Neuropharmacology 61 (2011) 1275–1281

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

The PDE10A inhibitor, papaverine, differentially activates ERK in male and female rat striatal slices

Yu-Tien Hsu [a], Guanghong Liao [b], Xiaoning Bi [b], Tetsushi Oka [c], Shigeki Tamura [c], Michel Baudry [a,*]

[a] Neuroscience Program, University of Southern California, Los Angeles, CA, USA
[b] Basic Medical Sciences, COMP, Western University of Health Sciences, Pomona, CA 91766, USA
[c] Asubio Pharma Co., Ltd., Kobe 650-0047, Japan

ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form
12 July 2011
Accepted 20 July 2011

Keywords:
Striatum
PDE10A
ERK
Dopamine
Estrogen
Gender

ABSTRACT

The phosphodiesterase 10A (PDE10A) is highly expressed within dopaminoreceptive medium spiny neurons (MSNs) of the striatum, which are implicated in various neurodegenerative diseases and psychiatric disorders, such as Huntington's disease and schizophrenia. With its dual action on cAMP and cGMP, PDE10A has been proposed to affect several signaling cascades in the corticostriatothalamic circuits. In particular, papaverine, a selective PDE10A inhibitor has been shown to activate/phosphorylate ERK in striatum. We used acute rat striatal slices to further characterize the effects of papaverine on ERK activation/phosphorylation in D1- and D2-responsive striatal neurons. Incubation of striatal slices from male rats with papaverine increased the levels of phospho-ERK1/2 (p-ERK), an effect enhanced with a D1 agonist or a D2 antagonist, but decreased with a D1 receptor antagonist or a D2 receptor agonist. Papaverine-induced increase in p-ERK was localized in striatal neurons receiving D1-enriched presynaptic terminals, as well as in postsynaptic D2-enriched neurons in striatal slices. Interestingly, papaverine had almost no stimulatory effects on ERK1/2 phosphorylation in slices prepared from female rats. In striatal slices prepared from ovariectomized female rats, papaverine treatment stimulated ERK1/2 phosphorylation to levels similar to those in slices from male rats. Moreover, estrogen was found to regulate the levels of D2 but not D1 receptors in striatum. These results indicate that circulating levels of female hormones, and in particular estrogen, regulate the effects of PDE10A inhibition on ERK1/2 phosphorylation in medium spiny neurons, an effect possibly linked to estrogen's regulation of D2 receptors. Considering the variety of events modulated by ERK1/2 activity, these findings suggest that sex difference needs to be taken into consideration for the further investigation of the effects of PDE10A inhibitors.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

A number of extracellular signals mediate their intracellular signaling effects through modifications of the levels of the cyclic nucleotides, cAMP and cGMP. The phosphodiesterase (PDE) family includes several members that are involved in the hydrolysis of cAMP and/or cGMP, and has been extensively studied because of the critical roles these enzymes are playing under physiological or pathological conditions. High levels of PDEs are expressed in brain, and understanding the roles of various PDEs has been intensively pursued, because they represent potential therapeutic targets against CNS disorders (Menniti et al., 2006). Among the PDE family, PDE10A has been extensively investigated due to its dual-substrate (cAMP and cGMP) enzymatic activity and its almost exclusive localization within medium spiny neurons (MSNs) of the striatum (Seeger et al., 2003; Coskran et al., 2006; Xie et al., 2006). Striatal MSNs receive inputs from cortical, thalamic and midbrain dopaminergic pathways (Surmeier et al., 2007), and dysfunction of these pathways has been found in many neurological disorders. Therefore, PDE10A has been viewed as a potential target for treating several neurological disorders, such Huntington's disease (Giampa et al., 2010; Kleiman et al., 2011), schizophrenia (Schmidt et al., 2008; Siuciak, 2008; Zhang, 2010), and addiction (Hebb and Robertson, 2007).

PDE10A has been proposed to regulate the functions of two of the major striatal pathways: 1) the direct pathway involving D1 receptor-enriched striatonigral neurons, as D1 receptor activation

Abbreviations: cAMP, 3′,5′-cyclic adenosine monophosphate; *cGMP*, Guanosine 3′,5′-cyclic mononucleotide; MSNs, medium spiny neurons; PDE10A, phosphodiesterase 10A; p-ERK, phosphorylated extracellular-signal-regulated kinases.

* Corresponding author. HNB534, USC, Los Angeles, CA 90089 2520, USA. Tel.: +1 213 740 9188; fax: +1 213 740 5687.
E-mail address: baudry@usc.edu (M. Baudry).

