

Adenosine A₁ Receptor Agonists Block the Neuropathological Changes in Rat Retrosplenial Cortex after Administration of the NMDA Receptor Antagonist Dizocilpine

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Noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine ((+)-MK-801) is known to induce neurotoxicity in rat retrosplenial cortex after systemic administration. The present study was undertaken to examine the effects of adenosine A₁ receptor agonists on the neurotoxicity in rat retrosplenial cortex after administration of dizocilpine. Pretreatment with adenosine A₁ receptor agonists, 2-chloro-N⁶-cyclopentyladenosine (CCPA) (0.1, 0.3, 1, or 3 mg/kg, intraperitoneally (i.p.)), or N⁶-cyclopentyladenosine (CPA) (1, 3, or 10 mg/kg, i.p.), attenuated neurotoxicity by dizocilpine (0.5 mg/kg, i.p.), in a dose-dependent manner. Coadministration with adenosine A₁ receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 3 mg/kg, i.p.) significantly blocked the protective effects of CCPA for dizocilpine-induced neurotoxicity. Furthermore, pretreatment with CCPA (3 mg/kg) attenuated significantly the dizocilpine-induced expression of HSP-70 protein, which is known as a sensitive marker of reversible neuronal damage, and coadministration with DPCPX (3 mg/kg) blocked the inhibitory effects of CCPA for marked expression of HSP-70 protein by administration of dizocilpine. Moreover, pretreatment with CCPA (3 mg/kg, i.p.) significantly suppressed the increase of extracellular acetylcholine (ACh) levels in the retrosplenial cortex by administration of dizocilpine (0.5 mg/kg). In contrast, local perfusion of CCPA (1 μM) into the retrosplenial cortex via the dialysis probe did not alter the ACh levels by administration of dizocilpine (0.5 mg/kg), suggesting that the locus of action of CCPA is not in the retrosplenial cortex. These findings suggest that adenosine A₁ receptors agonists could protect against neuropathological changes in rat retrosplenial cortex after administration of the NMDA receptor antagonist dizocilpine.

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INTRODUCTION

Several lines of evidence have suggested that a dysfunction in glutamatergic neurotransmission via the N-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (Coyle, 1996; Javitt and Zukin, 1991; Olney and Farber, 1995; Tamminga, 1998). This hypothesis evolves from clinical findings that non-competitive NMDA receptor antagonists such as phencyclidine (PCP) and its congener ketamine induce a schizophrenia-like psychosis representing negative and positive symptoms as well as cognitive dysfunction in humans (Javitt and Zukin, 1991; Krystal *et al*, 1994; Luby *et al*, 1959). Rodents treated with NMDA receptor

antagonists have been widely used in preclinical research on the neurobiology of schizophrenia (Gainetdinov *et al*, 2001). As part of this research, it has been demonstrated that NMDA receptor antagonists such as PCP and dizocilpine ((+)-MK-801) cause neuropathological changes in the rat retrosplenial cortex (Olney and Farber, 1995). Interestingly, it has been reported that the antipsychotic drugs clozapine and zotepine, but not haloperidol, block dizocilpine-induced neuropathological changes in the rat retrosplenial cortex at therapeutic doses (Farber *et al*, 1996; Fujimura *et al*, 2000; Hashimoto *et al*, 2000; Okamura *et al*, 2003). Therefore, determination of the potential to reverse dizocilpine-induced neuropathological changes in rat retrosplenial cortex may provide a beneficial model of screening for clozapine-like antipsychotic drugs (Morimoto *et al*, 2002; Olney and Farber, 1995; Olney *et al*, 1999; Sharp *et al*, 2001).

