Iptakalim protects against MPP⁺⁺-induced degeneration of dopaminergic neurons in association with astrocyte activation

Yan-Jing Yang¹², Shu Zhang*, Jian-Hua Ding¹, Fang Zhou¹ and Gang Hu¹

¹ Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, Nanjing, Jiangsu, P.R. China
² Dental Research Institute, Nanjing Medical University, Nanjing, Jiangsu, P.R. China

Abstract

Astrocyte activation observed in the MPTP mouse model and Parkinson’s disease patients participates in the cascade of deleterious events that ultimately leads to death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The present study aimed to elucidate whether inhibiting astrocyte activation was involved in the protective effects of iptakalim (Ipt), a novel ATP-sensitive potassium channel opener, on MPP⁺⁺-induced degeneration of dopaminergic neurons. The results showed that Ipt could decrease MPP⁺⁺-induced TNF-α release and p38 MAPK activation in reactive astrocytes. The effects of Ipt were reversed by the mitochondrial K ATP blocker, 5-hydroxydecanoate, indicating that mitochondrial K ATP channels participate in the regulation of astrocyte activation. Moreover, systematic administration of Ipt could significantly alleviate MPP⁺⁺-induced behavioural symptoms in motor coordination, the loss of dopaminergic neurons, and the activation of astrocyte and microglia in the SNpc. Together, these findings suggest that Ipt may protect against MPP⁺⁺-induced degeneration of dopaminergic neurons by inhibiting astrocyte activation and subsequent release of pro-inflammatory factors.

Received 19 March 2008; Reviewed 6 May 2008; Revised 7 July 2008; Accepted 9 July 2008; First published online 13 August 2008

Key words: ATP-sensitive potassium channel opener, astrocyte activation, iptakalim, Parkinson’s disease, TNF-α.

Introduction

Astrocytes, the major population in the brain, play a crucial role in maintaining normal brain physiology during development and in adulthood (Volterra and Meldolesi, 2005). Increasing evidence has demonstrated that astrocyte activation was involved in the pathogenesis of many neurodegenerative disorders, such as Parkinson’s disease (PD) (Marchetti and Abbracchio, 2005; Teismann et al., 2003). Indeed, activated astrocytes produce a number of neurotoxic molecules, including pro-inflammatory factors, reactive oxygen species and reactive nitrogen species (Aschner, 1998; Lau and Yu, 2001). These factor-mediated inflammatory processes contribute to neurodegenerative disorders (Ikeda and Murase, 2004; Minghetti, 2005). Thus, the drugs that can inhibit astrocyte activation and subsequent inflammatory processes may provide therapeutic benefits for neurodegenerative disorders such as PD (Seifert et al., 2006).

ATP-sensitive potassium (K ATP) channels, which link cell metabolism to its membrane potential, belong to a class of inwardly rectifying potassium channels that are widely distributed in the brain and localized in the basal ganglia with higher levels (Dunn-Meynell et al., 1998). Emerging evidence, including from our laboratory, has revealed a novel neuronal protective role of opening K ATP channels in PD models both in vivo and in vitro (Tai et al., 2003; Wang et al., 2005; Yang et al., 2004, 2006a). Since most developed K ATP channel openers fail to pass through the blood–brain barrier, there are few effective K ATP channel openers to be used in vivo to investigate the role of K ATP channels in neurodegenerative disorders. Therefore, novel K ATP
channel openers, which can permeate the blood–brain barrier and have low toxic side-effects, are considered as potential neuroprotectants (Wang et al., 2006; Yang et al., 2005).

Iptakalim (Ipt), a lipophilic para-amino compound with low molecular weight, can freely cross the blood–brain barrier and has been demonstrated to be a novel K<sub>ATP</sub> channel opener by pharmacological, electrophysiological, biochemical studies, and receptor binding tests (Wang, 2003; Wang et al., 2004; Xie et al., 2005). Ipt was initially designed and synthesized as a novel antihypertensive drug (Wang, 1998). Based on the technical requirement for novel antihypertensive drug approval, the pre-clinical investigation of Ipt has been completed and clinical trials are currently underway (Wang, 2003). Furthermore, our previous studies provided compelling support for the neuroprotective effects of Ipt on behavioural recovery and neuronal survival in various animal models, such as stroke and PD, at doses not affecting normal blood pressure (Wang et al., 2004, 2005; Yang et al., 2004, 2005). However, the mechanism involved in the protective effect of Ipt requires further study.

