Neuropharmacology 58 (2010) 444–451

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Alterations in gene regulation following inhibition of the striatum-enriched phosphodiesterase, PDE10A

Christine A. Strick*, Larry C. James, Carol B. Fox, Thomas F. Seeger, Frank S. Menniti [1], Christopher J. Schmidt

Neuroscience Research Unit, Pfizer Global Research and Development, Groton/New London Laboratories, Eastern Point Road 8220/405, Groton, CT 06340 USA

ARTICLE INFO

Article history:
Received 11 January 2008
Received in revised form
31 August 2009
Accepted 8 September 2009

Keywords:
Phosphodiesterase
PDE10A
Regulation of striatal gene expression
Striatum
cfos
Neurotensin
Substance P
Enkephalin

ABSTRACT

PDE10A is a member of the phosphodiesterase superfamily highly enriched within medium spiny neurons (MSN) in mammalian striatum. We have used inhibitors of PDE10A and quantitative measures of mRNA to demonstrate that PDE10A controls striatal gene expression by regulating MSN cyclic nucleotide signaling pathways. Acute treatment with PDE10A inhibitors produces rapid and transient transcription of the immediate early gene cfos in rat striatum. Although inhibition of PDE10A causes accumulation of both cAMP and cGMP, the increase in striatal cfos expression appears to depend on changes in cAMP, since the increase is present in mice deficient in nNOS which fail to increase cGMP in response to PDE10A inhibition. Consistent with its expression in a majority of striatal MSN, PDE10A inhibition significantly induces expression of both substance P and enkephalin, neuropeptide markers for the direct and indirect striatal output pathways, respectively. These findings support the hypothesis that PDE10A modulates signal transduction in both striatal output pathways and suggest that PDE10A inhibitors may offer a unique approach to the treatment of schizophrenia.

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are critical players in the regulation of cellular signal transduction. Through their ability to degrade cAMP and cGMP, PDEs modulate intracellular levels of these second messengers to influence the degree and duration of cellular responses to multiple stimuli. There are 21 genes in the human genome that are classified as cyclic nucleotide PDEs. These genes cluster into 11 families, based on similarities in amino acid sequence and biochemical characteristics (reviewed in: Conti and Jin, 1999; Soderling and Beavo, 2000; Francis et al., 2001; Lugnier, 2006). PDE10A is the single member of one of the more recently identified PDE families and has been cloned from human, mouse and rat (Loughney et al., 1999; Fujishige et al., 1999a,b; Soderling et al., 1999). PDE10A is a dual-substrate PDE, with the ability to catalyze the hydrolysis of both cAMP and cGMP in vitro and in vivo (Schmidt et al., 2008).

The distribution of PDE10A is uniquely restricted within the body, with high levels of PDE10A mRNA found only in brain and testis (Fujishige et al., 1999a,b; Soderling et al., 1999). Within the brain, both PDE10A mRNA and protein are specifically enriched in the medium spiny neurons (MSNs) of striatum (Seeger et al., 2003), with virtually no expression in striatal interneurons (Xie et al., 2006). As the primary input nucleus of the corticostriatothalamic loop, the striatum integrates glutamatergic and dopaminergic signaling to coordinate the execution of relevant cognitive and motor patterns with the suppression of inappropriate or less relevant options (Graybiel, 2000). The striatal MSNs are GABAergic projection neurons about evenly distributed between two anatomical and neurochemically distinct pathways. MSNs projecting directly to the substantia nigra and endopenducular nucleus inhibit the GABAergic output from these nuclei to the thalamus to facilitate behavioral responses. The opposite behavioral effect is produced by activation of the second striatal output pathway which projects initially to the globus pallidus, and ultimately increases nigrothalamic inhibition of activity (Surmeier et al., 2007). Projection neurons in these two pathways can be differentiated on the basis of distinct gene expression profiles. For example, a majority of neurons of the direct or striatonigral pathway express the D1 dopamine receptor and the neuropeptides dynorphin and substance P. D1 receptors are positively coupled to adenylyl cyclase and promote cortical activation of the direct pathway MSNs. In contrast, MSNs of the indirect or striatopallidal pathway express the neuropeptide enkephalin and D2 dopamine receptors which

* Corresponding author. Tel.: +1 860 441 4350.
E-mail address: christine.a.strick@pfizer.com (C.A. Strick).
[1] Present address: cyclicM LLC, 10 Reynolds Hill Rd., Mystic, CT 06355, USA.

