**Neuropsychopharmacology (2008) 33,** 1336–1342

**www.neuropsychopharmacology.org**

# $K_{ATP}$ Channel Openers Facilitate Glutamate Uptake by GluTs in Rat Primary Cultured Astrocytes

**Xiu-Lan Sun[1,2], Xiao-Ning Zeng[1,2], Fang Zhou[1], Cui-Ping Dai[1], Jian-Hua Ding[1] and Gang Hu*[,1]**

[1]*Laboratory of Neuropharmacology, Department of Anatomy, Histology & Pharmacology, Nanjing Medical University, Nanjing, Jiangsu, P.R. China*

Increasing evidence, including from our laboratory, has revealed that opening of ATP sensitive potassium channels ($K_{ATP}$ channels) plays the neuronal protective roles both *in vivo* and *in vitro*. Thus $K_{ATP}$ channel openers (KCOs) have been proposed as potential neuroprotectants. Our previous studies demonstrated that $K_{ATP}$ channels could regulate glutamate uptake activity in PC12 cells as well as in synaptosomes of rats. Since glutamate transporters (GluTs) of astrocytes play crucial roles in glutamate uptake and $K_{ATP}$ channels are also expressed in astrocytes, the present study showed whether and how $K_{ATP}$ channels regulated the function of GluTs in primary cultured astrocytes. The results showed that nonselective KCO pinacidil, selective mitochondrial KCO diazoxide, novel, and blood–brain barrier permeable KCO iptakalim could enhance glutamate uptake, except for the sarcolemmal KCO P1075. Moreover pinacidil, diazoxide, and iptakalim reversed the inhibition of glutamate uptake induced by 1-methyl-4-phenylpyridinium ($MPP^+$). These potentiated effects were completely abolished by mitochondrial $K_{ATP}$ blocker 5-hydroxydecanoate. Furthermore, either diazoxide or iptakalim could inhibit $MPP^+$-induced elevation of reactive oxygen species (ROS) and phosphorylation of protein kinases C (PKC). These findings are the first to demonstrate that activation of $K_{ATP}$ channel, especially mitochondrial $K_{ATP}$ channel, improves the function of GluTs in astrocytes due to reducing ROS production and downregulating PKC phosphorylation. Therefore, the present study not only reveals a novel pharmacological profile of KCOs as regulators of GluTs, but also provides a new strategy for neuroprotection.
*Neuropsychopharmacology* (2008) **33,** 1336–1342; doi:10.1038/sj.npp.1301501; published online 4 July 2007

**Keywords:** $K_{ATP}$ channel opener; glutamate uptake; mitochondria; iptakalim

## INTRODUCTION

ATP-sensitive potassium ($K_{ATP}$) channels originally were discovered in heart cardiac tissue. Uptill now, they have been found to be widely expressed in metabolically active tissues, and in many excitable cells including cardiac myocytes, hormone-secreting cells, skeletal and smooth muscle, and neurons (Ashcroft, 1988; Seino, 1999; Zawar *et al*, 1999). $K_{ATP}$ channels play a critical role in coupling cellular metabolism to electrical activity, and have been shown to participate in some important biological processes such as insulin secretion, synaptic transmission, and excitability of cardiac, vascular, and nonvascular smooth muscle. It is generally agreed that $K_{ATP}$ channels in cardiac muscle and neurons have a protective effect during cellular suffering from injury, such as ischemia and anoxia (Zini *et al*, 1993; Prensa and Parent, 2001). Within the brain, $K_{ATP}$ channels exist in both pre- and post-synaptic membrane, as well as in glial cells. Activation of post-synaptic $K_{ATP}$ channels causes membrane hyperpolarization, which limits neuronal excess excitation; activation of presynaptic $K_{ATP}$ channels can directly modulate neurotransmitter release from nerve terminals. Therefore, $K_{ATP}$ channel openers (KCOs) serve as efficient tools that can adjust cell excitability, and exhibit beneficial effects under pathological conditions such as ischemia, stroke, and neurodegenerative diseases (Yao and Gross, 1994; Sargent *et al*, 1993; Liss *et al*, 2005). Emerging evidence including from our laboratory has revealed that the opening of $K_{ATP}$ channels play dominant protective roles in various models of neurodegenerative diseases such as Parkinson's disease (PD) (Tai *et al*, 2003; Wang *et al*, 2006; Yang *et al*, 2004).

It is well-known that astrocytic glutamate transporters (GluTs) perform the majority of glutamate uptake from the extracellular space in the brain, and play an important role in maintaining extracellular glutamate concentrations below neurotoxic levels (Matute *et al*, 2006; Schousboe and Waagepetersen, 2005). The dysfunction of astrocytic GluTs leads to an excessive extracellular glutamate concentration, thereby inducing excitotoxicity and neurodegeneration (Ferrarese *et al*, 2000). Thus, it has been proposed that improvement of glutamate uptake might be a potential strategy for neuroprotection (Wadiche and Gersdorff, 2006; Genoud *et al*, 2006; Matsugami *et al*, 2006; Takayasu *et al*, 2006). Our previous studies have revealed that KCOs could