1-methyl-4-phenylpyridinium ion (MPP<sup>+</sup>), a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in astrocytes, blocks mitochondrial complex I and finally triggers selective dopaminergic degeneration, which is similar to the pathology found in PD (Tipton and Singer, 1993). Interestingly, a recent study demonstrated that an increase in the number of GFAP-immunopositive astrocytes was also found in the striatum and substantia nigra of MPTP-treated mice, suggesting that reactive astrocytes may play a key role in the regions damaged by MPTP (Smeyne et al., 2005; Vila et al., 2001). Thus, the drugs that can regulate astrocyte activation may exert neuroprotective effect against the toxicity of MPTP/MPP<sup>+</sup>. Mitochondrial complex I inhibitors, such as rotenone and MPP<sup>+</sup>, result in astrocyte- and microglia-mediated neuroinflammation and K<sub>ATP</sub> channels are considered as potential downstream targets of mitochondrial complex I inhibition (Liss et al., 2005). p38 MAPK protein, as a targeted signalling molecule in neurodegeneration, can be phosphorylated to initiate a signal cascade that regulates synthesis of a variety of pro-inflammatory factors, such as TNF-α (Kumar et al., 2003; Saklatvala, 2004). We recently reported that Ipt could alleviate rotenone-induced degeneration of dopaminergic neurons by inhibiting p38 MAPK-mediated microglia activation (Zhou et al., 2007). In the present study, our main aim was to investigate whether Ipt could inhibit MPP<sup>+</sup>-induced astrocyte activation via regulating p38 MAPK signalling transduction and thus prevent degeneration of dopaminergic neurons.

**Methods**

**Animals and reagents**

A total of 75 adult male Sprague–Dawley rats, weighing 200–250 g at the start of the experimental procedure, were used. All animals were housed in groups of five per cage under standard laboratory conditions with free access to food and water, constant room temperature of 22°C, 50–60% humidity, and a 12-h day–night cycle (lights on 12:00 hours). All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985) and the Guidelines for the Care and Use of Animals in Neuroscience Research by the Society for Neuroscience and approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University).

MPP<sup>+</sup> was purchased from Sigma (St Louis, MO, USA). Ipt hydrochloride (99.9%) was synthesized and provided by the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences of China. Ipt and MPP<sup>+</sup> were dissolved in sterile saline. Diazoxide was dissolved in 100% DMSO as vehicle and diluted with sterile saline to a concentration of 1 mg/ml (0.2% DMSO) for administration to rats. All other reagents were provided by standard commercial institutes.

**Surgery and drug administration**

The rats were randomly allocated to five groups (groups A–E), with 15 rats in each group. Rats of groups C–E were pretreated with Ipt (1.5 or 3.0 mg/kg.d, orally) or diazoxide (3.0 mg/kg.d, orally) for 3 d, simultaneously rats of groups A and B were pretreated with sterile saline containing 0.2% DMSO. After 3 d, rats of groups B–E were intrastriatally injected with MPP<sup>+</sup>. Rats of group A (control rats) were intrastriatally injected with saline. Groups C–E were administered Ipt or diazoxide and groups A and B were administered vehicle in the following 7 d.

Rats were anaesthetized using 2% sodium pentobarbital (40 mg/kg i.p.) and placed in a stereotaxic apparatus (Stoelting Instruments, Wood Dale, IL, USA). Unilateral injection of MPP<sup>+</sup> (26.73 mg/1.8 μl, 90 nmol) was performed in the right striatum (coordinates from bregma: AP +0.9 mm, ML −3.0 mm, DV −5.0 mm) by means of a Hamilton syringe (0.46 mm diameter) at a rate of 1 μl/min, the needle was left in place for 10 min. Sham-lesioned rats were...
infused with 1.8 μl saline into the right striatum and served as controls.

