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G_z proteins are functionally coupled to dopamine D2-like receptors in vivo

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Abstract

The receptors that couple to the G protein G_z in vivo are still relatively unknown. In this study, we investigated the effects of various dopamine receptor agonists in a mouse deficient in the α subunit of G_z. The dopamine D1-like receptor agonist SKF38393 stimulated comparable locomotor activity in both wildtype mice and mice lacking $G\alpha_z$. In contrast, the dopamine D2-like receptor agonist quinpirole suppressed locomotor activity in both groups of mice, but this suppression was significantly smaller in $G\alpha_z$ knockout mice. Consistent with these behavioural observations, quinpirole inhibition of dopamine release in the forebrain nucleus accumbens evoked by electrical stimulation of dopamine axons was significantly attenuated in mice lacking $G\alpha_z$. In addition, hypothermia and adrenocorticotropic hormone release resulting from activation of dopamine D2-like receptors were also significantly reduced in $G\alpha_z$ knockout mice. However, adrenocorticotropic hormone secretion induced by corticotrophin releasing hormone and the serotonin 1A receptor agonist 8-hydroxy-dipropylamino-tetralin were similar between wildtype and $G\alpha_z$ knockout mice. Western blot analysis showed that the expression levels of $G\alpha_i$, $G\alpha_o$, $G\alpha_s$, $G\alpha_q$ and $G\beta$ were the same in the brains of mice of both genotypes. Overall, our data suggest that G_z proteins are functionally coupled to dopamine D2-like receptors in vivo.

Keywords: $G\alpha_z$ knockout mouse; Quinpirole; Psychostimulant; Amperometry

1. Introduction

The effects of dopamine on nervous tissues are exerted through cell surface receptors coupled to heterotrimeric G proteins. To date, five dopamine receptors have been cloned in mammals, and they have been classified into two families (Missale et al., 1998). The dopamine D1-like receptor family consists of D1 and D5 receptors coupled to G proteins from the G_s family to mediate stimulation of adenylate cyclase (Sidhu and Niznik, 2000). The dopamine D2-like receptor family comprises the alternatively spliced D2 and D3 long and short receptors (Monsma et al., 1989), as well as D4 receptors (Oak et al., 2000). All of these receptors are coupled to members of the G_i G protein family that mediate inhibition of adenylate cyclase (Obadiah et al., 1999; Sidhu and Niznik, 2000).

Most of the studies on G protein coupling of dopamine receptors have been performed either in cell lines by transfection with receptors and G protein α subunits or in disrupted membrane preparations. Although these methods are very useful for preliminary identification of possible G proteins that a receptor

Abbreviations: 7OH-DPAT, 7-hydroxy-dipropylamino-tetralin; 8OH-DPAT, 8-hydroxy-dipropylamino-tetralin; ACTH, adrenocorticotropic hormone; CRH, corticotropin releasing hormone.

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can couple to, the results obtained must be confirmed in intact cells from body tissues. This is essential since the presence of cellular compartments can restrict receptors from accessing G proteins in other parts of the cell (Allgeier et al., 1997; Degtiar et al., 1997; Ostrom et al., 2000). The level of expression of various components in the G protein signaling cascade is also known to be tightly regulated in vivo (Mende et al., 1998).

The most abundant brain G protein G_o (Sternweis and Robishaw, 1984) has been proposed to be the predominant G protein that couple to most members of the dopamine D2-like receptor family in the brain (Jiang et al., 2001). This is based on the inability of dopamine to displace ^{125}I-sulpiride binding in brain sections from $G\alpha_o$-knockout mice when guanosine triphosphate is present (Jiang et al., 2001) and similar affinities of these compounds for all D2-like receptors (Missale et al., 1998). However, the high affinity receptor binding state of some D2-like receptors are known to be critically dependent on subtle changes in assay conditions, such as magnesium concentrations (Bancroft et al., 1998). Moreover, standard radiobinding techniques currently employed lack sufficient sensitivity to detect dopamine receptor coupling to other less abundant G proteins, such as G_z.

G_z is a member of the G_i G protein family whose in vivo functions remain unknown. Locomotor activity induced by the psychostimulant cocaine has been shown to be significantly enhanced in mice lacking the α subunit of G_z (Yang et al., 2000). Since cocaine-elicited locomotor activity relies on the integrity of dopaminergic system (Uhl et al., 2002), alterations in dopamine receptor functions in $G\alpha_z$ knockout mice may account for this augmented psychostimulant-induced behavioural response. In addition, we have recently shown that $G\alpha_z$ knockout mice exhibit an enhanced sensitivity to the disruption of prepulse inhibition of acoustic startle induced by treatment with amphetamine and apomorphine, as well as enhanced sensitivity to the locomotor activating effects of amphetamine (van den Buuse et al., 2005). The present study was therefore conducted to examine dopamine receptor functions in $G\alpha_z$ knockout mice.

2. Materials and methods

2.1. Animals

Mutant $G\alpha_z$ knockout mice were derived from heterozygous founders generated originally by gene targeting in the C57BL/6 mouse strain as previously described (Hendry et al., 2000). Mice used for the experiments, although of pure C57BL/6 origin, have been crossed for eight generations into the C57BL/6 background to remove any random mutations that might have occurred in the parental C57BL/6 stem cells used for electroporation. The animals were housed in a temperature-controlled room, with food and water available ad libitum. All experiments were performed on age matched adult C57BL/6 mice, using protocols approved by the Animal Experimentation Ethics Committee of the Australian National University and Macquarie University. Male mice were used in all experiments, except for the experiment on measurement of adrenocorticotropic hormone (ACTH) released by various drugs, where equal numbers of wildtype and $G\alpha_z$ knockout females were included to make up for insufficient mouse numbers. Where possible, mice of different genotypes were evaluated in parallel in the experiments. When this was not possible, due to shortage of resources, wildtype and $G\alpha_z$ knockout mice were tested alternately.

