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Western blot analysis

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We use this protocol and it's working



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Abstract

In order to examine the link between LRRK2 activity and motor behaviors sensitive to D2R signaling, we evaluated whether the effects of blocking D2R signaling by haloperidol are influenced by LRRK2 inhibition. Haloperidol, a typical antipsychotic used to treat psychosis, is known to cause Parkinsonian-like side effects, including catalepsy in rodents and dyskinesia in humans. We hypothesized that if LRRK2 kinase activity plays a significant role in D2R signaling in the striatum, its inhibition may interfere with the cataleptic response induced by haloperidol.

Troubleshooting

Sample preparation

- 1 After receiving the drug treatments striatal tissues were extracted and homogenized in 1x cell lysis buffer (Cell Signaling Technologies) supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific) using pellet pestles(30 seconds)
- 2 Protein concentration was determined by BCA protein assay (Thermo Scientific)

Western blot

- 3 30 µg of total lysate were separated by 4–12% NuPage Bis-Tris PAGE (Invitrogen) and transferred to membranes using the iBlot nitrocellulose membrane Blotting system (Invitrogen) by following manufacturer protocol
- 4 The membranes were incubated with primary antibodies specific for phospho-Rab12 (1:1000, Abcam), total Rab12 (1:1000) Proteintech and β -actin(1:3000, Sigma) at 4C overnight.
- 5 The membranes following probing with secondary anti-mouse or and anti-rabbit antibodies (1:2000, Thermo Scientific) for 1 hour at room temperature
- 6 The membranes were incubated with Immobilon ECL Ultra Western HRP Substrate (Millipore) for 3 min prior to image acquisition
- 7 Chemiluminescent blots were imaged with iBright CL1000 imaging system (Thermo Fisher Scientific).

Western blot analysis

- 8 Images acquired with iBright CL1000 imaging system were uploaded to ibright analysis software.
- 9 The ibright analysis software detected the areas of interest automatically and manually adjusted them if needed.
- 10 Absolute volume and intensity were detected. Further statistical analyses were done using GraphPad Prism 10.1 software