

The role of D₁ Dopamine Receptors and Phospho-ERK in Mediating Cytotoxicity. Commentary.

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Striatal neurodegeneration observed in several neurological diseases, occurs through unknown mechanisms. Recent evidence suggests that its pathogenesis may be linked, in part, to high synaptic levels of dopamine (DA). DA may cause neurotoxicity of striatal neurons through mitogen-activated protein kinases (MAPKs). Here we comment on the role of extracellular signal-regulated kinase (ERK) activation in the cytotoxicity mediated upon activation of the D₁ DA receptor, and describe a possible mechanism for phospho-ERK (p-ERK) in inducing cytotoxicity.

Keywords: D₁ Dopamine receptors; Neurodegeneration; Extracellular signal-regulated kinases; Mitogen activated protein kinases;

Abbreviations

DA, dopamine; MAPKs, mitogen activated protein kinases; ERK1/2, extracellular signal-regulated kinases 1 and 2; JNK, c-Jun N-terminal kinase; NOS, nitric oxide synthases; GPCRs, G protein-coupled receptors.

INTRODUCTION

Postsynaptic striatal neurodegeneration occurs through unknown mechanisms, but is linked to high extracellular levels of synaptic dopamine (DA). Accumulating evidence in primates indicates that blockade of D₁ DA receptors with antagonists, may have strong neuroprotective and anti-Parkinsonian effects (Andringa *et al.*, 1999; Cools *et al.*, 2002). Moreover, many studies have shown that blockade of D₁ DA receptors with its selective antagonist, SCH 23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine], attenuates methamphetamine neurotoxicity

(Bronstein and Hong, 1995; Takaki *et al.*, 2001). In both rat striatal primary cultures (Wersinger *et al.*, 2004) and in SK-N-MC neuroblastoma cells (Chen J *et al.*, 2003), which endogenously express a functional D₁ DA receptor (Sidhu and Fishman, 1990), we have recently shown that the chronic treatment of these cells with DA promotes increased expression of the nitric oxide synthases (NOS), neuronal NOS and inducible NOS, accompanied by increased NO production, oxidative stress and cytotoxicity. Both DA autooxidation and D₁ receptor activation independently cause an increase in cytotoxicity, suggesting that DA-induced neurotoxicity is mediated by two distinct pathways: through extracellular autooxidation of DA to toxic metabolites and through chronic activation of the D₁ DA receptor.

Further investigation of the pro-cytotoxic signaling pathways modulated via D₁ DA receptors revealed the involvement of MAPKs, the down-stream mediators of signal transduction from the cell surface receptors to the nucleus. MAPKs have been implicated in a wide variety of cellular processes such as proliferation, differentiation, and apoptotic cell death (reviewed in Chang and Karin, 2001; Pearson *et al.*, 2001). In mammals, three major groups of MAPKs have been identified: extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase, (JNK). JNK and p38 MAPK are stimulated by cellular stresses, such as free radicals and inflammatory agents, leading to apoptotic cell death. The ERK-linked signaling pathways are stimulated by receptor tyrosine kinases and G protein-coupled receptors (GPCRs), and generally lead to a mitogenic and proliferative response. In particular, ERK is involved in neuronal development, memory formation, survival and adaptation (Xia *et al.*, 1995; English and Sweatt, 1996; Segal and Greenberg, 1996; Atkins *et al.*, 1998; Davis, 2000; Sweatt, 2001). Yet, emerging evidence shows that