Adenosine receptors can be divided into four subtypes (A₁, A_{2A}, A_{2B}, and A₃) (Fredholm, 1995; Fredholm *et al*, 2001). Adenosine A₁ and A_{2A} receptors occur ubiquitously in the brain, and these receptors play a role in a variety of effects, for example, as anticonvulsants, anti-nociceptives,

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sedatives, muscle relaxants, and inhibitors of opiate withdrawal in morphine-dependent mice (Fredholm, 1995; Stone, 1991). Adenosine A₁ receptors play an important part in both the neuromodulatory role at the synaptic level and the homeostatic role of controlling the cell metabolism and ionic current (Cunha, 2001). In addition to their neuroprotective effect in ischemic models (Von Lubitz *et al.*, 1999; Jacobson *et al.*, 1996), it is reported that adenosine affects the behavioral and physiological effects of NMDA receptor antagonists. Adenosine A₁ receptor agonists decreased the electroencephalographic abnormalities induced by dizocilpine in rats (Popoli *et al.*, 1997), and blocked the abnormality of prepulse inhibition of acoustic startle response induced by PCP in rats (Browne and Welch, 1992; Sills *et al.*, 1999).

The heat shock or stress genes are induced by a wide variety of stimuli, including heat, ischemic stress, calcium ionophores, and certain toxins. Heat shock proteins may play important roles in cellular repair and/or protective mechanisms (Brown, 1995). Heat shock protein HSP-70, which is known as a sensitive marker of reversible neuronal damage, was induced in the retrosplenial cortex of the rat brain after administration of noncompetitive NMDA receptor antagonists such as dizocilpine, PCP, ketamine, and memantine (Sharp *et al.*, 1991, 2001; Hashimoto *et al.*, 1996, 1997; Tomitaka *et al.*, 1996, 2000). Furthermore, it has been reported that the NMDA receptor hypofunction state induced by administering NMDA receptor antagonist dizocilpine causes excessive release of acetylcholine (ACh) in retrosplenial cortex, and that γ -aminobutyric acid A (GABA_A) agonists or α_2 -adrenergic agonists prevent such release (Kim *et al.*, 1999). Studying the circuitry and receptor mechanisms of neurotoxicity of dizocilpine, Farber *et al.* (2002) hypothesized that dizocilpine-induced neurotoxicity involves increased glutamatergic and cholinergic activity at the retrosplenial cortex, and it is reasonable to also hypothesize that activation of adenosine A₁ receptors, which inhibit the release of neurotransmitters including ACh, would have a neuroprotective effect in this model.

Given these previous findings, the present study was undertaken to examine the effect of the adenosine A₁ receptor agonists CCPA and CPA on the neurotoxicity and expression of HSP-70 protein in rat retrosplenial cortex after administration of dizocilpine. We also used *in vivo* microdialysis methods to study the effects of adenosine A₁ receptor agonist on dizocilpine-induced increase of extracellular ACh levels in retrosplenial cortex.

METHODS

Animals

Female Sprague-Dawley rats (SLC, Hamamatsu, Shizuoka, Japan, 10–11 weeks old, 190–245 g) were housed separately under a 12-h light/12-h dark cycle with free access to food and water. We used female rats for all experiments in this study, as female rats are known to be more sensitive than male rats to the neurotoxic effects of NMDA receptor antagonists (Farber *et al.*, 1995; Fix *et al.*, 1995; Matsuki *et al.*, 2001). All experiments were carried out in accordance with the *Guide for Animal Experimentation*, Chiba University Graduate School of Medicine.

Drugs

The following drugs were purchased from the following sources: dizocilpine maleate, 2-chloro-N⁶-cyclopentyladenosine (CCPA), N⁶-cyclopentyladenosine (CPA), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Sigma-RBI, St Louis, MO, USA), and 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680) (Tocris Cookson Ltd., Bristol, UK). Other chemicals of analytical grade were purchased commercially.

Drug Treatment

Animals were injected intraperitoneally (i.p.) with vehicle (0.15% Tween 80; 2 ml/kg), CCPA (0.1, 0.3, 1, or 3 mg/kg), CPA (1, 3, or 10 mg/kg), CGS 21680 (1 mg/kg), or CCPA (1 or 3 mg/kg) plus DPCPX (1 or 3 mg/kg). After 15 min, the animals were injected i.p. with vehicle (distilled water; 1 ml/kg) or dizocilpine (0.5 mg/kg).

Neuropathology

At 4 h after administration of dizocilpine, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After 10 min, the animals were transcardially perfused with 100 ml of isotonic saline, followed by 400 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Subsequently, the brain was removed, processed by graded ethanol dehydration, embedded in paraffin, then sectioned into 4- μ m-thick coronal slices, and stained with hematoxylin and eosin. We used the brain level of bregma –5.80 mm from the atlas of the rat brain (Paxinos and Watson, 1998). We counted the number of vacuolized neurons in layers III and IV of the retrosplenial cortex of each side of the brain and averaged the number of vacuolized neurons for each subject from two slides per subject. Counting was performed blind with respect to the treatment group.

