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Stimulation of D_3 receptors activates Akt/GSK-3β and mTOR signalling pathways in the nucleus accumbens and striatum: a pharmacological and gene knockout characterisation

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No conflict of interest

INTRODUCTION

➢ Increasing evidences suggest a role for the serine/threonine kinase Akt and its downstream substrate glycogen synthase kinase-3β (GSK-3β) in the etiology of schizophrenia, a psychiatric disorder characterized by a deregulation of the dopaminergic system.

➢ mTOR (mammalian Target Of Rapamycin), a serine/threonine kinase activated by Akt, acts as a regulator of the CNS neuroplasticity playing a role in cognition and neuroprotection. Inhibition of the mTOR pathway was shown to improve cognitive functions which are impaired in schizophrenic patients (Ehninger et al., 2008).

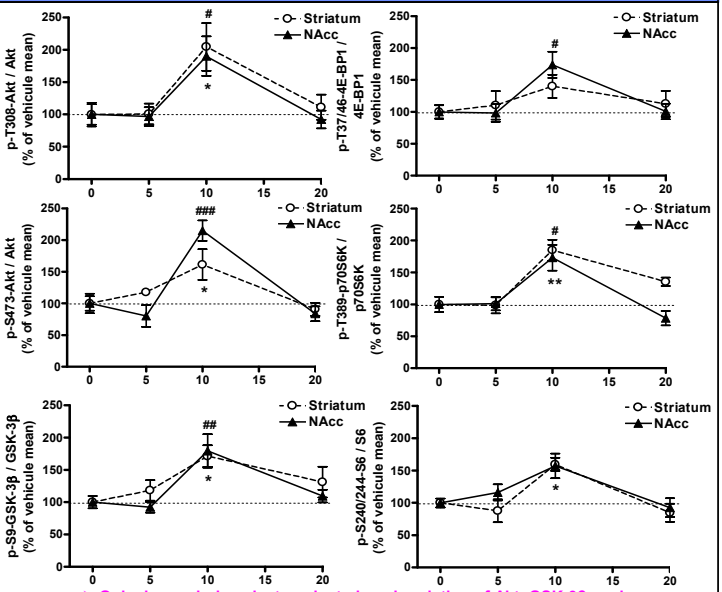
➢The papers about the effects of stimulation of D2-type receptors on the regulation of Akt and GSK-3β are apparently contradictory. This stimulation was reported to increase Akt and GSK-3β phosphorylation in various cell lines expressing D2-type receptors, such as striatal neurons (Brami-Cherrier et al., 2002), PC12, EM4 and CHO cells (Zapata et al., 2007; Nair and Olanow, 2008, Chen et al., 2009; Mannoury la Cour et al., 2011). In contrast, it was found to decrease Akt and GSK-3β phosphorylation in the striatum in vivo (Beaulieu et al., 2007).

➢ However, the activation of Akt/GSK-3β signaling in the cell models displayed a quick onset (few mins) whereas the *in vivo* inhibition slowly developed with a maximum 90 min after the beginning of stimulation. In the present study, we investigated the possibility of a transient activation of Akt/GSK-3β *in vivo* shortly after stimulation of dopamine D_2 and D_3 receptors. Our aims were :

▪ to examine the kinetic of short-term effects on Akt and its effectors, GSK-3β and mTOR pathways after acute treatment with quinelorane, a D_2/D_3 receptor agonist, in the rat striatum and nucleus accumbens.

▪ to determine the role of dopamine D_3 receptor subtype in the short-term effects of quinelorane.

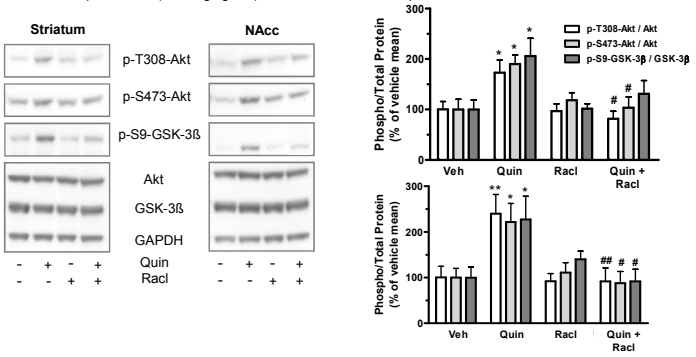
Quinelorane induced a transient activation of Akt/GSK-3β and mTOR signaling in the rat striatum and nucleus accumbens

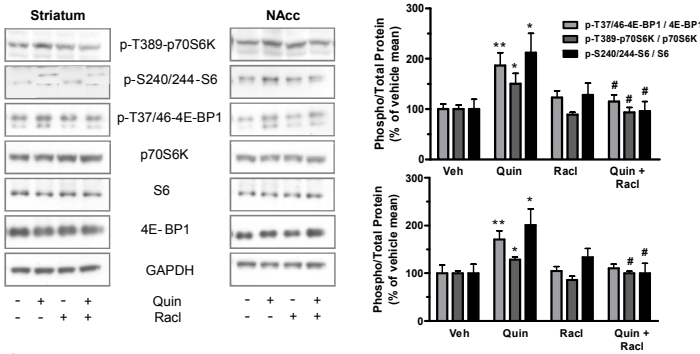


➢ Quinelorane induced a transient phosphorylation of Akt, GSK-3β, and mTOR downstream targets, with a maximal effect 10 min after injection.

