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Toxicology in Vitro 21 (2007) 656–664
Toxicology in Vitro
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# Inhibition by cocaine of G protein-activated inwardly rectifying $K^+$ channels expressed in *Xenopus* oocytes

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Received 3 August 2006; accepted 5 January 2007
Available online 20 January 2007

**Abstract**

Cocaine, a commonly abused psychostimulant, interacts with not only transporters for dopamine, serotonin and norepinephrine but also several receptors and channels. However, the molecular mechanisms underlying the various effects of cocaine remain to be clarified. Using the *Xenopus* oocyte expression assay, we investigated the effects of cocaine on G protein-activated inwardly rectifying $K^+$ (GIRK) channels, which regulate neuronal excitability and the heart rate. In oocytes injected with mRNAs for GIRK1/GIRK2, GIRK2 or GIRK1/GIRK4 subunits, cocaine reversibly reduced basal GIRK inward currents. The inhibition by cocaine at the toxic levels was concentration-dependent, but voltage-independent and time-independent during each voltage pulse. However, methylphenidate, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) at their toxic concentrations had little effect on the channels. Additionally, Kir1.1 and Kir2.1 channels were insensitive to all of the drugs. The inhibition by cocaine, which exists mainly in a protonated form at pH 7.4, was not affected by extracellular pH 9, at which the proportion of the uncharged form increases, suggesting the inhibition by both forms with similar effectiveness, and at physiological pH the effect being predominantly due to the protonated cocaine. Our results suggest that inhibition of GIRK channels by cocaine may contribute to some of its toxic effects.
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*Keywords:* Cocaine; Psychostimulant; GIRK channel; Kir channel; *Xenopus* oocyte

## 1. Introduction

Cocaine is well known as an abused psychostimulant drug (O'Brien, 2001). Also, cocaine clinically exerts its local anesthetic effect by blocking voltage-gated $Na^+$ channels, but its clinical use has decreased because of toxicity, namely, cardiovascular, neurologic and psychiatric complications such as arrhythmias, seizures and delirium, and its properties leading to addiction and abuse (O'Brien, 2001; White and Lambe, 2003). Cocaine binds transporters for dopamine, serotonin and norepinephrine (DAT, SERT and NET, respectively), and inhibits the reuptake of monoamines (O'Brien, 2001). The resulting potentiation of the monoaminergic neurotransmission systems is thought to play an important role in the physiological and behavioral effects (O'Brien, 2001). It has also been shown that cocaine interacts with several receptors and channels, namely, nicotinic acetylcholine (Karpen et al., 1982), γ-aminobutyric acid type A ($GABA_A$), glycine (Ye et al., 1999; Ren et al., 1999), serotonin type 3 ($5\text{-}HT_3$) (Breitinger et al., 2001),

*Abbreviations:* DAT, dopamine transporter; $GABA_A$, γ-aminobutyric acid type A; GIRK, G protein-activated inwardly rectifying $K^+$ channel; hK, high-potassium; 5-HT, serotonin; Kir channel, inwardly rectifying $K^+$ channel; MAP, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MPH, methylphenidate; ND96, high-sodium; NET, norepinephrine transporter; $n_H$, Hill coefficient; SERT, serotonin transporter.

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doi:10.1016/j.tiv.2007.01.009

muscarinic (Sharkey et al., 1988b) and σ (Sharkey et al., 1988a) receptors, and voltage-gated $Ca^{2+}$ and $K^+$ channels (Kimura et al., 1992; Premkumar, 2005). These actions of cocaine with relatively low potencies might also be involved in the molecular mechanisms underlying some of the various pharmacological effects of cocaine.

G protein-activated inwardly rectifying $K^+$ (GIRK) channels (also known as Kir3 channels) are members of a family of inwardly rectifying $K^+$ (Kir) channels that includes seven subfamilies (Reimann and Ashcroft, 1999). Four GIRK channel subunits have been identified in mammals (Kubo et al., 1993b; Lesage et al., 1995; Krapivinsky et al., 1995). Neuronal GIRK channels are predominantly heteromultimers composed of GIRK1 and GIRK2 subunits in most brain regions or homomultimers composed of GIRK2 subunits in the substantia nigra and ventral tegmental area (Kobayashi et al., 1995; Lesage et al., 1995; Liao et al., 1996), whereas atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4 subunits (Krapivinsky et al., 1995). A variety of G-protein-coupled receptors, such as $M_2$ muscarinic, $\alpha_2$ adrenergic, $D_2$ dopaminergic, 5-$HT_{1A}$, μ-, δ- and κ-opioid, nociceptin/orphanin FQ and $A_1$ adenosine receptors, activate GIRK channels through the direct action of G protein βγ-subunits released from $G_{i/o}$ proteins (Dascal, 1997; Kobayashi and Ikeda, 2006). In addition, ethanol activates GIRK channels independently of G-protein-coupled signaling pathways (Kobayashi et al., 1999). Activation of GIRK channels causes membrane hyperpolarization, and thus the channels play an important role in reducing neuronal excitability and the heart rate (Dascal, 1997; Signorini et al., 1997; Bettahi et al., 2002). Previously, it was shown that bupivacaine and lidocaine, local anesthetics, inhibited cloned GIRK channels expressed in *Xenopus* oocytes (Zhou et al., 2001). Cocaine, which is structurally related to these local anesthetics, inhibited muscarinic $K^+$ currents in ferret cardiac myocytes (Xiao and Morgan, 1998), suggesting inhibition of GIRK channels in the heart. In the present study, we investigated the effects of cocaine on brain-type and cardiac-type GIRK channels composed of cloned GIRK subunits by using the *Xenopus* oocyte expression assay. Furthermore, the effects of some of chemically different psychostimulants: methamphetamine (MAP), 3,4-methylenedioxymethamphetamine (MDMA) and methylphenidate (MPH), on GIRK channels were also examined.

