Expression of the Immediate Early Gene c-fos in Basal Ganglia: Induction by Dopaminergic Drugs

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ABSTRACT: Expression of the immediate early gene c-fos is increased in mammalian neurons by a number of stimuli and the usefulness of this gene as a marker of neuronal activation has been demonstrated in several systems. Directly-acting dopamine agonists of the D1-type (SKF 38393, CY 208-243) and indirectly-acting dopamine agonists (amphetamine, cocaine) all produce a rapid and transient increase in Fos protein levels in varying patterns in striatum and cerebral cortex. Directly-acting dopamine agonists only produce c-fos activation in denervated (supersensitive) striatum whereas cocaine and amphetamine activate c-fos in striatum in naive animals. Remarkably, D2 selective antagonists such as haloperidol, albeit in high doses, also activate c-fos expression. Activation of c-fos and other immediate early genes may play a part in the development of such long-term dopamine-related effects as dyskinetic movements and addiction.

RESUME: Expression du gene immediat precoce c-fos dans les ganglions de la base : induction par les agents dopaminergiques. L’expression du gene precoce immediat c-fos est augmentee dans les neurones des mammiferes par un certain nombre de stimuli et l’utilite de ce gene comme marqueur de l’activation neuronale a ete demontrée dans plusieurs systemes. Des agonistes dopaminergiques de type D1 (SKF 38393, CY 208-243) agissant directement et des agonistes dopaminergiques agissant indirectement (amphetame, cocaïne) produisent tous une augmentation rapide et transitoire dans les niveaux de protéine Fos selon des patrons variés dans le striatum et le cortex cerebral. Des agonistes dopaminergiques agissant directement produisent seulement une activation de c-fos dans le striatum dénervé (hypersensible), alors que la cocaine et l’amphetamine activent c-fos dans le striatum chez des animaux naifs. Il est remarquable d’observer que des antagonistes selectifs pour D2 tel l’haloperidol, administre a hautes doses, activent egalement l’expression de c-fos. L’activation de c-fos et d’autres genes immediats precoces peut jouer un role dans l’apparition d’effets a long terme reliés a la dopamine tels les mouvements dyskinétiques et la toxicomanie.

produce this activation. In the rat, the dopaminergic neurotoxin, 6-hydroxidopamine, when injected into the substantia nigra on one side, will denervate dopamine innervated structures on that side. In such animals, L-Dopa and the D1-selective agonists SKF 38393 and CY 208-243 produce a rapid increase in production of nuclear immunostaining for Fos protein in medium-sized neurons throughout the caudate-putamen. Fos-like immunostaining is seen after as little as 30 minutes and persists for 6-10 hours. In many systems, the Fos protein is only present for 2-4 hours; Fos-related antigens of undetermined functions may account for the immunostaining seen at longer survival times.

There is also a clear relationship between the degree of receptor supersensitivity and activation of c-fos expression. These unilaterally-6-OHDA-treated rats turn rapidly when given dopaminergic drugs, and this rotational behaviour can be used to assess the degree of supersensitivity of the dopamine receptors. Animals with incomplete lesions do not turn as rapidly as those with complete lesions. Similarly, animals with incomplete lesions show little or no Fos immunostaining while those which turn rapidly exhibit dramatic staining for Fos. Infusion of D1-dopamine agonists directly into the striatum of normal rats has no effect on c-fos expression. However, if the animals have been depleted of dopamine with either the dopaminergic neurotoxin 6-hydroxidopamine or the monamine depleting drug reserpine, such direct infusions of D1 agonists produce a robust c-fos activation.

It is most important to note that Fos production is independent of rotation per se. We know this because we can dissociate rotation and c-fos activation in several ways. First, although dopamine agonists of both D1 and D2 types produce similar amounts of rotation in unilaterally denervated animals, only D1 agonists activate c-fos expression in the caudoputamen. Second, D1-agonist induced Fos production is observed in animals that are anaesthetized prior to receiving the D1-agonist. Thirdly, rats will also rotate towards the denervated side if a D1-agonist is injected into the substantia nigra on the lesioned side; in such animals, there is no activation of c-fos in the caudoputamen.

As only D1-selective agonists activate c-fos in vivo, and the D1 dopamine receptor has traditionally been associated with adenyl cyclase, it was not surprising to find that infusion of the directly-acting adenyl cyclase activator forskolin into the striatum in rats produced a robust activation of Fos synthesis. Immunostaining for Fos occurs in medium sized neurons that make up more than 90% of striatal neurons. Retrograde tracer studies suggest that many of the neurons expressing c-fos in response to D1 agonist stimulation project to the substantia nigra. At present, the characteristics of the Fos positive neurons are incompletely understood and studies are under way to determine whether these neurons also contain such D1 receptor markers as DARPP-32 and which neuropeptides they express.

Interestingly, whether a rat is newly treated with 6-OHDA or had been treated with 6-OHDA 9 months previously, the Fos immunostaining after L-Dopa challenge seems similar. By extrapolation, this suggests that patients with Parkinson’s disease who take L-Dopa up to 4 times/day for years and often for decades may be activating c-fos gene expression with each dose.

What might the consequences of this continued, repeated activation of immediate early genes be? One possibility is that this activation might be associated with the development of dyskinesias. Evidence from non-human primate models of Parkinson’s disease suggests that dyskinesias are associated largely, if not exclusively, with D1-type agonist treatment. Why, then, do some patients not develop dyskinesias when treated with L-Dopa? It may be that the patients who do not develop dyskinesias are incompletely denervated and do not produce Fos in response to the L-Dopa. The relationship between the activation of immediate early genes and development of dyskinesias needs to be explored further in animal models.

