Iptkalim inhibits cocaine challenge-induced enhancement of dopamine levels in nucleus accumbens and striatum of rats by up-regulating Kir6.1 and Kir6.2 mRNA expression[1]

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KEY WORDS ATP-sensitive potassium channel opener; iptkalim; dopamine; glutamates; ATP-sensitive potassium channel subunits; gene expression regulation; messenger RNA

ABSTRACT

AIM: To investigate the effect and mechanism of novel ATP-sensitive potassium channel opener (KCO) iptkalim (IPT) on acute and cocaine challenge-induced alterations in the levels of dopamine (DA) and glutamate (Glu) from nucleus accumbens (NAc), striatum, and prefrontal cortex (PFC) in rats. **METHODS:** The levels of DA and Glu were assayed using high performance liquid chromatography (HPLC) combined with amperometric and fluorescent detection, respectively. The mRNA levels of Kir6.1, Kir6.2, SUR1, and SUR2 were measured by semiquantitative reverse transcription polymerase chain reaction (RT-PCR). **RESULTS:** IPT did not affect acute cocaine (30 mg/kg, ip)-induced elevations in either DA levels from NAc and striatum or Glu levels from NAc and PFC. An acute cocaine challenge (30 mg/kg, ip) on d 21 after withdrawal caused an elevation in DA levels in NAc and striatum. Moreover, the same treatment also increased Glu levels in PFC and NAc of cocaine-pretreated rats. Repeated IPT injections reversed cocaine challenge-induced DA increase in NAc and striatum. Cocaine challenge increased Kir6.1 and Kir6.2 mRNA expression in striatum and NAc and only elevate Kir6.2 expression in PFC in both cocaine-pretreated rats and rats pretreated with IPT plus cocaine. Moreover, expression of Kir6.1 and Kir6.2 mRNA was augmented in rats pretreated with IPT plus cocaine compared to rats pretreated with cocaine alone. No significant change was found in the SUR1 and SUR2 expression of all four groups. **CONCLUSION:** IPT inhibited cocaine challenge-induced enhancement of DA levels in NAc and striatum by up-regulating Kir6.1 and Kir6.2 mRNA expression.

INTRODUCTION

Substantial evidence indicates that meso-corticolimbic system plays an important role in cocaine addiction. The mesocorticolimbic system comprises dopaminergic cells in VTA and their projections to both PFC and NAc of the rats[1]. Cocaine is among the most commonly abused psychostimulants and inhibits all three monoamine transporters – DA, serotonin, and norepinephrine, especially for dopamine transporter (DAT), thereby potentiating monoaminergic transmission. ATP-sensitive potassium (K_{ATP}) channels,

[1] Project supported by the State Key Project of New Drug Research and Development Funds of China (No 969010101) and the National Natural Science Foundation of China (No 39970846).
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Received 2003-01-06 Accepted 2003-03-28

which are distributed widely in the brain and directly couple the metabolic state of a cell to its electrical activity, comprise heteromultimers of a pore-forming subunit of the inward rectifier (Kir) family and a regulatory sulfonylurea receptor (SUR) subunit[2]. Two Kir genes (Kir6.1 and Kir6.2) and two SUR genes [SUR1 and SUR2A/SUR2B (different isoforms of SUR2)] have been identified. The pore is thought to confer ATP inhibition and determine conductance, whereas the SUR is considered the primary target for sulfonylureas, KCO, and nucleotide diphosphonate (NDP). Moreover, K_{ATP} channels may modulate DA and Glu transmission and theoretically are helpful to the treatment of drug addiction[3,4].

We developed a new compound iptkalim hydrochloride, which has been demonstrated to be a novel K_{ATP} channel opener by pharmacological, electrophysiological, biochemical studies and receptor binding test[4]. Notably, IPT is only one of the KCOs that can pass through blood-brain-barrier and so as to make it possible to investigate its effects systematically[4]. Previous studies have indicated that IPT was relevant to the regulation of DA and Glu release[4], the very two neurotransmitters playing a critical role in cocaine addiction. But there is no report on the relationship between K_{ATP} channels and cocaine addiction hitherto. In the present study, we investigated the effects of IPT on cocaine-induced changes of DA and Glu transmission in striatum, PFC, and NAc so as to determine K_{ATP} as a potential target for treating cocaine addiction. To demonstrate the mechanism whereby IPT imposed on DA and Glu transmission, the expression of K_{ATP} channel subunits (Kir6.1, Kir6.2, SUR1 and SUR2) of rats receiving acute, repeated cocaine injections and repeated IPT plus cocaine injections was also studied.

