the high K^+ alone group and the CGS21680 group. **B** The effect of PIA and CGS21680 on high K^+-evoked taurine release. Note that perfusion with 20 μM CGS21680 enhanced high K^+-evoked taurine release, when compared with the high K^+ alone group ($P < 0.05$)

contrast, perfusion with 20μM CGS21680 dramatically increased taurine release to a maximal value of 899.32 ± 134.56% (n = 5).

When compared with the control (100.00 ± 14.98%; n = 9), perfusion with 2μM PIA did not change the total net amount of taurine release (113.90 ± 23.34%; n = 5) for 60min. In contrast, perfusion with 20μM CGS21680 significantly increased taurine release (182.36 ± 38.46%; n = 5) ($P < 0.05$) (Fig. 2B).

Effect of adenosine receptor antagonists on high K^+-evoked release of taurine

As shown in Fig. 3A, perfusion with 1μM DPCPX, the selective adenosine A_1 receptor antagonist, dramatically increased taurine release to a maximal value of 627.66 ± 91.85% (n = 5). In contrast, perfusion with 1mM DMPX, the selective adenosine A_2 receptor antagonist, did not affect taurine release (a maximal value of 394.54 ± 63.36%; n = 5).

Perfusion with 1μM DPCPX significantly increased the total net amount of taurine released over the 60min to 285.22 ± 48.17% (n = 5) ($P < 0.02$), when compared with the control (100.00 ± 13.26%; n = 5). In contrast, perfusion with 1mM DMPX did not affect taurine release (136.63 ± 35.79%; n = 5) (Fig. 3B).

Discussion

The major findings of the present study are summarized as follows: PIA, the selective adenosine A_1 receptor agonist, did not change the high K^+-evoked taurine release from rat hippocampus *in vivo*. DPCPX, the selective adenosine A_1 receptor antagonist, increased taurine release. In contrast, CGS21680, the selective adenosine A_{2A} receptor agonist, enhanced it. DMPX, the selective adenosine A_2 receptor antagonist, did not affect taurine release. These results, for the first time, demonstrate that adenosine enhances high K^+-evoked taurine release via activation of adenosine A_{2A} receptors. This study extends our previous reports that the increase in endogenous adenosine by adenosine transport inhibitors enhanced the high K^+-evoked taurine release (Kaku et al., 1994; Hada et al., 1996).

The present *in vivo* findings are in accordance with both an *in vitro* report of Madelian et al. (1988) that adenosine stimulates cAMP-mediated taurine release from cultured glial cells and an *in vivo* report of Miyamoto and Miyamoto (1996) that exogenous adenosine increases taurine release from rabbit brain regions, including the hippocampus. High K^+-evoked taurine release may originate from both neurons and glial cells, because Schousboe and Pasantes-Morales (1989) reported in cultured cerebellar neurons and astrocytes that high K^+ stimulated taurine release was associated with a swelling process. The increase in high K^+-evoked taurine release may be mediated via the adenosine A_{2A} receptors predominantly located in the hippocampus, which are different from the striatal adenosine A_{2A} receptors (Cunha et al., 1996).