0028-3908/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2011.07.030

stimulates adenylate cyclase activity and 2) the indirect pathway comprising D2 receptor-enriched striatopallidal neurons, as D2 receptor activation results in inhibition of adenylyl cyclase activity (Stoof and Kebabian, 1981). Moreover, the extracellular-signal-regulated kinase (ERK) pathway, which is associated with cell survival and proliferation, has been shown to be regulated by both D1 (Chen et al., 2009) and D2 receptors in the striatum (Pozzi et al., 2003; Bertran-Gonzalez et al., 2008, 2009). Papaverine is a relatively specific PDE10A inhibitor and has been shown to increase phosphorylation of ERK1/2 in mouse neostriatal slices (Nishi et al., 2008) and in mice *in vivo* (Siuciak et al., 2006a).

An increasing number of studies indicates that estrogen exerts a profound influence on the nigrostriatal dopaminergic pathway (Becker and Beer, 1986). In particular, the incidence of Parkinson's disease is greater in men than in women (Shulman, 2007), and there are significant gender differences in drug addiction and in response to various drugs acting on the striatum. These findings indicate that the female sexual hormone, estrogen, is likely to modify the responses to various drugs acting in the striatum. However, whether or not the different hormonal status of males and females modifies papaverine-induced increase in ERK phosphorylation has not been determined. In the present study, we set to examine potential sex differences in response to papaverine and to characterize the effects of papaverine on dopaminergic signaling, and in particular on the direct and indirect pathways. Our results indicate that the stimulatory effect of papaverine on ERK is absent in striatal slices prepared from intact female rats, but is present in ovariectomized female rats. In addition, the effect of papaverine is predominant in postsynaptic D2 receptor-enriched MSNs, and we propose that circulating levels of estrogen prime the activation of D2 receptors in striatum.

2. Materials and methods

2.1. Acute striatal slice preparation

Animals were treated in accordance with the principles and procedures of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*; all protocols were approved by the Institutional Animal Care and Use Committee of the University of Southern California. Striata were rapidly dissected from four-to six-week-old Sprague–Dawley rats, transferred to oxygenated, ice-cold cutting medium containing (in mM): sucrose (103), NaCl (62), $NaHCO_3$ (26), glucose (10), KCl (2.9), KH_2PO_4 (1.25), $MgSO_4$ (2.25), $CaCl_2$ (1.7), and cut into slices (0.25 × 0.25 × 2.00 mm^3) for biochemical experiments or (250 μm thick) for immunohistochemistry staining, with a McIlwain tissue slicer or a Leica vibratome. Slices were then maintained in a recovery chamber with oxygenated normal Krebs-Ringer-Bicarbonate medium (KRB), containing (in mM): NaCl (118), KCl (4.7), $CaCl_2$ (1.3), $MgSO_4$ (1.5), KH_2PO_4 (1.2), 25 $NaHCO_3$ (25), glucose (11.7), for 60 min at 36 °C in a 95% O_2/5% CO_2 atmosphere; after this step, the medium was replaced with freshly oxygenated KRB solution. Slices were then transferred into screw-cap microfuge tubes containing 2 ml KRB medium with various drugs; 10 μM papaverine, 2 μM SKF38393, 1 μM SCH23390, 2 μM quinpirole, and 1 μM sulpiride and further incubated at 36 °C for 10 min; at the end of incubation and after homogenization in lysis buffer, aliquots of homogenates were processed for western blot analysis. We selected 10 μM papaverine to test the role of PDE10A since this compound has been widely used in the literature, has been shown to be selective for PDE10A, and exerts maximal effect at this concentration (Nishi et al., 2008). Concentrations for drugs acting on dopamine receptors were also selected because of their maximal effects on the receptors. All chemicals were purchased from Sigma, St. Louis, MO.

2.2. Ovariectomy

Two-to-three week-old female Sprague–Dawley rats were ovariectomized and were sacrificed two weeks after surgery for acute slice experiment. Surgery was performed under anesthesia with ip injection of ketamine (50 mg/kg, Phoenix, MO) and xylazin (10 mg/kg, Akorn, IL). The two-week time period was selected because it corresponds to the time of maximal clearance of 17-β-estradiol (E2) from blood and other tissues, including brain.