2.2. Measurement of locomotor activity

Mice were accustomed to the testing room for about 2 h prior to the start of each experiment. Following subcutaneous (s.c.) injection of either a drug (SKF 38393: 10 or 100 mg/kg; quinpirole: 0.3, 1 or 2 mg/kg, all salt weight) or saline vehicle (10 μl/g), each mouse was placed immediately into a cage (29×18 cm) fitted with two pairs of infra-red photocells positioned 1.5 cm above the floor and spaced 10 cm apart. Eight mice (four wildtype and four $G\alpha_z$ knockouts) were evaluated simultaneously in eight cages occluded visually from one another. The locomotor activity of the mice was monitored for 4 h after administration of SKF 38393 and for 90 min after quinpirole administration. Locomotor activity measurements always occurred between 1330 hours and 1800 hours.

2.3. Constant potential amperometry

Each mouse was anaesthetized with an injection of 1.5 g/kg (i.p.) urethane, and then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) containing a mouse head-holder adaptor (Stoelting, Kiel, WI, USA). The body temperature of the mouse was maintained with a heating pad (TC-1000; CWE Inc., NY, USA), which was set at 40 ± 0.5 °C. Three holes were drilled through the animal's skull: one for a silver/silver chloride reference/auxiliary combination electrode, another for a stimulating electrode and a third hole for both the recording electrode and an adjacent 31 g stainless-steel guide cannula. The tip of a concentric bipolar stimulating electrode (SNE-100; Rhodes Medical Co., Woodland Hills, CA, USA) was positioned in the medial forebrain bundle (anteroposterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates: AP −2.1 mm from bregma, ML +1.0 mm, and about −4.3 mm DV from the dura) (Franklin and Paxinos, 1997). The recording electrode, which had a carbon fiber (10 μm outer diameter and 250 μm long, Thornel Type P, Union Carbide, USA) as the active recording surface, was implanted into the core region of the ipsilateral nucleus accumbens (coordinates: AP +1.5 mm from bregma, ML +1.0 mm, and between −3.3 and −3.5 mm DV from the dura) (Franklin and Paxinos, 1997). The depths of the stimulating and recording electrodes were slightly adjusted for each experiment to maximize dopamine release. For drug infusion, a guide cannula was implanted as close to the recording electrode as possible (0.2–0.3 mm), with its end positioned 1 mm above the tip of the recording electrode, so that when the infusion cannula (100 μm outer diameter, Polymicro Tech. Inc., AZ, USA) was inserted, its tip was approximately in line with the middle of the recording electrode's carbon fiber.

Constant potential amperometric recordings were made within a custom-made Faraday cage to increase the signal-to-noise ratio. Following implantation of all electrodes and the guide cannulae, a fixed positive potential (+0.6 V) was applied to the carbon fiber recording electrode and oxidation current monitored continuously (10,000 samples/s) with an electrometer (Powerlab system, ADInstruments, Sydney NSW, Australia), filtered at 50 Hz (Dommett et al., 2005; Forster and Blaha, 2003). After at least 60 min of implantation of the recording electrode, a baseline electrical stimulation-evoked dopamine response profile for each mouse was acquired by applying a train of monophasic 0.5 ms duration pulses (15 pulses at 50 Hz, 500 μA) once every 30 s for 2.5 min, via an optical isolator and programmable pulse generator (Iso-Flex/Master-8, AMPI, Jerusalem, Israel). Six stimulation-evoked responses were recorded automatically by a Macintosh computer running the 'Chart' program (ADInstruments, Castle Hill, NSW, Australia) and were subsequently averaged to obtain a mean baseline response. Thereafter, 100 ng of quinpirole in a volume of 1 μl was infused slowly into the nucleus accumbens through the injection cannula via a 10 μl microsyringe over a time period of 4 min. At 15-minute time intervals from the start of infusion, a mean dopamine release profile was obtained by averaging the responses from six stimulating pulse trains, following exactly the same protocol as was used for obtaining the baseline response. Recordings continued for at least 90 min after the start of quinpirole infusion.

Upon the completion of each experiment, an iron deposit was made in the medial forebrain bundle stimulation site by passing direct current (100 μA for 10 s) through the stimulating electrode. The mouse was then euthanized with an overdose of urethane (3 g/kg i.p.). The brain was removed, immersed

overnight in 10% buffered formalin containing 0.1% potassium ferricyanide (Lancaster Inc., Eastgate, UK), and then stored in a solution containing 30% sucrose and 10% formalin until sectioning. After fixation, 40 μm coronal sections were cut on a freezing microtome. A Prussian blue spot, which is produced from a redox reaction of ferricyanide, marked the stimulation site. The placements of the amperometric recording electrode, stimulating electrode and microinfusion cannulae were determined under a light microscope and recorded on representative coronal diagrams (Franklin and Paxinos, 1997).

2.4. Measurement of body temperature

Mice were housed individually in separate cages for about 2 h prior to the start of each experiment, which took place between 1330 hours and 1530 hours in the afternoon. A rectal themister probe (Physitemp Instruments, Clifton, NJ, USA) was used for body temperature measurement. The probe was inserted into the rectum to a depth of about 3–3.5 cm, which we found gave a reliable measure of the animal's core body temperature. To habituate the mice to the testing procedure, the body temperature of the mice was measured every 15 min for 1 h prior to drug administration (quinpirole: 0.1, 0.3, or 1 mg/kg s.c., 7-hydroxy-dipropylamino-tetralin (7OH-DPAT): 0.5, 1, or 3 mg/kg s.c., all salt weight, or saline vehicle 10 μl/g s.c.). Following drug administration, the body temperature of the mice was monitored every 15 min for another hour.

