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The Ras Homolog Rhes Affects Dopamine D1 and D2 Receptor-Mediated Behavior in Mice

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Abstract

Dopamine activates five different receptor subtypes and a complex array of intracellular signaling pathways. Rhes is a striatally-expressed GTP-binding protein involved in dopamine signaling. Here we have used mutant mice to test whether Rhes is involved in D1 and D2 dopamine receptor-mediated behaviors. Rhes was not necessary for the expression of normal D1/D2 receptor synergism, as measured by apomorphine-induced stereotypy. The stereotypic responses to D1/D2 co-stimulation and to D2 stimulation alone were significantly increased in mice lacking Rhes, but D1 receptor-mediated grooming was reduced in these mice. These results suggest that Rhes is normally inhibitory to behaviors induced by D1/D2 receptor co-stimulation and by D2 receptor stimulation alone. However, Rhes appears to facilitate the D1-specific behavior of grooming.

Keywords

stereotypy; grooming; GTP-binding protein; D1 dopamine receptor; D2 dopamine receptor; striatum; Ras

Introduction

Dopamine (DA) is an important neurotransmitter that participates in motor activity, cognition, learning, and affect [1]. Its effects are mediated through different G protein-coupled receptors, the D1-like (D_1 and D_5) and the D2-like (D_2, D_3, and D_4) [2,3]. This abundance of receptors for DA creates the potential for a complex interplay among receptor subtypes and signaling pathways to which they couple. For example, under normal conditions, both D1 and D2 type receptors must be activated in order to elicit most DA-mediated responses, a phenomenon referred to as requisite synergism [4]. This requisite D1/D2 synergism is absent in denervated supersensitive striatum [5], which also displays activation of alternate signaling pathways [6]. Furthermore, in addition to the traditional G_αs/olf and G_αi/o coupling, evidence now exists for the coupling of DA receptors to G_αq [7]. It is likely that accessory proteins contribute to the adaptations of DA receptors and to the ability to couple to multiple signaling pathways.

The GTP binding protein Rhes is one such accessory protein that has been shown to be involved in DA-related signaling and behavior. Rhes (*R*as *H*omolog *E*nriched in *S*triatum) is an intermediate-size GTP binding protein encoded by a gene that is highly expressed in rodent

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striatum [8,9]. It is similar to Ras proteins from residues 17–205 and contains the conserved GTP/GDP binding domains [8], but it has an extended, positively charged C-terminal region [10]. Since Ras proteins respond to extracellular stimuli by interacting with intracellular signaling pathways [11], the homology with Ras suggests that Rhes may also interact with intracellular signaling pathways. The initial observation suggesting a role for Rhes in DA signaling was the finding that *rhes* mRNA is specifically decreased under conditions in which animals are supersensitive to DA, such as after 6-OHDA lesion or DA depletion with reserpine treatment [9]. Recent studies with *rhes* mutant mice have shown that mice lacking Rhes protein show alterations in both D1 and D2 receptor-related effects. Rhes knockout (KO) mice are more sensitive to locomotor activation by D1 agonists than wild type (WT) mice are, and they are more sensitive to D2 antagonism [12]. We report here the results of studies using Rhes KO mice designed to test the hypothesis that the level of Rhes protein is causally related to the state of D1/D2 synergism and the degree of D1 and D2 receptor sensitivity.

Methods

Rhes mutant mice [13] that have been backcrossed for 10 generations on the C57/BL6 background were bred at the University of New Orleans. Mice were housed in same-sex cages with free access to food and water. Artificial lighting was provided from 07:00 to 19:00h. All procedures were in strict accordance with the Guidelines for the Care and Use of Animals in Research (Public Health Service, U.S.A.), and were approved by the University of New Orleans Institutional Animal Care and Use Committee. Every effort was made to minimize animal discomfort and to reduce the number of animals used.

Species-specific stereotyped behavior was recorded under each of the four following conditions: (1) stimulation of both D1 and D2 receptors [injection of saline 30 min prior to injection of the mixed D1/D2 agonist apomorphine (3 mg/kg)], (2) stimulation of D1 receptors alone [injection of the selective D2 antagonist eticlopride (0.3 mg/kg) 30 min. prior to injection of apomorphine (3.0 mg/kg)], (3) stimulation of D2 receptors alone [injection of the D1 antagonist SCH 23390 (0.1 mg/kg) 30 min. prior to injection of apomorphine (3 mg/kg)], (4) stimulation of neither receptor subtype [two saline injections at the appropriate time points]. All injections were intraperitoneal (IP). This treatment regimen was used rather than selective agonists in order to eliminate the effects of endogenous DA at the heterotypic receptor. All mice were subjected to all conditions using a Latin Square design in four sessions, each separated by 72–96 h.

