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Inhibition of GSK3 attenuates dopamine D1 receptor agonist-induced hyperactivity in mice

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Abstract

Recent evidence suggests a critical role for the intracellular signaling protein glycogen synthase kinase-3 (GSK3) in hyperactivity associated with dopaminergic transmission. Here, we investigated whether activation of GSK3 is necessary for the expression of behaviors specifically produced by dopamine D1 receptor activation. To assess the role of GSK3 in dopamine D1 receptor-induced hyperactivity, mice were pretreated with the selective GSK3 inhibitor SB 216763 (0.25–7.5 mg/kg, i.p.) or its vehicle prior to administration of the dopamine D1 receptor full agonist SKF-82958 (1.0 mg/kg, i.p.) or saline control. Inhibition of GSK3 via SB 216763 dose-dependently reduced ambulatory and stereotypic activity produced by SKF-82958. These data implicate a role for GSK3 in the behavioral manifestations associated with dopamine D1 receptor activation.

Keywords

Dopamine; glycogen synthase kinase-3; ambulation; stereotypy; activity

Introduction

The behavioral and neuromodulatory effects of dopamine are mediated via D1-like and D2-like dopamine receptors, members of the G-protein coupled receptor family [21]. The dopamine D1 receptor is highly expressed in striatal regions of the brain [18,26] and is coupled to stimulatory G-proteins (G_s/G_{olf}). Activation of dopamine D1 receptors results in the activation of adenylate cyclase and subsequent increases in cAMP [14,23]. Increases in cAMP following dopamine D1 receptor stimulation can regulate a number of intracellular signaling cascades via activation of protein kinase A and subsequent phosphorylation of cAMP response element binding protein (CREB) and dopamine- and cAMP-regulated phosphoprotein – 32 kd (DARPP-32) [15,24].

Previous studies have assessed the role of the dopamine D1 receptor in locomotor behaviors. Administration of the D1 receptor full-agonist SKF-82958 or partial agonist SKF-38393 increases locomotor activity in mice [9,13]. In addition, antagonism of the dopamine D1

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receptor prevents cocaine-induced hyperactivity [4]. Thus, given the importance of dopamine D1 receptors in locomotion, the goal of the present study was to identify intracellular signaling molecules involved in locomotor activity produced by dopamine D1 receptor activation.

One kinase that has recently gained attention as being critical to the behavioral and neurochemical manifestations of dopamine and dopaminergic receptors is glycogen synthase kinase-3 (GSK3) [1]. GSK3 is widely expressed in the adult rat brain [17] and is inactivated via phosphorylation of the serine 21 (α -isoform) or serine 9 (β -isoform) residues [12]. GSK3 is selectively inhibited by maleimide derivatives such as SB 216763 [7]. Inhibition of GSK3 by SB 216763 attenuates hyperactivity in mice lacking the dopamine transporter [1]. SB 216763 also attenuates cocaine-induced hyper-locomotion and prevents the development of cocaine-induced locomotor sensitization [20]. Based on previous studies indicating the importance of GSK3 in hyper-locomotor responses associated with dopamine, we investigated whether inhibition of GSK3 would alter ambulatory and stereotypy responses following direct dopamine D1 receptor stimulation.

Materials and Methods

Animals

Male CD-1 mice (8 weeks old) were obtained from Charles River Laboratories (Wilmington, MA). Mice were housed five per plastic cage ($28 \times 18 \times 14$ cm) without additional enrichment objects in a temperature- and relative humidity-controlled room with a 12-hr light/dark cycle (lights on at 7:00 a.m.). Animals were housed for seven days prior to behavioral testing and were handled and weighed daily. All animals had access to standard laboratory chow and tap water *ad libitum*. All animal testing was conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and with an approved protocol from Temple University Institutional Animal Care and Use Committee.

Compounds

($\pm$)-SKF-82958 (($\pm$)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetra-hydro-1H-3-benzazepine HBr) [22] (Sigma; St. Louis, MO) was dissolved in sterile saline (0.9% NaCl). SB 216763 (Tocris; Ellisville, MO) was dissolved in propylene glycol and brought up to volume in distilled water (70:30). Saline or propylene glycol vehicle (70%) was used for vehicle control injections.

Behavioral Testing/Drug Administration

Activity was measured using the Digiscan DMicro system (Accuscan, Inc., Columbus, OH). The activity monitors consist of transparent plastic boxes ($45 \times 20 \times 20$ cm) set inside metal frames that are equipped with 16 infrared light emitters and detectors. The number of photocell beam breaks is recorded by a computer interface. Ambulation was recorded as consecutive beams breaks resulting from horizontal movement, while stereotypy was recorded by repetitive beam breaks. Animals were placed in locomotor activity monitors for 30 minutes. Following the 30 minute acclimation period, mice were pretreated with vehicle or SB 216763 (0.25–7.5 mg/kg, i.p.) followed by an injection of saline or SKF-82958 (1.0 mg/kg, i.p.) 5 minutes later. Activity was measured for 90 minutes following the second injection.