## 2. Materials and methods

### 2.1. Preparation of specific mRNAs

Plasmids containing the entire coding sequences for the mouse GIRK1, GIRK2, and GIRK4 channel subunits were obtained by using the polymerase chain reaction method as described previously (Kobayashi et al., 1995, 1999). In addition, cDNAs for rat Kir1.1 in pSPORT and mouse Kir2.1 in pcDNA1 were provided by Dr. Steven C. Hebert and Dr. Lily Y. Jan, respectively (Ho et al., 1993; Kubo et al., 1993a). These plasmids were linearized by digestion with the appropriate enzyme as described previously (Ho et al., 1993; Kubo et al., 1993a; Kobayashi et al., 1999); and the specific mRNAs were synthesized *in vitro* by using the mMESSAGE mMACHINE™ *in Vitro* Transcription Kit (Ambion, Austin, TX, USA).

### 2.2. Electrophysiological analysis

Adult female *Xenopus laevis* frogs were purchased from Nippon Bio-supply Center (Tokyo, Japan) and maintained in the laboratory. Several ovarian lobes were surgically removed from the frogs. The frogs were cared for and treated humanely in accordance with our institutional animal experimentation guidelines. Individual oocytes (Stages V and VI) were manually dissected from the ovary and maintained in Barth's solution (Kobayashi et al., 2004). *Xenopus laevis* oocytes were injected with mRNA(s) for GIRK1/GIRK2 or GIRK1/GIRK4 combinations (each ~0.6 ng), GIRK2 (~5 ng), Kir1.1 (~1 ng) or Kir2.1 (~0.5 ng). The oocytes were incubated at 19 °C in Barth's solution after treatment with 0.7 $mg\,ml^{-1}$ collagenase for 1 h, and defolliculated by manual dissection. Whole-cell currents of the oocytes were recorded from 3 to 6 days after the injection with a conventional two-electrode voltage clamp amplifier, GeneClamp 500 (Axon Instruments, Foster City, CA, USA) with a MacLab A/D interface (ADInstruments Pty Ltd., Castle Hill, NSW, Australia) (Kobayashi et al., 1999, 2004). The membrane potential was held at −70 mV, unless otherwise specified. Microelectrodes were filled with 3 M KCl. The oocytes were placed in a 0.05 ml narrow chamber and superfused continuously with a high-potassium (hK) solution (composition in mM: KCl 96, NaCl 2, $MgCl_2$ 1, $CaCl_2$ 1.5 and HEPES 5, pH 7.4, adjusted with KOH) or with a high-sodium (ND96) solution (composition in mM: NaCl 96, KCl 2, $MgCl_2$ 1, $CaCl_2$ 1.5 and HEPES 5, pH 7.4, adjusted with NaOH). In the hK solution, the $K^+$ equilibrium potential was close to 0 mV; and inward K+ current flow through Kir channels was observed at negative holding potentials (Kobayashi et al., 2006). Data were fitted to a standard logistic equation by using KaleidaGraph (Synergy Software, Reading, PA, USA) for analysis of concentration-response relationships. The $EC_{50}$ value, which is the concentration of a drug that produces 50% of the maximal effect for that drug; the $IC_{25}$ and $IC_{50}$ values, which are the concentrations of a drug that reduces control current responses by 25% and 50%, respectively; and the Hill coefficient ($n_H$) were obtained from the concentration-response relationships.

### 2.3. Drugs

Cocaine hydrochloride, methamphetamine hydrochloride (MAP) and methylphenidate hydrochloride (MPH) were obtained from Takeda Chemical Industries Ltd. (Osaka, Japan), Dainippon Pharmaceutical (Osaka, Japan) and Sigma (St. Louis, MO, USA), respectively; and 3,

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