Each mouse was placed in a Plexiglas cylinder (measuring 22 cm high, 10.2 cm diameter) with a thin layer of wood chip bedding on the floor. After 30 minutes of habituation, the appropriate pre-treatment injection was given, followed 30 min later by injection of apomorphine (or saline). The testing session was terminated 60 min. later. Behavior was recorded with a Sony digital video camera for subsequent analysis and rated on a scale of 0 to 5 every 5 minutes during a 30-sec observation period. The scorer was unaware of genotype or treatment. This rating scale (modified for mice from [14]) has been previously employed in other mutant mouse studies [15] and is defined as follows: 0 = still; 1 = grooming or normal exploration; 2 = discontinuous unfocused stereotypy (e.g. brief episodes of strong sniffing); 3 = continuous unfocused stereotypy behavior (i.e. stereotypy directed to multiple objects and/or surfaces); 4 = continuous focused sniffing (i.e., sniffing of one object or within a highly circumscribed area); 5 = continuous focused oral stereotypy (i.e., licking/chewing of one object or within a highly circumscribed area).

In order to test for a D1 receptor-specific behavior, grooming in response to the D1 agonist SKF 38393 was examined. Male mice 2–4 months old were placed in the Plexiglas cylinders for 30 minutes of acclimation, and behavior was recorded as above. After an injection of SKF

38393 (10 mg/kg, IP), mice were immediately returned to the Plexiglas cylinders, and their behavior was recorded for an additional 60 minutes. The amount of grooming (total seconds spent grooming every 5 minutes during a 60-sec observation period) was recorded by an experimenter unaware of genotype.

Results

We tested whether *rhes* gene dosage affected D1/D2 synergism and DA receptor sensitivity by measuring stereotypy in response to dopaminergic drug administration. Stereotypy was used since it is a well-characterized behavioral measure of D1/D2 synergism [16]. Mice of all genotypes displayed normal requisite D1/D2 synergism; that is, stimulation of both D1 and D2 receptors was required for a full response. Figure 1 shows stereotypy scores averaged across the 60 minute observation period. Two-way repeated measures ANOVA (genotype × drug) of stereotypy scores gave significant main effects of drug ($F = 271.4$, $p < 0.0001$) and genotype ($F = 3.75$, $p < 0.05$), and a significant drug × genotype interaction ($F = 4.94$, $p < 0.001$). For each genotype, the D1+D2 condition differed significantly from saline, D1, and D2 treatment conditions, thus indicating that D1/D2 synergism is intact regardless of *rhes* gene dosage. However, some genotype differences were evident in the sensitivity of the response to the individual drug treatments. For both KO and heterozygous (Het), but not for WT mice, scores for the D1 treatment condition were significantly lower than the saline treatment condition. Furthermore, KO and Het mice showed a significantly higher response than WT mice in the D1/D2 condition.

In order to investigate further the nature of the genotype effects on sensitivity to dopaminergic drug treatment, we examined the time courses of the responses to drug stimulation. Figure 1 (inset) shows the response over time of each genotype to the combined activation of D1 and D2 receptors. Two-way repeated measures ANOVA (genotype × time) indicated significant main effects of time ($F=60.95$, $p< 0.0001$) and genotype ($F=4.94$, $p < 0.05$), but no time × genotype interaction.

Apomorphine-induced stereotypy is a behavior resulting from the synergistic effects of D1/D2 receptor activation. We also tested whether Rhes affects behaviors mediated by either receptor subtype alone. Although selective agonist stimulation of D2 receptors does not elicit sustained stereotypy, it does result in an early and brief burst of stereotyped behavior, an effect that is highly consistent across studies [14,15]. In the present study, the mice differed significantly in this effect as a function of genotype (Figure 2). Two-way ANOVA of the entire time course (genotype × time) indicated significant main effects of time ($F = 102.2$, $p < 0.0001$) and genotype ($F = 3.65$, $p < 0.05$), and a significant time × genotype interaction ($F = 4.41$, $p < 0.0001$). Analysis of stereotypy ratings at 5 and 10 min. post-agonist (the time frame during which this D1-independent D2 effect occurs) showed that KO mice were significantly more sensitive to D2 receptor stimulation compared with WT and Het mice ($F = 13.9$, $p<0.0001$ by one-way ANOVA of average totals for 5 and 10 minutes; Figure 2, inset).