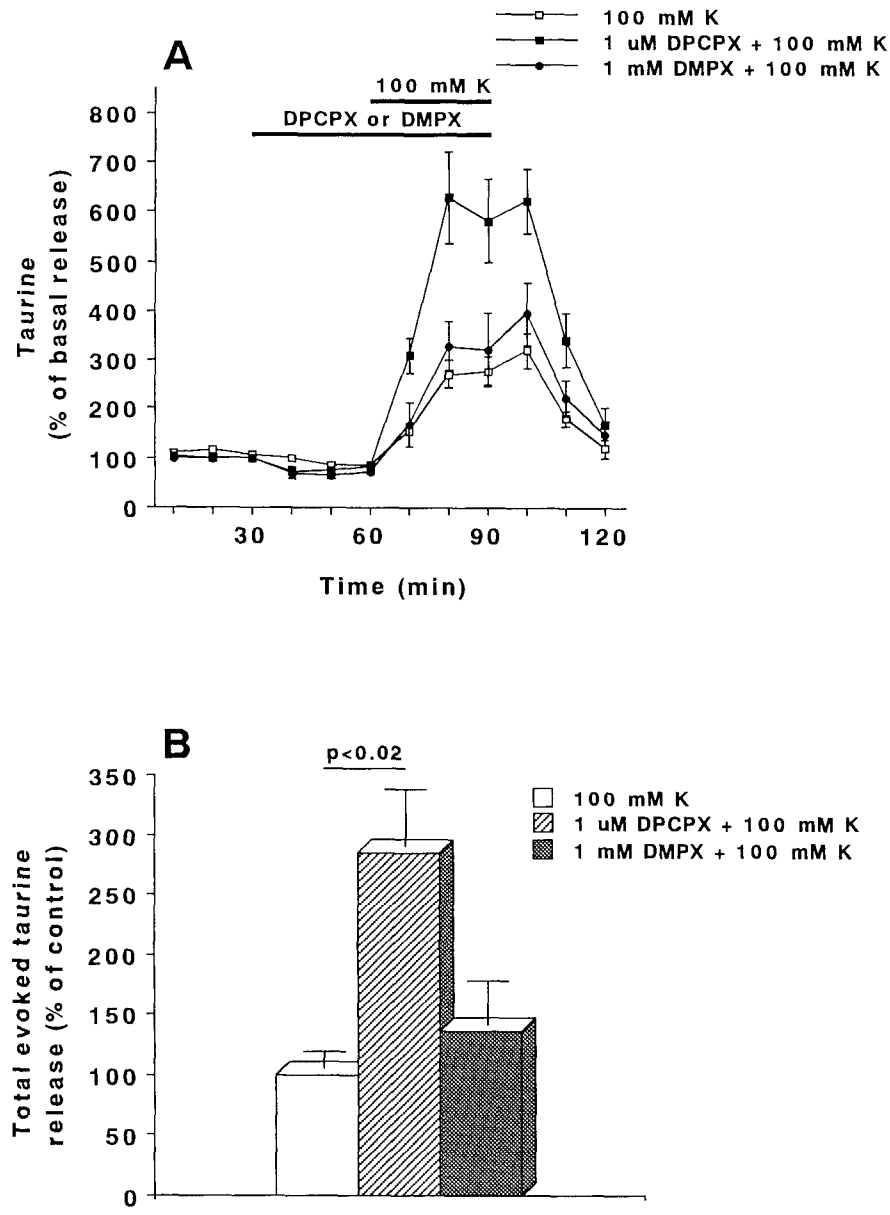

Fig. 3. A Time course of taurine release evoked by perfusion with 100 mM K^+ alone (control) (□), 1 μM DPCPX + 100 mM K^+ (■) or 1 mM DMPX + 100 mM K^+ (●). A mixed type ANOVA with repeated measures design was carried out to analyse the time course of taurine release. The main effects of both group and time were significant [$F(2/6) = 9.525$, $P < 0.02$ and $F(11/66) = 57.763$, $P < 0.0001$, respectively]. The interaction between group and time was also significant [$F(22/66) = 6.973$, $P < 0.0001$]. Fisher's protected least significant difference post-hoc test showed a significant group difference ($P < 0.01$) between the high K^+ alone group and the DPCPX group. **B** The effect of DPCPX and DMPX on high K^+-evoked taurine release. Note that perfusion with 1 μM DPCPX enhanced high K^+-evoked taurine release, when compared with the high K^+ alone group ($P < 0.02$)

The increase in taurine release by adenosine may occur to compensate the osmotic imbalance triggered by adenosine-induced astroglial swelling via cAMP accumulation (Bourke et al., 1981). It is not clear why and how adenosine causes astroglial swelling. However, it may be associated with increases in brain tissue uptake of water, Na^+, Cl^-, and K^+ (Bourke et al., 1978). An alternative explanation is that taurine release may not always be related to cellular swelling. For example, taurine release evoked by NMDA application does not seem to be related to cellular swelling, at least in the hippocampus *in vivo*, because the increase in taurine release was not inhibited by perfusion with hyperosmotic sucrose medium (Menendez et al., 1990).