MATERIALS AND METHODS

Animals and reagents Male Sprague-Dawley rats (250 g±20 g for acute experiment, 180 g±20 g for chronic experiment, Grade II, Certificate No 2001-0006) were housed at 20-22 °C on a 12 h:12 h light: dark schedule, with free access to food and water. Cocaine hydrochloride (99.9 %) was provided by Qinghai Pharmaceutical Factory. Iptkalim hydrochloride (99.9 %) was synthesized and provided by the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences of China. *O*-Phthaldialdehyde was purchased from Fluka AG CH-9740 Buchs (Switzerland). Both 1-heptanesulfonic and 2, 5-dihydroxybenzoic acid (2, 5-DHBA) were purchased from Sigma Chemical Co (USA). RNasin, dNTP primer and M-MLV reverse transcriptase were obtained from Promega Corporation (USA). Trizol reagent was purchased from Gibco Industries, Inc (USA). Oligo $(dT)_{15}$ and *Taq* DNA polymerase were purchased from Nanjing Sunshine Biotechnology Ltd. All other reagents were obtained from standard commercial sources.

Medication In the acute experiment, rats of four groups received saline (2 mL/kg), cocaine (30 mg/kg) and IPT (0.75, 1.5 mg/kg) followed by cocaine (30 mg/kg) 5 min later, respectively. For the chronic experiment, all rats were assigned to one of four treatment groups: 1) rats receiving a daily saline injection for 6 d, followed by a challenge dose of saline (SAL/SAL group); 2) rats receiving a daily saline injection for 6 d, followed by a challenge dose of cocaine hydrochloride (SAL/COC group); 3) rats receiving 15 mg/kg cocaine on the first day and 30 mg/kg cocaine on the following 5 d, followed by a challenge dose of cocaine (COC/COC group); 4) rats receiving IPT 0.75 mg/kg followed by 15 mg/kg cocaine 5 min later on the first day and IPT 0.75 mg/kg plus cocaine 30 mg/kg on the next 5 d, followed by a challenge dose of cocaine (IPT +COC/COC group). Challenge cocaine injections were administered at 30 mg/kg on d 21 and challenge doses of saline injections were also administered on d 21. All injections were given intraperitoneally.

Quantification of DA and Glu The concentration of DA was determined using HPLC with amperometric detection[5]. The rats were decapitated 30 min after the last injection in both acute and chronic experiment. The striatum, NAc, and PFC were removed rapidly on ice and homogenized in ice-cold solution A consisting of $HClO_4$ 0.1 mo1/L, edetic acid 0.1 mmo1/L, and $Na_2S_2O_3$ 0.15 % (pH 8.6). The homogenate was centrifuged at 10 000×*g* for 15 min to get the supernatant (S1). After Al_2O_3 was washed to neutral point by solution B (Tris-HCl 0.1 mo1/L, edetic acid 0.1 mmol/L, $Na_2S_2O_3$ 0.15 %, pH 8.6), S1 (100 μL), solution C 100 μL (Tris-HCl 1 mol/L, edetic acid 0.1 mmo1/L, $Na_2S_2O_3$ 0.15 %, pH 8.9) and 10 μL DHBA 10 mmo1/L were added into the test tubes containing Al_2O_3. Consequently, the mixture was centrifuged at 5000×*g* for 5 min. The supernatant was discarded and the precipitate was washed with 200 μL distilled water twice. Then the supernatant was discarded again. Solution A (100 μL) was added into the precipitate and centrifuged

at 5000×*g* for 5 min. All the centrifugations were done at 4 °C. The resulting supernatant (S2) was frozen at -70 °C for later HPLC analysis. HPLC-amperometric detector system consisted of an amperometric detector LC-4C, a reverse phase C_{18} column (Ultrasphere ODS 4.6 mm×250 mm, 5 μm, Japan), a chromatograph interface DA-5. The working electrode potential was set at 0.75 V. Samples were detected at 37 °C using flow rate of 1.0 mL/min. The mobile phase consisted of citrate 0.1 mo1/L, $Na_2HPO_4 \cdot 12H_2O$ 0.075 mo1/L, edetic acid 0.1 mo1/L, *1*-heptanesulfonic acid 1.0 mmo1/L and methanol 10 %; pH was adjusted to 3.9.