Immunohistochemistry

HSP-70 immunohistochemistry was performed using a Vectastain[®] Elite ABC kit (Vector Laboratories, Burlingame, CA, USA), as described previously (Hashimoto *et al.*, 1997). At 24 h after administration of dizocilpine (0.5 mg/kg), the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After 10 min, the animals were transcardially perfused with 100 ml of isotonic saline, followed by 400 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed and postfixed overnight at 4°C in the same fixative, and then 50- μ m-thick coronal sections of brains were cut in the ice-cold 0.01 M phosphate buffer saline (PBS; pH 7.4) using a vibrating blade microtome (VT1000S, Leica Microsystems Nussloch GmbH, Nussloch, Germany). The slices were washed twice in ice-cold 0.01 M PBS. Free-floating sections were placed in 0.01 M PBS buffer containing 2% horse serum, 0.2% Triton X-100, and 0.1% bovine serum albumin (HS-PBST) for 1 h at room temperature. The samples were incubated for 48 h at 4°C with a primary antibody to HSP-70 (Stressgen Biotechnologies Corp., Victoria, BC, Canada) diluted 1/1000 in HS-PBST. The sections were washed twice

in PBS, incubated for 1 h with a second antibody (biotinylated horse anti-rabbit immunoglobulin G; diluted 1/200), and then incubated in an avidin-biotinylated horse-radish peroxidase solution prepared from a kit for 1 h at room temperature. The sections were washed twice in ice-cold PBS, and an antibody reaction was developed with 3,3'-diaminobenzidine (0.015%) and 0.001% hydrogen peroxide in 50 mM Tris-HCl (pH 7.4). Following several rinses in PBS, sections were mounted on gelatinized slides, dehydrated through an ethanol gradient and cleared in xylene, and coverslipped with Permount[®] (Fisher Scientific, Fair Lawn, NJ, USA). We counted the number of neurons expressing HSP-70 immunoreactivity in the section containing layers III–V of the retrosplenial cortex, and averaged the number of HSP-70 immunoreactive neurons from two sections for each subject. Counting was performed blind with respect to the treatment group.

In vivo Microdialysis

Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame. A concentric I-type dialysis probe (0.22 mm diameter; 1.5 mm exposed membrane; Eicom Co., Kyoto, Japan) was implanted into the retrosplenial cortex ($A = 5.8$ mm, $L = 0.5$ mm, $D = 1.5$) relative to the bregma. The perfusion experiments were started 36–48 h after the rats had recovered from anesthesia. The perfusion rate was always 2 μ l/min, using modified Ringer's solution composed of Na⁺ 147 mM, K⁺ 4 mM, Ca²⁺ 2.3 mM, and Cl[−] 155.6 mM containing 2×10^{-4} M eserine sulfate. Dialysate was collected every 30 min and stored at -80°C .

The high-performance liquid chromatography (HPLC) system used for the determination of ACh was equipped with an electrochemical detector system (ECD-300, Eicom Co., Kyoto, Japan). The mobile phase contained 0.1 M Na₂HPO₄ and 65 mg/ml tetramethylammonium chloride, 200 mg/l sodium 1-decanesulfonate, pH 8.5 (adjusted with phosphoric acid), and a flow rate of 0.6 ml/min. Samples were injected manually into an analytical column (EICOM-PAK AC-GEL, 150 mm \times 4.6 mm diameter; Eicom). A postcolumn enzyme reactor (AC-ENZYM-PAK, 5 mm \times 4.6 mm diameter; Eicom) containing choline oxidase and acetylcholinesterase was used to convert ACh and choline to hydrogen peroxide before detection across a platinum electrode (WE-PT; Eicom) held at 450 mV relative to a reference electrode (RE-100; Eicom).