**Behavioural test**

The rotarod test, developed for rats by Rozas (Rozas et al., 1998), is well suited to distinguish persistent motor deficits from temporary inactivity. The rats were kept in the rotarod apparatus at a constant speed of 5 rpm for 3 d before the surgical procedure. Those that were able to remain on the rotating rod for 300 s were selected for the test. The rats were placed on the rod and sequentially tested at 5, 10, 15, 20, 25 and 30 rpm, for maximum of 300 s at each speed. Animals were given at least 5 min rest between successive speeds to alleviate stress and fatigue. Time-on-the-rod at each speed [i.e. the maximum time (up to 300 s) that each animal was able to stay on the revolving rod] was plotted against each corresponding speed of rotation. The overall rod performance (ORP) score for each animal was calculated as the area under the curve in the plot of time-on-the-rod against rotation speed. All tests commenced at about the same time.

**Immunohistochemistry and stereology**

The remaining animals were anaesthetized by chloral hydrate and perfused first with 0.9% saline and then with cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were dissected out and maintained in 4% paraformaldehyde overnight. Brains were cryopreserved in 30% sucrose in phosphate-buffered saline (PBS) and stored at 70 °C until required. Free-floating sections encompassing the entire midbrain were prepared using a cryostat. Sections were processed for tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) immunohistochemistry as follows. Following incubation for 1 h in 10% bovine serum albumin (BSA) with 0.3% Triton X-100 in 0.01 M PBS, tissue sections (30 μm) were incubated with primary antibodies overnight at 4 °C. Primary antibodies used in this study were as follows: mouse anti-TH (1:4000; Sigma), mouse anti-GFAP (1:300; Sigma) and mouse anti-ED1 (1:100; Serotec, Raleigh, NC, USA). Immunostaining was visualized by using 3,3'-diaminobenzidine and sections were then counterstained with haematoxylin.

All counts were performed by researchers blind to the experimental status of the animals. The total number of TH-immunoreactive (IR) neurons and GFAP-IR astrocytes in substantia nigra pars compacta (SNpc) were counted from six rats per group by using the optical fractionator method (West, 1993), an unbiased method of cell counting that is not affected by either the volume of reference (i.e. SNpc) or the size of the counted elements (i.e. neurons) (Stereo Investigator software, Microbrightfield, Colchester, VT, USA). In agreement with this method, TH-IR neurons and GFAP-IR astrocytes were counted in the intact and lesioned SNpc of every fourth section (30 μm) throughout the entire extent of the SNpc. Each midbrain section was viewed at low power (×10 objective), and the SNpc was outlined according to the established anatomical landmarks (the rat brain atlas of Paxinos and Watson, 1997). Then at a random start, the number of TH-IR neurons and GFAP-IR astrocytes were counted at high power (×100 oil). To avoid double counting of cells with unusual shapes, TH-IR neurons and GFAP-IR astrocytes were counted only when their nuclei were optimally visualized, which occurred only in one focal plane. After all of the TH-IR neurons and GFAP-IR astrocytes were counted, the total numbers of TH-IR neurons and GFAP-IR astrocytes in the SNpc were calculated by using the formula described by West (1993). Sampling grid dimensions were 120 × 120 × 5 μm (x, y, z axes).

**Astrocyte cell culture**

Rat primary astrocyte cultures were prepared from the midbrain of 2-d-old Sprague-Dawley rats and plated on poly d-lysine pre-coated cell-culture flasks containing DMEM (10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO2/95% air. Under these conditions, confluent cultures were achieved after 10 d in vitro and the cells were >96% positive for GFAP as assessed by immunocytochemical staining.

**GFAP immunocytochemistry**

Astrocytes were fixed in 4% paraformaldehyde in 0.1 M PBS for 15 min at room temperature, permeabilized with 0.1% Triton X-100, and rinsed three times, each for 10 min with PBS containing 0.2% BSA. The primary antibodies, mouse anti-GFAP antibodies (1:300; Sigma), were applied to the samples and kept at 4 °C overnight. The following day the cells were rinsed three times with PBS. Secondary antibodies were applied to samples and kept at 37 °C for 1 h. Samples were then rinsed three times with PBS and immunostaining was visualized by using 3,3'-diaminobenzidine.

**TNF-α assay**

The amount of TNF-α in the peripheral blood and culture medium was determined 24 h after treatment.
with a rat TNF-α enzyme-linked immunosorbent assay (ELISA) kit (BioSource International, Camarillo, CA, USA).