2.5. Measurement of ACTH release induced by different drugs

Mice were housed individually overnight in separate cages. The collection of blood samples via retro-orbital bleeding always occurred between 0900 hours and 1000 hours, and was performed within 40 s from initial disturbance of the cage. Mice were bled either without any drug injection (basal measurement), or 15 min after subcutaneous injection with saline vehicle, 1 mg/kg quinpirole (salt weight), 0.5 mg/kg 8-hydroxy-dipropylamino-tetralin (8OH-DPAT, salt weight), or 30 min after i.p. injection with saline vehicle or 10 μg/kg corticotropin releasing hormone (CRH). About 100 μl of blood was collected into ice-cold tubes containing 50 Kallikrein inhibitor units of aprotinin (Sigma, St. Louis, MO, USA) and 2.5 μl of 6% ethylenediaminetetraacetic acid (Sigma, St. Louis, MO, USA). The blood was centrifuged at 246 g for 20 min at 4 °C, and the plasma stored in a −70 °C freezer until ready for analysis. The level of ACTH in blood plasma was determined using a radioimmunoassay kit obtained from ICN Biomedicals (Aurora, OH, USA). As ACTH levels did not differ when blood samples were collected either 15 or 30 min after s.c. or i.p. saline injections, respectively, the vehicle data were combined for mice of each genotype.

2.6. Drugs

SKF 38393 was obtained from Tocris Cookson (Bristol, UK). Quinpirole, 7OH-DPAT, 8OH-DPAT, haloperidol, and CRH were all obtained from Sigma, St. Louis, MO, USA. All drugs (with the exception of haloperidol) were made up to the required concentration in physiological saline (0.9%) and injected in a volume of 10 μl/g mouse weight. Haloperidol was dissolved in a drop of acetic acid and diluted with saline.

2.7. Western blotting

Mouse brain homogenates were prepared at a concentration of 20% (w/v) in SDS (sodium dodecyl sulphate) sample buffer (10% glycerol, 2.5% dithiothreitol, 3% SDS, bromophenol blue, 0.5 M Tris–HCl, pH 6.8) and spun in a refrigerated centrifuge at 10,000 g for 20 min. Forty microliter of supernatant containing 0.5 mg protein were loaded onto a 9% SDS-polyacrylamide gel. After gel electrophoresis, the resolved proteins were electro-transferred onto a nitrocellulose support. The nitrocellulose was blocked with 5% skim milk at room temperature for 1 h, and then incubated with antibodies specific for $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{o}$, $G\alpha_{s}$, $G\alpha_{q}$, $G\alpha_{z}$, and $G\beta_{common}$ (see Crouch and Hendry, 1993; Crouch et al., 1994) at 5 °C overnight. The blot was rinsed four times with phosphate buffered saline containing 0.1% (v/v) Tween 20 (PBST) for 15 min each, then incubated with horseradish peroxidase conjugated secondary antibody (Amersham Pharmacia, United Kingdom) at room temperature for 1 h. This was followed by extensive washing with PBST for 4 h, with four changes. The bands were detected using the Enhanced Chemiluminescence kit (Amersham Pharmacia, United Kingdom) and visualized on a Phosphoimager (Fuji, Japan).

2.8. Data analysis

Analysis of Variance (ANOVA) was performed using the Statistical Package for the Social Sciences software (SPSS Inc., Chicago, USA). Drug dose and genotype were between-subject factors, and time was a within-subject factor. For data that did not meet the sphericity assumption of repeated measures ANOVA, the Huynh–Feldt correction was applied. Post hoc multiple comparisons were performed using Fisher's least significant difference procedure.

3. Results

3.1. The locomotor response of $G\alpha_z$ knockout mice to SKF 38393 and quinpirole

We investigated the locomotor response of $G\alpha_z$ knockout and wildtype mice to the dopamine D1-like receptor agonist, SKF 38393. SKF 38393 caused a significant enhancement of locomotor activity in both wildtype and $G\alpha_z$ knockout mice (Fig. 1A) ($F(2,38) = 16.5$, $p < 0.001$). However, there was no significant difference in the magnitude of locomotor responses between mice of the two genotypes ($F(1,38) < 1$, NS).

In contrast to the stimulation of locomotor activity by dopamine D1 receptors, activation of presynaptic D2-like autoreceptors is known to depress locomotor activity, through inhibition of dopamine release from dopaminergic terminals (Starr and Starr, 1986; Usiello et al., 2000). As expected, the D2-like receptor agonist quinpirole caused a significant reduction in the locomotor activity of both genotypes ($F(3,59) = 67.4$, $p < 0.001$) (Fig. 1B). It is important to note, however, that the locomotor suppressive effects of quinpirole was attenuated significantly less in mice lacking $G\alpha_z$ ($F(1,41) = 12.5$, $p < 0.01$), suggesting an impairment of D2-like autoreceptor function in the $G\alpha_z$ knockout mice.