Statistical Analysis

The differences between multiple groups of neuronal vacuolization and immunohistochemistry were examined by means of one-way analysis of variance (ANOVA), followed by Bonferroni/Dunn's *post hoc* test. The criteria for significance were $*p < 0.05$, $**p < 0.01$. The data were presented as the mean \pm standard error of mean (SEM). The ED₅₀ values for neuronal vacuolization were calculated by conventional graphical techniques by means of the iterative nonlinear least-squares method.

For data analysis of *in vivo* microdialysis, ACh output was expressed as a percentage of basal concentration. For each rat, a basal ACh concentration was determined by obtaining

the mean value for the first three samples collected before administration of a drug. The extracellular ACh concentration of each sample was divided by the calculated basal concentration and multiplied by 100 to obtain a percentage value. The differences between the control group and the drug-treated group were evaluated by two-way ANOVA seeking a significant interaction between treatment condition and time. Statistical comparison at each measurement time between groups was made by the Fisher's protected least significant difference (Fisher's PLSD) following one-way ANOVA. The criteria for significance were $*p < 0.05$, $**p < 0.01$ at the corresponding time point. The data were presented as the mean \pm SEM.

RESULTS

Behavioral responses occurred within 5 min after administration of dizocilpine (0.5 mg/kg). In accordance with previous reports (Koek *et al*, 1988; Tricklebank *et al*, 1989), a single administration of dizocilpine (0.5 mg/kg) to female rats produced head weaving and biting in a stationary posture, and increased locomotion within 15 min of injection; rats then exhibited occasional movements in the flat-body posture until the end of the experiment (4 h after injection). Pretreatment with CCPA (0.1, 0.3, 1, or 3 mg/kg) or CPA (1, 3, or 10 mg/kg) did not alter the abnormal behavioral changes induced in rats by dizocilpine, although we did not evaluate it quantitatively.

The administration of dizocilpine (0.5 mg/kg, i.p.) caused neuronal vacuolization in layers III–IV of the retrosplenial cortex of the rat brain 4 h after administration. One-way ANOVA showed significant differences among groups ($F = 19.43$ [4, 39], $p < 0.0001$; Figure 1), and pretreatment with CCPA (0.1, 0.3, 1, or 3 mg/kg) decreased the number of vacuolized neurons in the retrosplenial cortex after administration of dizocilpine, in a dose-dependent manner (Figure 1). The ED₅₀ value of CCPA was 0.72 mg/kg (95% confidential limits; 0.59–0.88 mg/kg). Another selective

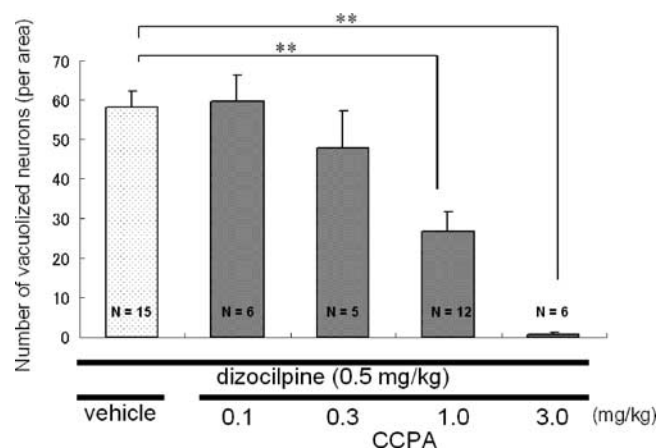


Figure 1 Effects of pretreatment with CCPA on dizocilpine-induced neurotoxicity in the rat retrosplenial cortex. At 15 min after i.p. administration of vehicle (2 ml/kg) or CCPA (0.1, 0.3, 1, or 3 mg/kg), dizocilpine (0.5 mg/kg) was administered to rats. The number of vacuolized neurons in the rat retrosplenial cortex was counted 4 h after administration of dizocilpine. Each value is the mean \pm SEM of 5–15 rats. $**p < 0.01$ as compared with the vehicle-treated group.

adenosine A₁ receptor agonist, CPA (1, 3, or 10 mg/kg), also decreased the number of vacuolized neurons after administration of dizocilpine. One-way ANOVA showed significant differences among groups ($F = 17.68$ [3, 20], $p < 0.0001$; Figure 2), and its effect in a dose-dependent manner. The ED₅₀ value of CPA was 1.56 mg/kg (95% confidential limits; 1.23–1.91 mg/kg).

