Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1058 (2005) 189 – 192
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Short Communication

Muscarinic receptor blockade attenuates reserpine-mediated Fos induction in the rat striatopallidal pathway

Alexia E. Pollack*, Michael R. Angerer

Department of Biology, University of Massachusetts-Boston, 100 Morrissey Blvd., Boston, MA 02125, USA

Accepted 22 July 2005
Available online 8 September 2005

Abstract

Acute administration of the dopamine-depleting agent reserpine (10 mg/kg) induces Fos expression in striatopallidal neurons of intact rats—an effect that is blocked by pretreatment with the D2 agonist quinpirole (0.5 mg/kg). Systemic administration of the muscarinic antagonist scopolamine (50 mg/kg) partially attenuates reserpine-mediated striatal Fos expression. These data suggest that muscarinic receptors, either within the striatum or in extrastriatal sites, regulate D2 receptor-mediated Fos expression in rat striatopallidal neurons.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Interaction between neurotransmitters

Keywords: D2 dopamine receptor; Scopolamine; Striatum; Immediate early gene

Voluntary movement involves the activity of several brain regions including the structures of the basal ganglia. The input structure of basal ganglia, the striatum, receives glutamatergic innervation from the cerebral cortex and dopaminergic projections from the substantia nigra pars compacta in the midbrain [1]. Dopamine acting at D1 receptors excites the neurons of the striatonigral (direct) pathway, while dopamine acting at D2 receptors inhibits striatopallidal (indirect) pathway neurons [5]. Consequently, administration of D2 antagonists induces the expression of the immediate early gene (IEG) product c-Fos in rat striatopallidal neurons [4,15]. In a similar manner, striatal Fos expression is induced 3 h following acute administration of reserpine (10 mg/kg), an agent that depletes vesicular stores of dopamine [3]. This response occurs in striatopallidal neurons [13] and is completely blocked by prior treatment with the D2 agonist quinpirole (0.5 mg/kg) [3,13], suggesting that it is the removal of dopamine (by reserpine) acting at D2 receptors that leads to Fos expression in striatopallidal neurons.

In the striatum, enkephalin (ENK)-containing striatopallidal neurons express D2 dopamine receptors [5] as well as several subtypes of muscarinic acetylcholine (ACh) receptors [2,18]. In turn, striatal cholinergic interneurons express D2 dopamine receptors [8,18] whose stimulation inhibits potassium-evoked release of ACh [16]. Therefore, D2-mediated regulation of striatopallidal neurons may occur by several possible mechanisms: *direct action* through D2 receptors expressed by striatopallidal neurons themselves, or *indirect action* through D2-mediated inhibition ACh release, which in turn affects striatopallidal neurons that express muscarinic receptors [2,18]. The latter mechanism suggests that administration of a muscarinic antagonist should be able to prevent or reverse D2-mediated effects in striatopallidal neurons. In support of this idea, administration of the muscarinic antagonist scopolamine attenuates striatal Fos expression following administration of a D2 antagonist [6,7], and blocks elevations in striatal ENK mRNA following 6-hydroxydopamine lesion (6-OHDA) [10,14] or chronic D2 antagonist administration in intact rats [14].

Abbreviations: ACh, acetylcholine; ENK, enkephalin; Fos-LI, Fos-like immunoreactivity; 6-OHDA, 6-hydroxydopamine; PBS, phosphate buffered saline; PFA, paraformaldehyde

* Corresponding author. Fax: +1 617 287 6650.
E-mail address: alexia.pollack@umb.edu (A.E. Pollack).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.07.043

Table 1
Effect of scopolamine on reserpine-mediated striatal Fos expression

Treatment group	(*N*)	Striatal Fos-LI
Water/Vehicle	(5)	35 ± 7[a]
Water/Reserpine (10)	(7)	573 ± 45[b]
Quinpirole (0.5)/Reserpine (10)	(6)	30 ± 7[a]
Scopolamine (10)/Reserpine (10)	(4)	660 ± 74[b]
Scopolamine (10)/Vehicle	(4)	58 ± 5[a]
Scopolamine (50)/Reserpine (10)	(8)	375 + 32[a,b]
Scopolamine (50)/Vehicle	(4)	58 ± 8[a]

Rats were pretreated with water, quinpirole (0.5 mg/kg) or scopolamine (10 or 50 mg/kg) 30 min prior to injection with vehicle or reserpine (10 mg/kg). Values in parentheses in Treatment Groups represent doses of drugs (mg/kg). *N* = number of animals in each group. Striatal Fos-LI represents the number of cells (mean ± SEM) expressing Fos-LI in a 1.2-mm^2 rectangular box in the dorsal striatum.

[a] Different from Water/Reserpine group, $P < 0.01$.