As expected, selective agonist stimulation of D1 receptors did not elicit stereotyped behavior (see Figure 1). Although KO and Het mice displayed lower scores on the stereotypy rating scale than WT littermates, the amount of behavior induced under these conditions is so low that a more meaningful D1-mediated behavior—namely grooming in response to the D1 agonist SKF 38393—was chosen to test whether the genotypes differed in D1 receptor sensitivity. As shown in Figure 3, KO mice displayed less grooming during a 60-minute post-drug observation period than WT mice did. Two-way repeated measures ANOVA (genotype × treatment) indicated a significant main effect of treatment ($F = 90.5$, $p<0.0001$) and a significant treatment × genotype interaction ($F = 4.9$, $p<0.05$). Post-hoc tests indicated no

differences in baseline grooming, but a significant difference ($p<0.01$) between WT mice and KO mice in response to SKF 38393 administration.

Discussion

Although there is substantial evidence that Rhes is involved in dopamine-mediated events, the extent of participation in D1 and/or D2 receptor activation is not known. The fact that *rhes* mRNA is detected in both striatopallidal and striatonigral neurons [12, our unpublished observations] suggests the possibility of interaction with both D1 and D2 class receptors, and therefore we have tested the involvement of Rhes in behavior mediated by both receptor classes, alone and in combination. Our findings indicate that Rhes is not necessary for intact dopamine D1/D2 receptor synergism. However, Rhes appears to be normally inhibitory to the stereotypy induced by co-activation of D1 and D2 receptors and to the brief burst of stereotypy induced by activation of D2 receptors alone. Surprisingly, D1-mediated grooming behavior was significantly decreased in the absence of Rhes, suggesting a facilitative role for Rhes in this behavior.

Behavioral, biochemical, and electrophysiological data suggest that under normal conditions, DA-mediated effects require activation of both D1 and D2 type receptors [4]. The mechanism of this phenomenon is unknown, but dopamine depletion causes a loss of this D1/D2 synergism and a profound DA receptor supersensitivity [4,5]. Since DA depletion is accompanied by a decrease in *rhes* mRNA [9] and Rhes protein (manuscript in preparation), we tested whether Rhes plays a role in behavioral D1/D2 synergism. The results presented here show that, like normal mice, mice with a complete loss of Rhes require stimulation of both D1 and D2 receptors in order to manifest a sustained stereotypic response. Thus, barring any developmental compensation in the mutant mice, these data strongly suggest that Rhes does not play a role in D1/D2 synergism as measured by behavioral stereotypy.

The sensitivity of the response to DA receptor activation was, however, affected by the presence or absence of Rhes. The increased response to D1/D2 receptor co-activation by apomorphine in KO mice suggests that Rhes is normally inhibitory toward this response. Previous studies have also suggested an inhibitory role for Rhes in DA-mediated events. For example, Rhes KO mice show an increased locomotor response to the D1 agonist SKF 81297, and increased G_{α}olf levels and GluR1 phosphorylation [12]. In a heterologous expression system, Rhes was shown to inhibit the activation of G_{α}s-coupled receptors at a point proximal to G protein activation [17]. The results for D2 receptor activation are less clear, with Rhes KO mice showing reduced D2-mediated G protein activation and increased sensitivity to D2 antagonists [12]. Our results indicate that for stereotypy, a behavior elicited only by concomitant stimulation of D1 and D2 receptors, Rhes is inhibitory. In an effort to determine the effects of Rhes on activation of D1 or D2 receptors individually, we tested behavior induced by each receptor subtype alone. Activation of D2 receptors gave a supersensitive early stereotypy response in KO mice, thus suggesting an inhibitory effect of Rhes on activation of this receptor. Activation of D1 receptors alone did not produce stereotypy, but the scores on the rating scale were reduced in KO and Het mice relative to WT. This effect is likely due to an enhanced sensitivity to the D2 antagonist eticlopride, which was given 30 minutes prior to apomorphine in the D1 treatment group, and is in agreement with the findings of Errico et al [12] of enhanced cataleptic response to haloperidol in Rhes KO mice.