*Correspondence: Dr G Hu, Laboratory of Neuropharmacology, Department of Anatomy, Histology & Pharmacology, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, P.R. China, Tel: +86 25 86863169, Fax: +86 25 86863108,
E-mail: ghu@njmu.edu.cn
[2]These authors contributed equally to this work.
Received 8 March 2007; revised 6 May 2007; accepted 6 June 2007

decrease extracellular glutamate concentrations and reduce excitotoxicity in PD animal and cellular models (Tai *et al*, 2003; Yang *et al*, 2004; Wang *et al*, 2004; Busija *et al*, 2004; Liss *et al*, 1999). We also demonstrated that KCOs could regulate glutamate uptake in PC12 cells and in synaptosomes from normal and PD rats (Inagaki *et al*, 1995). Since $K_{ATP}$ channels are expressed in astrocytes and astrocytic GluTs play key roles in maintaining extracellular glutamate levels, whether activation of $K_{ATP}$ channels plays a neuroprotective role via regulating the functions of GluTs is essential to be elucidated and is hitherto unknown. In the present study, we examined the effects of KCOs on glutamate uptake in primary cultured astrocytes to determine (1) whether activation of $K_{ATP}$ channels would regulate GluTs function, (2) whether KCOs could protect astrocytes against neurotoxin-induced dysfunction of GluTs, and (3) the possible mechanisms by which $K_{ATP}$ channels modulate glutamate uptake.

## MATERIALS AND METHODS

### Primary Astrocyte Cultures

Confluent primary astrocyte cultures were prepared from cerebral cortex of newborn Sprague–Dawley rats as described previously (Ivanova and Beyer, 2003), with minor modifications as listed below. All animal procedures were performed according to the NIH Guide for Animal Care and approved by the institutional animal care and use committee. Briefly, neonatal rats were killed by rapid decapitation, the cerebral cortices were removed and separated from meninges and basal ganglia, and tissue was dissociated with 0.25% trypase (Amresco) at 37°C and terminated by Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL Life Technologies) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. After centrifugation at 1500 r.p.m. for 5 min, the cell pellets were resuspended and seeded on poly-lysine-coated (Sigma) flask. The cultures were maintained at 37°C in a humidified 5% $CO_2$–95% air atmosphere. Culture medium was replaced 24 h later and then changed every 2–3 days. Before experiments, astrocytes were replated on poly-lysine-coated 6- or 24-well plates. Immunocytochemistry showed that ~98% of the cells stained positively for the astrocytic marker glial fibrillary acid protein (GFAP).

### Assays of Glutamate Uptake

Glutamate uptake determination was performed according to the procedure as described (Yao *et al*, 2005). Following pinacidil (Pina), diazoxide (Dia), P1075 or iptakalim (Ipt) administration for 48 h, uptake assays were initiated by adding [$^3$H]D, L-glutamate (190 nM, final concentration) to reaction flasks covered with astrocytes, and incubated for 15 min at 37°C in 24-well plates. Nonspecific uptake was determined from samples incubated in the presence of the $Na^+$-free buffer, and these values were subtracted from the total. All the reactions were terminated by rinsing thrice with ice-cold 0.9% NaCl. Then, the cells were lysed immediately with 0.3 M NaOH. After centrifugation at 10 000 r.p.m. for 15 min, supernatant was abstracted and radioactivity was determined by liquid scintillation counter. Protein level of each sample was detected using dye coomassie brilliant blue G-250.

### Measurement of ROS generation

Formation of reactive oxygen species (ROS) was evaluated using 2′,7′-dichlorofluorescin diacetate (DCFH-DA), a membrane-permeable probe de-esterified intracellularly. The non-fluorescent dye freely penetrates cells, is then hydrolyzed by intracellular esterases to 2′,7′-dichlorodihydrofluororescein (DCFH), and trapped inside the cells. Upon oxidation by ROS, DCFH yields the highly fluorescent product dichlorofluorescein (DCF).

Treated with drugs for 48 h, cells were loaded with DCFH-DA (50 μM final concentration) in DMEM for 60 min in the dark and fixed by 4% formaldehyde. After rinsing cells twice with PBS solution (137 mM NaCl, 2.7 mM KCl, 9.5 mM $Na_2HPO_4 \cdot 12H_2O$, 1.5 mM $KH_2PO_4$, pH 7.20), fluorescence was read at the excitation wavelength (Ex) of 488 nm and the emission wavelength (Em) of $530 \pm 20$ nm. DCFH-DA was initially dissolved to 40–50 mM in ethanol and stored at −20°C. A final concentration of ethanol (0.6% or less) did not alter the fluorescence measurement.

### PepTag Assay for Non-Radioactive Detection of PKC Activity

Following KCOs treatment for 48 h, the cells were rinsed with ice-cold PBS solution and harvested by scraping on ice into 0.2 ml of cold protein kinases C (PKC) extraction buffer. The lysates were subjected to mild sonication and followed by centrifugation at 4°C, $14\,000 \times g$ in a microcentrifuge for 5 min. Then the supernatant was used for the PepTag assay, which is generally performed within 24 h.

According to the manufacturer's (Promega) protocol, the cell samples were incubated with PKC reaction mixture (25 μl) at 30°C for 30 min and the reactions were stopped by placing the tubes in a boiling water bath. After adding 80% glycerol (1 μl), the samples were loaded on a 0.8% agarose gel at 100 V for 20 min. The bands were visualized and photographed under UV light. The units of kinase activity in each slice of agarose were calculated according to the manufacturer's instructions.

### Statistical Analysis

All data were presented as the mean ± SEM. Statistical analysis for multiple comparisons was performed by one-way ANOVA test with Bonferroni's corrections using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). The level of statistical significance is defined as $p < 0.05$.