**Western blotting**

Cells were washed twice with ice-cold PBS and homogenized in 200 µl lysis buffer. After incubation for 20 min on ice, cell lysates were centrifuged (10000 g for 10 min at 4 °C) and protein concentration in the extracts was determined by the Bradford assay (Kruger, 1994). Proteins in cell extracts were denatured with SDS sample buffer and separated by 10% SDS–PAGE. Proteins were transferred to nitrocellulose membranes using a Bio-Rad miniprotein-III wet transfer unit. The membranes were incubated with 5% BSA dissolved in TBST (pH 7.5; 10 mM Tris–HCl, 150 mM NaCl, and 0.1% Tween-20) at room temperature for 1 h, washed three times and incubated with different antibodies (p38 and phospho-p38 at 1:1000, Cell Signaling Technology Inc., Beverly, MA, USA). The membranes were washed three times with TBST buffer and incubated with the secondary antibody (1:2000) for 1 h followed by four washings. Signal detection was performed with an enhanced chemiluminescence kit (Cell Signaling Technology).

**Statistical analysis**

All values were expressed as mean ± standard error of the mean (S.E.M.). The significance of the difference between control and samples treated with various drugs was determined by one-way ANOVA followed by the post-hoc least significant difference test. Differences were considered significant at \( p < 0.05 \).

**Results**

*Ipt alleviates MPP⁺-induced behavioural symptoms in rotarod motor performance*

In order to assess the effect of Ipt on motor function in rats, the rotarod test was examined on day 7 following vehicle or MPP⁺ treatment. It was shown that vehicle-treated animals learned the task quickly and, after a short training period, were able to remain on the rod at different speeds (5–20 rpm). They stepped voluntarily from the hand of the experimenter onto the rotarod, except at very high rotation speeds. The ORP scores of control animals was about 6694 s. However, unilateral injection of MPP⁺ (26.73 mg/1.8 µl, 90 nmol) in the right striatum induced significant decline in rotarod performance in rats. The ORP scores of MPP⁺-treated rats were significantly reduced. Rats pretreated with Ipt (1.5, 3.0 mg/kg.d) or diazoxide (3.0 mg/kg.d) exhibited higher ORP scores compared with the MPP⁺-treated rats (Figure 1), suggesting that Ipt can alleviate MPP⁺-induced behavioural symptoms in motor coordination.

*Ipt suppresses MPP⁺-induced dopaminergic neuronal degeneration in rat SNpc*

Stereological count of TH-IR cells in SNpc was used to explore the effects of Ipt on MPP⁺-induced degeneration of dopaminergic neurons. As illustrated in Figure 2(a,b), the dopaminergic neurons in the SNpc were intensely immunoreactive to TH in the control group. Treatment with MPP⁺ induced a dramatic loss of unilateral dopaminergic neurons by 55% compared with the control group, indicated by the clearly reduced number of TH-IR neurons. Ipt (1.5, 3.0 mg/kg.d) or diazoxide (3.0 mg/kg.d) could markedly decrease MPP⁺-induced dopaminergic neuron degeneration by 26%, 30% and 33%, respectively. However, no significant diversity of TH-IR neurons of the contralateral side was observed. These results suggest that Ipt can delay MPP⁺-induced degeneration of dopaminergic neurons in SNpc.

*Ipt inhibits MPP⁺-induced astrocyte and microglia activation in rat SNpc*

GFAP immunostaining was performed to analyse astrocyte activation in the SNpc. As shown in Figure 3(a,b), administration of MPP⁺ (26.73 mg/1.8 µl, 90 nmol) in the right striatum could induce notable astrocyte activation in rat SNpc, indicated by

---

**Figure 1.** Effect of iptakalim (Ipt) on MPP⁺-induced the overall rod performance (ORP) scores. The ORP score for each animal was calculated as the area under the curve in the plot of time-on-the-rod against rotation speed. Data are means ± S.E.M. (\( n = 10 \)). ** \( p < 0.01 \) vs. control group; # \( p < 0.05 \) vs. MPP⁺ model group. DZ, Diazoxide.

