Neuropharmacology 89 (2015) 335–341

Contents lists available at ScienceDirect

Neuropharmacology

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

Protein kinase C beta regulates the D_2-Like dopamine autoreceptor

Kathryn D. Luderman [a], Rong Chen [a, 1], Mark J. Ferris [b], Sara R. Jones [b], Margaret E. Gnegy [c, *]

[a] Department of Pharmacology, University of Michigan, 1150 West Medical Center Drive, Ann Arbor, MI, 48109-5632, USA
[b] Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina, 27157-1083 USA
[c] Department of Pharmacology, University of Michigan, 1150 West Medical Center Drive, 2220E MSRB III, Ann Arbor, MI, 48109-0632, USA

ARTICLE INFO

Article history:
Received 21 August 2014
Received in revised form
3 October 2014
Accepted 7 October 2014
Available online 19 October 2014

Keywords:
Dopamine
Autoreceptor
Exocytosis
Quinpirole
Synaptosomes

ABSTRACT

The focus of this study was the regulation of the D_2-like dopamine autoreceptor (D_2 autoreceptor) by protein kinase Cβ, a member of the protein kinase C (PKC) family. Together with the dopamine transporter, the D_2 autoreceptor regulates the level of extracellular dopamine and thus dopaminergic signaling. PKC regulates neuronal signaling via several mechanisms, including desensitizing autoreceptors to increase the release of several different neurotransmitters. Here, using both PKC$\beta^{-/-}$ mice and specific PKCβ inhibitors, we demonstrated that a lack of PKCβ activity enhanced the D_2 autoreceptor-stimulated decrease in dopamine release following both chemical and electrical stimulations. Inhibition of PKCβ increased surface localization of D_2R in mouse striatal synaptosomes, which could underlie the greater sensitivity to quinpirole following inhibition of PKCβ. PKC$\beta^{-/-}$ mice displayed greater sensitivity to the quinpirole-induced suppression of locomotor activity, demonstrating that the regulation of the D_2 autoreceptor by PKCβ is physiologically significant. Overall, we have found that PKCβ downregulates the D_2 autoreceptor, providing an additional layer of regulation for dopaminergic signaling. We propose that in the absence of PKCβ activity, surface D_2 autoreceptor localization and thus D_2 autoreceptor signaling is increased, leading to less dopamine in the extracellular space and attenuated dopaminergic signaling.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Tight regulation of extracellular dopamine is crucial for normal dopaminergic signaling and is primarily achieved at the presynaptic terminal by activation of the dopamine transporter (DAT) and the D_2-like dopamine autoreceptor (D_2 autoreceptor). The primary function of the DAT is to take up dopamine from the extracellular space, terminating dopaminergic signaling (Giros et al., 1996). The D_2 autoreceptor regulates extracellular dopamine levels through a negative feedback mechanism (L'Hirondel et al., 1998). Both the D_2-like dopamine receptor (D_2R) and DAT are substrates for the widely expressed serine/threonine kinase protein kinase C (PKC) (Foster et al., 2002; Namkung and Sibley, 2004).

In addition to its regulation of D_2R and DAT, PKC impacts neurotransmitter exocytosis. Activation of PKC by phorbol esters increases the release of various neurotransmitters, including dopamine, following a depolarizing stimulus (Cubeddu et al., 1989; Huang et al., 1989; Barrie et al., 1991). PKC affects exocytosis through several different mechanisms, including inhibiting potassium channels, blocking calcium channel inactivation through G-proteins, increasing the size and replenishing rates of vesicle pools, and increasing the availability of the Soluble NSF Attachment Protein Receptor (SNARE) complex proteins involved in vesicle fusion (Barrett and Rittenhouse, 2000; Leenders and Sheng, 2005; Majewski and Iannazzo, 1998; Tanaka and Nishizuka, 1994). Cubeddu et al. (1989) demonstrated that PKC activation with a phorbol ester reduced the ability of the D_2 autoreceptor to inhibit evoked dopamine release. Additionally, inhibition of D_2Rs by phospholipase C and calcium-dependent PKCs has been shown in ventral tegmental dopaminergic neurons (Nimitvilai et al., 2013). PKC phosphorylates D_2R, resulting in internalization and desensitization of the receptor (Namkung and Sibley, 2004; Morris et al.,

Abbreviations: DAT, dopamine transporter; D_2R, D_2-like dopamine receptor; D_2 autoreceptor, D_2-like dopamine autoreceptor; SNARE, Soluble NSF Attachment Protein Receptor; PKC, protein kinase C; KRB, Krebs-Ringer buffer; 4AP, 4-aminopyridine; QP, quinpirole; HPLC, high-performance liquid chromatography.

* Corresponding author. Tel.: +1 734 763 5358.
E-mail addresses: luderman@umich.edu (K.D. Luderman), rchen@wakehealth.edu (R. Chen), mferris@wakehealth.edu (M.J. Ferris), srjones@wakehealth.edu (S.R. Jones), pgnegy@umich.edu (M.E. Gnegy).
[1] Present address: Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina, 27157-1083 USA.

http://dx.doi.org/10.1016/j.neuropharm.2014.10.012
0028-3908/© 2014 Elsevier Ltd. All rights reserved.