## RESULTS

### KCOs Facilitated Glutamate Uptake in Rat Primary Astrocyte Cultures in a Concentration-Dependent Manner

Astrocytes in culture exhibited a high-affinity glutamate uptake after 48 h of incubation in DMEM with $V_{max}$ calculated to $10.66 \pm 0.06$ pM mg$^{-1}$ min$^{-1}$ (Figure 1a). As shown in Figure 1a, the uptake was significantly increased

(117, 126, and 129% of the control) when exposed to pinacidil (Pina, 1, 10, and 100 μM respectively). Preincubation with 100, 200, or 500 μM diazoxide (Dia), a mitochondrial $K_{ATP}$ (mito$K_{ATP}$) channel opener, increased glutamate uptake to 120, 136, and 149%, respectively, of the control in a concentration-dependent manner (Figure 1b). However, compared with control group, no significant effect on astroglial glutamate uptake was detected in the presence of P1075 ($p > 0.05$, Figure 1c), a selective sarcolemmal $K_{ATP}$ (sarc$K_{ATP}$) channel opener. As a novel KCO, iptakalim (Ipt) increased glutamate uptake to 118 and 114% at the concentration of 1 and 10 μM, respectively (Figure 1d). These findings indicated that the KCOs could facilitate glutamate uptake by GluTs in astrocytes.

### KCOs Alleviated $MPP^+$-Induced Glutamate Uptake Inhibition in Rat Primary Astrocyte Cultures

After incubation with 1-methyl-4-phenylpyridinium ($MPP^+$, 150 μM) for 48 h, astrocytic glutamate uptake was remarkably decreased to $5.13 \pm 0.46$ pmol $mg^{-1}$ $min^{-1}$ compared with control group ($7.08 \pm 0.22$ pmol $mg^{-1}$ $min^{-1}$, Figure 2a). As shown in Figure 2a, preincubation with Pina concentration-dependently (0.1–100 μM) alleviated glutamate uptake impairment in $MPP^+$ cotreated astrocytes, which was completely blocked by glibenclamide (Glib, 10 μM) and mito$K_{ATP}$ channel blocker 5-hydroxydecanoate (5-HD, 250 μM) but not HMR1098 (20 μM, a sarc$K_{ATP}$ channel blocker, Figure 2b). These results indicated that the prevention of the uptake improvement with Glib or 5-HD was due to the blockade of the mito$K_{ATP}$ channel, because Glib or 5-HD alone had no effects on glutamate uptake (Figure 2h). To examine further the involvement of mito$K_{ATP}$ channel in the glutamate uptake, Dia or P1075 was preincubated with $MPP^+$. Dia (10, 100, 200 or 500 μM) and the novel KCO Ipt (0.1, 1, 10 or 100 μM) could concentration-dependently attenuated uptake inhibition induced by $MPP^+$ (Figure 2c and e), P1075 (1, 10, 20, 50 or 100 μM) had no effect on astrocytic glutamate uptake ($p > 0.05$, Figure 2g). The facilitating effects of Dia (100 μM) and Ipt (10 μM) on glutamate uptake were completely abolished by 5-HD (250 μM, Figure 2d and f), indicating that the protective role of Dia and Ipt in astrocitic glutamate clearance was actually due to the opening of mito$K_{ATP}$ channels.

### KCOs Inhibited $MPP^+$-Triggered ROS Elevation in Rat Primary Astrocyte Cultures

After, preincubation with different KCOs for 30 min, then followed by incubation with 150 μM $MPP^+$ for 48 h, the production of ROS was measured with the DCFH-DA probe. $MPP^+$ (Figure 3b) increased the ROS output to 472% of control group (Figure 3a). Either Dia (100 μM, Figure 3c) or Ipt (10 μM) (Figure 3e) could inhibit ROS production significantly. Furthermore, the effects of Dia and Ipt were reversed by 5-HD (250 μM) (Figure 3d and f). P1075 failed to reverse $MPP^+$-induced ROS accumulation (Figure 3g).

### KCOs Suppressed PKC Activity in $MPP^+$-Treated Astrocyte Cultures

After preincubation with Dia (100 μM), Ipt (10 μM), P1075 (20 μM), and 5-HD (250 μM) for 30 min followed by exposure to $MPP^+$ for 48 h, astroglial protein kinases C