3.2. Quinpirole-induced inhibition of accumbal dopamine release in $G\alpha_z$ knockout mice

Quinpirole has been reported to suppress locomotor activity by acting on dopamine D2-like receptors in the core of the nucleus accumbens (Swanson et al., 1997). To confirm that accumbal presynaptic D2-like receptor function has been compromised in the $G\alpha_z$ knockout mouse, the inhibition of electrically evoked dopamine release by quinpirole in the nucleus accumbens was measured. As shown in Fig. 2, infusion of quinpirole (100 ng/μl) into the core of the accumbens inhibited stimulated dopamine release by $79.5 \pm 1.8\%$ in wildtype mice ($n = 7$) within 15 min, whereas the same dose of quinpirole caused only $60.6 \pm 4.2\%$ inhibition in $G\alpha_z$ knockout mice ($n = 8$) ($t(13) = 3.88$, $p < 0.01$). At 30 min post-infusion, the differences between the genotypes remained significant ($t(13) = 2.67$, $p < 0.05$), but gradually diminished at 45 min post-drug infusion due to variability caused mainly by rapid

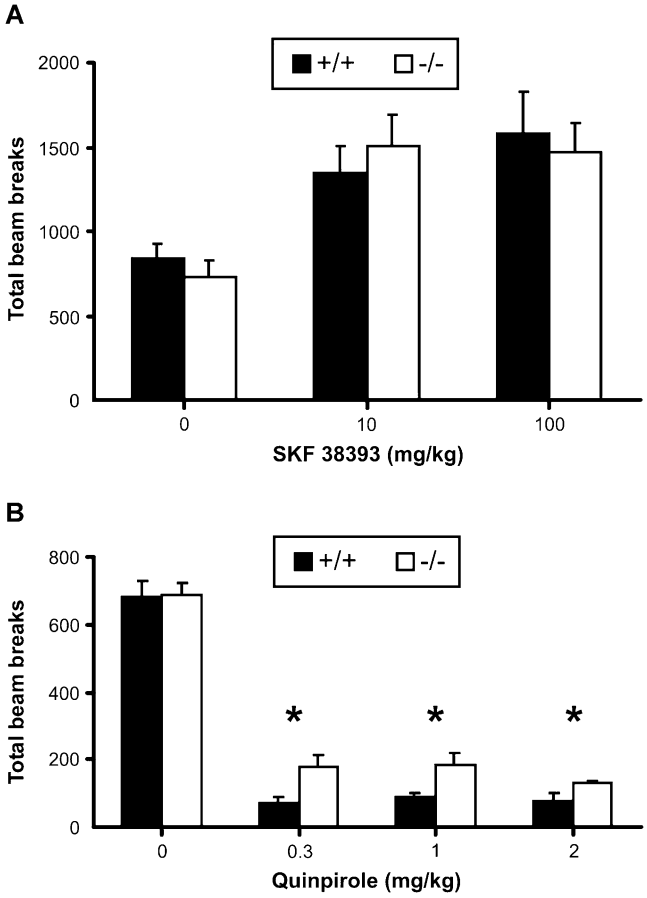

Fig. 1. Locomotor responses of wildtype and Gα_z knockout mice to the D1 and D2-like receptor agonists SKF 38393 and quinpirole, respectively. Means ± SEM are shown. (A) Wildtype (+/+) and Gα_z knockout mice (−/−) were administered (s.c.) saline vehicle (0 mg/kg, $n = 10$/group), 10 mg/kg ($n = 6$/group) or 100 mg/kg ($n = 6$/group) SKF 38393, or (B) saline vehicle (0 mg/kg, $n = 10$/group), 0.3 mg/kg (+/+, $n = 5$; −/−, $n = 7$), 1 mg/kg (+/+, $n = 11$; −/−, $n = 12$) or 2 mg/kg (+/+, $n = 5$; −/−, $n = 7$) quinpirole. Total locomotor activity was monitored over 4 h (SKF 38393) and 90 min (quinpirole); +/+ vs −/−: $^*p \leq 0.05$.

baseline recovery of one wildtype mouse. Intra-accumbens infusion of haloperidol (1 μg/μl) 15 min before quinpirole infusion prevented quinpirole inhibition of electrically stimulated dopamine release (data not shown), suggesting the observed inhibitory effects were specific to activation of dopamine D2-like receptors.

The correct placement of recording electrodes, stimulating electrodes and cannulae were confirmed by histology. Placements of the amperometric electrode recording surfaces and drug infusion cannulae were confined to the medial aspect of the core of the nucleus accumbens in the range of +1.54 to +1.34 mm anterior to bregma (Fig. 2). The tips of the stimulating electrodes were accurately positioned within the medial forebrain bundle ranging from −1.94 to −2.18 mm posterior to bregma (Fig. 2).

3.3. Hypothermia induced by quinpirole and 7OH-DPAT

Stimulation of dopamine D2 receptors is well known to elicit hypothermia in mice (Boulay et al., 1999a). We evaluated the thermic responses of wildtype and Gα_z knockout mice to the dopamine D2-like receptor agonists, quinpirole and 7OH-DPAT. In Gα_z knockout mice, the decrease in body temperature produced by both quinpirole ($F(1,38) = 27.3$, $p < 0.001$) and 7OH-DPAT ($F(1,48) = 23.12$, $p < 0.001$) was found to be significantly attenuated (Fig. 3).

3.4. Quinpirole-induced ACTH release

Dopaminergic pathways and D2-like receptors also participate in the regulation of pituitary hormone release. Systemic administration of quinpirole results in a dose-dependent increase in plasma ACTH levels, which can be antagonized by the D2-like antagonist, sulpiride (Borowsky and Kuhn, 1992). This quinpirole-induced increase in ACTH levels is also significantly attenuated in Gα_z knockout mice ($F(1,25) = 5.36$, $p < 0.05$) (Fig. 4). The mechanism by which D2-like selective agonists produce stimulation of ACTH release remains obscure, but may depend on CRH secreted by hypothalamic neurons. As an additional control, we also examined CRH-stimulated ACTH release. Intraperitoneal administration of 10 μg/kg CRH produced a significant rise in plasma ACTH ($F(1,28) = 12.5$, $p < 0.01$), as previously described (Muglia et al., 2000). However, there was no significant difference observed between wildtype and Gα_z knockout mice ($F(1,28) < 1$, NS) (Fig. 4). The serotonin 1A receptor agonist, 8OH-DPAT, is also known to stimulate plasma ACTH via hypothalamic CRH (Serres et al., 2000). As expected, 0.5 mg/kg of 8OH-DPAT caused a significant rise in plasma ACTH 15 min after injection (Fig. 5) ($F(1,29) = 50.5$, $p < 0.001$). However, there was no difference between wildtype and Gα_z knockout mice ($F(1,29) < 1$, NS), suggesting that CRH receptors involved in ACTH secretion are not impaired in Gα_z knockout mice.