Coadministration of DPCPX (1 or 3 mg/kg) with CCPA (3 mg/kg) significantly attenuated the protective effect of CCPA, and one-way ANOVA showed significant differences among the four groups ($F = 28.63$ [3, 29]), $p < 0.0001$; Figure 3). In addition, administration of the vehicle (2 ml/kg), CCPA (1 mg/kg), or DPCPX (1 mg/kg) alone did not

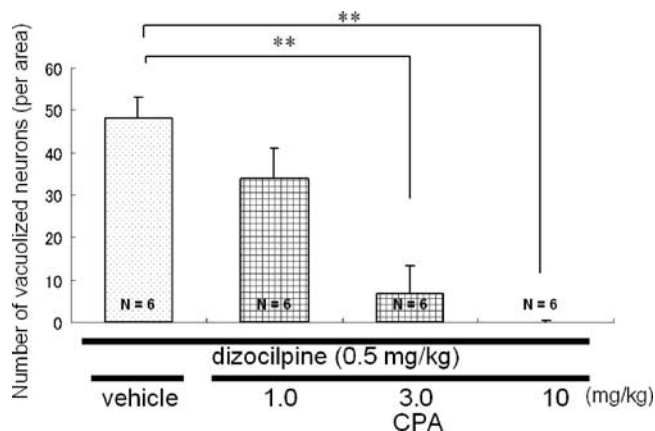


Figure 2 Effects of pretreatment with CPA on dizocilpine-induced neurotoxicity in the rat retrosplenial cortex. At 15 min after i.p. administration of vehicle (2 ml/kg), CPA (1, 3, or 10 mg/kg), the rats were administered dizocilpine (0.5 mg/kg, i.p.) and killed 4 h after administration of dizocilpine. The number of vacuolized neurons in the rat retrosplenial cortex was counted. Each value is the mean \pm SEM of six rats. $^{***}p < 0.01$ as compared with the vehicle-treated group.

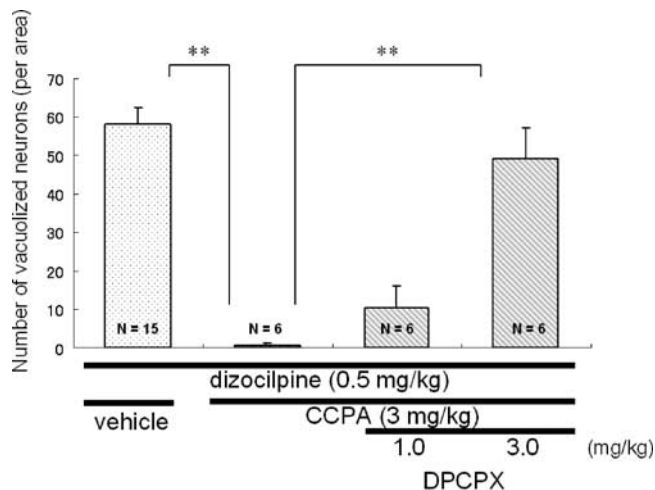


Figure 3 Effects of DPCPX on the protective effects of CCPA for dizocilpine-induced neurotoxicity in the rat retrosplenial cortex. At 15 min after i.p. administration of vehicle (2 ml/kg), CCPA (3 mg/kg), or CCPA (3 mg/kg) plus DPCPX (1 or 3 mg/kg), the rats were administered dizocilpine (0.5 mg/kg, i.p.) and killed 4 h after administration of dizocilpine. The number of vacuolized neurons in the rat retrosplenial cortex was counted. Each value is the mean \pm SEM of six or 15 rats. $^{**}p < 0.01$ as compared with the CCPA-treated group.

produce neuronal vacuolization. Pretreatment with adenosine A_{2A} receptor agonist CGS 21680 (1 mg/kg) did not significantly alter the number of neurons vacuolized after administration of dizocilpine (0.5 mg/kg).