[b] Different from Water/Vehicle group, $P < 0.01$.

However, it is not clear whether the cholinergic system participates in reserpine-mediated striatal Fos expression or whether this response is due solely to D2-mediated effects on striatopallidal neurons themselves. Therefore, we tested whether administration of the muscarinic antagonist scopolamine could block reserpine-mediated striatal Fos expression in intact rats.

Male, Sprague–Dawley rats (200 g; Charles River, Wilmington, MA) were injected (i.p.) with pretreatment drugs 30 min prior to injection with reserpine (10 mg/kg). Rats were pretreated with water, D2 agonist quinpirole (0.5 mg/kg) or muscarinic antagonist scopolamine hydrobromide (10 or 50 mg/kg)—using doses of quinpirole and scopolamine that have been used previously [13,14]. All drugs were dissolved in water, except for reserpine (2.5 mg/ml), which was dissolved in 100μl acetic acid and brought to final volume with water (vehicle) before being injected at a dose of 10 mg/kg. A group of control animals were injected with water 30 min prior to vehicle, while two other groups of animals were injected with scopolamine (10 or 50 mg/kg) 30 min prior to vehicle. Rats were treated in a manner conforming to University of Massachusetts Policies and Procedures and the National Institute of Health Guide for Care and Use of Laboratory Animals.

Three hours following injection of vehicle or reserpine, rats were anesthetized with pentobarbital (50 mg/kg) and perfused transcardially with saline followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brains were removed, placed in 4% PFA overnight, and stored in 30% sucrose for 1–2 days at 4 °C. Sixty-micron coronal brain sections were cut using a sliding microtome and stored in phosphate buffered saline (PBS) at 4 °C. Fos immunohistochemistry was performed as described previously [13] using a c-Fos polyclonal antibody (PC35, Oncogene Science, Cambridge, MA). For quantification of Fos-like immunoreactivity (Fos-LI), 1–3 brain sections/rat from the rostral striatum (corresponding to plates 12–14 [12]) were analyzed using a Scion Image Analysis System by one observer blind to the treatment groups (M.R.A.). A rectangular box (1.2 mm^2) was placed over the dorsal striatum and all cells expressing Fos-LI were counted within this area. Data are expressed as the number of Fos-LI positive cells (mean ± SEM) in 1.2 mm^2 of the dorsal striatum. Comparisons between treatment groups were made using an ANOVA followed by Dunnett's post hoc test, with $P < 0.05$ considered significant.

Three hours following injection of rats with Water/Vehicle there were only a few scattered cells expressing Fos-LI in the dorsal striatum (Table 1; Fig. 1A). In contrast, 3 h following

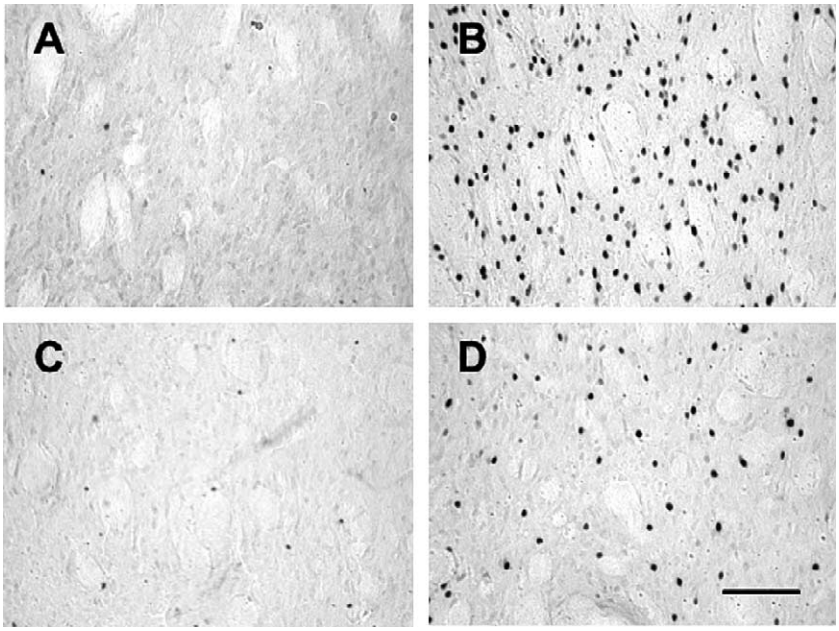

Fig. 1. Photomicrographs of striatal Fos-LI 3 h following administration of: (A) Water/Vehicle, (B) Water/Reserpine (10 mg/kg), (C) Quinpirole (0.5 mg/kg)/Reserpine and (D) Scopolamine (50 mg/kg)/Reserpine. Scale bar = 0.1 mm.