Data Analysis

Time-course behavioral data were analyzed by a repeated measures ANOVA with treatment as the between group factor and time as the within group factor. Cumulative behavioral data

were analyzed using two-way ANOVA with pre-treatment and treatment factors. Significant ANOVAs were followed by a Bonferroni test for multiple comparisons (GraphPad Prism 4, La Jolla, CA). EC50 values were determined using nonlinear regression as the mean effect vs. dose [25].

Results

Inhibition of GSK3 attenuated acute SKF-82958-induced ambulation

Ambulatory activity was measured following pretreatment with the GSK3 inhibitor SB 216763 (0.25–7.5 mg/kg, i.p.) followed by an injection of the D1-like receptor agonist SKF-82958 (1.0 mg/kg) 5 minutes later. The data displayed in Figure 1A represent cumulative ambulatory counts recorded over the first 60 minutes post SKF-82958 or saline injections. Two-way ANOVA indicated a significant treatment effect of SB 216763 on SKF-82958-induced ambulatory activity (Interaction: $F(5,84)=1.867$, $p=0.108$; Pretreatment: $F(5,84)=1.974$, $p=0.090$; Treatment: $F(1,84)=97.24$, $p<0.0001$). Bonferroni post-hoc analyses indicated that SKF-82958 significantly increased ambulatory activity compared with saline-injected controls (vehicle/saline vs. vehicle/SKF, $p<0.001$). Pretreatment with SB 216763 at doses of 2.5 and 7.5 mg/kg significantly attenuated SKF-82958-induced ambulatory activity (2.5 mg/kg SB 216763/SKF vs. vehicle/SKF, $p<0.01$; 7.5 mg/kg SB 216763/SKF vs. vehicle/SKF, $p<0.001$). Nonlinear regression analysis of the mean effect versus dose yielded an EC50 value of $0.70 \text{ mg/kg} \pm 0.66 \text{ mg/kg}$ for SB 216763 in attenuating SKF-82958-induced ambulation. SKF-82958-induced ambulatory activity was not significantly changed following pretreatment with the lower doses of SB 216763 (0.25–1.0 mg/kg, $p>0.05$). SB 216763 had no effect on baseline ambulatory activity at any dose tested (vehicle/saline vs. all SB 216763 doses/saline, $p>0.05$).

The time course of ambulatory activity is shown in Figure 1B for mice injected with SB 216763 (7.5 mg/kg) or vehicle prior to SKF-82958 (1.0 mg/kg) or saline. Two-way ANOVA with time and treatment factors showed significant differences between the treatment groups (Interaction: $F(54,589)=1.352$, $p=0.052$; Time: $F(18,589)=1.257$, $p=0.210$; Treatment: $F(3,589)=180.9$, $p<0.0001$). Posthoc analysis revealed that SKF-82958 significantly increased locomotor activity compared to saline-injected control (vehicle/SKF vs. vehicle/saline; $p<0.001$). Pretreatment with SB 216763 significantly reduced SKF-82958-induced locomotion at the times shown in Fig. 1B (SB 216763/SKF vs. vehicle/SKF, $p<0.05$ – 0.01). SB 216763 had no significant effect on baseline activity (SB 216763/saline vs. vehicle/saline, $p>0.05$).

Inhibition of GSK3 attenuated acute SKF-82958 induced stereotypy

Stereotypic activity was also measured following pretreatment with the GSK3 inhibitor SB 216763 (0.25–7.5 mg/kg, i.p.) and injection of SKF-82958 (1.0 mg/kg) 5 minutes later. The data displayed in Figure 2A represent cumulative stereotypy counts recorded over the first 60 minutes post SKF-82958 injection. Two-way ANOVA indicated significant pretreatment and treatment effects (Interaction: $F(5,84)=1.683$, $p=0.148$; Pretreatment: $F(5,84)=3.202$, $p=0.011$; Treatment: $F(1,84)=71.31$, $p<0.0001$). Bonferroni post-hoc analysis revealed that SKF-82958 (1 mg/kg) significantly increased stereotypy as compared with saline controls (vehicle/saline vs. vehicle/SKF, $p<0.001$). Administration of SB 216763 at doses of 1.0, 2.5, or 7.5 mg/kg significantly attenuated SKF-82958-induced stereotypic activity (vehicle/SKF vs. 1.0 mg/kg SB 216763/SKF, $p<0.01$; vehicle/SKF vs. 2.5 mg/kg SB 216763/SKF, $p<0.05$; vehicle/SKF vs. 7.5 mg/kg SB 216763/SKF, $p<0.001$). Nonlinear regression analysis of the mean effect versus dose yielded an EC50 value of $1.04 \text{ mg/kg} \pm 0.55 \text{ mg/kg}$ for SB 216763 in attenuating SKF 82958-induced stereotypy. SKF-82958-induced stereotypic activity was not significantly changed following pretreatment with the lower

