

Decreased Cocaine Self-Administration in Kir3 Potassium Channel Subunit Knockout Mice

Andrew D Morgan¹, Marilyn E Carroll¹, Annemarie K Loth¹, Markus Stoffel² and Kevin Wickman^{*3}

¹Department of Psychiatry, University of Minnesota, Minneapolis, MN, USA; ²Laboratory of Metabolic Diseases, The Rockefeller University, New York, NY, USA; ³Department of Pharmacology, University of Minnesota, Minneapolis, MN, USA

Multiple G protein-linked neurotransmitter systems have been implicated in the behavioral effects of cocaine. While actions of certain neurotransmitter receptor subtypes and transporters have been identified, the role of individual G protein-regulated enzymes and ion channels in the effects of cocaine remains unclear. Here, we assessed the contribution of G protein-gated, inwardly rectifying potassium (Kir3/GIRK) channels to the locomotor-stimulatory and reinforcing effects of cocaine using knockout mice lacking one or both of the key neuronal channel subunits, Kir3.2 and Kir3.3. Cocaine-stimulated increases in horizontal locomotor activity in wild-type, Kir3.2 knockout, Kir3.3 knockout, and Kir3.2/3.3 double knockout mice, with only minor differences observed between the mouse lines. In contrast, Kir3.2 and Kir3.3 knockout mice exhibited dramatically reduced intravenous self-administration of cocaine relative to wild-type mice over a range of cocaine doses. Paradoxically, Kir3.2/3.3 double knockout mice self-administered cocaine at levels significantly higher than either single knockout alone. These findings suggest that Kir3 channels play significant and complex roles in the reinforcing effect of cocaine. *Neuropsychopharmacology* (2003) **28**, 932–938, advance online publication, 5 March 2003; doi:10.1038/sj.npp.1300100

Keywords: cocaine; locomotor activity; mouse; self-administration; potassium

INTRODUCTION

Many studies have implicated G protein-dependent signaling systems in the locomotor-stimulatory and reinforcing effects of cocaine (Koob *et al*, 1998; Nestler, 2001; Sealfon, 2000). As such, one or more G protein-regulated effectors could mediate the key cellular responses underlying these effects of cocaine. Indeed, compelling evidence indicates that alterations in the cAMP/adenylyl cyclase cascade are correlated with escalating intake of several drugs of abuse, including cocaine (Nestler, 2001). In contrast, the significance of other G protein-regulated enzymes and ion channels to the effects of cocaine administration is less clear. A better understanding of the roles played by individual G protein-regulated effectors in the cellular, physiological, and behavioral effects of cocaine and other drugs of abuse could lead to the design of more specific and effective pharmacological interventions for the treatment of drug addiction.

G protein-gated potassium (Kir3/GIRK) channels are found in excitable cells of the heart and central nervous

system, and consist of hetero- and homotetrameric complexes composed of members of a family of four inwardly rectifying potassium channel subunits (Kir3.1–4; Dascal, 1997). Based on multiple expression studies and because Kir3.4 mRNA has been detected in only a few neuron populations, neuronal Kir3 channels are thought to be formed primarily by various combinations of Kir3.1, Kir3.2, and Kir3.3 subunits (Chen *et al*, 1997; Dascal, 1997; Isomoto *et al*, 1997; Karschin *et al*, 1996; Wickman *et al*, 2000). Although Kir3 channels formed by different combinations of Kir3 subunits generally exhibit similar functional properties, it should be noted that Kir3.1 homomultimeric complexes do not form functional channels (Disssmann *et al*, 1996; Duprat *et al*, 1995; Hedin *et al*, 1996; Jelacic *et al*, 2000, 1999; Krapivinsky *et al*, 1995a,b; Velimirovic *et al*, 1996).

Kir3 channels are potently activated by ligand-bound, G_{i/o}-coupled receptors for several neurotransmitters and drugs of abuse (North, 1989). Several observations suggest that Kir3 channel function may contribute to the cellular and behavioral consequences of cocaine administration. First, Kir3.1, Kir3.2, and Kir3.3 are expressed in the rodent nigrostriatal and mesocorticolimbic dopaminergic projection systems (Chen *et al*, 1997; Inanobe *et al*, 1999; Karschin *et al*, 1996). Second, the activation of Kir3 channels by D₂, D₃, and D₄ dopamine receptors has been demonstrated in expression systems (Gregerson *et al*, 2001; Inanobe *et al*, 1999; Kuzhikandathil *et al*, 1998; Pillai *et al*, 1998; Saugstad *et al*, 1996). Third, the *weaver* mutant mouse, which harbors

*Correspondence: Dr K Wickman, Department of Pharmacology, University of Minnesota, 6-120 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA, Tel: +1 612 624 5966, Fax: +1 612 625 8408, E-mail: wickm002@tc.umn.edu

Received 4 June 2002; revised 16 October 2002; accepted 18 October 2002

Online publication: 25 October 2002 at <http://www.acnp.org/citations/Npp102502416>

a mutation in the *Kir3.2* gene, exhibits a dramatic degeneration of nigrostriatal dopaminergic neurons and a pronounced deficit in locomotor activity and coordination (Oo *et al*, 1996; Roffler-Tarlov and Graybiel, 1984; Schmidt *et al*, 1982). Finally, Kir3.2 knockout mice were reported to exhibit a transient hyperactive locomotor activity phenotype consistent with improper dopaminergic signaling (Blednov *et al*, 2001b).