3.5. Absence of compensation by other G protein α subunits

Finally, we examined whether the alteration of dopamine D2-like receptor function could be explained by changes in the expression levels of other G protein α or β subunits. As expected, western blot analysis revealed the complete absence of the G protein α_z subunit in Gα_z knockout mice. In addition, this analysis also indicated a lack of significant differences between Gα_z knockout and wildtype mice in the expression levels of all other G protein subunits (Fig. 5).

4. Discussion

In the present study, mice deficient in the α subunit of G_z were used to investigate the role of G_z in dopamine D2-like receptor signaling in the intact animal. G_z is a member of the inhibitory guanine nucleotide binding protein family. It is widely found in many regions of the brain where dopamine receptors are located, including the cerebral cortex, amygdala, caudate nucleus, putamen, nucleus accumbens, substantia nigra, hypothalamus and hippocampus (Glick et al., 1998; Hinton et al., 1990; Serres et al., 2000; Wang et al., 1998).

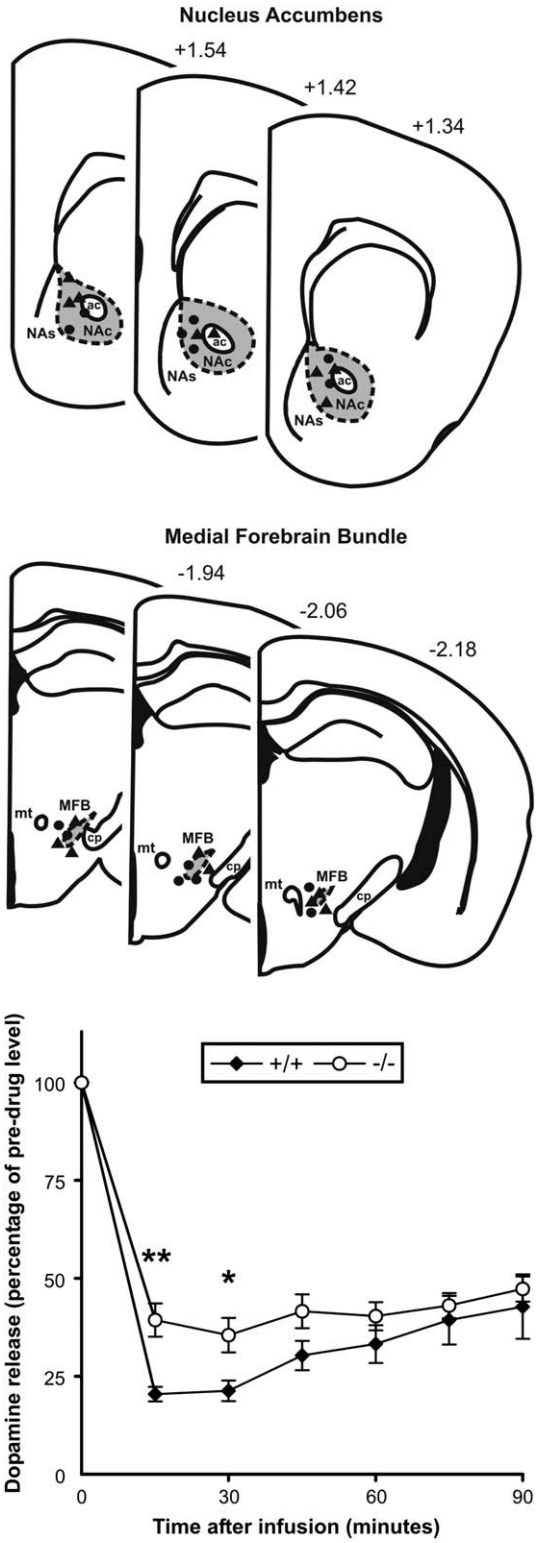

Fig. 2. Representative coronal sections of the brain showing placement of amperometric recording electrodes in the nucleus accumbens (gray area) and stimulating electrodes in the medial forebrain bundle (gray area) of wildtype (closed circles) and Gα_z knockout (closed triangles) mice. The numbers correspond to distance (mm) from Bregma. Abbreviations: NAc, nucleus accumbens core; NAs, nucleus accumbens shell; ac, anterior commissure; MFB, medial forebrain bundle; mt, mammillothalamic tract; cp, cerebral peduncle. The figures are adapted from Franklin and Paxinos (1997). Quinpirole inhibition of dopamine release is significantly attenuated in the nucleus accumbens of the Gα_z knockout mouse. The release of dopamine from synaptic terminals in the core of the nucleus accumbens was electrically evoked by stimulation of their axons in the medial forebrain bundle. After recording pre-drug baseline responses, 100 ng of quinpirole was microinfused via a cannula into the nucleus accumbens, and dopamine release in the nucleus accumbens core was recorded every 15 min for a total duration of 90 min. The mean ± SEM responses from wildtype (+/+, $n = 7$) and Gα_z knockout mice (−/−, $n = 8$) mice are shown; +/+ vs −/−: $^*p < 0.05$, $^{**}p < 0.01$.