---

Y.-J. Yang et al.
the large number of GFAP-IR cells (increased to 603\% of control) and larger cell body and hypertrophic processes. Ipt (1.5, 3.0 mg/kg.d) or diazoxide (3.0 mg/kg.d) pretreatment resulted in significant suppression of astrocyte activation in rat SNpc, indicated by the small number of GFAP-IR cell (suppressed by 228\%, 221\% and 229\% of control respectively) and thin, clearly distinguishable processes. Simultaneously, intrastriatal injection of saline resulted in few increase in ED1-positive cells in the SNpc, but the number of ED1-positive cells increased significantly in rats treated with MPP\(^+\). The proliferation of microglia in response to MPP\(^+\) treatment was reduced by Ipt or DZ (Figure 3c). These results suggest that Ipt can inhibit MPP\(^+\)-induced astrocyte and microglia activation, and this inhibitory effect may be involved in the protective effects of Ipt.

Ipt suppresses the increase of TNF-\(\alpha\) production from reactive astrocytes and the activation of p38 MAPK induced by MPP\(^+\)

Rat primary-cultured astrocytes were used for in-vitro study to further investigate the inhibitory effect of Ipt on astrocyte activation. In the present study, GFAP immunocytochemistry was performed to analyse astrocyte activation in the primary culture. As shown in Figure 4, the administration of MPP\(^+\) could induce notable astrocyte activation, indicated by larger cell body and hypertrophic processes. Either Ipt or diazoxide pretreatment significantly suppressed astrocyte activation, indicated by thin, clearly distinguishable processes, which was abolished by 5-hydroxydecanoate (5-HD).

As shown in Figure 5a, incubation with MPP\(^+\) (150 \(\mu\)M) for 24 h could significantly increase the production of TNF-\(\alpha\) from reactive astrocytes (\(p<0.05\) vs. control). Pretreatment with different concentrations of Ipt (0.01, 0.1, 1, 10, 100 \(\mu\)M) 30 min prior to addition of MPP\(^+\) produced a concentration-dependent decrease in TNF-\(\alpha\) production with a minimum effective dose of 1 \(\mu\)M (Figure 5b). The selective mitochondrial (mito)-K\(_{\text{ATP}}\) channel opener diazoxide (100 \(\mu\)M) also decreased MPP\(^+\)-induced TNF-\(\alpha\) production by 73\% of control. Notably, 30-min pre-incubation with the selective mito-K\(_{\text{ATP}}\) channel blocker 5-HD (250 \(\mu\)M) could reverse the inhibitory effects of Ipt and diazoxide (Figure 5c). These results suggest that Ipt can inhibit MPP\(^+\)-induced production of pro-inflammatory factors from reactive astrocytes by opening mito-K\(_{\text{ATP}}\) channels.

Astrocytes were treated with MPP\(^+\) (150 \(\mu\)M) for different time intervals to determine the involvement of p38 MAPK after MPP\(^+\) stimulation. Treatment with
MPP⁺ led to a rapid and transient phosphorylation of p38, indicating activation of p38 MAPK pathways, with the peak level of phospho-p38 occurring at 30 min (Figure 6a). The result suggests that the p38 MAPK signalling pathway was activated in response to MPP⁺ treatment in astrocytes. Next, we detected whether Ipt could regulate MPP⁺-induced p38 MAPK phosphorylation at the 30-min time-point. As shown in Figure 6b, pretreatment with 10 µM Ipt or 100 µM diazoxide could suppress MPP⁺-induced increase of phospho-p38 level by >65%, and the suppressive effects of Ipt and diazoxide were reversed by 5-HD (250 µM). These data further suggest that Ipt can inhibit MPP⁺-induced pro-inflammatory factor production from reactive astrocytes by regulating the p38 MAPK signalling pathway.

Discussion

The present study shows that systematic administration with Ipt significantly alleviated MPP⁺-induced behavioural symptoms in motor coordination, the loss of dopaminergic neurons, and the activation of astrocytes and microglia in the SNpc. Ipt also decreased MPP⁺-induced TNF-α release and p38 MAPK activation in reactive astrocytes, which was abolished by the selective mito-K⁺ATP channel blocker 5-HD.