The purpose of the present study was to test the hypothesis that intact Kir3 channel function is required for the locomotor-stimulatory and reinforcing effects of cocaine. Accordingly, the performance of wild-type, Kir3.2 knockout, Kir3.3 knockout, and Kir3.2/3.3 double knockout mice was evaluated in horizontal locomotor activity and intravenous cocaine self-administration paradigms. Our findings suggest that Kir3 channels contribute to the reinforcing effects of cocaine, and as such, could constitute novel targets for drug development strategies aimed at treating cocaine addiction.

METHODS

Behavioral Subjects

Generation of the Kir3.2 knockout, Kir3.3 knockout, and Kir3.2/3.3 double knockout mouse lines has been described (Signorini *et al*, 1997; Torrecilla *et al*, 2002). To generate the wild-type and single subunit knockout mice used in this study, breeding groups were established consisting of heterozygous F1 generation parents harboring null *Kir3.2* or *Kir3.3* mutations backcrossed 5–11 rounds against the C57BL/6J mouse strain. To generate the Kir3.2/Kir3.3 double knockout mice, breeding groups were established consisting of animals homozygous for the *Kir3.3* null allele (two rounds of backcrossing against the C57BL/6J mouse strain) and heterozygous for the *Kir3.2* null allele (six rounds of backcrossing against the C57BL/6J mouse strain). Kir3.3 knockout and Kir3.2/3.3 double knockout offspring from these crosses were used in the behavioral studies. Mouse genotyping was performed by PCR as described (Wickman *et al*, 1998). Specific PCR primer sequences and thermal cycle parameters are available upon request.

Separate cohorts of drug-naïve, male mice were used for the locomotor and self-administration tests. Offspring from 2 to 7 breeding groups per mouse line were studied to minimize the influence of unique, parent-specific traits. All mice were maintained on a 12L:12D (light phase; 0600–1800 h) schedule. Kir3.2 knockout and Kir3.2/3.3 double knockout mice typically died between 4 and 9 months of age. Although the causes of death were not determined in these cases, spontaneous seizures and shortened lifespan have been reported in Kir3.2 knockout mice (Blednov *et al*, 2001b; Signorini *et al*, 1997). Given the lifespan consideration, all behavioral tests in this study were performed on mice ranging from 12 to 18 weeks of age (24–32 g). No seizure activity was observed in the animals during the collection of the reported data.

The use of animals for the studies detailed below was approved by the University of Minnesota Institutional Animal Care and Use Committee (0201A14632). Laboratory facilities were accredited by the American Association

for the Accreditation of Laboratory Animal Care (AAA-LAC). Behavioral studies were performed in a quiet, temperature- and humidity-controlled environment to minimize extraneous stress. The experimenter was blind to subject genotype throughout the studies and analysis of the behavioral data. No significant differences in self-administration or locomotor activity were detected between the Kir3.3 knockout mice derived from the F1 heterozygous crosses and the Kir3.3 knockout mice derived from the double knockout breeding groups. Similarly, no significant differences in self-administration or locomotor activity were detected between the wild-type mice derived from crosses of F1 *Kir3.2* heterozygous and F1 *Kir3.3* heterozygous parents. As such, the behavioral data were collapsed to form single wild-type and Kir3.3 knockout test groups.

Behavioral Apparatus

The locomotor activity study was carried out in a custom-made Plexiglas chamber measuring $0.60 \times 0.51 \times 0.30 \text{ m}^3$, and divided into two chambers. Each individual chamber was divided into $15\text{--}0.102 \text{ m}^2$ sections for observation. The locomotor activity chamber was shrouded with a 0.91 m tall black curtain that provided sound and light attenuation. A standard 8 mm video camera was mounted above a small hole placed 0.91 m above the chambers. The food and intravenous cocaine self-administration study was performed in standard Plexiglas operant chambers housed in sound attenuating cubicles (Med-Associates; St Albans, VT). The operant chambers were equipped with a 3.33 RPM syringe pump for drug delivery, a food pellet delivery system, two ultrasensitive mouse levers, and two stimulus lights. A 28 V, 100 mA house light located at the top of the cage was illuminated during experimental sessions.

Locomotor Activity Measurements

Mice were habituated to the activity chamber for 1 day (day –1) prior to testing with saline injection to obtain a baseline locomotor measurement. Following the habituation day, mice were injected daily (1000–1400) with saline (day 0) or cocaine (15 mg/kg; days 1–5). Mouse activity was recorded by videotape for 31 min following each injection. The locomotor activity values presented herein were determined by summing total line crosses during the following intervals postinjection: 2–6, 10–11, 15–16, and 30–31 min. Each count reflects the crossing of a line by the most rostral mouse tail section. Data were analyzed by a two-way ANOVA (genotype and day) between subjects, followed by individual comparisons using Tukey's HSD test. Significance in all tests was defined as $p < 0.05$.

Food-Maintained Behavior

Subjects were initially trained to lever press for 20 mg food pellets (Noyes Precision Pellets; Research Diets, Inc., New Brunswick, NJ) under a fixed-ratio 1 (FR 1) schedule. During the daily 3-h sessions (0800–1100 or 1130–1430), correct responding on the active (left) lever resulted in stimulus light illumination for 5 s and a delivery of one

pellet to a food hopper located between the two levers. Responses on the inactive (right) lever were counted but had no programmed consequences. The criteria for acquisition of food self-administration were discrimination (3:1) between the active and inactive levers, and delivery of ≥ 30 pellets for 3 consecutive days. All animals were placed on a restricted diet 18 h prior to the start of the first food self-administration session. Behavioral subjects were restricted to 3 g of food daily plus the amount earned in session (3–5 g total per day) throughout the food self-administration test, which lasted 3–7 days. The food restriction procedure resulted in no more than a 10% decrease in body weight. Following the successful completion of the food self-administration test and prior to the beginning of the cocaine self-administration test, a period which lasted 5–9 days, food was provided *ad libitum* and body weights returned to prestudy values. During this period, subjects displayed evidence of behavioral extinction, exhibiting response levels on the active lever that were 60–77% lower than observed during the food self-administration sessions.