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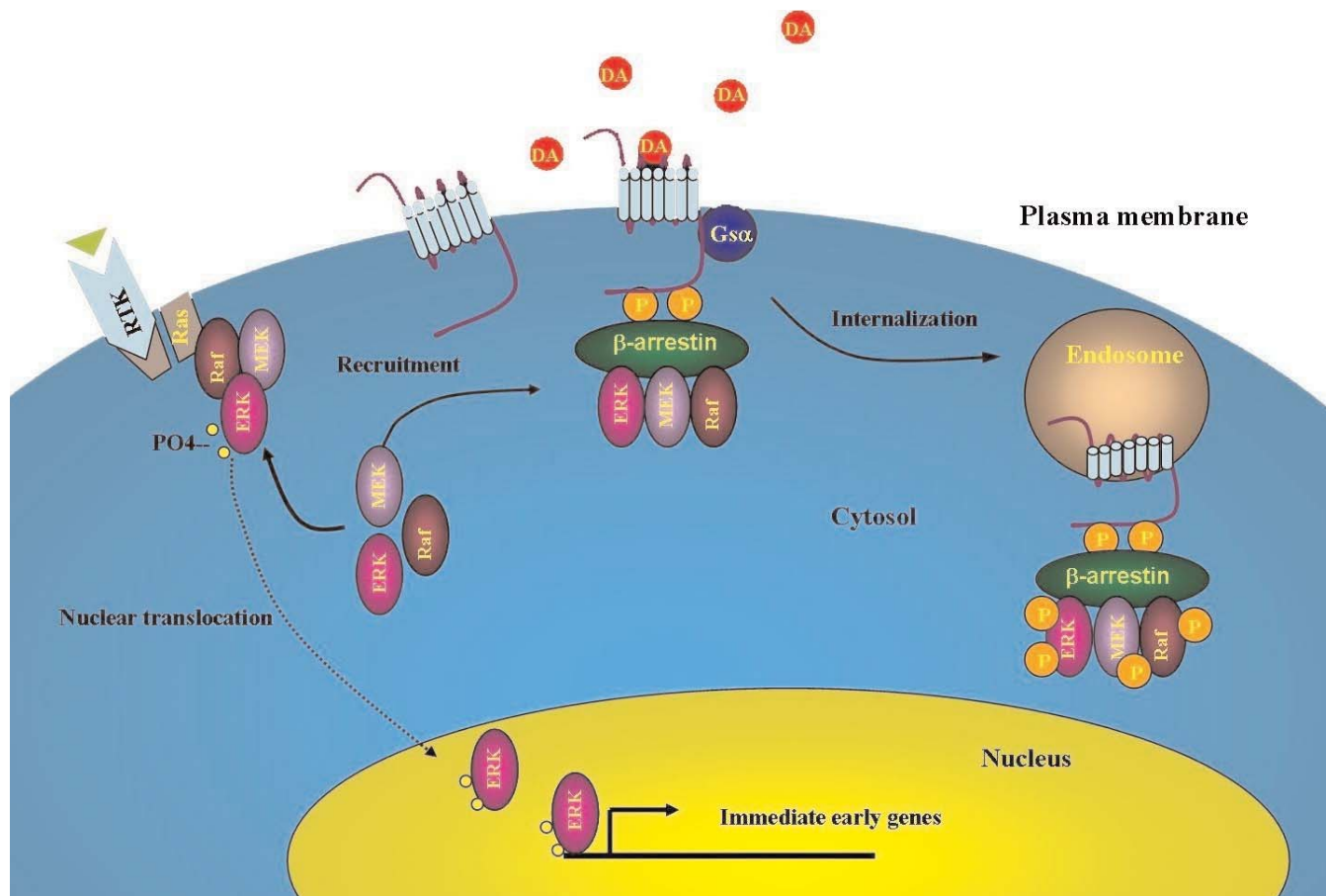


FIGURE 1 Schematic representation of β -arrestin scaffolding of D₁ DA receptors and p-ERK. After DA stimulates D₁ DA receptors, the receptors are phosphorylated. This, in turn, leads to β -arrestin recruitment to the receptors. The receptors are then internalized into acidic endosome. β -arrestin acts as scaffold protein bringing with Raf, MEK and ERK. This association facilitates the activation of ERK and causes cytosolic retention of p-ERK, thereby decreasing nuclear ERK signaling.

ERK activation may also be linked to neuronal death (reviewed in Grewal *et al.*, 1999; Colucci-D'Amato *et al.*, 2003; Chu *et al.*, 2004), suggesting that ERK signaling may have an alternate function in the mediation of neurotoxic events.

In this *commentary*, we provide evidence for the selective participation of p-ERK activation in the early events underlying the genesis of neuronal cytotoxicity upon stimulation of D₁ DA receptors.

STIMULATION OF D₁ DA RECEPTORS ACTIVATES ERK AND PROMOTES NEURONAL CELL DEATH

In SK-N-MC cells and in rat primary striatal neurons we found that the direct activation of D₁ DA receptors, induced a sustained (up to at least 2 h) stimulation of the p-ERK signaling cascade, followed by cell death. The increase in p-ERK activation and cell death induced by DA (in the presence of an antioxidant to prevent autooxidation) and SKF R-38393 [1-phenyl-2,3,4,5-tetrahy-

dro-(1H)-3-benzazepine-7,8-diol], a D₁ DA receptor-selective agonist, was blocked by SCH 23390 and by an inhibitor of MEK1/2, the upstream dual-specificity kinase of ERK. Moreover, suppression of p-ERK upon co-transfection with the catalytically defective MEK1 mutant, MEK1-K97M, prevented the SKF R-38393- and the DA-induced increases in p-ERK activation, relieving the D₁ DA receptor-mediated cytotoxicity. Together, these combined data confirmed that stimulation of the D₁ DA receptor leads to ERK activation and promotes cytotoxicity.