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.09.008

are negatively coupled to cAMP production (Gerfen et al., 1990; Robertson and Jian, 1995). It is this segregation of differentially coupled dopamine receptors between the two functionally opposed output systems which allows changes in extracellular dopamine concentrations to signal the selection or rejection of a behavioral response.

As one might predict from its localization, disruption of the PDE10A gene or systemic administration of a PDE10A inhibitor significantly impacts basal ganglia function. Biochemical evidence indicates that inhibition of PDE10A augments both cGMP and cAMP signaling pathways within MSNs. Downstream consequences of PDE10A inhibition also include increased phosphorylation of the MAP kinase ERK, the transcriptional activator CREB (Siuciak et al., 2006b), and the regulatory protein DARPP-32 (Nishi et al., 2008). PDE10A knockout ($PDE10A^{-/-}$) mice show reduced spontaneous locomotor activity while pharmacological inhibition of PDE10A reduces spontaneous and stimulant-induced increases in locomotion. These effects are similar to those produced by D2 receptor inhibition and are consistent with enhanced output of the indirect pathway. Recent studies have confirmed a preferential effect of PDE10 inhibitors on activity within the indirect pathway (Nishi et al., 2008; Threlfell et al., 2009). However, the presence of PDE10A in all MSNs suggests the potential to modulate signal transduction in both striatal projections in a parallel fashion. An effect on activation of both pathways would predict that PDE10A inhibition will have distinct effects on basal ganglia circuitry and unique therapeutic potential. We investigated this possibility in the present set of experiments, using striatal gene expression as an index of MSN activity. Specifically, we measured changes in expression of the immediate early gene cfos and the neuropeptide neurotensin (NT), as well as expression of the genes for substance P and enkephalin, markers for the direct and indirect striatal output pathways, respectively, as a consequence of PDE10A inhibition. The effects of PDE10A inhibition on striatal gene expression were compared to those induced by a D1 receptor agonist and a D2 antagonist, which were previously evaluated using in situ hybridization and immunohistochemistry (Angulo, 1992; Augood et al., 1993; Robertson and Jian, 1995; Robertson et al., 1995; Wang and McGinty, 1997; Lipska et al., 2003). The results confirm that PDE10A inhibition enhances signal transduction and consequently gene expression in medium spiny neurons of both the direct and indirect striatal output pathways.

2. Methods

2.1. Animals

Male Sprague–Dawley CD rats (200–250 g) and CD-1 mice (30–35 g) were from Charles River Laboratories (Kinston, NY). Male nNOS wild type (C57BL6/J) and $nNOS^{-/-}$ mice (25–30 g) were from Jackson Laboratories (Bar Harbor, ME). $PDE10A^{-/-}$ mice on a DBA1LacJ background were created as described in Siuciak et al. (2006a). All animals were housed under a 12-h light–dark cycle with lights on at 0600. Food and water was provided ad libitum and animals were acclimated for minimum 1 week. Animals were handled and cared for according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and all procedures were performed with the approval of the Pfizer Animal Care and Use Committee.

2.2. Drug administration

Rats and mice were pre-handled prior to the study to minimize stress. SKF81297 (Sigma), papaverine (Research Biochemical Int., Ontario, Canada), PQ-10 (Chappie et al., 2007) and MP-10 (Schmidt et al., 2008) were prepared in 40% 2-hydroxypropyl beta-cyclodextrin (Sigma) acidified with acetic acid (60 μl 1 N/ml solution). Haloperidol (Sigma) was prepared in 0.03% tartaric acid. Dosing was as follows: papaverine, 32 mg/kg; PQ-10, 17.8 mg/kg; MP-10, 3.2 mg/kg; SKF81297, 3.2 mg/kg; haloperidol, 0.32 mg/kg, or as described in the Figures. Rats were dosed at 1–2 ml/kg; mice at 10 ml/kg.

2.3. In situ hybridization

Rats were killed by decapitation at 1, 3, or 6 h after dosing. Brains were rapidly removed, frozen in isopentane on dry ice, and stored at −80 °C until use. Brains were serially sectioned at 20 microns onto glass slides, fixed in a 4% paraformaldehyde solution for 20 min, and then dehydrated through a series of ethanol solutions (ending in 100% EtOH), dried, and stored at −20 °C until hybridization.