Cocaine Self-Administration

Following the food sessions, mice were anesthetized with ketamine (100 mg/kg) and acepromazine (2.5 mg/kg), and implanted with chronic indwelling silastic catheters (0.30 mm ID \times 0.64 mm OD; Dow Corning, Midland, MI) in their right external jugular veins. The catheter exited dorsally through an incision mid-scapulae and was connected to a 28 gauge cannula connector (Plastics One Inc., Roanoke, VA) imbedded in dental acrylic and positioned subcutaneously near the caudal portion of the skull. After at least a 2- to 3-day recovery period, mice were trained in daily 3-h acquisition sessions under an FR 1 schedule of reinforcement using a cocaine dose (0.5 mg/kg) similar to previously described procedures (Caine *et al*, 1999; Rocha *et al*, 1998b). During the acquisition sessions, responding on the active lever resulted in an infusion of drug and illumination of the stimulus light for the duration of infusion. Drug infusion times (2.7–4.2 s) and volumes (60–90 μ l) varied according to subject's body weight. Responses on the inactive lever were counted, but had no programmed consequences. The criteria for acquisition of cocaine self-administration were discrimination ($> 3:1$) between active and inactive levers, and a mean daily intake of ≥ 5 mg/kg cocaine for 3 consecutive days. Following the acquisition phase of the test, five cocaine doses (0.125, 0.25, 0.5, 1.0, 2.0 mg/kg) were presented in nonsystematic order, the only restriction being that the lowest dose never followed the highest dose. Cocaine dose was changed when responding stabilized to less than 15% variation for at least 3 consecutive days. The criteria for including mice in the analyses included the successful completion of at least three doses of cocaine and a patent catheter. Catheter patency was confirmed by the rapid loss of the righting reflex upon delivery of sodium methohexital (5 mg/kg, i.v.). Data were analyzed by two-way ANOVA (genotype and dose) between subjects, followed by individual comparisons using Tukey's HSD test. Significance in all tests was defined as $p < 0.05$.

RESULTS

Wild-type and Kir3 subunit knockout mice were subjected to tests of baseline and cocaine-induced horizontal locomotor activity. Following saline injection (day 0), baseline horizontal locomotor activities of the mutant mice were lower than those of the wild-type controls, although the differences were not statistically significant ($F_{3,32} = 1.44$, $p = 0.26$; Figure 1). Following a single injection of 15 mg/kg cocaine (day 1), absolute horizontal locomotor activity levels increased significantly in all test groups ($F_{1,29} = 21.4$, $p < 0.001$). In addition, all mouse lines tested exhibited an increased locomotor-stimulatory effect of repeated cocaine administration, manifested as significant increases in cocaine-induced locomotor activity on days 1–3 ($F_{1,87} = 31.7$, $p < 0.001$). Interestingly, while locomotor activities of wild-type, Kir3.3 knockout, and Kir3.2/3.3 double knockout lines appeared to plateau after day 3, Kir3.2 knockout mice continued to exhibit significant increases in cocaine-induced locomotor activity across all days ($F_{3,145} = 12.97$, $p < 0.001$, genotype; $F_{5,145} = 22.6$, $p < 0.001$, day; $F_{15,145} = 2.0$, $p < 0.05$, genotype \times day).

Separate cohorts of wild-type and Kir3 subunit knockout mice were subjected to a two-component test of food- and cocaine-maintained responding. In the first component of the test, mice were trained to press a lever to receive a food pellet under an FR 1 schedule of reinforcement. As shown in Table 1, no differences were observed between wild-type, Kir3.2 knockout, Kir3.3 knockout, or Kir3.2/3.3 double knockout mice with respect to rate of acquisition of food self-administration ($F_{3,32} = 0.81$, $p = 0.50$). Similarly, there were no statistically significant differences between groups with respect to the intake of food (g/kg) earned in session ($F_{3,32} = 2.6$, $p = 0.07$).

In the second component of the test, a jugular catheter was implanted in each mouse. After a 2- to 3-day recovery period, the animals were trained in daily 3-h acquisition sessions under an FR 1 schedule of reinforcement with cocaine (0.5 mg/kg). As shown in Table 1, no differences

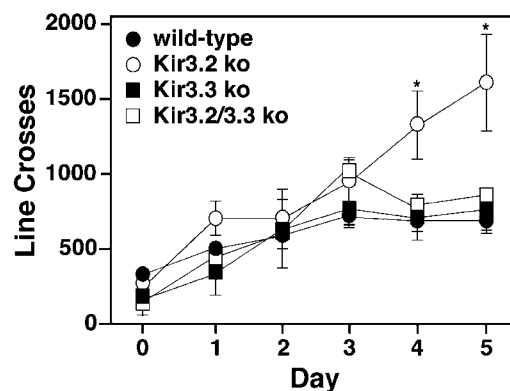


Figure 1 Baseline and cocaine-induced locomotor activity in wild-type and Kir3 subunit knockout (ko) mice. Line crosses in wild-type (black circles; $n = 10$), Kir3.2 ko (open circles; $n = 10$), Kir3.3 ko (black squares; $n = 10$), and Kir3.2/3.3 ko (open squares; $n = 3$) are presented for saline (day 0) or 15 mg/kg cocaine (days 1–5) pretreatment. The y-axis represents the total line crosses occurring in a distributed 8 min sample of a 31 min postinjection session. * $p < 0.05$, Kir3.2 ko vs wild-type, Kir3.3 ko, and Kir3.2/3.3 ko mice.