doses of SB 216763 (0.25 or 0.50 mg/kg, $p > 0.05$). Administration of SB 216763 had no effect on baseline stereotypic activity at any dose tested (vehicle/saline vs. all SB doses/saline, $p > 0.05$).

The time-course of changes in stereotypy produced by SB 216763 (7.5 mg/kg) followed by SKF-82958 (1.0 mg/kg) are shown in Figure 2B. Results from the two-way ANOVA demonstrate that there were significant differences between treatment groups (Interaction: $F(54,589)=1.288$, $p=0.087$; Time: $F(18,589)=0.955$, $p=0.511$; Treatment: $F(3,589)=171.8$, $p < 0.0001$). Posthoc analyses showed that SKF-82958 significantly increased stereotypic behaviors (vehicle/SKF vs. vehicle/saline, $p < 0.001$). Pretreatment with SB 216763 (7.5 mg/kg) prior to SKF-82958 significantly attenuated SKF-82958-induced stereotypic activity at the times indicated on Fig. 2B (SB 216763/SKF vs. vehicle/SKF, $p < 0.05-0.001$).

Discussion

Previous studies have implicated the importance of dopamine D1 receptors in animal models of hyperactivity [9,13,19]. Our data support previous reports demonstrating that acute administration of the dopamine D1 receptor agonist SKF-82958 yields a hyperactive response in rodents consisting of increases in both ambulatory and stereotypic behaviors [9,13,19]. In the present study, the selective GSK3 inhibitor, SB 216763, attenuated ambulatory and stereotypic behaviors produced by a D1 receptor agonist. These data are the first to demonstrate that the increases in ambulatory and stereotypy responses produced by stimulation of dopamine D1 receptors are dependent in part on GSK3 activity.

Previous studies indicate the importance of GSK3 in behaviors associated with altered dopaminergic transmission. The selective GSK3 inhibitors SB 216763 and AR-A014418 reduce hyperactivity produced by the indirect dopaminergic agonists, amphetamine [11,16] and cocaine [20]. Non-selective inhibitors of GSK3 such as lithium [8] and valproate [5] attenuate the hyper-locomotor phenotype of mice lacking the dopamine transporter [1] and the hyperactivity produced by cocaine [20]. These previous studies demonstrate the importance of GSK3 in dopamine-mediated hyperactivity, although they do not indicate which dopamine receptor(s) may be involved.

Multiple lines of evidence suggest that dopamine receptors signal through the Akt/GSK3 pathway in the striatum. Akt negatively regulates GSK3 activity by phosphorylating GSK3. Mice lacking the dopamine transporter demonstrate a persistent elevation of striatal extracellular dopamine [10] and have reduced Akt activity with concomitant increases in the activity of GSK3 in the striatum [1]. Previous studies investigating the regulation of GSK3 activity by dopamine D1 receptors are contradictory and suggest that regulation is contingent upon spatial and temporal perturbations of the receptor and kinases upstream of GSK3. For example, mice lacking D1 receptors have normal baseline levels of GSK3 β phosphorylation in the striatum [2]. Likewise, the increase in GSK3 activity seen in the striatum of mice lacking the dopamine transporter is not blocked by the D1 receptor antagonist SCH 23390 [1]. In contrast to those findings, it has been shown that stimulation of the dopamine D1 receptor with SKF-38393 increases Akt phosphorylation in striatal neurons, and overexpression of a dominant negative form of Akt inhibits CREB phosphorylation induced by dopamine D1 receptor stimulation [3]. Further support for the regulation of GSK3 by D1 receptors comes from studies demonstrating that amphetamine modulation of GSK3 β phosphorylation is dependent on DARPP-32, a key downstream signaling molecule for dopamine receptors [24]. Our data provide support that D1 receptors signal through GSK3, since selective inhibition of GSK3 attenuated SKF-82958-induced hyperactivity.