Slide-mounted frozen brain sections were incubated in proteinase K (1 mg/ml) for 5 min, rinsed in RNAse-free water and suspended in 0.1 M triethanolamine at pH 8.0 and acetylated by addition of 0.25% acetic anhydride over 5 min. A rat cfos antisense probe consisting of a 587 nucleotide fragment of coding sequence corresponding to nucleotides 309–895 of NM_022197 was generated by in vitro transcription and labeling from a T7 promoter according to suppliers' protocol (Ambion). Probe was applied to the slide-mounted sections in a volume of 15–25 μl/section, containing 0.6–1 × 10^6 CPM of ^{33}P per section. All sections were incubated overnight in a humid environment at 50 °C, and then rinsed in 2× SSC, treated with RNAse A to destroy single-stranded RNA, rinsed in a standard series of washes, and dehydrated in a graded series of ethanols (30–100%). The resulting slides were apposed to beta-max film (Amersham) in standard x-ray cassettes, and exposed for 5–10 days. The film was developed in D-19 developer (Kodak) and Kodak Fixer at room temperature. Films were quantified with an Imaging Research MCID image analyzer.

2.4. RNA quantification

Animals were killed by decapitation (rats) or cervical dislocation (mice) at various times after injection, as described in the Figures. Striata were dissected out and frozen at −80 °C until use. Total RNA was harvested from frozen striata using Qiagen RNeasy Mini Kit with tissue protocol and DNase treatment. For quantitative RT-PCR analysis, 1 μg of total RNA was converted to cDNA using Sprint PowerScript Reverse Transcriptase (BD Biosciences) primed with random hexamers (100 ng) followed by RNase H (Invitrogen) treatment. The cDNA was diluted 3–5-fold with distilled water, and 3 μl was used per PCR reaction.

Real time quantitative PCR for cfos (mouse, Mm00487425_m1, RefSeq NM_010234; rat, Rn02396759_m1, RefSeq NM_022197), NT (mouse, Mn00481140_m1, RefSeq NM_024435; rat, Rn01503265_m1, RefSeq NM_001102381), and ribophorin (mouse, Mm00505837_m1, RefSeq NM_133933; rat, Rn00565052_m1, RefSeq NM_013067) was performed using Applied Biosystems Taqman Assays-on-Demand and ABI 7700 or ABI 7900 HT Sequence Detector. PCR reactions were performed in triplicate on each sample. Mouse or rat ribophorin was used as control to correct for variation in mRNA quality and quantity. Expression of cfos, NT and ribophorin was measured using a standard curve generated from serial dilution of cDNA (Livak and ABI Prism 7700 Sequence Detection System. User Bulletin no. 2 PE Applied Biosystems, 1997). Statistical analyses were carried out as described in Figure Legends.

Rat substance P (preprotachykinin), enkephalin, and ubiquitin mRNA levels were measured using Invader RNA Assay (Eis et al., 2001; Third Wave Technologies (TWT), Madison, WI). Assays for ubiquitin (#91–093) and preprotachykinin (#91–167) were available from TWT. An assay for enkephalin was custom designed based on rat sequence NM_017139, and included probe oligo 5′-AACGAGGCG-CACCGTACCAGG-NH_2C_7-3′, invader oligo 5′-AGGAAGTTGATGTCGCCGGGAA-3′, stacker oligo 5′-CGGUAGCUGCAUUUAG-3′, and arrestor oligo 5′-CCUGGUACG-GUGCGC-3′. Invader assays were conducted in biplex format with ubiquitin using 20–50 ng of RNA per well in triplicate according to TWT protocol. Target mRNA was quantitated using standard curves constructed from known amounts of in vitro transcripts either provided with the kits or generated in house with RiboMax Large Scale RNA Production System-T7 (Promega). Results were normalized against ubiquitin to control for variation in quantity or quality of input RNA, and are expressed as fold change, with vehicle treated group set to 1. Statistical analysis was carried out as described in Figure legend.

3. Results

Our experiments were designed to test the hypothesis that PDE10A modulates signal transduction in MSNs of both the direct and indirect striatal output pathways. To accomplish this we investigated changes in gene expression subsequent to inhibition of PDE10A, and contrasted these effects to those resulting from activation of dopamine D1 receptors (direct pathway activation) or blockade of dopamine D2 receptors (indirect pathway disinhibition). A difficulty in these types of experiments is to choose a relevant dose of each experimental compound. The PDE10A inhibitors used in this study vary over a potency range of approximately 100-fold (IC_{50} = 36 nM, 4.0 nM, and 0.48 nM for papaverine, PQ-10, and MP-10, respectively) and in selectivity over other PDEs (at least 9-fold, 49-fold, and 3000-fold for papaverine, PQ-10, and MP-10,