Grooming was measured as a D2 receptor-independent D1 receptor-mediated behavior [18–21]. Rhes KO mice displayed a significantly decreased grooming response to SKF 38393, suggesting that Rhes is necessary for full expression of this response. The facilitative action of Rhes on this D1-mediated behavior contrasts with its inhibitory action measured in other assays of D1 receptor activation [12]. These effects—i.e. positive versus negative regulation

—may depend on such factors as the parameter being measured, the complement of DA receptors involved, and the particular G protein coupling and signal transduction pathways involved. The behaviors which display increased expression in Rhes KO mice, namely locomotion and stereotypy, require a concomitant activation of both D1 and D2 receptors, whereas grooming does not require D2 receptors. Furthermore, although Rhes has been shown to be inhibitory to $G_{\alpha}s$ activation [17] and to $G_{\alpha}olf$ expression [12], D1 receptors are also known to couple to $G_{\alpha}q$ [22], and Rhes could affect this coupling. Indeed, there is likely to be a $G_{\alpha}q$ component to grooming, as this behavior can be induced by the D1 agonist SKF 83959, which activates $G_{\alpha}q$, but not $G_{\alpha}s/olf$, pathways [23,24]

Conclusions

The GTP-binding protein Rhes does not appear to be necessary for dopamine D1/D2 receptor synergism. However, it exerts complex effects on D1 and D2 receptor sensitivity, depending on the cohort of receptors activated. Rhes inhibits behaviors resulting from concomitant stimulation of D1/D2 receptors and from stimulation of D2 receptors alone. Its effects on D1 receptors are more complex. Although it has been shown previously to be inhibitory to $G_{\alpha}s$ activation and $G_{\alpha}olf$ expression, its presence is necessary for full expression of the D1-mediated behavior of grooming.

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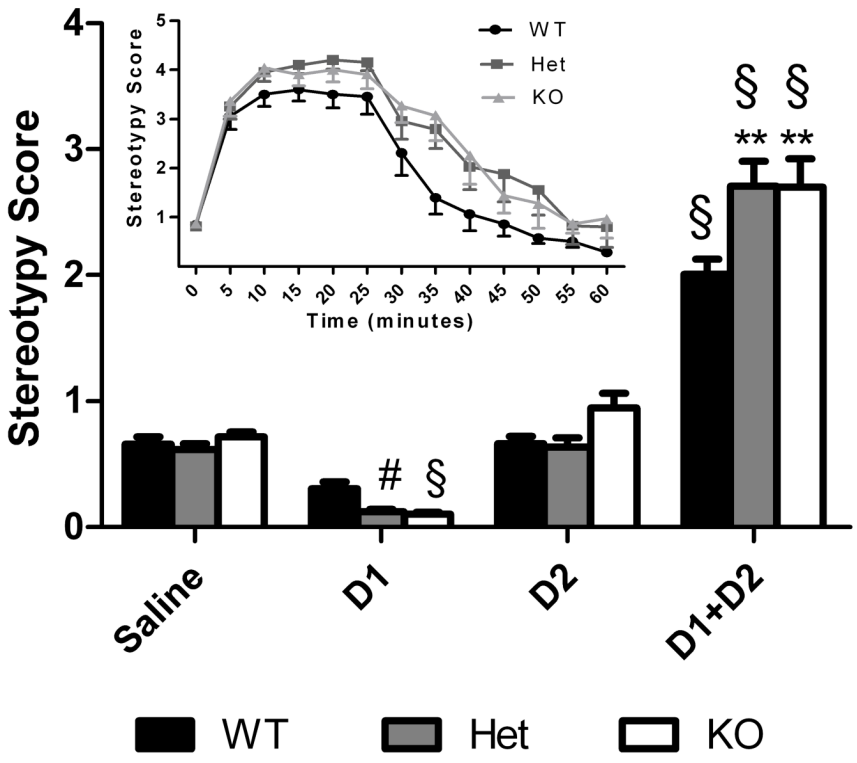

Figure 1.

Apomorphine (3 mg/kg)-induced stereotypy is increased in KO and Het mice. Main figure: Stereotypy was measured as described in Methods, and scores averaged across all of the time points are depicted. Bars represent mean ± SEM. ** $p<0.001$ compared with the WT group under the same treatment condition; # $p<0.01$ compared with saline treatment in the same genotype; § $p<0.001$ compared with saline treatment in the same genotype. Inset: Time course of stereotypy response under D1+D2 conditions for the 60 minutes immediately following apomorphine injection. n = 11–12.