The concentration of Glu was determined using HPLC with fluorescent detection[6]. NAc and PFC were homogenized in ice-cold 0.4 mol/L $HClO_4$ solution and centrifuged at 10 000×*g* for 15 min. The supernatant (S3) was neutralized with 0.75 volumes of $KHCO_3$ 2.0 mo1/L. After being recentrifuged at 4000×*g* for 5 min, supernatant (S4) was frozen at -70 °C for later HPLC analysis. All the centrifugations were done at 4 °C. HPLC-fluorescent detector system consisted of Shimadzu HPLC, a reverse phase C_{18} column (Ultrasphere ODS 4.6 mm×250 mm, 5 μm, Japan), a fluorescence HPLC monitor RF-530 and a liquid chromatograph LC-6A. The emission wavelength was set at 425 nm and the excitation wavelength at 338 nm. The mobile phase consisted of $Na_2HPO_4 \cdot 12H_2O$ 0.1 mo1/L, edetic acid 0.1 mmo1/L, and methanol 30 %; pH was adjusted to 6.04. The pre-column derivatization reaction was made by adding equivalent volume of sample S4 to derivative fluid containing *o*-phthaldialdehyde 20 mmol/L, β-mercaptoethanol 2 mmo1/L, tetraborate 25 mmol/L, and 50 % methanol (pH 9.6) for 3 min. The reaction solutions were used to assay the Glu at 37 °C using flow rate of 0.8 mL/min.

RNA preparation and semiquantitative reverse transcription polymerase chain reaction (RT-PCR) Semiquantitative RT-PCR with β-actin as an internal control was performed to examine the expression of messenger RNA for the K_{ATP} subunits, Kir6.1, Kir6.2, SUR1, and SUR2 in PFC, striatum, and NAc[7]. The rats were decapitated 30 min after the last injection and the striatum, NAc, and PFC were removed rapidly on ice. Immediately upon removal, tissue samples were rinsed in sterile saline, snap-frozen in liquid nitrogen, and stored at -70 °C until RNA extraction. RNA was isolated from frozen tissue by homogenization in Trizol Reagent. For cDNA synthesis, 20 μL reverse transcription mixture containing total RNA 1 μg , dNTP 10 mmol/L, oligo $(T)_{15}$ primer 100 pg, RNasin 20 IU and M-MLV reverse transcriptase 200 IU was incubated at 42 °C for 1 h and then the reverse transcriptase was inactivated by heating the reaction mixture to 95 °C for 10 min.

PCR amplification was carried out with 1 μL cDNA product in a 20 μL reaction volume containing 3 pmol of each specific oligonucleotide primer (Tab 1), dNTP 10 mmol/L, and *Taq* DNA polymerase 1.5 IU. For all of the reactions, preliminary experiments were performed to determine the number of PCR cycles at which saturation occurred, and the experiments mentioned were carried out with a number of cycles that preceded saturation. PCR cycles were as follows. Kir 6.1 primers: 94 °C, 4 min; 94 °C, 30 s; 57 °C, 30 s; 72 °C, 30 s; 72 °C, 5 min (27 cycles). Kir 6.2 primers: 94 °C, 4 min; 94 °C, 30 s; 64 °C, 30 s; 72 °C, 30 s; 72 °C, 5 min (33 cycles). SUR1 primers: 94 °C, 4 min; 94 °C, 30 s; 64 °C, 30 s; 72 °C, 30 s; 72 °C, 5 min (29 cycles). SUR2 primers: 94 °C, 4 min; 94 °C, 30 s; 59 °C, 30 s; 72 °C, 30 s; 72 °C, 5 min (35 cycles). β-Actin primers: 94 °C, 4 min; 94 °C, 30 s; 57 °C, 30 s; 72 °C, 30 s; 72 °C, 5 min (23 cycles). The absence of contamination was routinely checked by RT-PCR on negative control samples in which either the RNA samples were replaced with sterile water or the M-MLV reverse transcriptase was omitted. No specific cDNA was obtained using these conditions. PCR products (5 μL) were separated by electrophoresis on a 2 % agarose gel and visualized after ethidium bromide staining over UV light. This semiquantitative measure was expressed as ratios compared with β-actin.