Recent experiments in vitro have shown that G_z can couple to many of the same receptors as $G_{i/o}$, including dopamine D2-like receptors (Ho and Wong, 1998; Obadiah et al., 1999; Sidhu and Niznik, 2000). However, coupling of these G proteins to D2-like receptors within specific dopaminergic systems remains to be demonstrated in vivo. The data presented here demonstrate for the first time that G_z is functionally coupled to D2-like receptors in vivo.

A common criticism of gene knockout studies is that there may be compensations in other parts of the system as a result of inactivation of a specific gene. In the case of G_z, some members of the $G_{i/o}$ family, which share similar receptors and effectors as G_z (Ho and Wong, 1998), may increase their expression to compensate for the absence of G_z protein. However, western blot analysis of whole brain from Gα_z knockout mice did not reveal any changes in the levels of other G protein α subunits. Functional compensation by other G proteins is predominantly observed when receptor G protein coupling is studied in broken membrane preparations. However, under these in vitro conditions, the observed compensation is likely an artifact as a result of disruptions to membrane compartments which play an important role in determining signaling specificity in vivo (Hur and Kim, 2002; Tsunoda and Zuker, 1999). Indeed, in membranes prepared from rat RINm5F neuroendocrine cells, galanin and somatostatin receptors show promiscuous coupling to various G_i and G_o isoforms (Degtiar et al., 1997; Schmidt et al., 1991). In contrast, when G protein coupling of galanin and somatostatin receptors are examined in whole RINm5F cells by electrophysiology, galanin receptors couple specifically to G_{o1} and somatostatin receptors to G_{o2} (Degtiar et al., 1997). Similarly, in dog thyroid membranes, stimulation of thyrotropin receptors cause incorporation of [α-^{32}P]-GTP-γ-azidoanlide into Gα_s, Gα_i, and G$\alpha_{q/11}$, suggesting that activation of the thyrotropin receptor would normally stimulate these G proteins (Allgeier et al., 1997). However, in the same experiment, when intact thyrocytes were used, the thyrotropin receptor could only couple to G_s and G_i (Allgeier et al., 1997). This indicates that although the thyrotropin receptor can recognize $G_{q/11}$ in disrupted membranes, membrane compartmentalization in an intact cell prevents the receptor from interacting with $G_{q/11}$ in vivo.

Over the dose range tested, systemic injection of 7OH-DPAT decreased body core temperature to a greater extent than quinpirole in both wildtype and Gα_z knockout mice. In vitro binding studies suggest a 16-fold and eightfold selectivity for D3 receptors, compared to D2 receptors, for 7OH-DPAT and quinpirole, respectively (Malmberg and Mohell, 1995; Sautel et al., 1995; Morris et al., 2000) suggesting