2.3. Western blots

Protein lysates were prepared at a final concentration of 2 μg/μl by homogenization of rat striatal slices in cold lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 mM NaF, and 1 mM Na_3VO_4), with 1:100 protease inhibitor cocktail (Sigma, MO.). After sample preparation, 20 μg proteins were electrophoresed on 10% SDS polyacrylamide gels. After separation, proteins were transferred to PVDF membranes. PVDF membranes were blocked with 5% non-fat milk for 1 h at room temperature then probed with different primary antibodies overnight at 4 °C with 1:10,000 dilution of anti-actin (Millipore), and with 1:1000 dilution of anti-phospho-ERK1/2, anti-ERK1/2 (Cell Signaling), anti-phospho-mTOR and anti-phospho-CREB (Cell Signaling), followed by horseradish peroxidase-conjugated secondary antibodies (1:10,000) and enhanced by chemiluminescence detection reagent (Amersham Biosciences). The specificity of the D1 receptor antibody has been clearly demonstrated in Levey et al. (1993), and these antibodies have been used in many studies, further indicating their specificity (Scott et al., 2002; Takeuchi et al., 2002; Fiorentini et al., 2003; Takeuchi and Fukunaga, 2004; Park et al., 2005). The specificity of the D2 receptor antibodies has been demonstrated (Takeuchi et al., 2002; Takeuchi and Fukunaga, 2004) and these antibodies have been used in many studies (Scott et al., 2002; Park et al., 2005).

2.4. Immunohistochemistry

Striatal slices were fixed in 2% paraformaldehyde in phosphate buffer overnight at 4 °C. After incubation with 20% sucrose, striatal slices were sectioned on a microtome at 20 μm. Free-floating tissue sections were blocked with a blocking buffer (0.2% bovine serum albumin, 2% horse serum, 0.02% Triton X-100 in 1× phosphate buffered saline (PBS)) for 1 h before being probed with primary antibodies. Primary antibodies were: D1 receptor (1:100, made in rat, Sigma, St. Louis, MO), D2 receptor (1:50, made in goat, Santa Cruz, CA), and phospho-ERK (Thr202/Tyr204) (1:1000, made in rabbit, Cell Signaling Technology, Danvers, MA), overnight at 4 °C. After wash with 1× PBS 3 times with 10 min intervals, sections were incubated with fluorescent-conjugated secondary antibodies: Alexa Fluor®594 anti-rat, Alexa Fluor®488 anti-rabbit, or Alexa Fluor®488 anti-goat (all from Invitrogen, Carlsbad, CA) at room temperature for 1 h. Slices were washed again with 1× PBS (3 × 10 min) and sealed with mounting medium (Vectashield; Vector Laboratories, Inc., Burlingame, CA) containing 4′, 6′-diamidino-2-phenylindole (DAPI) to stain nuclei. Immunofluorescent signal was detected with a Nikon confocal microscope (Nikon TE 2000U with D-Eclipse C1 system; Melville, NY). No fluorescent signal was detected when the primary antibodies were omitted from the procedures.

2.5. Statistical analysis

Statistical comparisons were made using Student's *t* test and one-way analyses of variance (ANOVA) followed by Tukey's multiple comparison *post-hoc* tests were used for pair-wise comparisons between experimental treatments. Results were generally calculated as means ± standard error of the mean (SEM) from the indicated number of independent experiments and expressed as percent of the indicated control. p values >0.05 were regarded as not significant.

3. Results

3.1. Effects of papaverine, a D1 receptor agonist (SKF38393), and a D2 receptor antagonist (sulpiride) on ERK, CREB and mTOR activation in striatal slices

Striatal slices were prepared from male rats and were incubated for 10 min in the absence or presence of papaverine (10 μM), or a D1 receptor agonist (SKF38393, 2 μM), or a D2 receptor antagonist (sulpiride, 1 μM) or their combinations. Levels of double-phosphorylated ERK1/2 (p-ERK) (Fig. 1), p-CREB and p-mTOR were determined by immunoblots (Figs. S1 and S2). Following treatment with 10 μM papaverine, levels of p-ERK were increased by about 50%; similarly, incubation with 2 μM SKF38393 or 1 μM sulpiride resulted in significant increases in p-ERK levels. Combining papaverine with either SKF38393 or sulpiride resulted in significantly higher p-ERK levels than papaverine alone. Similar results were observed with p-CREB (Fig. S1) and p-mTOR (Fig. S2). Thus, the effects of papaverine on signaling pathways downstream of cAMP are similar to those of a D1 receptor agonist or of a D2 receptor antagonist.

3.2. Effects of papaverine on ERK activation/phosphorylation in striatal slices from male and female rats

Striatal slices were prepared from both male and female rats and incubated for 10 min in the absence or presence of 10 μM

Download English Version:

https://daneshyari.com/en/article/5815710

Download Persian Version:

https://daneshyari.com/article/5815710

Daneshyari.com