Data analysis All values were presented as mean±SD. Student's two-tailed *t*-test was used for comparison and the difference was considered significant if $P<0.05$.

RESULTS

Effects of iptkalim on acute cocaine-induced changes of DA and Glu levels from NAc, striatum, and PFC in rats Acute injection of cocaine increased the levels of DA in striatum and NAc by 23.6 % and 25.1 %, respectively($P<0.05$). Pretreatment with IPT 0.75, 1.5 mg/kg tended to decrease cocaine-induced elevation of DA in striatum and NAc, but the diffference was not significant compared to rats receiving acute cocaine injection ($P>0.05$). Acute injection of cocaine resulted in a significant elevation of Glu levels in NAc

Tab 1. Specific primer pairs.

Primer name	Primer sequence	Product size/bp	GenBank Acc No
Kir6.1	Sense: GAGTGAACTGTCGCACCAGA Antisense: CGATCACCAGAACTCAGCAA	247	D 42145
Kir6.2	Sense: TCCAACAGCCCGCTCTAC Antisense: GATGGGGACAAAACGCTG	167	D 50581
SUR1	Sense: GGAGCAATCCAGACCAAGAT Antisense: AGCCAGCAGAATGATGACAG	248	L 40624
SUR2	Sense: ACCTGCTCCAGCACAAGAAT Antisense: TCTCTTCATCACAATGACCAGG	144	D 83598
β-actin	Sense: CACGATGGAGGGGCCGGACTCATC Antisense: TAAAGACCTCTATGCCAACACAGT	240	NM_031144

and PFC by 18.9 % and 16.0 %, respectively (P<0.05). Pretreatment with IPT showed the tendency of decreasing cocaine-evoked increase of Glu, but the diffference was not significant (P>0.05). Neither acute cocaine injection nor coinjection of IPT and cocaine could change levels of Glu in striatum (Tab 2).

Effects of iptkalim on cocaine challenge-induced changes of DA and Glu levels from NAc, striatum, and PFC in rats After a cocaine challenge on d 21, the levels of DA in striatum and NAc in saline-pretreated rats (SAL/COC) were increased by 23.4 % and 33.4 % respectively compared to controls (SAL/SAL). DA levels in striatum and NAc from cocaine-pretreated rats (COC/COC) were increased by 50.8 % and 73.0 % respectively compared to controls. Moreover, cocaine challenge-induced elevation in DA levels in cocaine-pretreated rats was greater than that in saline-pretreated rats (P<0.05). DA levels in striatum and NAc from rats pretreated with IPT plus cocaine were decreased compared to rats pretreated with cocaine alone (P<0.01), and difference was not significant compared with controls. The levels of Glu in PFC and NAc in cocaine-pretreated rats were increased by 38.2 % and 34.0 % respectively after challenge compared with controls. Pretreatment of IPT plus cocaine tended to decrease the elevation of Glu levels from PFC and NAc in rats after cocaine challenge, but the difference was not significant compared with rats pretreated with cocaine alone. The difference of Glu levels in striatum was not significant among any groups (Tab 3).

Tab 2. Effects of iptkalim (IPT) on acute cocaine 30 mg/kg-induced changes of dopamine and glutamate levels from nucleus accumbens (NAc), striatum, and prefrontal cortex (PFC) in rats. Mean±SD. n= 9. ^{a}P>0.05, ^{b}P<0.05 *vs* control group. ^{d}P>0.05 *vs* cocaine group.

Groups	DA/nmol· g^{-1} (wet weight)	Glu/μmol· g^{-1} (wet weight)
Striatum		
Control	65±12	9.3±2.3
Cocaine	80±13^{b}	9.3±1.6^{a}
COC+IPT 0.75 mg/kg	71±12^{ad}	9.4±1.7^{ad}
COC+IPT 1.5 mg/kg	70±14^{ad}	9.7±1.1^{ad}
NAc		
Control	30±8	4.2±0.5
Cocaine	37±3^{b}	5.0±0.5^{b}
COC+IPT 0.75 mg/kg	34±8^{ad}	4.7±0.6^{ad}
COC+IPT 1.5 mg/kg	33±8^{ad}	4.7±0.1^{ad}
PFC		
Control	-	11.0±1.8
Cocaine	-	12.7±1.7^{b}
COC+IPT 0.75 mg/kg	-	11.6±1.6^{ad}
COC+IPT 1.5 mg/kg	-	11.4±1.2^{ad}