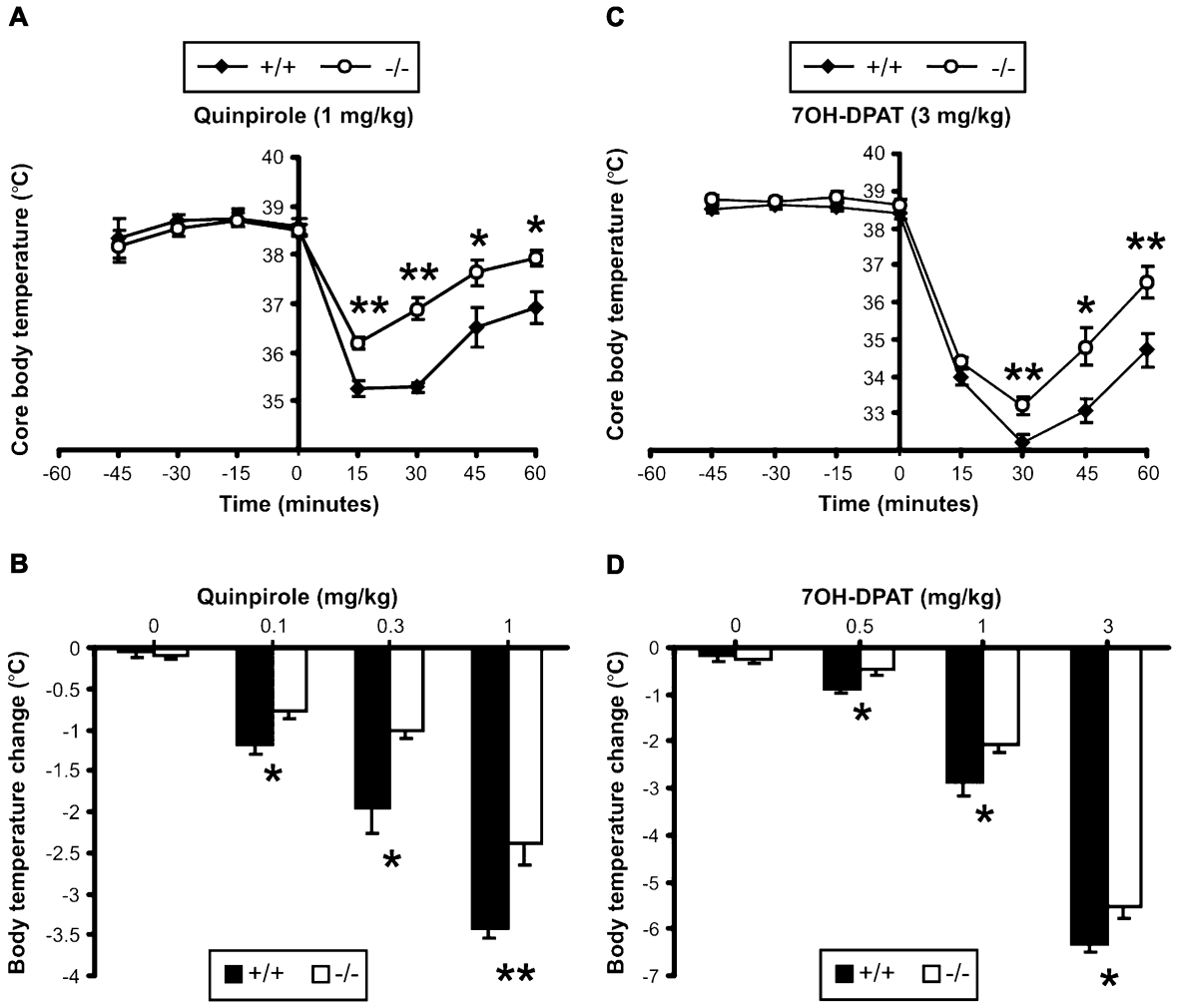

Fig. 3. Hypothermia induced by the dopamine D2-like receptor agonists, quinpirole and 7-hydroxy-dipropylamino-tetralin (7OH-DPAT) is significantly reduced in $G\alpha_z$ knockout (−/−), compared to wildtype (+/+) mice. Mice were administered (s.c.) saline vehicle (0 mg/kg) or various doses of either quinpirole or 7OH-DPAT at time 0, and their body temperatures were measured using a rectal thermistor probe. Hypothermic changes in body temperature of +/+ and −/− mice in response to 1 mg/kg quinpirole (A), and following various doses of quinpirole 15 min after injection (B). Hypothermic changes in body temperature of +/+ and −/− mice in response to 3 mg/kg 7OH-DPAT (C), and following various doses of 7OH-DPAT 30 min after injection (D). The plotted values are the mean body temperatures ± SEM ($n \geq 7$/group); +/+ vs −/−: *$p < 0.05$, **$p < 0.01$.

that the hypothermic effects of these D2/D3 agonists may be mediated by D3 receptors. However, the relative selectivity of these putative D3 preferring agonists has been shown in vivo to be highly dose-dependent. For example, Levant et al. (1996) and others (Zuch and Cory-Slechta, 2001) have shown that stimulation of D2 receptors contributes to the pharmacological effects of 7OH-DPAT when administered at doses above 0.3 mg/kg (s.c.). It is important to note that in the present studies the dose range for this drug was 0.5−3 mg/kg (s.c.). In addition, a number of recent pharmacological studies in rats and selective dopamine receptor knockout mice suggest a more prominent role for D2 receptors in mediating D2-like agonist-induced hypothermia, including their locomotor attenuating effects. Administration of the D2/D3 agonist PD128907 has been shown to induce hypothermia and hypolocomotion with similar efficacy and potency in wildtype and D3 knockout mice, while 7OH-DPAT has been shown to be ineffective in eliciting these effects in D2 knockout mice (Boulay et al., 1999a,b). Moreover, administration of the selective D2 antagonist L-741,626 prevents hypothermia induced by PD128907 in rats, whereas the selective D3 antagonist A-437203 is ineffective in preventing this effect (Chaperon et al., 2003). Together, these data strongly argue that the hypothermic and hypolocomotor effects of these D2-like receptor agonists are best attributed to an action at central D2 receptors.

The significant blunting, but not complete abolition of D2-like receptor mediated behavioural responses in $G\alpha_z$ knockout mice indicates that dopamine D2-like receptors couple to more than one G protein in vivo. As noted above, activation of dopamine D2 receptors likely mediate the hypothermic and hypolocomotor effects of D2-like agonists. The present observations of significant attenuations in both the hypothermic and locomotor depressing effects of D2-like agonists suggest that G_z is coupled to D2 receptors to partly mediate these effects. As these responses were not completely abolished (as reported in D2 receptor knockout mice), our results suggest that dopamine D2 receptors also couple to other G proteins. The dopamine D2 receptor can exist as two alternately spliced

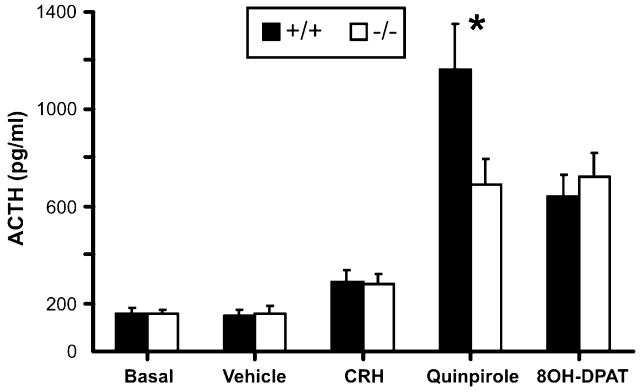

Fig. 4. Quinpirole stimulation of plasma ACTH is significantly attenuated in the $G\alpha_z$ knockout (−/−), compared to wildtype (+/+) mice. Mice were administered (s.c.) saline (vehicle, $n = 8$/group), 10 μg/kg corticotropin releasing hormone (CRH) ($n = 8$/group), 1 mg/kg quinpirole (+/+, $n = 7$; −/−, $n = 6$), or 0.5 mg/kg 8-hydroxy-dipropylamino-tetralin (8OH-DPAT) (+/+, $n = 8$; −/−, $n = 9$), and blood was collected via the retro-orbital route either 15 (all drugs except CRH) or 30 min (CRH) after the injection. Non-disturbed (basal), saline (vehicle), or drug-induced changes in ACTH levels in blood plasma were determined using a commercial RIA kit. The mean plasma ACTH levels ± SEM are shown. +/+ vs −/−: $^*p < 0.05$.