Astrocyte activation is a prominent occurrence following injury to the CNS (Marchetti and Abbracchio, 2005; Teismann et al., 2003). Enhancement in the immunoreactivity of GFAP is found in the striatum and substantia nigra of PD patients and mice treated with MPTP (Mirza et al., 2000; Smeyne et al., 2005). In the MPTP mouse model, astrocyte activation is concomitant with the death of neurons (Przedborski et al., 2000). Therefore, it is possible that reactive astrocytes play a key role in regions damaged by MPTP (Watanabe et al., 2005). Cytotoxic factors produced by reactive astrocytes can damage local neural cells, and reactive astrocytes can actively contribute to secondary degeneration after CNS insults or in response to inflammatory signalling cues (Hirsch, 2000; Liu et al., 2002). Therefore, inhibition of astrocyte activation and subsequent release of pro-inflammatory factors may offer prospective clinical therapeutic benefit for PD.

Ipt, a novel K⁺ATP channel opener, exhibited significant neuroprotective effect in preventing rotenone-induced behavioural alterations and dopamine depletion, as well as protecting SH-SY5Y cells against death (Hu et al., 2005; Yang et al., 2004, 2005, 2006b). We also found that Ipt could alleviate rotenone-induced degeneration of dopaminergic neurons by inhibiting microglia-mediated neuroinflammation (Zhou et al., 2007). Liberatore et al. (1999) have reported that changes in MAC-1 expression were evident after 12 h,
reached a maximum by 24–48 h, and were no longer different from control samples by day 7 after MPTP injection. In contrast to MAC-1 expression, changes in GFAP expression were only noticeable by 24 h, were maximal by 4–7 d, and showed a trend towards returning to control levels at 21 d after MPTP injection. Therefore, we measured the effects of Ipt at 7 d after MPP+ treatment. In the present study, we found that treatment with Ipt could recover MPP+-induced deficit in rotarod motor performance, and delay the degeneration of dopaminergic neurons. Ipt could also relieve activation of astrocytes in the substantia nigra induced by MPP+. Consistently, Ipt could suppress MPP+-induced elevation of TNF-α released from reactive astrocytes by down-regulating p38 phosphorylation, suggesting that p38 MAPK signalling transduction is involved in the inhibitory effect of Ipt on MPP+-induced astrocyte activation. Combining this with our previous finding that Ipt inhibited MPP+-induced astrocyte apoptosis by regulating the levels of JNK and ERK1/2 phosphorylation (Zhang et al., 2007), we propose that Ipt may protect astrocytes by regulating MAPK signalling transduction. The function of early glial response following brain injury may be part of the repair and protective response, but the presence of site-specific microglia and astrocyte activation in human neurodegenerative diseases such as PD (Teismann et al., 2003; Forno et al., 1992) raises the question of whether this response could contribute to the developing injury. Indeed, blocking of microglia activation decreases TH-IR neuronal loss triggered by MPTP or lipopolysaccharides (Lu et al., 2000; Wu et al., 2002). Similarly, suppression of astrocyte response with arundic acid [(R)-(–)-2-propyloctanoic acid, ONO-2506] reduced the number of TUNEL-positive apoptotic cells in the perinfract area, prevented delayed infarct expansion and ameliorated neurological deficits after permanent middle cerebral artery occlusion, indicating a deleterious effect of reactive astrocytes (Tateishi et al., 2002). The present findings indicate that inhibition of astrocyte activation by Ipt might be involved in its protective effects on MPP+-induced degeneration of dopaminergic neurons in rats.

The present study also showed that the selective mito-KATP channel opener diazoxide had a similar protective effect to that of Ipt. Moreover, the inhibitory effect of Ipt or diazoxide on inflammatory factor release from activated astrocytes was abolished by the selective mito-KATP channel blocker 5-HD. These findings suggest that Ipt may regulate TNF-α released from reactive astrocytes via opening mito-KATP channels in astrocytes and subsequently exert neuroprotective effects. Accumulating evidence has shown that the amount of mito-KATP channels located in brain cells is at least 6-fold higher than that in heart cells (Baigar et al., 2001; Lacza et al., 2003), indicating an essential role of mito-KATP channel in the physiology and pathology of the CNS. Previous studies have shown that KATP channel openers, especial mito-KATP channel openers, can provide protective effects for neurons, and neuroblast against ischaemia, trauma and toxic reagents such as rotenone and MPP+-induced cell damage (Ardehali and O’Rourke, 2005;
Nakagawa et al., 2005; Wang et al., 2004; Yang et al., 2006b). There are only a few studies that reveal the role of KATP channels in the regulation of astrocyte functions. Rajapakse et al. (2003) reported that opening KATP channels could prevent astrocyte death induced by oxygen glucose deprivation and H2O2. Our previous study found that opening KATP channels could increase the glutamate uptake and subsequently decrease the extracellular accumulation of glutamate induced by MPP+ in astrocytes (Hu et al., 2005). Thus, opening KATP channels of astrocytes may exhibit neuronal protection by regulating astrocyte function.