In addition to the effects of dopamine D1 receptors on GSK3, recent evidence suggests that dopamine D2 and D3 receptors can regulate GSK3 activity. Administration of the dopamine D2 receptor antagonist raclopride to dopamine transporter knock-out mice decreases GSK3 activity in the striatum [1]. Mice lacking the D2 receptor show higher levels of phosphorylation of Akt and GSK3 β in the striatum than wild-type controls [2]. GSK3 β and Akt phosphorylation levels are also increased in the striatum of mice lacking the D3 receptor [2], although in the limbic forebrain, D3 receptor mutants show reduced levels of phosphorylated GSK3 with no change in basal Akt [6]. Thus, D3 receptors may regulate GSK3 in a brain region-specific manner.

Previous data from our laboratory demonstrate that selective inhibition of GSK3 with SB 216763 reduced acute cocaine-induced ambulatory and stereotypic activity in mice with EC50 values of 1.21 mg/kg $\pm$ 0.18 mg/kg for ambulation and 1.28 mg/kg $\pm$ 0.21 mg/kg for stereotypy [20]. An additional finding of that study was that 7.5 mg/kg SB 216763 reduced cocaine-stimulated activity to baseline levels [20]. In the present study, 7.5 mg/kg SB 216763 attenuated SKF-82958-induced ambulation by approximately 51% and stereotypy by 64%. This indicates that SB 216763 is more potent yet less effective at reducing dopamine D1 receptor-induced hyperactivity as compared to cocaine-induced hyperactivity.

The data presented herein demonstrate that administration of the selective GSK3 inhibitor SB 216763 attenuated the heightened ambulatory and stereotypy responses produced by dopamine D1 receptor stimulation. This suggests that a signaling pathway comprising GSK3 is activated by dopamine D1 receptors and is critical for the full expression of behavioral responses to D1 receptor agonists. These data provide evidence of a greater role of GSK3 in dopamine-mediated behaviors than previously appreciated and support further elucidation of the specific mechanisms by which this kinase functions to modulate dopaminergic behaviors.

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Figure 1. Inhibition of GSK3 attenuated dopamine D1 receptor-induced ambulation in mice
 Adult male CD-1 mice were pretreated with vehicle or the selective GSK3 inhibitor SB 216763 (0.25–7.5 mg/kg) 5 minutes prior to injection of SKF-82958 (1.0 mg/kg, i.p.) or saline and ambulatory activity (1A-B) was recorded. (1A) Cumulative ambulatory data for the first 60 minutes post SKF-82958 or saline are shown. SKF-82958 significantly increased ambulatory activity as compared with saline controls (# $p < 0.001$; vehicle/saline vs. vehicle/SKF-82958). Mice pretreated with SB 216763 (2.5 and 7.5 mg/kg) exhibited a significant attenuation of SKF 82958-induced ambulation (** $p < 0.01$, *** $p < 0.001$ SB 216763/SKF-82958 vs. veh/SKF-82958). SB 216763 alone had no effect on baseline ambulatory activity. Data points represent the means $\pm$ SEM of ambulatory counts over 60 minutes ($n = 6-12$ /group).
 (1B) The time course of ambulatory is shown for mice injected with SB 216763 (7.5 mg/kg) or vehicle prior to SKF-82958 (1.0 mg/kg) or saline. Pretreatment with SB 216763 significantly reduced SKF-82958-induced locomotion. (* $p < 0.05$, ** $p < 0.01$ SB 216763/SKF-82958 vs. veh/SKF-82958).

Figure 2. Inhibition of GSK3 attenuated dopamine D1 receptor-induced stereotypy in mice
 Adult male CD-1 mice were pretreated with vehicle or the selective GSK3 inhibitor SB 216763 (0.25–7.5 mg/kg) 5 minutes prior to injection of SKF-82958 (1.0 mg/kg, i.p.) or saline and stereotypic activity (2A-B) was recorded. (2A) Cumulative stereotypy data for the first 60 minutes following SKF-82958 are shown. SKF-82958 produced a significant increase in stereotypic activity as compared with saline controls ($^{\#}p < 0.001$; vehicle/saline vs. vehicle/SKF-82958). Mice pretreated with SB 216763 (1.0–7.5 mg/kg) exhibited a significant attenuation of SKF 82958-induced stereotypy. ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ SB 216763/SKF-82958 vs. vehicle/SKF-82958). SB 216763 alone had no effect on baseline stereotypic activity. Data points represent the means $\pm$ SEM of stereotypy counts over 60 minutes ($n = 6-12$ /group).
 (2B) The time course of changes in stereotypy is shown for mice injected with SB 216763 (7.5 mg/kg) or vehicle prior to SKF-82958 (1.0 mg/kg) or saline. SB 216763 (7.5 mg/kg) significantly attenuated stereotypy produced by SKF-82958. ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ SB 216763/SKF-82958 vs. vehicle/SKF-82958).