**Activation of c-fos and Other Immediate Early Genes by Indirectly-acting Dopaminergic Agonists**

Indirect dopamine agonists such as d-amphetamine and cocaine, which act to increase levels of endogenous dopamine, also activate the c-fos gene. However, there is a marked difference between the Fos response to direct and indirect agonists. Both amphetamine and cocaine induce expression of Fos protein in the intact striatum as well as in the 6-OHDA-denervated striatum of rats unilaterally lesioned with 6-OHDA. In fact, in 6-OHDA-treated rats, the preponderance of Fos-like immunoreactivity is found on the intact side following indirect agonist treatment. Interestingly, despite the fact that the effects of cocaine and amphetamine are in many ways indistinguishable behaviorally, these psychostimulant drugs produce strikingly different patterns of c-fos expression in the striosome-matrix compartments and limbic subdivisions of the striatum.

Animals given cocaine (25 mg/kg, i.p.) show widespread activation of c-fos throughout the striatum 2 hours later. In contrast, animals given injections of 5-10 mg/kg, i.p. of amphetamine and examined for striatal Fos-like immunoreactivity 2 hours later exhibit a vividly patchy pattern of c-fos expression. By comparing adjacent sections stained immunochemically for Fos-like immunoreactivity and calbindin D28k-like immunoreactivity, it was possible to show that the Fos-positive patches correspond to the calbindin-poor striosomes.

The striosomes and matrix are the major neurotransmitter-specific compartments of the caudoputamen and have different input-output connections. Striosomes and matrix are known to differ markedly in several important dopaminergic characteristics. For example, they have different levels of binding of D1 and D2 dopamine receptor-specific ligands, and monoamine uptake-site ligands, and they have different dopaminergic innervation. Interestingly, cocaine-induced Fos accumulation is completely eliminated by reserpine administration whereas amphetamine-induced Fos accumulation remains intact. These findings raise the possibility that there may be a relationship between reserpine-sensitive and reserpine-insensitive pools of releasable catecholamines and psychostimulant induction of c-fos.

The differential effects of cocaine and amphetamine on c-fos activation could conceivably relate to the different behavioural effects that these psychostimulant drugs produce. Although cocaine and amphetamine have many common features in terms of behaviour and neurotransmitter release, they do differ in some very significant ways, including neurotoxicity. Amphetamine and related compounds have marked neurotoxic effects, whereas cocaine appears to be devoid of such effects.
This idea has been reinforced by the finding that NMDA receptors play a role in D1 dopamine-mediated c-fos activation, it is known that the NMDA receptor plays a role in amphetamine neurotoxicity.

**Activation of c-fos and Other Immediate Early Genes by Dopamine Receptor Antagonists**

The finding that haloperidol and related D2 dopamine receptor antagonists will activate c-fos expression throughout the striatum was surprising, especially as the simplest explanation (increased dopamine release following autoreceptor inhibition) seems unlikely as the D1 receptor antagonist SCH 23390 will not prevent D2 antagonist-induced activation of c-fos. This strengthens the idea that there is normally a tonic D2-mediated inhibition of c-fos expression in the striatum. Moreover, the induction of c-fos expression can be overcome by administration of the D2-selective agonist LY-171555, as would be expected if this were an effect mediated by D2 dopamine receptors.

**New Developments**

Fos and other products of immediate early genes act as parts of DNA complexes. Fos itself is active in heterodimeric association with the protein products of c-jun and other members of the jun family, but is not active, at least in vitro, in homodimeric Fos-Fos complexes. For striatal Fos induced by dopaminergic drugs to be functional, other immediate early genes need to be stimulated in the striatum, or there must be constitutive expression of such genes. In situ hybridization evidence suggests that jun-B and other immediate early genes are indeed activated by cocaine and amphetamine. Interestingly, though high levels of striatal jun-B mRNA transcripts are induced by these drugs, c-jun mRNA transcripts are barely detectable at 1 hour post-injection. This suggests that specific patterns of transcriptional factor activation may be involved in the response of individual cell types to particular stimulations.

The work of Hunt and his colleagues was the first to suggest that c-fos activation might serve as a functional tracing technique with cellular resolution. This potential has been borne out by many findings including those on the dopaminergic system. One interesting application of this approach has been the use of c-fos to map out functional interactions between graft and host tissue in the striatum. Amphetamine and cocaine induce strong c-fos expression in neurons of intrastriatal grafts derived from embryonic rat striatal primordia and implanted into previously excitotoxin-damaged striatum of host rats. The induction is specific in that it is located in the regions of the grafts that have striatal characteristics and that project to the pallidum. Thus, the c-fos activation may be indicative of functionally-specific graft-host interactions.

In addition to using Fos activation as a technique to study responses to systemically administered drugs, we have studied the response of c-fos following direct infusions of drugs into the striatum. This procedure has many advantages and permits use of compounds that do not cross the blood-brain barrier.

The most important question to be answered now is what is the role of c-fos and other immediate early genes in dopaminergic function in the striatum. We have suggested the possibility that such long-term changes as priming drug addiction and dyskinesias might be related in some way to dopamine receptor mediated c-fos activation. The molecular mechanisms by which these changes are achieved remain to be determined. In other systems, c-fos and related immediate early genes appear to regulate synthesis of peptides and proteins such as enkephalin and nerve growth factor. Understanding of the role that the immediate early genes play in the basal ganglia will give us important insights into the function of this extensive neural system of the forebrain.

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**References**