Expression of Kir6.1, Kir6.2, SUR1, and SUR2 mRNA from PFC, striatum, and NAc in rats The specificity of RT-PCR products was confirmed by the results of agarose gel electrophoresis that yielded single product bands of the expected size. Negligible primer-dimer bands were produced during the amplification. Acute cocaine injection did not result in any changes in the expression of Kir6.1 and Kir6.2 mRNA in PFC, NAc, and striatum of rats. On d 21 after rats received con-

Tab 3. Effects of iptkalim (IPT) 0.75 mg/kg on cocaine 30 mg/kg challenge-induced changes of dopamine and glutamate levels from NAc, striatum, and PFC in rats. *n*=9. Mean±SD. [a]*P*>0.05, [b]*P* <0.05, [c]*P* <0.01 *vs* control group. [d]*P*>0.05, [e]*P*<0.05, [f]*P*<0.01 *vs* COC/COC group. [h]*P*<0.05 *vs* SAL/COC group.

Groups	DA/nmol· g^{-1} wet weight	Glu/μmol· g^{-1} wet weight
Striatum		
SAL/SAL	65±12	8.6±0.4
SAL/COC	80±11[b]	8.6±0.5
COC/COC	98±13[ch]	8.8±0.6[a]
IPT+COC/COC	62±13[af]	9.0±0.4[ad]
NAc		
SAL/SAL	25±5	4.5±0.8
SAL/COC	33±8[b]	5.4±0.9[b]
COC/COC	42±5[ch]	6.0±0.6[ch]
IPT+COC/COC	22±7[af]	5.7±0.8[bd]
PFC		
SAL/SAL	-	7.2±0.7
SAL/COC	-	8.3±1.3[b]
COC/COC	-	10.0±0.7[ch]
IPT+COC/COC	-	9.7±1.0[bd]

tinuous cocaine injections or IPT plus cocaine injections, a cocaine challenge resulted in higher Kir6.1 and Kir6.2 mRNA expression in striatum and NAc and elevated Kir6.2 expression in PFC compared to controls. Moreover, pretreatment with IPT plus cocaine caused a significant increase in expression of Kir6.1 and Kir6.2 mRNA compared to rats pretreated with cocaine alone. No significant change was found in the SUR1 and SUR2 expression of all four groups (Fig 1).

DISCUSSION

Considerable studies have demonstrated that DA is one of the essential neurotransmitters in the acute reinforcing effects of cocaine and neurobiological substrates for the reinforcing properties is associated with both mesolimbic dopamine system and nigrostriatal dopamine system[8,9]. The present result that acute injection of cocaine increased DA levels in striatum and NAc significantly supports this view. We also found that a challenge dose of cocaine significantly increased DA levels in NAc and striatum in cocaine-pretreated rats. The results are consistent with the idea that the augment of DA transmission in NAc and striatum plays a critical role for the development of cocaine addiction after the rats were administered repeatedly[10]. Notably, DA levels in rats pretreated with IPT plus cocaine after cocaine challenge were significantly decreased compared to the rats pretreated with cocaine alone and the difference was not significant compared with controls. These results indicate that K_{ATP} channels are involved in the development of cocaine addiction.

K_{ATP} channels couple cellular metabolic status to changes in transmembrane potassium fluxes and cellular excitability and play an important regulatory role under both physiological and pathological conditions[2]. K_{ATP} channel subunits are widely expressed throughout different brain regions such as cerebral cortex, hippocampus, striatum, substantial nigra, and nucleus accumbens[2]. These regions are closely associated with cocaine-induced neuroadaptation. It is reported that K_{ATP} channels may regulate neurotransmitter release and may serve a protective role in reducing levels of excitatory amino acids (EAAs) during brain ischemia and anoxia[11]. However, little is known about the effect of K_{ATP} channel activation on cocaine addiction. Present data show that coinjection of IPT plus cocaine exhibited the tendency of decreasing acute cocaine-induced DA level enhancement in striatum and NAc. It may result from the outflow of K^+ currents and hyperpolarization of presynaptic terminals caused by the opening of K_{ATP} channels after IPT combined with SUR1, which consequently decreased DA elevation and partly counteracted acute cocaine-induced DA level enhancement. However, the effect of IPT activating K_{ATP} channel may be weaker than that of cocaine increasing DA levels and so DA levels in NAc and striatum could not be reduced significantly. Interestingly, pretreatment with IPT could reverse the augment of DA levels induced by cocaine challenge in cocaine-pretreated rats. Furthermore, the up-regulation of Kir6.1 and Kir6.2 mRNA expression in striatum and NAc of rats pretreated with IPT plus cocaine may account for the reversing. Theoretically, the up-regulation of Kir6.1 and Kir6.2 mRNA expression probably increases the number of Kir6.1 and Kir6.2 subunits. Correspondingly, IPT opened more K_{ATP} channels and consequently cocaine challenge-induced DA augment was reversed.

