Neuropharmacology 113 (2017) 82–88

Contents lists available at ScienceDirect

Neuropharmacology

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

Divergent cAMP signaling differentially regulates serotonin-induced spinal motor plasticity

D.P. Fields [a], G.S. Mitchell [b, *]

[a] Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706, USA
[b] Department of Physical Therapy and McKnight Brain Institute, University of Florida, Gainesville, FL 32610, USA

ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form
11 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
Motor neuron
Phrenic
Spinal cord
Respiratory plasticity
5-HT7 receptor
5-HT2 receptor
Exchange protein activated by cAMP
Protein kinase A
EPAC
PKA

ABSTRACT

Spinal metabotropic serotonin receptors encode transient experiences into long-lasting changes in motor behavior (i.e. motor plasticity). While interactions between serotonin receptor subtypes are known to regulate plasticity, the significance of molecular divergence in downstream G protein coupled receptor signaling is not well understood. Here we tested the hypothesis that distinct cAMP dependent signaling pathways differentially regulate serotonin-induced phrenic motor facilitation (pMF); a well-studied model of spinal motor plasticity. Specifically, we studied the capacity of cAMP-dependent protein kinase A (PKA) and exchange protein activated by cAMP (EPAC) to regulate 5-HT2A receptor-induced pMF within adult male rats. Although spinal PKA, EPAC and 5-HT2A each elicit pMF when activated alone, concurrent PKA and 5-HT2A activation interact via mutual inhibition thereby blocking pMF expression. Conversely, concurrent EPAC and 5-HT2A activation enhance pMF expression reflecting additive contributions from both mechanisms. Thus, we demonstrate that distinct downstream cAMP signaling pathways enable differential regulation of 5-HT2A-induced pMF. Conditional activation of independent signaling mechanisms may explain experience amendable changes in plasticity expression (i.e. metaplasticity), an emerging concept thought to enable flexible motor control within the adult central nervous system.

Published by Elsevier Ltd.

1. Introduction

Serotonin elicits long-lasting motor plasticity via G protein-coupled receptors (GPCRs; Brunelli et al., 1976; Randić et al., 1993; Clark and Kandel, 1993), with different receptor subtypes giving rise to plasticity via independent signaling pathways (reviewed in Barbas et al., 2003). Concurrent activation of multiple serotonin receptor subtypes reveals inhibitory inter-receptor cross-talk interactions thereby regulating serotonin-induced plasticity (Seol et al., 2007; Treviño et al., 2012; Hoffman and Mitchell, 2013; MacFarlane et al., 2011). Although signaling pathways downstream from individual GPCRs are known to diverge, it is not known how signaling divergence differentially impacts serotonin-induced plasticity.

Serotonin-induced motor plasticity is a major feature of the neural system controlling breathing (Mitchell and Johnson, 2003; Feldman et al., 2003). For example, serotonin elicits plasticity in respiratory defense reflexes of gastropod mollusks (Glanzman et al., 1989; Mackey et al., 1989; Levy and Susswein, 1993), and enhances spinal respiratory motor control in mammals (Bach and Mitchell, 1996; Baker-Herman and Mitchell, 2002). In rats, selective activation of spinal Gq-coupled serotonin 2A receptors (5-HT2A; MacFarlane et al., 2011) or Gs-coupled serotonin 7 receptors (5-HT7; Hoffman and Mitchell, 2011) elicits long-lasting phrenic motor facilitation (pMF). When multiple spinal serotonin receptors are stimulated with non-specific serotonin, pMF expression exhibits a bell-shaped dose response curve; low serotonin doses elicit pMF through Gq associated 5-HT2 receptors, but high serotonin doses elicit pMF only when spinal Gs associated 5-HT7 receptors are blocked (MacFarlane and Mitchell, 2009). Thus, there is a poorly understood interplay between Gq and Gs-coupled serotonin receptors within the spinal motor network regulating the expression of serotonin-induced pMF (MacFarlane and Mitchell, 2009; Hoffman and Mitchell, 2013).

Due to differences in cAMP binding affinity (Dostmann and

* Corresponding author. Department of Physical Therapy, College of Public Health & Health Professions, University of Florida, 1225 Center Drive, PO Box 100154, Gainesville, FL 32610, USA.
E-mail address: gsmitche@phhp.ufl.edu (G.S. Mitchell).

http://dx.doi.org/10.1016/j.neuropharm.2016.09.018
0028-3908/Published by Elsevier Ltd.

Taylor, 1991; Ponsioen et al., 2004; Zhou et al., 2016), cell type and sub-cellular distribution (Seino and Shibasaki, 2005), cAMP can independently activate cAMP-dependent protein kinase A (PKA) versus exchange protein activated by cAMP (EPAC), thus enabling distinct functional outcomes from Gs-coupled receptor signaling. For example, netrin-1 receptors differentially activate PKA and EPAC to dynamically regulate spinal axonal growth (Murray et al., 2009). While netrin-1 induced, cAMP-dependent, EPAC signaling promotes growth cone extension early in development, PKA signaling predominates later in development switching netrin-1/cAMP effects to growth cone repulsion. Thus, EPAC and PKA underlie contrasting time-specific and context-specific functions within the developing nervous system.