Table 1 Group Data for the Self-Administration Study

Genotype	n	Age (days)	Weight (g)	Food acquisition		Cocaine acquisition	
				Days	Intake (g/kg)	Days	Intake (mg/kg)
Wild-type	10	106 ± 9	27.5 ± 0.9	3.6 ± 0.3	65.5 ± 7.2	4.4 ± 0.4	15.1 ± 2.6
Kir3.2 ko	8	85 ± 5	24.4 ± 1.2	4.2 ± 0.7	103.6 ± 26.9	4.7 ± 1.0	21.3 ± 8.4
Kir3.3 ko	10	90 ± 5	28.2 ± 1.5	4.6 ± 0.5	47.3 ± 9.8	4.4 ± 0.4	13.0 ± 2.1
Kir3.2/3.3 ko	8	108 ± 19	29.5 ± 1.8	4.8 ± 0.9	64.9 ± 10.7	3.5 ± 0.2	16.8 ± 4.0

Group sizes (n), ages, weights, and parameters related to the food and cocaine self-administration test are presented as the means ± SEM for the four groups of mice tested. Mouse age and weight data represent the values at the start of food self-administration sessions. There were no significant differences between groups with respect to age ($F_{3,32} = 1.17$; $p = 0.34$) or weight ($F_{3,32} = 2.26$; $p = 0.10$). For food and cocaine self-administration, 'days' refers to the interval required to meet the acquisition criteria, and 'intake' refers to the mean daily intake of either food pellets or cocaine over the last 3 days of acquisition. For the food component of the test, intake refers only to the amount of food earned by performing the operant task; all mice in the study were supplemented daily with 3 g of food.

were detected between the mouse groups with respect to rate of acquisition of cocaine self-administration ($F_{3,32} = 0.80$, $p = 0.50$) or mean daily cocaine intake (mg/kg; $F_{3,32} = 0.59$, $p = 0.62$).

Following successful acquisition of cocaine self-administration behavior, responding at multiple cocaine doses was measured. Wild-type mice exhibited significantly higher responding (active lever presses) at the three lowest doses of cocaine tested (0.125, 0.25, and 0.5 mg/kg) compared to Kir3.2 knockout ($F_{4,17} = 56.5$, $p < 0.001$) and Kir3.3 knockout ($F_{4,19} = 74.9$, $p < 0.001$) mice, and at the two lowest cocaine doses compared to Kir3.2/3.3 double knockout mice ($F_{4,17} = 59.7$, $p < 0.001$; Figure 2a,b). Interestingly, the response levels of the Kir3.2/3.3 double knockout mice were significantly higher than those of either the Kir3.2 knockout ($F_{4,15} = 6.91$, $p < 0.001$) or the Kir3.3 knockout mice ($F_{4,17} = 9.6$, $p < 0.001$) at the three lowest doses of cocaine tested.

Responses on the active lever during cocaine infusions were tabulated, but they did not result in additional infusion time. To determine whether differences in responding between groups resulted in differences in cocaine intake, the mean daily number of cocaine infusions and mean daily intake values (mg/kg) were tabulated for each mouse group and cocaine dose (Figure 2c–e). The differences observed between groups with respect to the number of drug infusions as a function of cocaine dose were comparable to those identified in comparisons of lever press behavior.

While response levels on the active lever were low in Kir3.2 knockout and Kir3.3 knockout groups, a comparison of responding on both the active and inactive levers shows that all four groups discriminated between these levers ($> 3:1$) at all cocaine doses tested (Figure 2f). In addition, there were no significant differences between groups with respect to the number of responses on the inactive lever ($F_{3,32} = 1.43$, $p = 0.237$).

DISCUSSION

The purpose of this study was to determine whether intact Kir3 function is required for the locomotor-stimulatory and reinforcing effects of cocaine. Our findings suggest that potassium channels formed by the neuronal Kir3 subunits, Kir3.2 and Kir3.3, are not strictly required for either behavioral effect of cocaine. Indeed, cocaine-stimulated

locomotor activity in mice lacking Kir3.2 and/or Kir3.3, and evoked progressive increases in locomotor activity upon repeated administration for all lines tested. Furthermore, mice lacking both Kir3.2 and Kir3.3 displayed a modest reduction in cocaine self-administration behavior compared to wild-type mice across a range of doses. Surprisingly, however, mice lacking either the Kir3.2 or Kir3.3 subunit exhibited dramatically reduced cocaine self-administration behavior compared to Kir3.2/3.3 double knockout and wild-type mice. Together, these findings suggest that Kir3 channels contribute in a complex manner to the expression of the reinforcing effect of cocaine.