Glu is an important regulator of DA cell activity in central nervous system. DA and Glu projections often innervate common postsynaptic targets[12]. Some recent investigations have established that the develop-

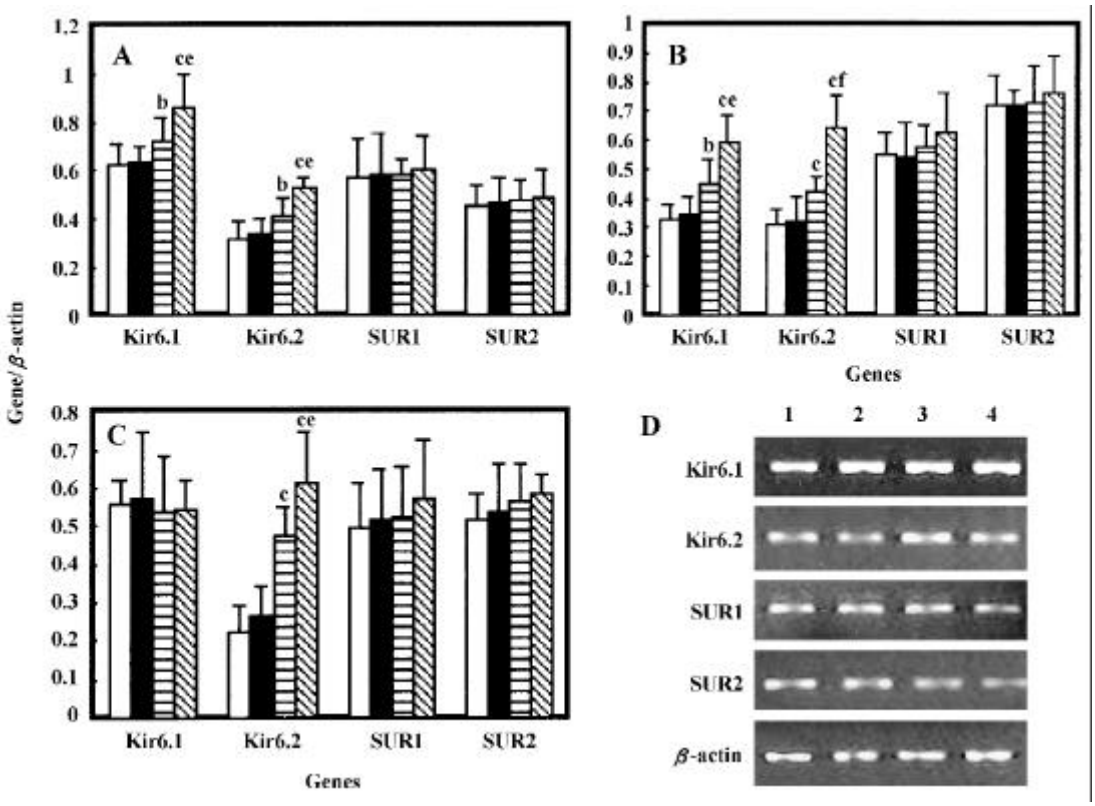

Fig 1. The ratios of Kir6.1/b-actin, Kir6.2/b-actin, SUR1/b-actin, and SUR2/b-actin were used to express the mRNA levels of Kir6.1, Kir6.2, SUR1, and SUR2 in rat 30 mg/kg striatum (A), NAc (B), and PFC (C), respectively. □: SAL/SAL; ■: SAL/COC; ▤: COC/COC; ▨: IPT+COC/COC; D) Representative of results obtained from agarose gel electrophoresis RT-PCR products. 1: SAL/SAL; 2: SAL/COC; 3: COC/COC; 4: IPT+COC/COC. *n*=8 for each group. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* COC/COC group.