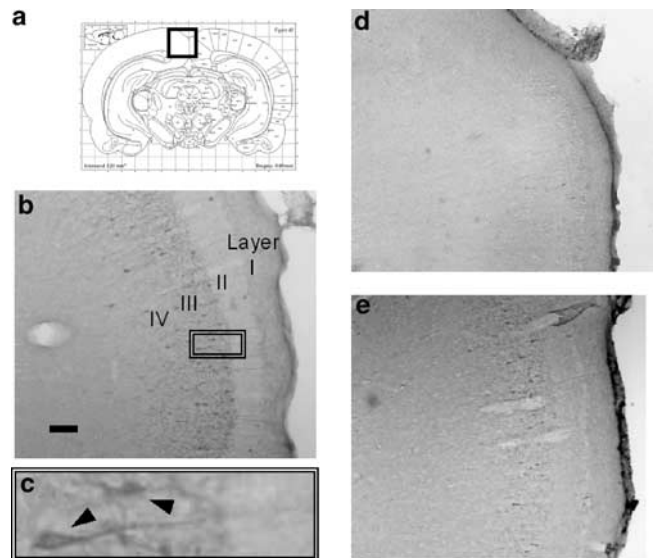


Figure 4 Effects of adenosine A₁ receptor agonist CCPA and antagonist DPCPX in the expression of HSP-70 in the rat retrosplenial cortex after administration of dizocilpine. At 15 min after administration of vehicle (2 ml/kg, i.p.), CCPA (3 mg/kg, i.p.), or CCPA (3 mg/kg, i.p.) plus DPCPX (1 or 3 mg/kg, i.p.), the rats were administered dizocilpine (0.5 mg/kg, i.p.). The HSP-70 immunohistochemistry in the rat brain was performed 24 h after administration of vehicle or dizocilpine. The HSP-70 immunohistochemistry in the rat brain was performed 24 h after administration of vehicle or dizocilpine. (a) Drawing of a coronal section of a rat brain including the retrosplenial cortex (Paxinos and Watson, 1998). (b) Vehicle + dizocilpine, (c) A magnified view of (b) (arrowhead shows HSP-70 positive neurons), (d) CCPA + dizocilpine, (e) CCPA and DPCPX + dizocilpine. Scale bar: 100 μ m.

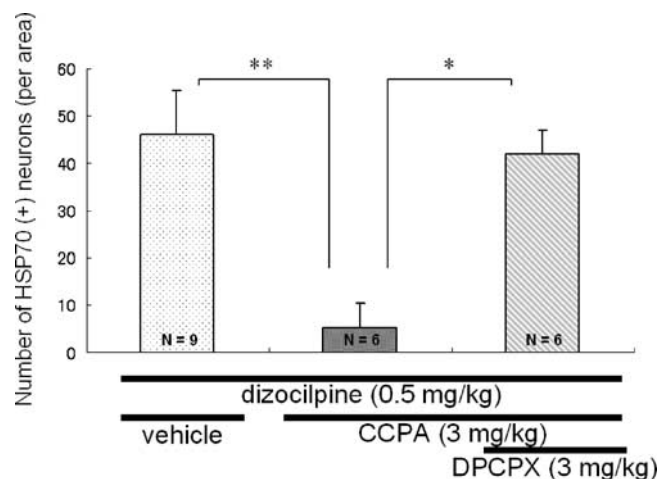


Figure 5 Effects of adenosine A₁ receptor agonist CCPA and antagonist DPCPX in the expression of HSP-70 in the rat retrosplenial cortex after administration of dizocilpine. At 15 min after i.p. administration of vehicle (2 ml/kg), CCPA (3 mg/kg), or CCPA (3 mg/kg) plus DPCPX (3 mg/kg), dizocilpine (0.5 mg/kg) was administered to rats. The number of HSP-70 immunoreactive neurons in the rat retrosplenial cortex was counted 24 h after administration of dizocilpine. Each value is the mean \pm SEM of six or nine rats. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with the CCPA-treated group.

The administration of dizocilpine (0.5 mg/kg) increased the expression of HSP-70 protein in layers III–V of the retrosplenial cortex of the rat brain (Figures 4 and 5). Pretreatment with CCPA (3 mg/kg) significantly inhibited the marked expression of HSP-70 protein by dizocilpine (Figure 5). One-way ANOVA showed significant differences among groups ($F=7.74$ [2, 18], $p=0.0038$), and pretreatment with CCPA (3 mg/kg) significantly inhibited the expression of HSP-70 protein in the retrosplenial cortex by dizocilpine (Figure 5). Furthermore, coadministration of DPCPX (3 mg/kg) with CCPA (3 mg/kg) significantly attenuated the protective effect of CCPA on the expression of HSP-70 protein by dizocilpine (Figures 4 and 5).