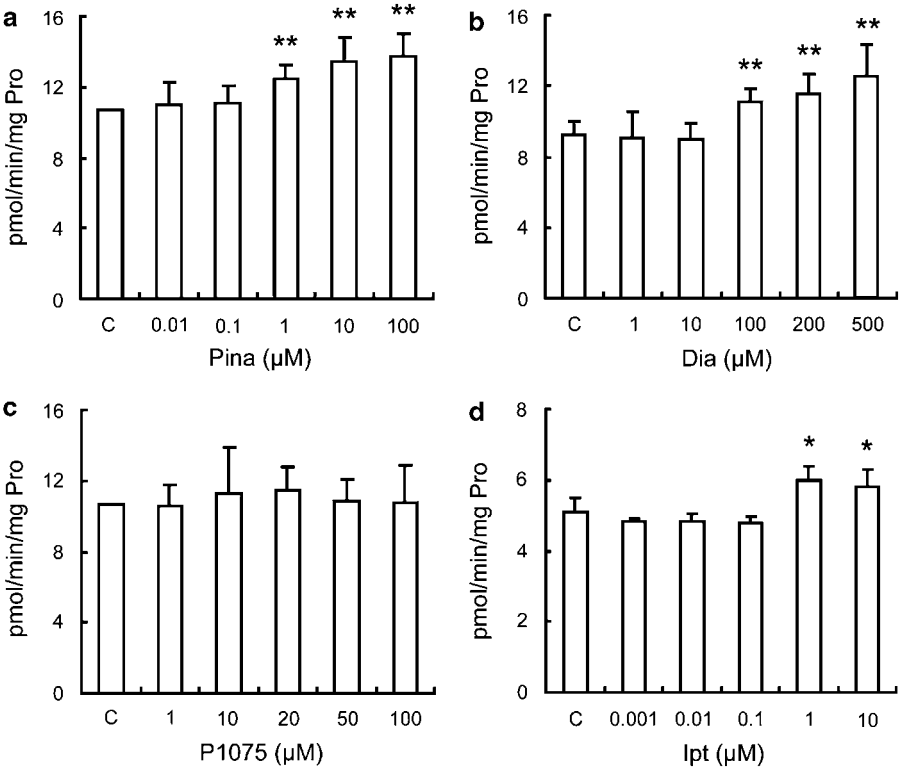


**Figure 1** Kinetics of glutamate uptake in differentially pretreated astrocyte populations. Values represented as mean ± SEM of at least four independent experiments performed in triplicate. C, control; Pina, pinacidil; Dia, diazoxide; Ipt, iptakalim. $^*p < 0.05$, $^{**}p < 0.01$ *vs* control group.

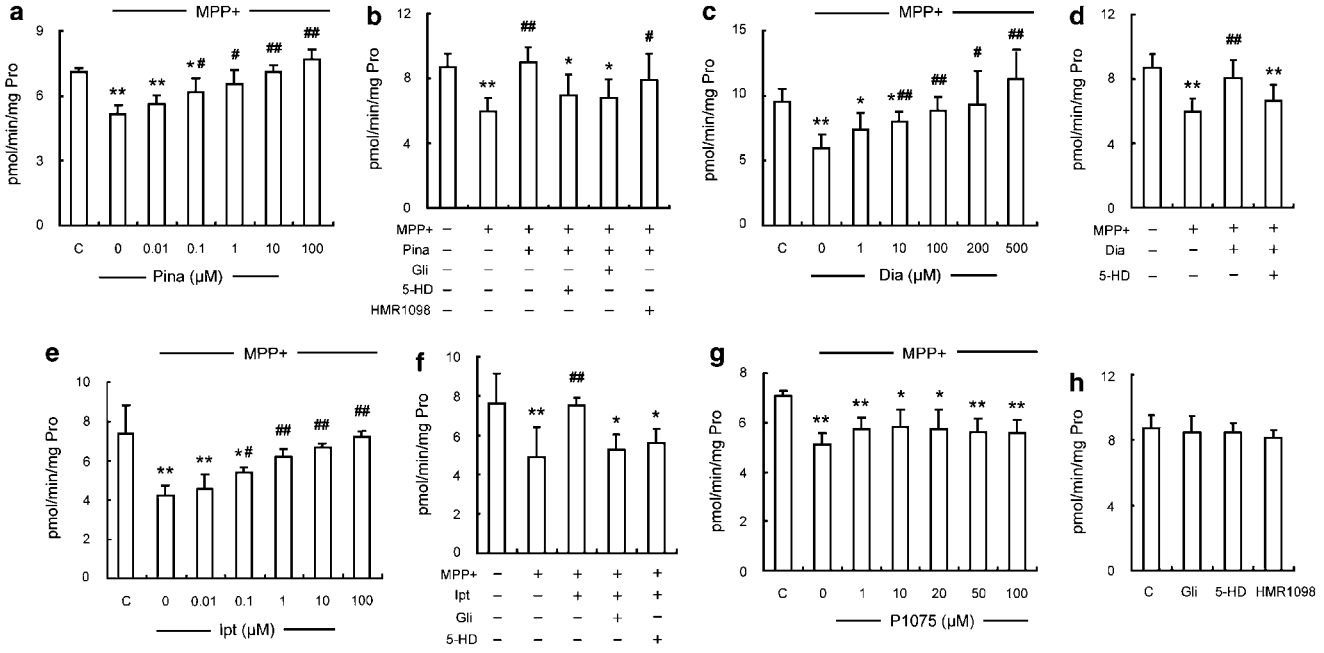


**Figure 2** Protective effects of KCOs on glutamate uptake inhibition induced by $MPP^+$ in primary cultured astrocytes. Results are mean ± SEM performed at least four independent experiments in triplicate. C, control; $MPP^+$, 1-methyl-4-phenylpyridinium; Pina, pinacidil; Dia, diazoxide; Ipt, iptakalim; Glib, glibenclamide; 5-HD, 5-hydroxydecanoate. $^*p<0.05$, $^{**}p<0.01$ *vs* unteated group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* $MPP^+$-treated group.