The unpredictable contribution of genetic background is frequently cited as a potential confound in the behavioral analysis of mutant mouse lines. The Kir3.2 and Kir3.3 knockout lines used in this study originated as an equal mixture of 129/Sv and C57BL/6 genetic backgrounds (Signorini et al, 1997; Torrecilla et al, 2002). Analysis of these two parental strains and F1 129 × C57 hybrid crosses indicated that the reinforcing and locomotor-stimulatory effects of cocaine are mediated by dominant C57-based loci (Miner, 1997). Since the Kir3.2 and Kir3.3 null mutant alleles had been backcrossed at least five times against the C57BL/6J strain prior to establishing the breeding groups that generated the wild-type and single subunit knockout subjects in the current study, the contribution of 129/Sv-based loci to the unique phenotypes in these groups was likely minor. Nevertheless, we cannot rule out the possibility that the phenotypes observed in this study were shaped in part by epistatic interactions between the targeted mutation(s) and the genetic background.

Recently, Kir3.2 knockout mice were reported to exhibit higher levels of locomotor activity in a novel environment compared to wild-type control mice (Blednov et al, 2001b). Here, we report that Kir3.2 knockout mice exhibited normal baseline locomotor activity. Since the same Kir3.2 knockout mouse line was used in both studies, the difference could reflect unique features of the experimental designs. Indeed, Blednov et al (2001b) monitored locomotor activity under fairly intense illumination, creating a more stressful environment. It is also possible that behavioral differences between the studies could be because of the greater degree of backcrossing of the Kir3.2 null mutation employed in this study.

Several observations indicate that the minimal cocaine self-administration behavior observed in the Kir3.2

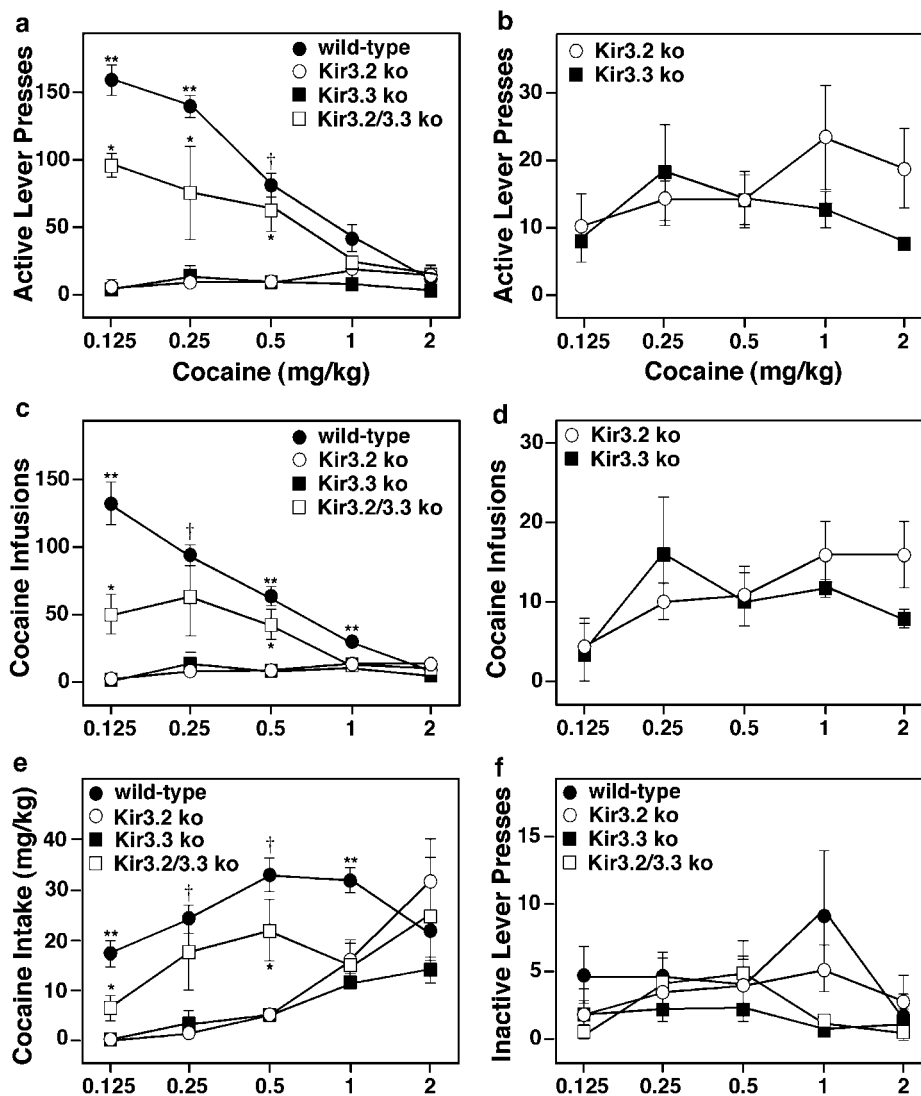


Figure 2 Self-administration behavior of wild-type and Kir3 subunit knockout (ko) mice as a function of cocaine dose. The data represent the means \pm SEM for the 3-h sessions measured on the last 3 days of stable responding at each cocaine dose. (a and b) The number of responses on the active lever for wild-type (black circles; $n = 10$), Kir3.2 ko (open circles; $n = 8$), Kir3.3 ko (black squares; $n = 10$), and Kir3.2/3.3 ko (open squares; $n = 8$) mice for each cocaine dose. Data for the Kir3.2 ko and Kir3.3 ko groups are plotted on an expanded graph (b) because of the low response levels measured relative to the wild-type and Kir3.2/3.3 ko groups. (c and d) The number of cocaine infusions received by the four mouse groups at each cocaine dose. Data for the Kir3.2 ko and Kir3.3 ko groups are plotted on an expanded graph (d) because of the low number of infusions recorded relative to the wild-type and Kir3.2/3.3 ko groups. (e) Daily cocaine intake values (mg/kg) for the four mouse groups at each cocaine dose. (f) The number of responses on the inactive lever for the four mouse groups at each cocaine dose. Statistical symbols and comparisons are as follows: **wild-type vs all three Kir3 ko groups, $p < 0.05$, †wild-type vs Kir3.2 ko and Kir3.3 ko groups, $p < 0.05$, * Kir3.2/3.3 ko compared to Kir3.2 ko and Kir3.3 ko groups, $p < 0.05$.