We have recently reported that the adenosine A_1 receptor antagonist, DPCPX, increased high K^+-evoked glutamate release due to blockade of an inhibitory action through the adenosine A_1 receptor and incidence of spreading depression (Kaku et al., 1997). In the present study, the increase in high K^+-evoked taurine release by DPCPX could be caused to compensate osmotic imbalance induced by spreading depression, because Na^+ and water flow into neurons and glial cells during spreading depression, resulting in cell swelling (Somjen et al., 1992). Moreover, the increase in glutamate release would stimulate taurine release from astrocytes through a swelling-triggered mechanism (Koyama et al., 1994). According to our unpublished observation, CGS21680 at 20μM did not change high K^+-evoked glutamate release, but reduced the occurrence of spreading depression. It is conceivable that the reduced incidence of spreading depression may be associated with both a decrease in glutamate release and an increase in taurine release by CGS21680.

We cannot explain exactly the reason(s) why PIA did not inhibit high K^+-evoked taurine release in this study. However, this finding is in accordance with a report of Madelian et al. (1988) showing that PIA caused a dose-dependent increase in cAMP accumulation and taurine release. We have reported that PIA decreases high K^+-evoked glutamate release and occurrence of spreading depression (Hada et al., 1995). Moreover, although PIA is highly selective for the adenosine A_1 receptor, it is not exclusively specific to the adenosine A_1 receptor and at high concentrations may have the adenosine A_2 receptor actions as well (Fredholm et al., 1994). Therefore, the possibility remains that no modulatory effect of PIA (2μM) on high K^+-evoked taurine release may be due to the adenosine A_2 receptor action. However, this issue remains to be solved in future.

Cellular swelling occurs when the brain becomes ischemic. It appears that taurine is released as one of the auto-defensive responses of brain tissue to minimize osmotic imbalances and cell swelling. The released taurine may exert an inhibitory effect on neighboring neurons and serve as a negative feedback mechanism in reducing neuronal hyperexcitation under pathological conditions.

The view that taurine has neuroprotective actions is supported by a large amount of evidence. For example, in our previous studies (Kaku et al., 1994; Hada et al., 1996), we have observed that an increase in endogenous adenosine by the use of adenosine transport inhibitors enhances high K^+-evoked taurine release from rat hippocampus and reduces the incidence of

spreading depression. A possible mechanism for the neuroprotective actions of taurine against spreading depression may proceed as follows: taurine may act on presynaptic terminals to both inhibit glutamate release (Kamisaki et al., 1993) and hyperpolarize hippocampal neurons (Zeise, 1985; Taber et al., 1986), resulting in the reduced occurrence of spreading depression.

In conclusion, the present results demonstrate that adenosine enhances high K^+-evoked taurine release via activation of adenosine A_{2A} receptors from both neurons and glial cells in rat hippocampus *in vivo*.

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References

Bourke RS, Kimelberg HK, Daza MA (1978) Effects of inhibitors and adenosine on (HCO_3^-/CO_2)-stimulated swelling and Cl^- uptake in brain slices and cultured astrocytes. Brain Res 154: 196–202

Bourke RS, Waldman JB, Kimelberg HK, Barron KD, San Filippo BD, Popp AJ, Nelson LR (1981) Adenosine-stimulated astroglial swelling in cat cerebral cortex *in vivo* with total inhibition by a non-diuretic acylaryloxyacid derivative. J Neurosurg 55: 364–370