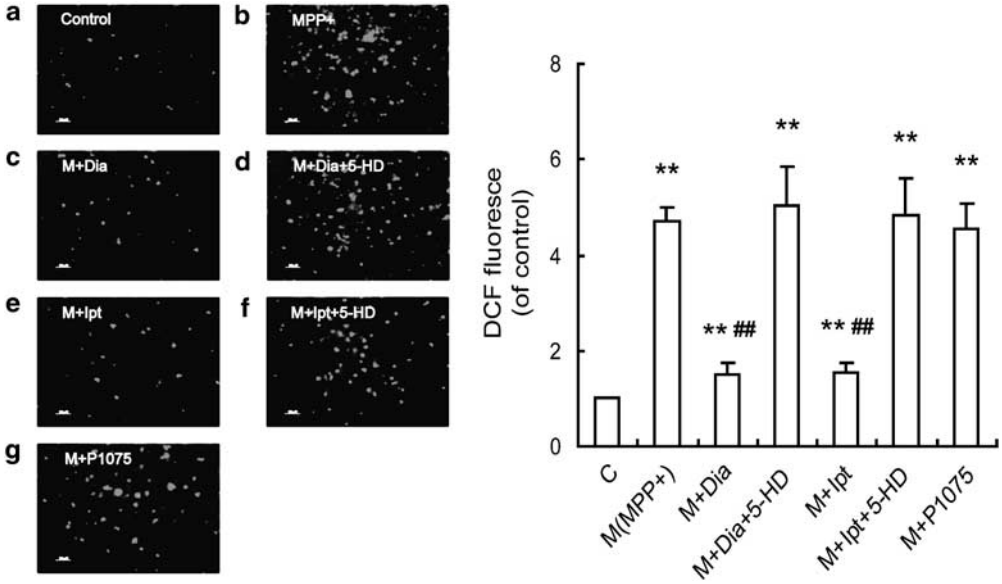


**Figure 3** Effects of KCOs on ROS production in astrocytes incubated with $MPP^+$. Values represented as mean ± SEM of four independent experiments performed in triplicate. C, control; M&$MPP^+$, 1-methyl-4-phenylpyridinium; Dia, diazoxide; Ipt, iptakalim; 5-HD, 5-hydroxydecanoate. $^{**}p<0.01$ *vs* control group; $^{\#\#}p<0.01$ *vs* $MPP^+$-treated group.

(PKC) activity was detected with PepTag non-radioactive protein kinase assays (Promega). As shown in Figure 4, $MPP^+$ (150 μM) increased the astroglial PKC activity to 23 nM $min^{-1}$ $gPro^{-1}$. Pretreatment with Dia and Ipt significantly inhibited PKC activity to 13 nM $min^{-1}$ $gPro^{-1}$ and 15 nM $min^{-1}$ $gPro^{-1}$ repectively. However, P1075 failed to influence the increase of cellular PKC activity induced by $MPP^+$.

## DISCUSSION

In the brain, $K_{ATP}$ channels do not open under physiological conditions, but they can be activated by either a specific opener or under many pathological conditions, such as acute ischemia/hypoxia, ischemic preconditioning or metabolic inhibition, thereby hyperpolarizing the membrane potential (Farkas *et al*, 2004; Thomzig *et al*, 2001; Nicholls

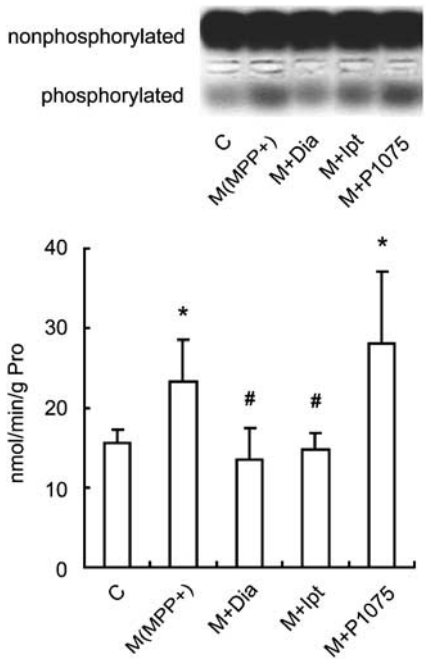


**Figure 4** Effects of various KCOs on $MPP^+$-induced PKC activation of primary cultured astrocytes. Compared with non-preconditioned cells, $MPP^+$ remarkably increased intracellular PKC activity, which could be restrained by Dia and Ipt. There was no significant change detected in P1075-treated group. Results are mean ± SEM of four independent experiments performed in triplicate. C, control; M&$MPP^+$, 1-methyl-4-phenylpyridinium; Dia, diazoxide; Ipt, iptakalim. *$p < 0.05$ *vs* control group; #$p < 0.05$ *vs* $MPP^+$-treated group.

and Attwell, 1990). $K_{ATP}$ channels are regulated by intracellular concentrations of ATP ($[ATP]_i$), being opened as $[ATP]_i$ decreases, and thus by linking neuronal metabolism to excitability, they are thought to play an important role in protecting cells from neuronal death caused by hypoxia, ischemia or metabolic inhibition (Tai *et al*, 2003; Yang *et al*, 2004; Busija *et al*, 2004; Liss *et al*, 1999).

$K_{ATP}$ channels are hetero-octameric proteins composed of pore-forming Kir6.x subunits (Kir6.1 and Kir6.2) and regulatory sulfonylurea receptor (SUR) subunits (SUR1, SUR2A, and SUR2B) (Seino, 1999; Inagaki *et al*, 1995), and located in various cellular compartments, including the surface of the plasmalemmal membrane (sarc$K_{ATP}$ channel) and the inner mitochondrial membrane (mito$K_{ATP}$ channel) (Farkas *et al*, 2004). In the brain, $K_{ATP}$ channels are enriched in neurons as well as in glial cells, but their subunit combinations of $K_{ATP}$ channels appear not to be homogeneous. For example, the subunits of $K_{ATP}$ channels in neurons are Kir6.2 and SUR1/SUR2B (Zawar *et al*, 1999; Thomzig *et al*, 2001), and $K_{ATP}$ channels in astrocytes are composed of Kir6.1 and SUR1 (Thomzig *et al*, 2001). These studies have raised the questions: what is the role of $K_{ATP}$ channels expressed in discrete cellular populations and how do they work in particular physiological/pathophysiological episodes?