knockout and Kir3.3 knockout lines did not result from a global disruption in dopaminergic signaling or structures critical for the execution of the tasks under consideration. First, all mice tested successfully completed the acquisition phase of the self-administration task with food as a reward. Second, the selective responding on the active lever observed for all mouse lines throughout the study indicated that all mice tested were capable of associative learning. Third, response levels on the inactive lever during the cocaine self-administration test, an indirect measure of mouse activity levels, were indistinguishable between the four mouse genotypes. Finally, baseline horizontal locomotor activity levels and cocaine-induced increases in horizontal locomotor activity were comparable across the four

genotypes tested. Given these observations, we conclude that the differences observed between mouse lines most likely reflect the significant and complex contributions of Kir3 channels formed by Kir3.2 and/or Kir3.3 subunits to the behaviors under consideration.

Although the dose range used in the self-administration study was chosen to reveal both the ascending and descending limbs of the biphasic cocaine dose-response curve (Chiamulera *et al*, 2001; Horger *et al*, 1991; Rocha *et al*, 1998a,b), the curve for the wild-type group consisted only of a partial descending limb. Similarly, curves obtained for the knockout groups were incomplete and/or difficult to discern given the relatively low levels of responding. For example, the lever-press behavior first increased slightly

and then appeared to plateau or slightly decrease at cocaine doses ≥ 1 mg/kg for both the Kir3.2 knockout and Kir3.3 knockout groups. These data, therefore, could reflect decreased sensitivities of the Kir3.2 knockout and Kir3.3 knockout mouse lines to the reinforcing effect of cocaine. However, in the absence of complete dose-response curves for each mouse line and given the relatively low levels of behavioral responding observed for the Kir3.2 knockout and Kir3.3 knockout groups, it is not possible to state with certainty that these mouse lines are less sensitive to cocaine. Indeed, the locomotor activity behavior of the Kir3.2 knockout mice, particularly upon repeated cocaine exposure, suggests an increased sensitivity of this line to cocaine.

An assessment of the relative cocaine sensitivities of the Kir3 knockout mouse lines is further complicated by the design of the self-administration test itself. While performance of the mice during the food component of the test served as a control for locomotor function and demonstrated the abilities of the previously uncharacterized mutant mice to learn a complex task, behavior acquired during the food training sessions could have impacted the performance of the mice during the cocaine sessions. Indeed, higher daily cocaine intake values were measured during the dose-response analysis at 0.5 mg/kg cocaine for wild-type (33 vs 15 mg/kg) and Kir3.2/3.3 double knockout mice (22 vs 17 mg/kg), relative to cocaine intake measured at this dose during the acquisition phase of the test (Table 1, Figure 2e). In contrast, markedly lower intake values were observed for the Kir3.2 knockout (5 vs 21 mg/kg) and Kir3.3 knockout (5 vs 13 mg/kg) groups during the dose-response analysis. The differences observed between groups suggest that the self-administration behavior measured during the dose-response analysis was shaped by the incomplete extinction of food-maintained lever press behavior. It is also possible that the differences observed between groups reflects the different amounts of cocaine earned throughout the study by each group and/or differences in innate sensitivities of the groups to cocaine (Deroche et al, 1999).

The cocaine self-administration behavior of the Kir3.2/3.3 double knockout mice does not represent a simple summation of the Kir3.2 knockout and Kir3.3 knockout phenotypes. Although perplexing, this observation could reflect the contribution of Kir3 channels of different subunit compositions to distinct neural circuits exerting opposing influence on self-administration behavior. Indeed, localization studies suggest that Kir3.2 and Kir3.3 subunits overlap in many brain regions, but they are also found in unique regions of the rodent nigrostriatal and mesocorticolimbic dopaminergic systems (Karschin et al, 1996). For example, Kir3.2 appears to be the predominant functional Kir3 subunit in the substantia nigra, while Kir3.3 appears to be the more abundant subunit in regions such as the olfactory tubercle, entorhinal cortex, and amygdala (Inanobe et al, 1999; Karschin et al, 1996). Since Kir3.2 and Kir3.3 mRNAs were not detected (or were detected at very low levels) in the rodent nucleus accumbens (Karschin et al, 1996; Chen et al, 1997), the decreased cocaine self-administration behavior observed in both the Kir3.2 knockout and Kir3.3 knockout lines probably does not reflect a simple genetic ablation of the dopaminergic projection from the ventral tegmental area to the nucleus accumbens, a pathway known to be important for cocaine self-administration (Maldonado et al,

1993; Roberts and Koob, 1982; Self et al, 1994). Furthermore, it should be noted that the observed behavioral effects could result from disruptions in nondopaminergic signaling that occur in circuitry downstream from the proximal target(s) of cocaine. Accordingly, the clear interpretation of the self-administration behavior observed in the mouse lines under consideration will require a definitive cellular and subcellular analysis of Kir3 subunit localization in the mouse dopaminergic projection systems, as well as a detailed understanding of the neurotransmitter system(s) linked to Kir3 channels in these circuitries.