Brundege JM, Dunwiddie TV (1997) Role of adenosine as a modulator of synaptic activity in the central nervous system. Adv Pharmacol 39: 353–391

Cunha RA, Johansson B, Constantino MD, Sebastiao AM, Fredholm BB (1996) Evidence for high-affinity binding sites for the adenosine A_{2A} receptor agonist [^{3}H]CGS21680 in the rat hippocampus and cerebral cortex that are different from striatal A_{2A} receptors. Naunyn-Schmiedeberg's Arch Pharmacol 353: 261–271

Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P, Williams M (1994) Nomenclature and classification of purinoceptors. Pharmacol Rev 46: 143–156

Hada J, Kaku T, Morimoto K, Hayashi Y, Nagai K (1995) Effects of L-PIA, an adenosine A_1 receptor agonist, on high K^+-evoked amino acid release and spreading depression. 4th IBRO World Congress of Neuroscience [Abstract]: 193

Hada J, Kaku T, Morimoto K, Hayashi Y, Nagai K (1996) Adenosine transport inhibitors enhance high K^+-evoked taurine release from rat hippocampus. Eur J Pharmacol 305: 101–107

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Kaku T, Hada J, Hayashi Y (1994) Endogenous adenosine exerts inhibitory effects upon the development of spreading depression and glutamate release by microdialysis with K^+ in rat hippocampus. Brain Res 658: 39–48

Kaku T, Hada J, Morimoto K, Hayashi Y (1997) Role of adenosine upon high K^+-evoked spreading depression and glutamate release from *in vivo* rat hippocampus. In: Okada Y (ed) The role of adenosine in the nervous system. Elsevier, Amsterdam, pp 157–164

Kamisaki Y, Maeda K, Ishimura M, Omura H, Itoh T (1993) Effects of taurine on depolarization-evoked release of amino acids from rat cortical synaptosomes. Brain Res 627: 181–185

Koyama Y, Ishibashi T, Tanaka K, Baba A (1994) L-Glutamate-stimulated taurine release from rat cerebral cultured astrocytes. J Neurosci Res 38: 75–80

Madelian V, Silliman S, Shain W (1988) Adenosine stimulates cAMP-mediated taurine release from LRM55 glial cells. J Neurosci Res 20: 176–181

Menendez N, Solis JM, Herreras O, Herranz AS, Martin del Rio R (1990) Role of endogenous taurine on the glutamate analogue-induced neurotoxicity in the rat hippocampus in vivo. J Neurochem 55: 714–717

Miyamoto AT, Miyamoto JK (1996) Effects of adenosine on taurine release in the central nervous system. Jpn J Physiol 46 [Suppl]: S179

Saransaari P, Oja SS (1992) Release of GABA and taurine from brain slices. Prog Neurobiol 38: 455–482

Schousboe A, Pasantes-Morales H (1989) Potassium-stimulated release of [^{3}H]taurine from cultured GABAergic and glutamatergic neurons. J Neurochem 53: 1309–1315

Somjen GG, Aitken PG, Czeh GL, Herreras O, Jing J, Young JN (1992) Mechanisms of spreading depression: a review of recent findings and a hypothesis. Can J Physiol Pharamacol 70: S248–S254

Taber KH, Lin C-T, Liu J-W, Thalmann RH, Wu J-Y (1986) Taurine in hippocampus: localization and postsynaptic action. Brain Res 386: 113–121

Von Lubitz DKJE, Carter M, Beenhakker M, Lin RC-S, Jacobson KA (1995) Adenosine: a prototherapeutic concept in neurodegeneration. Ann NY Acad Sci 765: 163–178

Zeise M (1985) Taurine on hippocampal slices: comparison to GABA and glycine, and antagonism by 4-aminopyridine. In: Oja SS, Ahtee L, Kontro P, Paasonen MK (eds) Taurine: biological actions and clinical perspectives. Alan R. Liss, New York, pp 281–287

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