It has been well-known that activation of $K_{ATP}$ channels can regulate release of neurotransmitters from neurons, and reduce neuronal excitability thereby protecting against excitotoxicity. How do $K_{ATP}$ channels regulate the functions of astrocytes? Traditionally, astrocytes were thought to play minor roles in neuronal function and in directing overall activities in the brain, serving only a maintenance role in regulating brain homeostasis (Nicholls and Attwell, 1990; Anderson and Swanson, 2000). Notably, recent studies challenge these assumptions and suggest that rather than being an innocuous bystander, astrocytes may play crucial roles in regulating neuronal activity and signal transmission; deficiencies in these functions may contribute to neurodegeneration (Haydon, 2001). One way astrocytes wield their effects on neuronal function is through GluTs to maintain stimulatory, but nontoxic low levels of free intrasynaptic L-glutamate in the area adjacent to neurons (Nicholls and Attwell, 1990; Robinson, 1998; Lipton and Rosenberg, 1994). Abnormalities in this process result in the accumulation of extracellular glutamate in synaptic clefts, and subsequently induce overexcitation, and death of neurons (Nicholls and Attwell, 1990; Robinson, 1998; Lipton and Rosenberg, 1994). Therefore, the present study is to show the effects of $K_{ATP}$ channel opening on glutamate uptake by GluTs in astrocytes.

We demonstrated that administration with Pina, Dia and Ipt increased glutamate uptake in rat primary cultured astrocytes, but sarc$K_{ATP}$ channel opener P1075 failed to affect astrocytic glutamate uptake. Furthermore, Pina, Dia and Ipt could also reverse the inhibition of glutamate uptake induced by $MPP^+$, and mito$K_{ATP}$ blocker 5-HD inhibited these effects. These findings suggest that activation of $K_{ATP}$ channels, especially mito$K_{ATP}$ channel, facilitated the function of GluTs in astrocytes. Ipt is a lipophilic *para*-amino compound with low molecular weight, which can freely cross the blood–brain barrier and has been shown to be a novel KCO by pharmacological, electrophysiological and biochemical studies, and receptor binding test (Wang, 2003; Xie *et al*, 2005). Ipt was initially designed and synthesized as a novel antihypertensive drug (Wang, 1998). In the view of the technical requirement for novel antihypertensive drug approval, the preclinical investigation of Ipt has been completed and clinical trials are currently underway (Wang, 2003). Our previous studies provided compelling supports for the neuroprotective effects of Ipt at the dosages not affecting blood pressure, via preventing the excitotoxic insults and anti-neuroinflammation (Wang *et al*, 2006, 2004; Yang *et al*, 2004). The present study revealed a new mechanism involved in the neuroprotective effects of the novel KCO.

It has been reported that mito$K_{ATP}$ channel opening could reduce the rate of ATP loss, and decrease the rate of adenine nucleotide degradation so that ADP is available for phosphorylation. The results from our previous studies also showed that activation of mito$K_{ATP}$ channel inhibited the decrease of ADP-dependent state 3/4 oxygen consumption and respiratory control index injured by $MPP^+$ in astrocytes. Consequently, activation of mito$K_{ATP}$ channel by KCOs preserves an adequate supply of ATP (Garlid *et al*, 2003). Glutamate uptake into astrocytes is driven by the electrochemical gradients of $Na^+$ and $K^+$, with a stoichiometry of 3 $Na^+$, and 1 $H^+$ in and 1 $K^+$ out with the uptake of each glutamate anion (Sung *et al*, 2003). The resulting increase in $[Na^+]_i$ might be corrected by a cycle of the $Na^+$ pump and ATP consumption. Therefore, mito$K_{ATP}$ channel

activation by KCOs might increase the supplementation of ATP, and thereby enhancing glutamate uptake by GluTs.

Activation of mito$K_{ATP}$ channel induces $K^+$ influx, which partially restores the potential back toward basal level and enhances mitochondrial respiration by mitochondria matrix swelling, and subsequently yields a mild uncoupling (Starkov, 1997). Matrix swelling triggered by mito$K_{ATP}$ channel activation changes the geometry between the inner and outer mitochondrial membranes, and thus decreases intermembrane space, inducing faster electron transport and decreases in ROS release (Starkov, 1997; Jezek *et al*, 2004). Thus, mito$K_{ATP}$ channel acts as a potent governor to mitochondrial ROS release (Brookes *et al*, 2004). It has been demonstrated that oxidative stress can reduce glutamate transporter activities, and antioxidants can reverse the impairment of glutamate uptake (Jayakumar *et al*, 2006). The data from our laboratory also showed that antioxidants glutathione and N-acetylcysteine could improve glutamate uptake in lipopolysaccharide-treated astrocytes (Zhou *et al*, 2006). In the present study, treatment with $MPP^+$, an inhibitor of mitochondrial complex I, that can induce ATP depletion, increased ROS in astrocytes, whereas diazoxide and iptakalim could inhibit ROS production. Taken together, inhibition of ROS production might be responsible for improvement of glutamate uptake by KCOs.