2007). While there is evidence showing that PKC affects the regulation of both extracellular dopamine and D_2R, it has yet to be determined which of the ten mammalian PKC isoforms interacts with D_2R to cause these changes.

We previously reported that the PKCβ isoform regulates DAT trafficking and activity in response to amphetamine (Johnson et al., 2005; Furman et al., 2009; Chen et al., 2009). Additionally, we have shown that PKCβ is present in dopaminergic neurons and co-localizes with DAT (O'Malley et al., 2010). More recently, we determined that PKCβ is crucial for coordinating the interaction between the D_2 autoreceptor and DAT (Chen et al., 2013). Because both D_2 autoreceptors and PKCβ are expressed in the dopaminergic neurons and D_2 autoreceptors are a known substrate of PKC, we hypothesized that PKCβ regulates the D_2 autoreceptor. In the present study we used mice genetically lacking PKCβ along with specific PKCβ inhibitors to determine the impact of this kinase on D_2 autoreceptor activity, which was assessed by measuring dopamine exocytosis following chemical stimulation of synaptosomes or electrical stimulation of brain slices, as well as measuring D_2R-mediated changes in locomotor activity in the presence of the D_2R/D_3R agonist quinpirole. We determined that PKCβ influences the D_2 autoreceptor by regulating its surface localization and activity. Coupled with our findings regarding the regulation of DAT by PKCβ, this work identifies a role for PKCβ as a key regulator of extracellular dopamine levels and thus dopaminergic signaling.

2. Materials and methods

2.1. Animals

All animal use and procedures were approved by the Institutional Animal Care and Use Committee at the University of Michigan and were in accordance with the National Institutes of Health guidelines. We made every effort to reduce the number of animals used and minimize animal suffering. The generation of PKC$\beta^{+/+}$ and PKC$\beta^{-/-}$ mice was previously described (Leitges et al., 1996). These mice were backcrossed with C57BL/6J mice more than ten times. Mice had free access to water and standard laboratory chow. Experimental mice were gender- and age-matched and were used between two and four months of age.

2.2. Chemicals

LY379196 was a generous gift from Eli Lilly (Indianapolis, Indiana). Enzastaurin was purchased from LC Labs (Woburn, MA). [^{3}H]-sulpiride was from PerkinElmer (Waltham, MA). Complete Mini protease inhibitor was purchased from Roche Diagnostics (Indianapolis, IN). All other chemicals, including 4-aminopyridine, quinpirole, sulpiride, and butaclamol, were purchased from Sigma Aldrich (St. Louis, MO).

2.3. Striatal dopamine release via suprafusion

Synaptosomes from whole striata were prepared as described previously (Chen et al., 2009). Briefly, mice were sacrificed by cervical dislocation. Striata were dissected on ice and homogenized in 0.32 M sucrose containing Complete Mini protease inhibitor cocktail. Homogenates were centrifuged at 4 °C (800 × g, 10 min) to remove cellular debris. The supernatant was centrifuged again (12,000 × g, 15 min, 4 °C). The pellet containing crude synaptosomes was resuspended in oxygenated Kreb-Ringer's Buffer (KRB) (145 mM NaCl, 2.7 mM KCl, 1.2 mM KH_2PO_4, 1.0 mM $MgCl_2$, 10 mM glucose, 24.9 mM $NaHCO_3$, 0.05 mM ascorbic acid, 0.05 mM pargyline, pH 7.4). Synaptosomes were loaded into the chambers of a Brandel suprafusion apparatus (Brandel Inc., Gaithersburg, MD). The samples were perfused with oxygenated KRB at approximately 800 µl/min. Following a 60-min wash to achieve a steady dopamine baseline, 14 fractions were collected at 1-min intervals. Dopamine release was stimulated at fractions seven and eight with 50 µM 4-aminopyridine (4AP) in the presence or absence of quinpirole or sulpiride. The PKCβ inhibitor LY379196 was applied during the 60-min wash period and continued throughout fraction collection. An internal standard solution composed of 50 mM perchloric acid, 25 µM EDTA, and 10 nM 2-aminophenol was added to each collected fraction. Dopamine content in each fraction was measured using high-performance liquid chromatography (HPLC) with electrochemical detection (Thermo Scientific/ESA, Sunnyvale, CA).