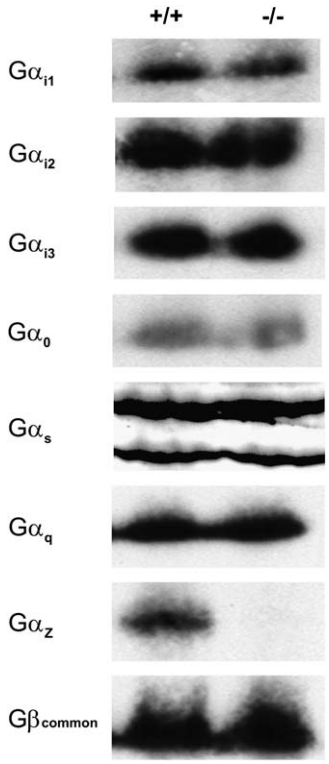

Fig. 5. Brain levels of $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$, $G\alpha_q$, $G\alpha_z$, and Gβ in wildtype (+/+) and $G\alpha_z$ knockout (−/−) mice. The proteins from the brains of wildtype and $G\alpha_z$ knockout mice were analysed by SDS-polyacrylamide gel electrophoresis, electro-transferred onto nitrocellulose, and blotted with antibodies specific for $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$, $G\alpha_q$, $G\alpha_z$, and $G\beta_{common}$. The experiment was repeated twice. There was some slight variation in the intensity of the protein bands due to protein transfer and blotting. However, no discernible differences, with the exception of $G\alpha_z$, could be observed between any of the Gα and Gβ subunits.

forms (Rouge-Pont et al., 2002; Usiello et al., 2000; Wang et al., 2000) and it can form homomers with itself or heteromers with other receptors (Franco et al., 2000). Moreover, these D2 receptor subtypes are all absent in the D2 receptor knockout mouse. Therefore, the particular dopamine D2 receptor subtype(s) that couple to G_z in vivo is presently unknown. However, our data suggest that other G proteins (including $G_{i/o}$, which is present in far greater quantities than G_z in the brain) do not couple to the G_z preferring dopamine D2 receptor in an effort to compensate for the absence of G_z.

Among other members of the D2-like receptor family, both D2 and D3 receptors are capable of functioning as autoreceptors to inhibit dopamine release. However, studies of D2 and D3 receptor knockout mice have found the D2 receptor to be prominent in this role (Benoit-Marand et al., 2001; L'hirondel et al., 1998; Rouge-Pont et al., 2002; Schmitz et al., 2001), with the D3 receptor making only a minor contribution (Joseph et al., 2002). While most of these studies have been performed in the dorsal striatum, D2 receptors are also present in the nucleus accumbens (Bancroft et al., 1998; Le Moine and Bloch, 1996; Levant, 1998), and are likely to perform the same role. A partial impairment in the function of D2 autoreceptors might therefore account for the observed inhibition of electrically evoked dopamine release by quinpirole in $G\alpha_z$ knockout mice. However, a role for D3 receptors cannot be entirely excluded at present. D3 receptors are also found in the nucleus accumbens (Bancroft et al., 1998), and they may be involved in the inhibition of dopamine release through postsynaptic receptors coupled to short-loop negative feedback pathways (Koeltzow et al., 1998).

In the present study, G_z has also been found to be involved in mediating the secretion of ACTH caused by activation of D2-like receptors. Pituitary secretion of ACTH is believed to occur via dopamine receptors in the hypothalamus (Borowsky and Kuhn, 1992). Cocaine, an indirect dopamine agonist at both D1 and D2-like receptors, has also been found to stimulate ACTH release (Rivier and Vale, 1987). The mechanism involves CRH from the hypothalamus as the effect of cocaine is blocked by a CRH antiserum (Rivier and Vale, 1987). Quinpirole-induced ACTH release may operate via a similar mechanism. Recent evidence shows that stimulation of cortical CRH receptors results in the incorporation of a photo-reactive form of GTP, [α-^{32}P]-GTP-γ-azidoanlide into $G\alpha_z$ (along with $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, and $G\alpha_o$) (Grammatopoulos et al., 2001). However, our data did not reveal any significant difference between wildtype and $G\alpha_z$ knockout mice in CRH-stimulated ACTH secretion suggesting that CRH receptors are not coupled to G_z to mediate ACTH secretion. Additionally, stimulation of ACTH release by the serotonin 1A agonist, 8OH-DPAT, which is known to occur via a CRH mechanism (Serres et al., 2000), also did not differ between mice of the two genotypes. This suggests that CRH receptors in the pituitary, and serotonin 1A receptors in the hypothalamus that mediate ACTH secretion, are not coupled to G_z. This is in contrast to the findings of Serres et al. (2000) where the employment of $G\alpha_z$ antisense oligonucleotides was found to partially counteract the rise in plasma ACTH levels

caused by 8OH-DPAT in rats. The discrepancy in these results may be attributed to species differences, general oligonucleotide toxicity or non-specificity in their $G\alpha_z$ antisense, which they have suggested in their experiments. Regardless, the present results suggest that pituitary CRH receptors are not impaired in the $G\alpha_z$ knockout mouse. Thus, the significant diminution of quinpirole-induced ACTH release in the $G\alpha_z$ knockout mouse is likely caused by impairment of D2-like receptor function as a result of the absence of $G\alpha_z$.

In summary, our data indicate that G_z is functionally coupled to brain D2-like receptors in vivo. Since disturbances in central dopaminergic networks are implicated in a number of debilitating neurodegenerative and psychopathological diseases, including Parkinson's disease, schizophrenia, and drug addiction, G_z might serve as a potential drug target when subtle fine tuning of dopaminergic functions are required.

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