In vivo microdialysis study indicated that the administration of dizocilpine (0.5 mg/kg) increased significantly the extracellular levels of ACh in rat retrosplenial cortex, as previously reported (Kim *et al*, 1999). Pretreatment with CCPA (1 or 3 mg/kg, i.p.) significantly suppressed the increase of ACh by dizocilpine, and there was a tendency of dose dependence (Figure 6). Next, we examined the effects of direct application of CCPA (1 μ M) into the retrosplenial cortex on the increase of ACh levels after systemic administration of dizocilpine (0.5 mg/kg). Perfusion of CCPA (1 μ M) into the retrosplenial cortex via the dialysis probe did not alter the extracellular levels of ACh in retrosplenial cortex after administration of dizocilpine (Figure 7).

DISCUSSION

The major findings of the present study are that the adenosine A₁ receptor agonist CCPA significantly decreased the number of vacuolized neurons and neurons expressing HSP-70 immunoreactivity in rat retrosplenial cortex in-

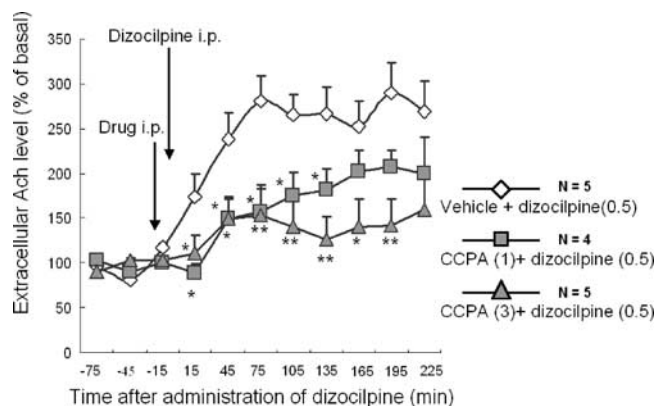


Figure 6 Effects of systemic administration of CCPA on increase of extracellular ACh levels in the retrosplenial cortex after administration of dizocilpine. Dialysate samples were collected every 30 min. Levels of ACh were calculated as described in the Methods section, and values are expressed as the percentage of basal ACh concentration (mean \pm SEM of four or five rats). At 15 min after i.p. administration of CCPA (1 or 3 mg/kg, i.p.) or vehicle (2 ml/kg, i.p.), dizocilpine (0.5 mg/kg, i.p.) was administered to rats. The systemic administration of CCPA resulted in a significant decrease in ACh concentration in the retrosplenial cortex as compared to that of the control group ($F=3.764$ [2, 20], $p<0.0001$). * $p<0.05$, ** $p<0.01$ compared to the vehicle-treated group at the corresponding time point.

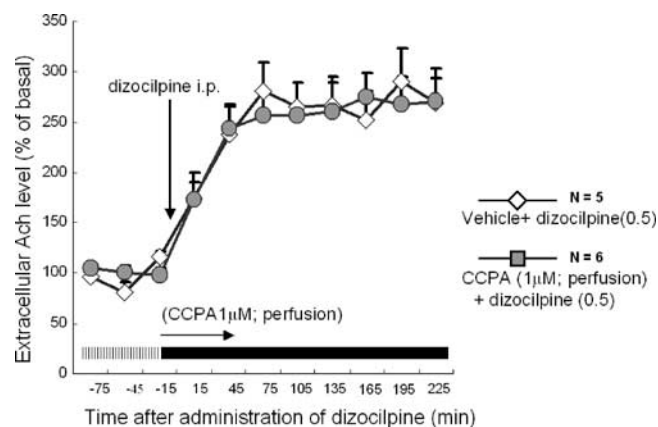


Figure 7 Effects of a local perfusion of CCPA on dizocilpine-induced elevation of extracellular ACh levels in the retrosplenial cortex. Dialysate samples were collected every 30 min. Levels of ACh were calculated as described in the Methods section, and values are expressed as percentage of basal ACh concentration (mean \pm SEM of 5 rats). At 30 minutes after starting a local perfusion of CCPA (1 μ M) directly into the retrosplenial cortex, dizocilpine (0.5 mg/kg, i.p.) was administered to rats. The local perfusion of CCPA resulted in no significant change in ACh concentration in the retrosplenial cortex as compared to that of the control group ($F=0.559$ [1, 10], $p=0.843$).