Diazoxide, a selective mito-KATP channel opener, was originally developed as an antihypertensive agent, but was found to induce hyperglycaemia by reducing insulin secretion. The clinical use of diazoxide has been hampered by its lack of potency and selectivity giving rise to side-effects (Hansen, 2006). It has been demonstrated that diazoxide conferred neuroprotective effect on various in-vivo cerebral ischaemia models and neuronal cell cultures exposed to oxygen–glucose deprivation (Domoki et al., 2004; Kis et al., 2003; Teshima et al., 2003). In comparison, Ipt possesses several advantages, such as free penetration through the blood–brain barrier and low toxic side-effects during systemic administration. Ipt also

Figure 5. ELISA analysis of the effect of iptakalim (Ipt) on increased production of TNF-α from reactive astrocytes induced by MPP+. (a) 150 µM MPP+ increased the release of TNF-α in astrocytes. (b) Various concentrations of Ipt decreased the release of TNF-α induced by MPP+ in astrocytes. (c) KATP channel blocker 5-hydroxydecanoate (5-HD) abolished the inhibitory effect of Ipt on the release of TNF-α induced by MPP+ (150 µM). Data are presented as the mean ± S.E.M. of three individual experiments. * p<0.05, ** p<0.01 vs. control groups; † p<0.05, ‡ p<0.01 vs. 150 µM MPP+ -treated alone groups; † p<0.05 vs. Ipt + MPP+ -treated groups; $ p<0.05 vs. diazoxide (DZ)+MPP+ -treated groups.

Figure 6. Western blotting analysis of the effect of iptakalim (Ipt) on expression of p38 MAPK phosphorylation induced by MPP+ in astrocytes. (a) MPP+ triggered an increased p38 MAPK phosphorylation, indicative of p38 MAPK activation. (b) Pretreatment with Ipt suppressed MPP+-induced p38 MAPK phosphorylation. Data are presented as the mean ± S.E.M. of three individual experiments. * p<0.05, ** p<0.01 vs. control groups; † p<0.05, ‡ p<0.01 vs. 150 µM MPP+ -treated alone groups; † p<0.05 vs. Ipt + MPP+ -treated groups; $ p<0.05 vs. diazoxide (DZ)+MPP+ -treated groups.
Iptakalim inhibits astrocyte activation

exerts neuroprotective effects in doses not affecting blood pressure (Wu et al., 2005). Therefore, Ipt is a promising compound that may protect neurons against neurodegenerative diseases. Further, Ipt may be the lead compound for medicinal chemistry refinements to synthesize drugs that have selective effects on glial K\(_{ATP}\) channels.

In conclusion, the present study demonstrates that inhibition of astrocyte activation and subsequent release of pro-inflammatory factors by opening mitochondrial K\(_{ATP}\) channels in astrocytes may be a prospective strategy for neuroprotection. As a novel and blood–brain barrier permeable K\(_{ATP}\) channel opener, Ipt can inhibit MPP\(^+\)-induced astrocyte activation thereby protecting against the degeneration of dopaminergic neurons.

Acknowledgements

These studies were supported by grants from the National Natural Science Foundation of China (No. 30625038 and No. 30572172), the Key Project of Natural Science Foundation of Jiangsu Educational Department (No. 05KJA31014 and No. 06KJA31029), and the National Key Basic Research Programme of China (No. 2006CB500706).

Statement of Interest

None.

References


Marchetti B, Abbraccchio MP (2005). To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? Trends in Pharmacological Sciences 26, 517–525.