Many studies have suggested that glutamate uptake may be regulated by a variety of signaling pathways (Chaudhry *et al*, 1995; Rothstein *et al*, 1994), and that protein phosphorylation plays important roles in glutamate uptake and the expression and redistribution of GluTs (Rothstein *et al*, 1994; Gegelashvili and Schousboe, 1997). However, the effects of protein phosphorylation on the function of GluTs remain controversial because different transporters could be regulated in opposite directions by the same signaling pathway (Chaudhry *et al*, 1995; Danbolt, 2001). In the present study, the primary astrocyte cultures were prepared from cerebral cortex of rats where GLT-1 represents the predominant glial transporter (O'Shea *et al*, 2006; Marco and Michael, 2004). It has been reported that activation of PKC caused internalization of GLT-1 and decreased in cell-surface expression of GLT-1 (Kalandadze *et al*, 2002); PKC activation also inhibited the traffic process of GLT-1 whereby PKC phosphorylation reduced the function of GLT-1 (Ganel and Crosson, 1998). The present study showed that treatment with diazoxide and iptakalim inhibited PKC phosphorylation, thus it is reasonable to presume that inhibiting PKC phosphorylation by KCOs facilitates glutamate uptake. However, the detailed pathways involved in KCO inhibiting PKC activity deserve to be defined.

In summary, it is the first study to demonstrate that activation of $K_{ATP}$ channel, especially mito$K_{ATP}$ channel, improves the function of GluTs in astrocytes. Reducing ROS production and downregulating PKC phosphorylation contribute to the facilitative effects of KCOs on glutamate uptake. Therefore, this study not only reveals a novel pharmacological profile of KCOs as regulators of GluTs, but also provides a new strategy for neuroprotection. Notably, Ipt, a novel and blood–brain barrier permeable KCO, may be a promising therapeutic agent for neurodegenerative diseases such as PD.

## ACKNOWLEDGEMENTS

We acknowledge the gift of HMR 1098 by Dr Juergen Puenter (Aventis, Frankfurt, Germany) for this research. These studies were supported in part by grants from the National Natural Science Foundation of China (Nos. 30625038 and 30572172), the Key Project of Natural Science Foundation of Jiangsu Educational Department (Nos. 05KJA31014, 05KJB310075 and 06KJA31029), the Key Project of Jiangsu Health Department (No. K200501).

## DISCLOSURE/CONFLICT OF INTEREST

There is no duality of interest that we should disclose.

## REFERENCES

Anderson CM, Swanson RA (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32**: 1–14.

Ashcroft FM (1988). Adenosine 5′-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* **11**: 97–118.

Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol* **287**: C817–C833.

Busija DW, Lacza Z, Rajapakse N, Shimizu K, Kis B, Bari F *et al* (2004). Targeting mitochondrial ATP-sensitive potassium channels—a novel approach to neuroprotection. *Brain Res Rev* **46**: 282–294.

Chaudhry FA, Lehre KP, van Lookeren Campagne M, Ottersen OP, Danbolt NC, Storm-Mathisen J (1995). Glutamate transporters in glial plasma membranes: highly differentiated localizations revealed by quantitativeultrastructural immunocytochemistry. *Neuron* **15**: 711–720.

Danbolt NC (2001). Glutamate uptake. *Prog Neurobiol* **65**: 1–105.

Farkas E, Institoris A, Domoki F, Mihaly A, Luiten PG, Bari F (2004). Diazoxide and dimethyl sulphoxide prevent cerebral hypoperfusion-related learning dysfunction and brain damage after carotid artery occlusion. *Brain Res* **1008**: 252–260.

Ferrarese C, Begni B, Canevari C, Zoia C, Piolti R, Frigo M *et al* (2000). Glutamate uptake is decreased in platelets from Alzheimer's disease patients. *Ann Neurol* **47**: 641–643.

Ganel R, Crosson CE (1998). Modulation of human glutamate transporter activity by phorbol ester. *J Neurochem* **70**: 993–1000.

Garlid KD, Dos Santos PZ, Xie J, Costa AD, Paucek P (2003). Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K(+) channel in cardiac function and cardioprotection. *Biochim Biophys Acta* **1606**: 1–21.

Gegelashvili G, Schousboe A (1997). High affinity glutamate transporters: regulation of expression and activity. *Mol Pharmacol* **52**: 6–15.

Genoud C, Quairiaux C, Steiner P, Hirling H, Welker E, Knott JW (2006). Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol* **4**: e343.

Haydon PG (2001). GLIA: listening and talking to the synapse. *Nat Rev Neurosci* **2**: 185–193.

Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G *et al* (1995). Reconstitution of $IK_{ATP}$: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270**: 1166–1170.

Ivanova T, Beyer C (2003). Estrogen regulates tyrosine hydroxylase expression in the neonate mouse brain. *J Neurobiol* **54**: 638–647.

Jayakumar AR, Panickar KS, Murthy ChR, Norenberg MD (2006). Oxidative stress and mitogen-activated protein kinase phosphorylation mediate ammonia-induced cell swelling and glutamate uptake inhibition in cultured astrocytes. *J Neurosci* **26**: 4774–4784.

Jezek P, Zackova M, Ruzicka M, Skobisova E, Jaburek M (2004). Mitochondrial uncoupling proteins–Facts and fantasies. *Physiol Res* **53**(Suppl 1): S199–S211.

Kalandadze A, Wu Y, Robinson MB (2002). Protein kinase C activation decreases cell surface expression of the GLT-1 subtype of glutamate transporter. Requirement of a carboxyl-terminal domain and partial dependence on serine 486. *J Biol Chem* **277**: 45741–45750.

Lipton SA, Rosenberg PA (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* **330**: 613–622.

Liss B, Bruns R, Roeper J (1999). Alternative sulfonylurea receptor expression defines metabolic sensitivity of K-ATP channels in dopaminergic midbrain neurons. *EMBO J* **18**: 833–846.

Liss B, Haeckel O, Wildmann J, Miki T, Seino S, Poeper J (2005). K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. *Nat Neurosci* **8**: 1742–1751.

