Rhes, a striatal-enriched small G protein, mediates mTOR signaling and L-DOPA–induced dyskinesia

Srinivasa Subramaniam1,7, Francesco Napolitano2,7, Robert G Mealer1, Seyun Kim1,6, Francesco Errico3, Roxanne Barrow1, Neelam Shahani3, Richa Tyagi1, Solomon H Snyder1,3,4,8 & Alessandro Usiello2,5,8

L-DOPA–induced dyskinesia, the rate-limiting side effect in the therapy of Parkinson’s disease, is mediated by activation of mammalian target of rapamycin (mTOR) signaling in the striatum. We found that Ras homolog enriched in striatum (Rhes), a striatal-specific protein, binds to and activates mTOR. Moreover, Rhes−/− mice showed reduced striatal mTOR signaling and diminished dyskinesia, but maintained motor improvement on L-DOPA treatment, suggesting a therapeutic benefit for Rhes-binding drugs.

The beneficial effects of l-3,4-dihydroxyphenylalanine (L-DOPA) in the therapy of Parkinson’s disease are markedly limited by the disabling dyskinetic effects of the drug. It was recently discovered that L-DOPA–induced dyskinesia is mediated by activation of mTOR in the striatum.1 The mTOR inhibitor rapamycin prevented dyskinesia, but not the therapeutic effects of L-DOPA on limb motion. We previously reported that Rhes, a striatal-enriched small G protein, accounts for the unique neuropathology of Huntington’s disease by enhancing mutant huntingtin sumoylation and toxicity.2 These findings predict that Rhes deletion should be neuroprotective, as has been reported in striatal cultures modeling Huntington’s disease3. Rhes also promotes cross-sumoylation of SUMO enzymes4. Rhes resembles Ras homolog enriched in brain (Rheb), the known physiologic activator of mTOR signaling. We found that Rhes directly binds and activates mTOR. Moreover, L-DOPA–induced mTOR activation and dyskinesia was substantially reduced in Rhes−/− mice (also known as Rasd2) deleted mice, whereas beneficial motor effects were not altered. The mediation by Rhes of L-DOPA–induced dyskinesia implies that drugs that bind and inactivate Rhes will have a therapeutic benefit.

In striatal cell lines, striatal tissue and HEK293 cells, Rhes bound mTOR (Fig. 1a,b and Supplementary Figs. 1 and 2). The mTOR-associated proteins Raptor, Rictor and Gβ1 also co-precipitated with Rhes. Purified Rhes bound directly to the purified catalytic domain5 of mTOR (Fig. 1c). Binding was highly selective, and much less binding occurred with Rheb and negligible binding occurred for other G proteins (Supplementary Fig. 2a). The GTP-binding activity of Rhes is required for its interactions with the mTOR complex, as binding was greatly reduced in Rhes-S33N, which cannot bind GTP (Supplementary Fig. 2b). Similar to other small G proteins, Rhes is anchored to membranes by farnesylation6. Rhes-C263S, which is resistant to farnesylation, showed reduced binding to the mTOR complex (Fig. 1a and Supplementary Fig. 2b).

Rhes activates signaling by mTOR complex 1 (mTORC1), which is comprised of mTOR, Raptor and Gβ1 (Fig. 1d). Thus, over-expression of Rhes stimulates phosphorylation of the mTORC1 targets S6-kinase, 4EBP1 and S6, but does not activate phosphorylation of Akt at Thr308, which is upstream of mTOR. Rhes appeared to also influence mTORC2, as it bound to Rictor (Fig. 1a and Supplementary Fig. 2), which was selectively associated with the mTORC2 complex, and elicited some increase in phosphorylation of Akt at Ser473, a target of mTORC2 (Fig. 1e). Moreover, the decline of pAkt-Ser473 on removal of serum was much less with Rhes than with vehicle. Although the actions of Rhes following mTORC1 signaling were abolished by rapamycin (Fig. 1e), the influences on pAkt-Ser473 resisted rapamycin treatment, consistent with the known rapamycin resistance of mTORC2. A direct influence of Rhes on mTOR was evident from in vitro experiments in which Rhes and Rheb similarly augmented phosphorylation of 4EBP1 (Fig. 1f).

As reported previously1, L-DOPA treatment of mice with unilateral 6-hydroxydopamine (6-OHDA) striatal lesions markedly augments striatal mTOR signaling. This increase was abolished in Rhes−/− mice treated with L-DOPA (Fig. 2a). Treatment of unilaterally lesioned mice for 3, 6 or 9 d with L-DOPA (10 mg per kg of body weight) elicited pronounced dyskinesia (Fig. 2b). Dyskinesia following L-DOPA (10 mg per kg) was markedly reduced in Rhes knockout mice (Fig. 2b). The dyskinetic influences of L-DOPA were similar at 20–60 min following injection and declined markedly by 2 h. The rate of decline was similar in wild-type and Rhes knockout mice, suggesting that Rhes deletion does not simply alter L-DOPA turnover (Fig. 2b). These observations establish that Rhes mediates both mTOR activation and L-DOPA–induced dyskinesia in the striatum. Stratal levels of Rhes, which also activates mTOR, were unaltered in Rhes−/− striatum (Supplementary Fig. 3a).