In conclusion, we present evidence supporting a role for Kir3 channels in the reinforcing effect of cocaine. Future studies will be aimed at identifying the neuroanatomical and neurochemical bases of the observed phenotypes, and determining whether Kir3 channel function contributes to the reinforcing effects of other drugs of abuse. Since Kir3 channels have been linked to opioid (μ , δ , κ) and cannabinoid receptors (CB₁) and ethanol (Blednov et al, 2001a; Henry and Chavkin, 1995; Henry et al, 1995; Ho et al, 1999; Ikeda et al, 1996, 1995; Kobayashi et al, 1999; McAllister et al, 1999), the Kir3 channel class could constitute a novel and useful target for pharmacological manipulations designed to prevent or treat drug addiction.

ACKNOWLEDGEMENTS

We thank Maria Roman, and Stephanie Cintora for assistance with mouse genotyping, and Megan Roth and Erin Larson for careful reading of the manuscript. This work was supported by NIH Grants T32 DA07097 (ADM), RO1 DA03240, K05 DA15267 (MEC), RO1 MH61933 (KW), and a PhRMA Foundation Research Starter Award (KW).

REFERENCES

- Blednov Y, Stoffel M, Chang S, Harris R (2001a). Potassium channels as targets for ethanol: studies of G-protein-coupled inwardly rectifying potassium channel 2 (GIRK2) null mutant mice. *J Pharmacol Exp Ther* 298: 521–530.
- Blednov YA, Stoffel M, Chang SR, Harris RA (2001b). GIRK2 deficient mice. Evidence for hyperactivity and reduced anxiety. *Physiol Behav* 74: 109–117.
- Caine S, Negus S, Mello N (1999). Method for training operant responding and evaluating cocaine self-administration behavior in mutant mice. *Psychopharmacology* 147: 22–24.
- Chen SC, Ehrhard P, Goldowitz D, Smye RJ (1997). Developmental expression of the GIRK family of inward rectifying potassium channels: implications for abnormalities in the weaver mutant mouse. *Brain Res* 778: 251–264.
- Chiamulera C, Epping-Jordan M, Zocchi A, Marcon C, Cottiny C, Tacconi S et al (2001). Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat Neurosci* 4: 873–874.
- Dascal N (1997). Signalling via the G protein-activated K⁺ channels. *Cell Signal* 9: 551–573.
- Deroche V, Le Moal M, Piazza P (1999). Cocaine self-administration increases the incentive motivational properties of the drug in rats. *Eur J Neurosci* 11: 2731–2736.
- Dissmann E, Wischmeyer E, Spaschus A, Pfeil DV, Karschin C, Karschin A (1996). Functional expression and cellular mRNA localization of a G protein-activated K⁺ inward rectifier isolated from rat brain. *Biochem Biophys Res Commun* 223: 474–479.