THE POSSIBLE MECHANISM FOR THE ACTIVATION OF ERK IN CYTOTOXICITY

In resting cells, ERK is kept in the cytoplasm by the microtubule cytoskeleton, which serves as a major docking matrix for up to 35% of cellular ERK (Reszka *et al.*, 1995). Other putative cytosol-anchoring proteins of ERK are the MAP kinase phosphatases (MKPs), in particular MKP3 (Camps *et al.*, 1998; Brunet *et al.*,

1999), and also MEK1/2 (Fukuda *et al.*, 1997). After phosphorylation by MEK1/2, p-ERK is detached from its cytosolic anchor(s) and is rapidly translocated into the nucleus (Chen RH *et al.*, 1992), in a process that requires homodimerization and phosphate incorporation into the regulatory Thr and Tyr residues of at least one of the ERKs in the dimer (Khokhlatchev *et al.*, 1998). Several sites on ERK have been identified to be important in both its nuclear translocation [residues 321-327] and its cytosolic retention [residues 312-320], with the three acidic residues Asp-316, Asp-319, and Glu-320, being especially important (Rubinfeld *et al.*, 1999). Once in the nucleus, activated ERK can phosphorylate several nuclear transcription factors, such as Elk-1 (Gille *et al.*, 1995), thereby transmitting signals originally received by cell surface receptors to the nucleus. The rapid nuclear translocation of activated ERK is essential for promoting cell cycle progression (reviewed in Pouyssegur *et al.*, 2003).

An interesting finding of our recent studies is that upon D₁-receptor stimulation, the majority of p-ERK was retained in the cytoplasm, with only a modest amount translocated into the nucleus. The failure of p-ERK to translocate into the nucleus, even after 2 h, coupled with its retention in the cytoplasm, provides some evidence for a possible mechanism by which p-ERK accumulation in the cytoplasm may trigger a cytotoxic, rather than a mitogenic, response upon activation of the D₁ DA receptor (FIG. 1). From co-immunoprecipitation studies, p-ERK was found to form a stable heterotrimeric complex with the D₁ DA receptor and β -arrestin2. The arrestin proteins enable the internalization and trafficking of certain phosphorylated GPCRs, including the D₁ DA receptor (Kim *et al.*, 2004), away from the plasma membrane, terminating receptor-dependent signals by precluding receptor-G protein coupling (Hall and Lefkowitz, 2002). Blockade of the expression of β -arrestin2 with its dominant negative mutant almost completely prevented the formation of the heterotrimeric complex, while simultaneously blocking the phosphorylation of ERK. Abolishing the formation of the heterotrimeric complex rescued D₁ DA receptor-mediated cell death.

If abnormally retained in the cytoplasm, p-ERK may phosphorylate multiple plasma membrane, cytoplasmic and cytoskeletal substrates (Pearson *et al.*, 2001), resulting in altered, and perhaps inappropriate, series of cellular events. The significance of cytosolic retention of p-ERK is underscored by recent findings in postmortem tissue of neurodegenerative diseases, where neuronal inclusion bodies were found to contain substantially high levels of aggregated p-ERK. Thus, from immuno-

histochemical analyses, granular precipitates of p-ERK were seen in the cytoplasm of neurons exhibiting early *tau* deposition in Alzheimer's disease, in neurons with Pick bodies in Pick's disease, and in neurons in progressive supranuclear palsy and corticobasal degeneration (Ferrer *et al.*, 2001a). Moreover, p-ERK, but not p-p38 MAPK or p-JNK, were found in Lewy bodies in Parkinson's disease and in dementia with Lewy bodies (Ferrer *et al.*, 2001b), which were shown to be located primarily in the cytoplasm and not in the nucleus (Zhu *et al.*, 2002). Therefore, elucidation of the precise mechanisms which cause cytosolic retention of p-ERK is likely to provide important insights into the etiology of multiple neurodegenerative diseases.

CONCLUSION

To conclude, there is growing evidence which implicates p-ERK in promoting cell death. DA may induce neurotoxicity upon stimulation of D₁ receptors, which causes sustained activation and cytosolic retention of p-ERK. The abnormal cytosolic retention of p-ERK may trigger a cascade of phosphorylation events in cytoplasm, which finally culminate in neurotoxicity and cell death.

Acknowledgements

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