duced by dizocilpine, and that coadministration with the adenosine A₁ receptor antagonist DPCPX could reverse the protective effects of CCPA for neuropathological damages and HSP-70 expression. Furthermore, CPA, another selective adenosine A₁ receptor agonist, was also found to decrease the number of vacuolized neurons, and the ED₅₀ values of CPA were higher than those of CCPA, which is also the case for their respective K_i values of adenosine A₁ receptors (Lohse *et al*, 1988). We also found that the adenosine A_{2A} receptor agonist CGS 21680 could not alter neuropathological changes in rat retrosplenial cortex after administration of dizocilpine. Thus, it seems that adenosine A₁ receptors play a role in the protective effects of CCPA against dizocilpine-induced neurotoxicity.

In the present study, CCPA decreased both the number of vacuolized neurons and the amount of ACh released in the retrosplenial cortex induced by dizocilpine. It has been reported that systemic administration of GABAergic agonists (diazepam and pentobarbital), the α_2 -adrenergic agonist clonidine, and the novel metabotropic glutamate receptor agonist LY 379268 significantly suppressed both dizocilpine-induced neuronal vacuolization and ACh release in the rat retrosplenial cortex (Kim *et al*, 1999; Okamura *et al*, in press). Interestingly, local injection of clonidine to the retrosplenial cortex did not reduce extracellular ACh levels or the number of vacuolized neurons, whereas local injection to the basal forebrain, where the majority of the cortical-projecting cholinergic neurons exist, significantly reduced the number of vacuolized neurons (Farber *et al*, 2002). These results indicate that there are certain pathways that mediate the neurotoxicity to this region. The existence of such complex circuitry is supported by the work of Tomitaka *et al* (2000), which demonstrated that expression of HSP-70 protein in the rat retrosplenial cortex was induced by bilateral injection of dizocilpine into the anterior thalamus, but was not induced by bilateral injection of dizocilpine into the retrosplenial

cortex. In the present study, local perfusion of CCPA did not affect extracellular Ach levels. The explanation for this result could be that the adenosine A₁ receptors with which CCPA interacts to suppress the release of Ach in the retrosplenial cortex do not locate within the retrosplenial cortex, but, rather, in the basal forebrain. Further detailed studies are needed to clarify the mechanism by which CCPA reduces the neurotoxicity of dizocilpine in rat retrosplenial cortex.

Several studies have suggested that adenosine plays a role in the behavioral or physiological effects of dizocilpine-treated rodents (Browne and Welch, 1982; Sills et al, 1999; Dall'Igna et al, 2003). Based on the findings in those reports, a possible state of adenosinergic hypoactivity in schizophrenia has been proposed (Lara, 2002). Furthermore, a clinical study indicated that dipyridamole, an adenosine uptake inhibitor, plus haloperidol is significantly more effective than haloperidol alone for decreasing psychopathological symptoms of patients with schizophrenia (Akhondzadeh et al, 2000), which is in line with the model of adenosine activity-deficit proposed for that disease (Lara, 2002). Those findings, taken with ours here, suggest that adenosine A₁ receptors may, in part, contribute to the pathophysiology of schizophrenia. The findings obtained here, however, were not in themselves sufficient to conclude that adenosine A₁ receptor agonists would be effective drugs for schizophrenia.

In conclusion, the results of the present study indicate that adenosine A₁ receptor agonists significantly decrease neuronal vacuolization and the expression of HSP-70 protein in the rat retrosplenial cortex following administration of the NMDA receptor antagonist dizocilpine. Based on the NMDA hypofunction hypothesis in schizophrenia, our findings suggest that adenosine A₁ receptors may play a role in the pathophysiology of schizophrenia.

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