Raclopride prevented quinelorane-induced phosphorylations of Akt, GSK-3β and downstream targets of mTOR

Pre-treatment : non-selective D_2/D_3 receptors antagonist, raclopride, (0.16 mg/kg, s.c) 30 min before quinelorane.
Treatment : quinelorane (0.16 mg/kg, s.c); Sacrifice : 10 min after quinelorane.

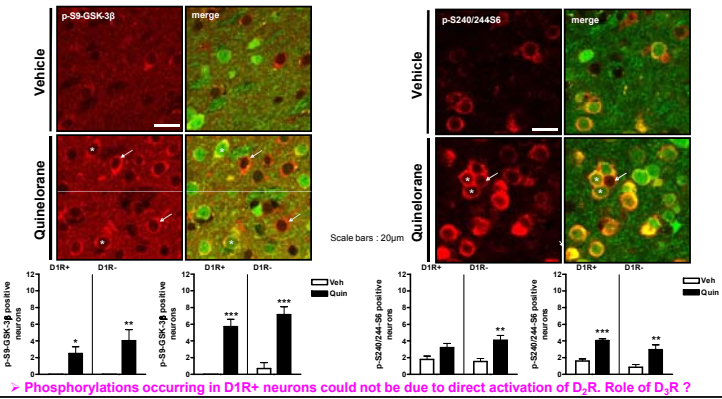




➢ Activation of the various pathways by quinelorane is mediated by D_2/D_3 receptors.

Quinelorane-induced phosphorylations occurred in both D1R+ and D1R- neurons

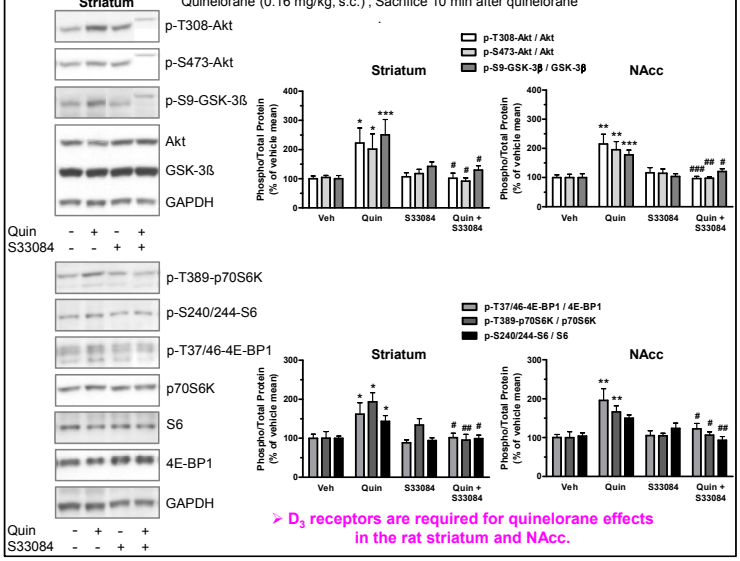
Transgenic mice expressing GFP in D1R-positive (D1R+) neurons
Quinelorane (0.63mg/kg, s.c.): Sacrifice after Quinelorane.



➢ Phosphorylations occurring in D1R+ neurons could not be due to direct activation of D_2R. Role of D_3R ?

Selective D_3 receptor antagonist S33084 blocked quinelorane-elicited effects upon Akt/GSK-3β and mTOR signaling

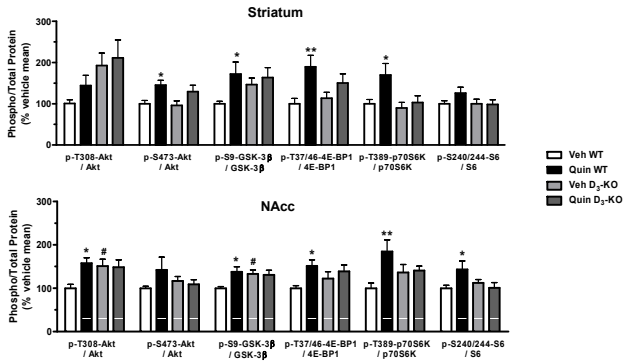
Pre-treatment: selective D_3 receptors antagonist, S33084, (0.16 mg/kg, s.c) 30 min before quinelorane
Quinelorane (0.16 mg/kg, s.c.) ; Sacrifice 10 min after quinelorane



➢ D_3 receptors are required for quinelorane effects in the rat striatum and NAcc.

Quinelorane failed to increase phosphorylation of Akt, GSK-3β and mTOR targets in D_3-KO mice

Quinelorane (0.63 mg/kg, s.c.); Sacrifice 10 min after quinelorane.



➢ D_3 receptors are required for quinelorane effects in the mouse striatum and NAcc.

CONCLUSIONS / PERSPECTIVES

Conclusions

➢Quinelorane induced a transient increase of phosphorylation of Akt, GSK-3β and mTOR downstream effectors in the rat striatum and nucleus accumbens with a maximal effect 10 min after injection. All protein phosphorylation levels returned to baseline at 20 min.

➢ Pharmacological and genetic approaches revealed a major role for D_3 receptors in the effects of quinelorane, though a contribution of D_2 receptors cannot be excluded .

Perspectives

➢ Testing the influence of the mTORC1 inhibitor, rapamycin, on quinelorane-induced activation of the mTOR pathway.

➢ Evaluating the action of quinelorane in the cortex, a structure involved in the pro-cognitive effects of D_3 receptor antagonists and rapamycin.

➢ Characterizing the molecular mechanisms involved in the recruitment of Akt by D_2 and D_3 receptor activation (e.g; role of Src, PI3-Kinase).