Marco IG, Michael BR (2004). Protein kinase C-dependent remodeling of glutamate transporter function. *Mol Interv* **4**: 48–58.

Matsugami TR, Tanemura K, Mieda M, Nakatomi R, Yamada K, Kondo T *et al* (2006). From the Cover: indispensability of the glutamate transporters GLAST and GLT1 to brain development. *Proc Natl Acad Sci USA* **103**: 12161–12166.

Matute C, Domercq M, Sanchez-Gomez MV (2006). Glutamate-mediated glial injury: mechanisms and clinical importance. *Glia* **53**: 212–224.

Nicholls D, Attwell D (1990). The release and uptake of excitatory amino acids. *Trends Pharmacol Sci* **11**: 462–468.

O'Shea RD, Lau CL, Farso MC, Diwakarla S, Zagami CJ, Svendsen BB *et al* (2006). Effects of lipopolysaccharide on glial phenotype and activity of glutamate transporters: evidence for delayed up-regulation and redistribution of GLT-1. *Neurochem Int* **48**: 604–610.

Prensa L, Parent A (2001). The nigrostriatal pathway in the rat: a single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. *J Neurosci* **21**: 7247–7260.

Robinson MB (1998). The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype. *Neurochem Int* **33**: 479–491.

Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D *et al* (1994). Localization of neuronal and glial glutamate transporters. *Neuron* **13**: 713–725.

Sargent CA, Sleph PG, Dzwonczyk S, Normandin D, Antonaccio MJ, Grover GJ (1993). Cardioprotective effects of the cyanoguanidine potassium channel opener P-1075. *J Cardiovasc Pharmacol* **22**: 564–570.

Schousboe A, Waagepetersen HS (2005). Role of astrocytes in glutamate homeostasis: implications for excitotoxicity. *Neurotox Res* **8**: 221–225.

Seino S (1999). ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* **61**: 337–362.

Starkov AA (1997). 'Mild' uncoupling of mitochondria. *Biosci Rep* **17**: 273–279.

Sung U, Apparsundaram S, Galli A, Kahlig KM, Savchenko V, Schroeter S *et al* (2003). A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. *J Neurosci* **23**: 1697–1709.

Tai KK, McCrossan ZA, Abbott GW (2003). Activation of mitochondrial ATP-sensitive potassium channels increases cell viability against rotenone-induced cell death. *J Neurochem* **84**: 1193–1200.

Takayasu Y, Iino M, Shimamoto K, Tanaka K, Ozawa S (2006). Glial glutamate transporters maintain one-to-one relationship at the climbing fiber-Purkinje cell synapse by preventing glutamate spillover. *J Neurosci* **26**: 6563–6572.

Thomzig A, Wenzel M, Karschin C, Eaton MJ, Skatchkov SN, Veh RW (2001). Kir6.1 is the principal pore-forming subunit of astrocyte but not neuronal plasma membrane K-ATP channels. *Mol Cell Neurosci* **18**: 671–690.

Wadiche JI, Gersdorff H (2006). Long-distance signaling via presynaptic glutamate transporters. *Nat Neurosci* **9**: 1352–1353.

Wang H (1998). Cardiovascular ATP-sensitive K+ channel as a new molecular target for development of antihypertensive drugs. *Acta pharmacologica Sinica* **19**: 397–402.

Wang H (2003). Pharmacological characteristics of the novel antihypertensive drug, iptakalim hydrochloride, and its molecular mechanisms. *Drug Develop Res* **58**: 65–68.

Wang H, Zhang YL, Tang XC, Feng HS, Hu G (2004). Targetin g ischemic stroke with a novel opener of ATP-sensitive potassium channels in the brain. *Mol Pharmacol* **66**: 1160–1168.

Wang S, Hu LF, Zhang Y, Sun T, Sun YH, Ding JH *et al* (2006). Effects of systemic administration of iptakalim on extracellular neurotransmitter levels in the striatum of unilateral 6-hydroxydopamine-lesioned rats. *Neuropsychopharmacology* **31**: 933–940.

Xie W, Wang H, Ding J, Wang H, Hu G (2005). Anti-proliferating effect of iptakalim, a novel KATP channel opener, in cultured rabbit pulmonary arterial smooth muscle cells. *Eur J Pharmacol* **511**: 81–87.

Yang Y, Liu X, Ding JH, Sun J, Long Y, Wang F *et al* (2004). Effects of iptakalim on rotenone-induced cytotoxicity and dopamine release from PC12 cells. *Neurosci Lett* **366**: 53–57.

Yao HH, Ding JH, Zhou F, Wang F, Hu LF, Sun T *et al* (2005). Enhancement of glutamate uptake mediates the neuroprotection exerted by activating group II or III metabotropic glutamate receptors on astrocytes. *J Neurochem* **92**: 948–961.

Yao Z, Gross GJ (1994). A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of KATP channels. *Circulation* **89**: 1229–1236.

Zawar C, Plant TD, Schirra C, Konnerth A, Neumcke B (1999). Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *J Physiol* **514**(Part 2): 327–341.

Zhou F, Yao HH, Wu JY, Yang YJ, Ding JH, Hu G (2006). Activation of Group II/III metabotropic glutamate receptors attenuates LPS-induced astroglial neurotoxicity via promoting glutamate uptake. *J Neurosci Res* **84**: 268–277.

Zini S, Tremblay E, Pollard H, Moreau J, Ben Ari Y (1993). Regional distribution of sulfonylurea receptors in the brain of rodent and primate. *Neuroscience* **55**: 1085–1091.