2.4. Striatal dopamine release via electrical stimulation

Brain slices were prepared as described previously (Mateo et al., 2005). Briefly, mice were decapitated, brains rapidly removed, and the coronal brain slices (400 µm thick) containing the nucleus accumbens were prepared using a vibrating tissue slicer. Slices were maintained at 32 °C in oxygen-perfused (95% O_2–5% CO_2) modified Kreb's buffer, which consisted of (in mM): NaCl, 126; $NaHCO_3$, 25; D-glucose, 11; KCl, 2.5; $CaCl_2$, 2.4; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; L-ascorbic acid, 0.4; pH 7.4. A capillary glass-based carbon-fiber electrode (active area ~100 µm long, 7 µm wide) was positioned approximately 75 µm below the surface of the slice in the nucleus accumbens core. Dopamine release was evoked every 5 min by a 4-ms, one-pulse stimulation (monophasic, 300 µA) from a bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) placed 100–200 µm from the carbon-fiber electrode.

Fast-scan cyclic voltammetry recordings were performed and analyzed using locally written software (Demon Voltammetry and Analysis; Yorgason et al., 2011). The electrode potential was linearly scanned as a triangular waveform from −0.4 to 1.2 V and back to −0.4 V (Ag vs. AgCl) using a scan rate of 400 V/s. Cyclic voltammograms were recorded at the carbon-fiber electrode every 100 ms by means of a potentiostat (Dagan, Minneapolis, MN, USA). Once the stimulated dopamine response was stable for at least three successive collections, baseline measurements were taken. Evoked extracellular concentrations of dopamine were assessed by comparing the current at the peak oxidation potential for dopamine with electrode calibrations of known concentrations of dopamine (1–3 µM). Data were modeled using Michaelis–Menten kinetics to determine dopamine released and V_{max} (Yorgason et al., 2011). The selective D_2R agonist (−)-quinpirole hydrochloride was used to induce autoreceptor activation. Quinpirole-induced decreases in electrically stimulated dopamine release were compared with pre-drug values (each animal served as its own control) to obtain a percent change in stimulated dopamine release. The PKCβ inhibitor enzastaurin (200 nM) was applied after stable baselines were obtained, 60 min before quinpirole was added, and continued throughout the experiment. Quinpirole dose–response curves were plotted as log concentration (*M*) of quinpirole vs. percent of control dopamine response.

2.5. D_2R binding

The D_2R binding protocol was modified from previously published methods (Namkung and Sibley, 2004). Briefly, striatal synaptosomes were prepared as described above and were resuspended in KRB. To measure surface D_2R binding, synaptosomes were treated for 5 min at 37 °C with vehicle or the PKCβ inhibitor enzastaurin. Then the synaptosomes were incubated with 10 nM [^{3}H]-sulpiride, a hydrophilic D_2 receptor antagonist, for 3.5 h on ice. Non-specific binding was determined in the presence of 10 µM (−)-butaclamol. Overall D_2R expression was determined using a membrane preparation from the striatal synaptosomes prepared above (Rubinstein et al., 1990). These synaptosomes were resuspended in 50 mM Tris–HCl (pH 7.4) and centrifuged at 40,000 × g for 15 min. The resulting membrane fraction was resuspended in KRB and then treated with vehicle or enzastaurin for 5 min at 37 °C. Membranes were incubated with 10 nM [^{3}H]-sulpiride ± 10 µM (−)-butaclamol for 90 min at room temperature (Namkung and Sibley, 2004). The reaction was terminated by filtering over GF/B Whatman filters and washing 3X with ice cold KRB. The radioactivity bound was quantified by scintillation counting. Data are expressed as the amount of specific [^{3}H]-sulpiride bound, normalized to the vehicle treated control.

2.6. Locomotor suppression by acute quinpirole treatment

Locomotor suppression following quinpirole treatment in a novel environment was measured using radiotransmitter implantation (Mini Mitter Co., Bend, OR) as previously described (Chen et al., 2007). Briefly, a radiotransmitter was implanted into the peritoneal cavity of each mouse. Following recovery, PKC$\beta^{+/+}$ and PKC$\beta^{-/-}$ mice were injected with saline and quinpirole (0.03, 0.1, or 0.3 mg/kg i.p.). Locomotor activity (gross activity count) was recorded immediately after the injection for 15 min and the data are expressed as the sum of the recorded locomotor activity as a percent of the saline control treatment.

2.7. Statistical analysis

Results were analyzed using GraphPad Prism 6 software (San Diego, CA) and are plotted as mean ± SEM. Statistical significance was set at $p < 0.05$. Comparisons between multiple groups or treatments were made using one-, two- or three-way ANOVA with Bonferroni post-test. Three-way ANOVA was performed using Systat (Chicago, IL). When only two groups were compared, a paired, two-tailed Student's *t*-test was used.

3. Results

3.1. Suppression of PKCβ activity increases the control of dopamine release by D_2 autoreceptors

The primary function of the D_2 autoreceptor is to control the amount of dopamine in the extracellular space through a negative feedback mechanism. Activation of the D_2 autoreceptor reduces dopamine exocytosis. We optimized a suprafusion assay to measure D_2 autoreceptor control of dopamine exocytosis. In this assay,

Download English Version:

https://daneshyari.com/en/article/5814310

Download Persian Version:

https://daneshyari.com/article/5814310

Daneshyari.com