A previous study noted that the therapeutic effects of L-DOPA, monitored by forelimb use of 6-OHDA–lesioned mice in the cylinder test, are not mediated by the mTOR pathway, as they resist rapamycin treatment1. In our experiments, forelimb use was enhanced by L-DOPA in both wild-type and Rhes mutant mice (Fig. 2c). Thus, Rhes mediates the adverse dyskinetic actions of L-DOPA, but not its therapeutic motor effects. Rhes mutants display very
Figure 1 Rhes binds and activates mTOR. (a) Endogenous mTOR, Raptor or Rictor and Gβl binding with overexpressed GST, GST-Rhes (wild type (WT) or C263S) in striatal cells. (b) Endogenous mTOR or Rictor binding with recombinant GST or GST-Rhes WT in striatal tissue. (c) Purified Rhes bound directly to purified mTOR-CD (catalytic domain). (d) Rhes increased phosphorylation of S6K (Thr389), S6 (Ser235/236), 4EBP1 (Ser65) and Akt (Ser473) in serum-containing and deprived (2 h) striatal cells. (e) Rapamycin blocked Rhes-induced phosphorylation of S6K (Thr389), but not Akt (Ser473), in HEK293 cells. (f) Rhes activated mTOR directly in vitro. All data are expressed as means ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001 compared with myc controls. n.s., not significant, P < 0.1512. Full-length blots are presented in Supplementary Figures 4–9.

little change in motor behavior and coordination, which would therefore not be likely to contribute notably to the altered L-DOPA responses that we observed.

Figure 2 Effect of Rhes deletion on mTOR striatal signaling and dyskinesia in unilaterally 6-OHDA-lesioned mice. (a) Phosphorylation levels of S6 and 4EBP1 at the Ser240/244 and Ser65 residues, respectively, in the striatum of Rhes+/+ and Rhes−/− mice lesioned with 6-OHDA (n = 10–12). Full–length blots are presented in Supplementary Figure 10. (b) Temporal evolution of abnormal involuntary movements (AIMs) in lesioned Rhes+/+ and Rhes−/− animals during chronic treatment with L-DOPA (10 mg per kg; Rhes+/+, n = 12; Rhes−/−, n = 10). The AIMs are also separately shown following L-DOPA treatment at day 9 as total score and time course over a 180-min test session. (c) Left forelimb use determined by the cylinder test in 6-OHDA-lesioned Rhes+/+ and Rhes−/− mice (n = 8 per genotype) before (left) and after (right) L-DOPA administration. All data are expressed as mean ± s.e.m. *P < 0.05 and ***P < 0.001, as compared with unlesioned striata (a) and wild-type controls (b). Experiments were conducted in conformity with protocols approved by the veterinary department of the Italian Ministry of Health and in accordance with the ethical and safety rules and guidelines for the use of animals in biomedical research, provided by the relevant Italian laws and European Union directives (n. 86/609/EC).
and DARPP-32 to Erk1/2, and GluR1 glutamate receptors, all upstream of mTOR\textsuperscript{1,8}. Rhes has previously been shown to modulate dopamine signaling through both D1 and D2 receptors\textsuperscript{7,9}. If Rhes’ effect on mTOR–induced dyskinesia occurred through dopamine signaling upstream of mTOR, then Erk1/2 and GluR1 phosphorylation should be altered in Rhes\textsuperscript{−−} mice. However, phosphorylation of Erk1/2 and GluR1-S845 increased similarly in wild-type and Rhes\textsuperscript{−−} mice following L-DOPA treatment, indicating that Rhes does not affect dopamine signaling and activation of Erk1/2-GluR1 to mediate the dyskinetic effects of L-DOPA (Supplementary Fig. 3c). Previous studies have shown that Rhes\textsuperscript{−−} mice display dopamine D1 receptor hypersensitivity\textsuperscript{7,9}. If Rhes acted primarily via the dopamine system, Rhes\textsuperscript{−−} mice would be predicted to manifest worsened L-DOPA dyskinesia, which is the opposite of what we observed. Taken together, these findings support the conclusion that Rhes’ mediation of L-DOPA-induced dyskinesia occurs via its activation of mTOR.

Our findings indicate that Rhes physiologically binds to and activates mTOR in the striatum, an action that has previously been manifested only by Rheb. A study reported that the Rag-GTPases bind mTOR and facilitate its translocation to lysosomes for activation by Rheb\textsuperscript{10}. Activation of mTOR signaling by Rhes has functional consequences, as it appears to mediate the dyskinetic effects of L-DOPA via enhancement of mTOR signaling, but not the therapeutic improvement of forelimb movement, which is not blocked by rapamycin or reduced in Rhes mutants.

The selective mediation by mTOR of dyskinetic, but not therapeutic effects of L-DOPA, implies that mTORC1 inhibitors such as rapamycin might diminish these adverse effects that markedly interfere with the therapy of Parkinson’s disease. These drugs may also be beneficial by preventing neuronal death in Parkinson’s disease\textsuperscript{11}. Unfortunately, rapamycin and related drugs are powerful inhibitors of protein synthesis with associated toxicity. Our findings indicate that drugs blocking Rhes binding to mTOR selectively might offer similar therapeutic benefits. However, as Rhes is highly enriched in the striatum, with negligible levels in peripheral tissues, drugs blocking Rhes–mTOR interactions may have much less potential for adverse effects, consistent with the lack of major abnormalities in Rhes\textsuperscript{−−} mice.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS
S.S. initiated the project and conducted the mTOR binding and activation experiments, which were further characterized by R.G.M. A.U. initiated the dyskinesia and mTOR experiments in mice. S.S. and S.H.S. further conceived and designed the experiments. F.N. and F.E. conducted the L-DOPA and behavioral experiments, under the direction of A.U. R.B. generated the Rhes constructs. S.K. performed the in vitro mTOR activity assay. R.T. and N.S. contributed to the mTOR activity and binding experiments. S.H.S. wrote the manuscript with input from R.G.M., S.S. and A.U.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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