Here, we tested the hypothesis that EPAC and PKA differentially regulate serotonin-induced pMF. Using recently available, highly selective, drugs to manipulate spinal cAMP signaling (Table 1), we investigated the functional significance of distinct downstream cAMP signaling mechanisms on 5-HT2A induced pMF. We demonstrate that whereas PKA constrains 5-HT2A induced pMF, EPAC and 5-HT2A co-activation exert additive effects, enhancing pMF expression. Thus, cAMP signaling differentially regulates serotonin-induced pMF. This is the first demonstration that downstream signaling from a single intracellular molecule enables differential regulation of plasticity within the adult nervous system. While the present studies do not conclusively confirm that downstream cAMP signaling divergence occurs within a single cell, or cell type (i.e. neuron vs astrocyte vs glia), these observations provide evidence that flexible signaling through distinct PKA vs EPAC mechanisms may explain a number of emergent properties of serotonin-induced neuroplasticity of spinal motor networks, including metaplasticity (Huang et al., 1992; Kirkwood et al., 1995; Abraham and Bear, 1996; Fischer et al., 1997; Mitchell and Johnson, 2003).

2. Materials/methods

2.1. Animals

Adult male Sprague-Dawley rats (2–5 months old; colony 218A, Harlan; Indianapolis, IN) were doubly housed, with food and water *ad libitum*, a 12 h light/dark cycle, and controlled humidity/temperature. The University of Wisconsin Institutional Animal Care and Use Committee approved all animal procedures.

2.2. Neurophysiology experiments

Anesthesia was induced with isoflurane in a closed chamber and then maintained via nose cone (3.5% isoflurane in 50% O2, balance N2). Rats were tracheotomized and pump ventilated (2.5 ml per breath; frequency adjusted to regulate end-tidal PCO_2 between 40 and 50 mmHg; Rodent Ventilator, model 683; Harvard Apparatus; South Natick, MA, USA) with an inspiratory mixture of 50% O_2; 2% CO_2; balanced N_2 Followed by bilateral vagotomy in the mid-cervical region to eliminate ventilator entrainment of breathing efforts. An arterial catheter was placed into the right femoral artery to enable blood sampling for blood-gas analysis during protocols. To enable intrathecal drug delivery, a dorsal laminectomy and durotomy (C1/C2) was performed, a silicone catheter (OD 0.6 mm; Access Technologies, IL, USA; primed with drug/vehicle) was inserted through a small hole in the dura and advanced caudally (~3 mm) until resting at the C3-C4 spinal region. To minimize unintended drug diffusion from the catheter it was not placed until the stabilization period at the end of surgical preparations. The left phrenic and left hypoglossal (XII) nerves were isolated via a dorsal approach, cut distally, de-sheathed, submerged in mineral oil and then placed on bipolar silver wire electrode. After nerve dissection, rats were slowly converted to urethane anesthesia (1.8 g/kg, i.v. via tail vein catheter). Rectal body temperature (Traceable™, Fisher Scientific; Pittsburgh, PA, USA) was maintained within ±1.0 of 37.5 °C using a custom temperature-controlled surgical table. A flow-through capnoguard with sufficient response time to measure exhaled CO_2 in rats (Capnoguard, Novametrix; Wallingford, CT; USA) was used to monitor and control end-tidal CO_2 (via adjustments to ventilator frequency). A heparinized plastic capillary tube (250 × 125 μl cut in half) was used to sample arterial blood to measure gas tensions (PaO_2, $PaCO_2$), pH and base excess (ABL 800Flex, Radiometer; Copenhagen, Denmark). Intravenous fluid infusions at a rate of 1mL/Hr (1:10:5 by volume of $NaHCO_3$/Lactated Ringer's/Hetastarch) were used to maintain blood pressure, acid/base and fluid balance from induction with isoflurane to euthanasia (overdose with urethane) following neurophysiological recordings.

Phrenic nerve activity was amplified (x10,000: A-M Systems, Everett, WA), band-pass filtered (100 Hz–10 kHz), full-wave rectified, processed with a moving averager (CWE 821 filter; Paynter, Ardmore, PA: time constant, 50ms) and analyzed using a WINDAQ data-acquisition system (DATAQ Instruments, Akron, OH). Peak integrated phrenic burst frequency, amplitude, and mean arterial blood pressure (MAP) were analyzed in 60sec bins prior to obtaining blood samples. Data were included only if $PaCO_2$ was maintained within ±1.5 mmHg of baseline (set by recruitment threshold; approx. 45 mmHg), base excess was within ±3mEq/L of 0mEq/L, MAP had decreased less than 30 mmHg of baseline values (approx. 120 mmHg), and PaO_2 decreased less than 50 mmHg from baseline (approx. 300 mmHg) while remaining above 150 mmHg for the entire protocol. There was no significant drift tendency in any of the physiological variables as assessed via 2-way ANOVA (Table 2).

One hour after conversion to urethane adequate levels of anesthesia were confirmed by an absence of response (movement, arterial blood pressure, phrenic nerve activity) to toe pinch. Rats

Table 1
Published selectivity of PKA/EPAC activators and inhibitors from cell culture assays. The volume:conc (concentration) values listed in column 1 were concentration and volume used for intrathecal injections in the present *in-vivo* study.

Drug (volume:conc)	EPAC Ka	PKA Ka	PKA Ki	EPAC Ki
6-Bnz-cAMP[a] (10 μL:100 μM)	NSa	2.7 μM	–	–
8-pCPT-2′-O-Me-cAMP[a] (10 μL:100 μM)	1.8 μM	190 μM	–	–
Rp-8-Br-cAMP[a] (10 μL:1 mM)	–	–	8.5 μM	NSi
ESI-05[b] (10 μL:2 M)	–	–	NSi	0.43 μM

[Ka] concentration for half of maximum cAMP induced response.
[Ki] concentration for inhibition of half maximum cAMP induced response.
[NSa] non-significant activating effect; ≥100 fold Ka difference.
[NSi] non-significant inhibitory effect; ≥100 fold Ki difference.
[a] Poppe et al., 2008.
[b] Tsalkova et al., 2012.