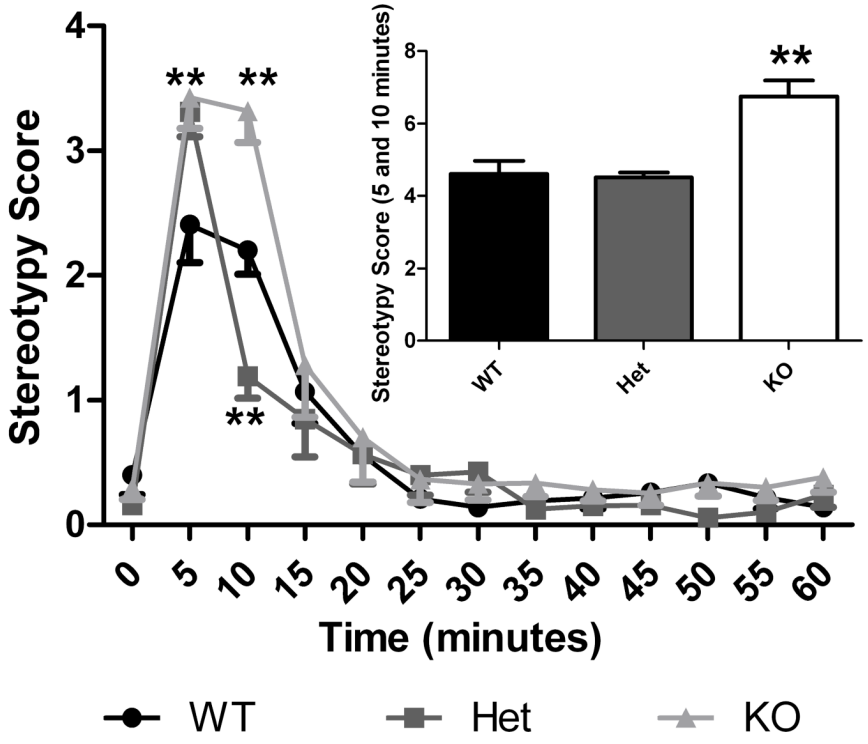

Figure 2.

Activation of D2 receptors causes an increased response in KO mice. Main figure: Mice (n = 11–12) were injected with the D1 antagonist SCH 23390 (0.1 mg/kg) followed 30 minutes later by apomorphine (3 mg/kg) in order to activate D2 receptors in the absence of D1 receptor activation. The stereotypy occurring at 5 and 10 minutes post-apomorphine was significantly greater in KO than WT mice. ** $p<0.01$ compared with WT mice given the same treatment. Inset: Scores for the 5- and 10-minute observation period were combined, and totals were analyzed by one-way ANOVA. **$p<0.0001$ compared with WT and Het.

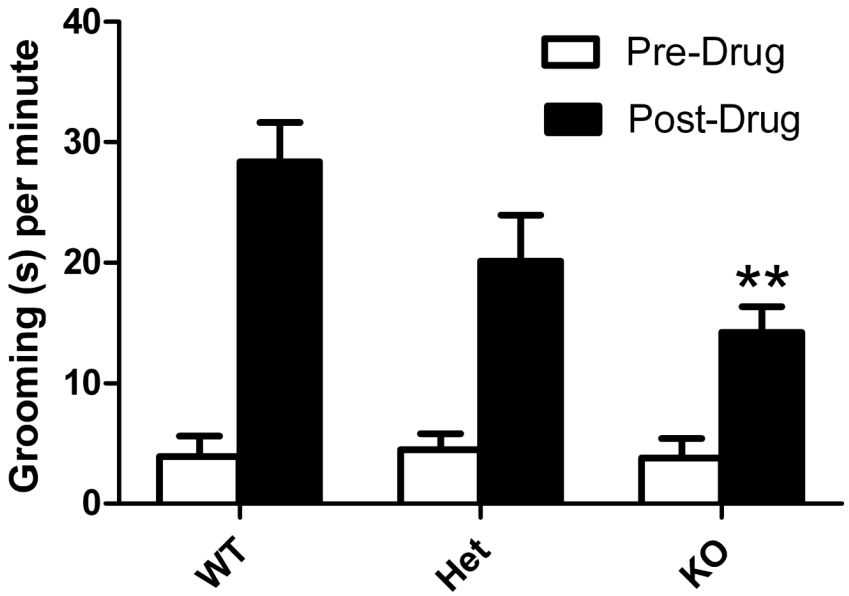

Figure 3.

Rhes KO mice display a decreased grooming response to the D1 agonist SKF 38393. Time spent grooming was recorded for 30 minutes before and 60 minutes after injection of the D1 agonist. All mice spent significantly more time grooming post-drug than pre-drug, but KO mice spent significantly less time grooming than WT mice after D1 agonist treatment. n = 5–7; ** $p<0.01$ compared with WT mice of the same treatment condition.