ment and maintenance of behavioral sensitization requires alterations in EAA transmission[12]. In our study, the result that acute cocaine injection resulted in a significant increase of Glu in NAc and PFC but not Glu level in striatum has been reported. Agreed with a critical role for EAAs in sensitization[13], a cocaine challenge resulted in an increase of Glu levels in PFC and NAc of cocaine-pretreated rats than those of saline-pretreated rats. In addition, pretreatment with IPT plus cocaine tended to decrease Glu levels in PFC and NAc. It is supposed that IPT decreased the cytoplasm Ca^{2+} concentration and consequently inhibited the elevation of Glu levels after binding to SUR. Obviously, the capacity of IPT decreasing DA levels is stronger than Glu. Some investigations have confirmed that K_{ATP} channels with SUR2 showed a lower sulfonylure sensitivity compared with the channels with SUR1[14]. Thus, it is hypothesized that K_{ATP} channels around glutamatergic inputs in NAc and outputs in PFC may express more SUR2 than SUR1, which makes them less sensitive to IPT and may be the reason for pretreatment with IPT failing to reverse the enhancement of Glu levels after cocaine challenge. Another possibility is the dissimilar mechanism whereby IPT inhibited the elevation of DA and Glu levels. Perhaps some other factors play more important roles than K_{ATP} does in regulating Glu levels. However, there is little evidence to support this hypothesis and it remains to be further studied.

In conclusion, the results in the present study suggest that as a novel KCO, IPT can reverse the cocaine challenge-induced enhancement of DA levels by up-regulating Kir6.1 and Kir6.2 mRNA expression in striatum and NAc.

REFERENCES

1 Chang JY, Janak PH, Woodward DJ. Neuronal and behavioral correlations in the medial prefrontal cortex and nucleus accumbens during cocaine self-administration by rats. Neuroscience 2000; 99: 433-43.

2 Liss B, Roeper J. Molecular physiology of neuronal K-ATP channels. Mol Membr Biol 2001; 18: 117-27.

3 Johnson DW, Eodice P, Winterbottom H, Mokler DJ. Decreased accumbens dopamine release after cocaine challenge

in behavioral sensitized female rats. Pharmacol Biochem Behav 2000; 65: 659-64.

4 Wang H. Pharmacological characteristics of the novel antihypertensive drug iptakalim hydrochloride and its molecular mechanisms. Drug Dev Res 2002; 54: 240-1.

5 Hu G, Wu YM, Jin GZ. (-)-Stepholidine enhances K^+ depolarization-induced activation of synaptosomal tyrosine 3-monooxygenase from rat striatum. Acta Pharmacol Sin 1997; 18: 49-54.

6 Tang XC, Rao MR, Hu G, Wang H. Alterations of amino acid levels from striatum, hippocampus, and cerebral cortex induced by global cerebral ischemia in gerbil. Acta Pharmacol Sin 2000; 21: 819-23.

7 Wang RG, Zhu XZ. Expression of angiopoietin-2 and vascular endothelial growth factor in mice cerebral cortex after permanent focal cerebral ischemia. Acta Pharmacol Sin 2002; 23: 405-11.

8 Koob GF. Circuits, drugs, and drug addiction. Adv Pharmacol 1998; 42: 978-82

9 Zhang Y, Loonam TM, Noailles PA, Angulo JA. Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat brain during acute, chronic, and early withdrawal conditions. Ann N Y Acad Sci 2001; 937: 93-120.

10 Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Rev 1997; 25: 192-216.

11 Sun XD, Lee EW, Wong EH, Lee KS. ATP-sensitive potassium channels in freshly dissociated adult rat striatal neurons: activation by metabolic inhibitors and the dopaminergic receptor agonist qunipirole. Pflügers Arch 2000; 440: 530-47.

12 Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog Neurobiol 1998; 54: 679-720.

13 Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 1996; 16: 1550-60.

14 Meyer M, Chudziak F, Schwanstecher C, Schwanstecher M, Panten U. Structural requirements of sulphonylureas and analogues for interaction with sulphonylurea receptor subtypes. Br J Pharmacol 1999; 128: 27-34.