- Duprat F, Lesage F, Guillemare E, Fink M, Hugnot JP, Bigay J *et al* (1995). Heterologous multimeric assembly is essential for K⁺ channel activity of neuronal and cardiac G-protein-activated inward rectifiers. *Biochem Biophys Res Commun* **212**: 657–663.
- Gregerson KA, Flagg TP, O'Neill TJ, Anderson M, Luring O, Horel JS *et al* (2001). Identification of G protein-coupled, inward rectifier potassium channel gene products from the rat anterior pituitary gland. *Endocrinology* **142**: 2820–2832.
- Hedin KE, Lim NF, Clapham DE (1996). Cloning of a *Xenopus laevis* inwardly rectifying K⁺ channel subunit that permits GIRK1 expression of I_{KACH} currents in oocytes. *Neuron* **16**: 423–429.
- Henry DJ, Chavkin C (1995). Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes. *Neurosci Lett* **186**: 91–94.
- Henry DJ, Grandy DK, Lester HA, Davidson N, Chavkin C (1995). Kappa-opioid receptors couple to inwardly rectifying potassium channels when coexpressed by *Xenopus* oocytes. *Mol Pharmacol* **47**: 551–557.
- Ho BY, Uezono Y, Takada S, Takase I, Izumi F (1999). Coupling of the expressed cannabinoid CB1 and CB2 receptors to phospholipase C and G protein-coupled inwardly rectifying K⁺ channels. *Receptors Channels* **6**: 363–374.
- Horger B, Wellman P, Morien A, Davies B, Schenk S (1991). Caffeine exposure sensitizes rats to the reinforcing effects of cocaine. *Neuroreports* **2**: 53–56.
- Ikeda K, Kobayashi T, Ichikawa T, Usui H, Abe S, Kumanishi T (1996). Comparison of the three mouse G-protein-activated K⁺ (GIRK) channels and functional couplings of the opioid receptors with the GIRK1 channel. *Ann NY Acad Sci* **801**: 95–109.
- Ikeda K, Kobayashi T, Ichikawa T, Usui H, Kumanishi T (1995). Functional couplings of the delta- and the kappa-opioid receptors with the G-protein-activated K⁺ channel. *Biochem Biophys Res Commun* **208**: 302–308.
- Inanobe A, Yoshimoto Y, Horio Y, Morishige KI, Hibino H, Matsumoto S *et al* (1999). Characterization of G-protein-gated K⁺ channels composed of Kir3.2 subunits in dopaminergic neurons of the substantia nigra. *J Neurosci* **19**: 1006–1017.
- Isomoto S, Kondo C, Kurachi Y (1997). Inwardly rectifying potassium channels: their molecular heterogeneity and function. *Jpn J Physiol* **47**: 11–39.
- Jelacic TM, Kennedy ME, Wickman K, Clapham DE (2000). Functional and biochemical evidence for G-protein-gated inwardly rectifying K⁺ (GIRK) channels composed of GIRK2 and GIRK3. *J Biol Chem* **275**: 36211–36216.
- Jelacic TM, Sims SM, Clapham DE (1999). Functional expression and characterization of G-protein-gated inwardly rectifying K⁺ channels containing GIRK3. *J Membr Biol* **169**: 123–129.
- Karschin C, Dissmann E, Stuhmer W, Karschin A (1996). IRK(1–3) and GIRK(1–4) inwardly rectifying K⁺ channel mRNAs are differentially expressed in the adult rat brain. *J Neurosci* **16**: 3559–3570.
- Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T *et al* (1999). Ethanol opens G-protein-activated inwardly rectifying K⁺ channels. *Nat Neurosci* **2**: 1091–1097.
- Koob G, Sanna P, Bloom F (1998). Neuroscience of addiction. *Neuron* **21**: 467–476.
- Krapivinsky G, Gordon EA, Wickman K, Velimirovic B, Krapivinsky L, Clapham DE (1995a). The G-protein-gated atrial K⁺ channel I_{KACH} is a heteromultimer of two inwardly rectifying K⁺-channel proteins. *Nature* **374**: 135–141.
- Krapivinsky G, Krapivinsky L, Velimirovic B, Wickman K, Navarro B, Clapham DE (1995b). The cardiac inward rectifier K⁺ channel subunit, CIR, does not comprise the ATP-sensitive K⁺ channel, I_{KATP}. *J Biol Chem* **270**: 28777–28779.
- Kuzhikandathil EV, Yu W, Oxford GS (1998). Human dopamine D3 and D2L receptors couple to inward rectifier potassium channels in mammalian cell lines. *Mol Cell Neurosci* **12**: 390–402.
- Maldonado R, Robledo P, Chover A, Caine S, Koob G (1993). D1 Dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacol Biochem Behav* **45**: 239–242.
- McAllister SD, Griffin G, Satin LS, Abood ME (1999). Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a *xenopus* oocyte expression system. *J Pharmacol Exp Ther* **291**: 618–626.
- Miner L (1997). Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. *Pharmacol Biochem Behav* **58**: 25–30.
- Nestler EJ (2001). Molecular neurobiology of addiction. *Am J Addict* **10**: 201–217.
- North A (1989). Drug receptors and the inhibition of nerve cells. *Br J Pharmacol* **98**: 13–28.
- Oo TF, Blazeski R, Harrison SM, Henchcliffe C, Mason CA, Roffler-Tarlov SK *et al* (1996). Neuron death in the substantia nigra of *weaver* mouse occurs late in development and is not apoptotic. *J Neurosci* **16**: 6134–6145.
- Pillai G, Brown NA, McAllister G, Milligan G, Seabrook GR (1998). Human D2 and D4 dopamine receptors couple through betagamma G-protein subunits to inwardly rectifying K⁺ channels (GIRK1) in a *Xenopus* oocyte expression system: selective antagonism by L-741,626 and L-745,870 respectively. *Neuropharmacology* **37**: 983–987.
- Roberts D, Koob G (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* **17**: 901–904.
- Rocha B, Odom L, Barron B, Ator R, Wild S, Forster M (1998a). Differential responsiveness to cocaine in C57BL/6J and DBA/2J mice. *Psychopharmacology* **138**: 82–88.
- Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B *et al* (1998b). Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci* **1**: 132–137.
- Roffler-Tarlov S, Graybiel A (1984). *Weaver* mutation has differential effects on the dopamine-containing innervation of the limbic and non-limbic striatum. *Science* **307**: 62–66.
- Saugstad JA, Segerson TP, Westbrook GL (1996). Metabotropic glutamate receptors activate G-protein-coupled inwardly rectifying potassium channels in *Xenopus* oocytes. *J Neurosci* **16**: 5979–5985.
- Schmidt M, Sawyer B, Perry K, Fuller R, Foreman M, Ghatti B (1982). Dopamine deficiency in the *weaver* mutant mouse. *J Neurosci* **2**: 376–380.
- Sealfon SC (2000). Dopamine receptors and locomotor responses: molecular aspects. *Ann Neurol* **47**: S12–S19; discussion S19–S21.
- Self D, Terwilliger R, Nestler E, Stein L (1994). Inactivation of G_i and G_o proteins in nucleus accumbens reduces both cocaine and heroin reinforcement. *J Neurosci* **14**: 6239–6247.
- Signorini S, Liao YJ, Duncan SA, Jan LY, Stoffel M (1997). Normal cerebellar development but susceptibility to seizures in mice lacking G protein-coupled, inwardly rectifying K⁺ channel GIRK2. *Proc Natl Acad Sci USA* **94**: 923–927.
- Torreclilla M, Marker C, Cintora S, Stoffel M, Williams J, Wickman K (2002). G protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. *J Neurosci* **22**: 4328–4344.
- Velimirovic BM, Gordon EA, Lim NF, Navarro B, Clapham DE (1996). The K⁺ channel inward rectifier subunits form a channel similar to neuronal G protein-gated K⁺ channel. *FEBS Lett* **379**: 31–37.
- Wickman K, Karschin C, Karschin A, Picciotto M, Clapham D (2000). Brain localization and behavioral impact of the G protein-gated K⁺ channel subunit, GIRK4. *J Neurosci* **20**: 5608–5615.
- Wickman K, Nemec J, Gendler SJ, Clapham DE (1998). Abnormal heart rate regulation in GIRK4 knockout mice. *Neuron* **20**: 103–114.