HHS Public Access

Author manuscript

Biochem J. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Biochem J. 2016 January 1; 473(1): 1–5. doi:10.1042/BJ20150934.

Cocaine Inhibits Store-Operated Ca^{2+} Entry in Brain Microvascular Endothelial Cells: Critical Role for Sigma-1 Receptors

G. Cristina Brailoiu[1], **Elena Deliu**[2], **Linda M Console-Bram**[2], **Jonathan Soboloff**[3], **Mary E Abood**[2,4], **Ellen M Unterwald**[2,5], and **Eugen Brailoiu**[2,*]

[1]Department of Pharmaceutical Sciences, Jefferson College of Pharmacy, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, United States

[2]Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, United States

[3]Fels Institute for Cancer Research and Molecular Biology and Department of Medical Genetics & Molecular Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, United States

[4]Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, United States

[5]Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, United States

Abstract

Sigma-1 receptor (Sig-1R) is an intracellular chaperone protein with many ligands, located at the endoplasmic reticulum. Binding of cocaine to Sig-1R has previously been found to modulate endothelial functions. In the present study, we show that cocaine dramatically inhibits store-operated Ca^{2+} entry (SOCE), a Ca^{2+} influx mechanism promoted by depletion of intracellular Ca^{2+} stores, in rat brain microvascular endothelial cells. Using either Sig-1R shRNA or pharmacological inhibition with the unrelated Sig-1R antagonists BD-1063 and NE-100, we show that cocaine-induced SOCE inhibition is dependent on Sig-1R. In addition to revealing new insight into fundamental mechanisms of cocaine-induced changes in endothelial function, these studies provide an unprecedented role for Sig-1R as a SOCE inhibitor.

[*]To whom correspondence should be addressed: Eugen Brailoiu, M.D., Center for Substance Abuse Research, Temple University School of Medicine, 3500 N. Broad Street, Room 848, Philadelphia, PA 19140, Tel: 215-707-2791, Fax: 215-707-9890, ebrailou@temple.edu.

Declaration of interest: The authors declare no competing financial interest.

Author contributions: G.C.B., E.D., L.C.B., J.S. and E.B. performed experiments and analyzed data. G.C.B. and E.D. drafted the manuscript. M.E.A., E.M.U., J.S. analyzed data and revised the manuscript critically for important intellectual content. E.B. designed the study. All authors reviewed the results, read and approved the final version of the manuscript.

Keywords

sigma-1 receptor; endothelial cell; calcium; calcium imaging; endoplasmic reticulum; store-operated calcium entry

Introduction

Sigma-1 receptors (Sig-1Rs) are chaperone proteins residing at the interface between endoplasmic reticulum (ER) and mitochondria [1, 2]; upon stimulation, they translocate to other cell regions [3, 4]. Sig-1Rs interact with various targets, including ion channels, G protein-coupled receptors, tyrosine kinase receptors, and kinases, to regulate cellular functions [3, 5], Stimulating factors include Sig-1R agonists and ER stress [2]. Moreover, activated Sig-1Rs typically associate with inositol 1,4,5-trisphosphate receptor type 3, favoring an increase in cytosolic Ca^{2+} concentration [2, 6].

Store-operated Ca^{2+} entry (SOCE) [7, 8] is an important mechanism for Ca^{2+} influx, described in a variety of cells, including the endothelium [8, 9]. Physiologically, SOCE is activated by depletion of intracellular Ca^{2+} pools following stimulation of plasma membrane receptors that couple to phosphoinositide hydrolysis and inositol 1,4,5-trisphosphate generation. These decreases in ER Ca^{2+} content are sensed by the ER Ca^{2+} sensor, stromal interaction molecule 1 (STIM1) [8, 10], resulting in activation of Ca^{2+} channels in the plasma membrane termed Orai1, also known as Ca^{2+} release-activated Ca^{2+} channels [8, 11].

Since cocaine is a Sig-1R agonist [12] and cocaine-induced activation of Sig-1Rs was reported to modulate the function of brain microvascular endothelium [13, 14], we examined the Sig-1R-mediated effect of cocaine on SOCE in rat brain microvascular endothelial cells (RBMVEC).

Experimental procedures

Chemicals

All chemicals were from Sigma Aldrich (St. Louis, MO), unless otherwise mentioned. Cocaine hydrochloride was generously supplied by NIDA; NE-100 hydrochloride was from Santa Cruz Biotechnology (Dallas, TX).

Cell Culture

RBMVEC from Cell Applications, Inc. (San Diego, CA, USA) were cultured in rat brain endothelial basal medium and rat brain endothelial growth supplement, according to the manufacturer's instructions (Cell Applications, Inc.). Cells were grown in T75 flasks coated with attachment factor (Cell Applications, Inc.) until 80% confluent. Cells were plated on round coverslips of 25 mm diameter coated with human fibronectin (Discovery Labware, Bedford, MA, USA) for measurements of cytosolic Ca^{2+} and membrane potential and on six-well plates for western blot analysis.

Cell transfections

RBMVEC were transfected 24 hours after plating with four unique 29mer shRNA constructs Oprs1 (Sig-1R) rat, with the following sequences: TF711128A (GCCATTCGGGACGATACTGGGCTGAGATT), TF711128B (ATCATCTCTGGCACTTTCCACCAGTGGAG), TF711128C (CCTGTTTCTGACTATTGTGGCGGTGCTGA), TF711128D (TTGCACGCCTCGCTGTCTGAGTACGTGCT), in pRFP-C-RS vector or scrambled negative control non-effective shRNA in pRFP-C-RS (OriGene Technologies Rockville, MD), using Cytofect endothelial cell transfection kit (Cell Applications, Inc.). Throughout the manuscript, the Sig-1R shRNA constructs were abbreviated Sig-1R shRNA (A), Sig-1R shRNA (B), Sig-1R shRNA (C) and Sig-1R shRNA (D).

Western blot analysis

Whole-cell lysates were separated on Mini-PROTEAN TGX 4–20% gels (Bio-Rad, Hercules, CA, USA) by sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblotting. Proteins were transferred to an Odyssey nitrocellulose membrane (Li-Cor Biosciences, Lincoln, NE, USA). After blocking with Odyssey blocking buffer, the membranes were incubated overnight with primary antibody against Sigma-1R (OPRS1, rabbit polyclonal, 1:1000, OriGene Technologies, Rockville, MD). An antibody against β-actin (mouse monoclonal, 1: 10,000; Sigma-Aldrich) was used to confirm equal protein loading. Membranes were washed with Tris-buffered saline-Tween 20 (TBST) and incubated with the secondary antibodies: IRDye 800CW conjugated goat anti-rabbit IgG, and IRDye 680 conjugated goat anti-mouse IgG (1 : 10,000, 1 h at 21°C). Membranes were then washed in TBST and scanned using an Li-Cor Odyssey Infrared Imager and analyzed using Odyssey software.

Cytosolic Ca^{2+} measurement

Intracellular Ca^{2+} levels were evaluated as previously described [15, 16]. Briefly, cells were incubated with 5 μM fura-2 AM (Molecular Probes, Eugene, OR, USA) in Hanks' balanced salt solution at 21 °C for 1 h and washed with dye-free Hanks' balanced salt solution. Coverslips were mounted in an open bath chamber (QR-40LP; Warner Instruments, Hamden, CT, USA) on the stage of an inverted microscope Nikon Eclipse TiE (Nikon Inc., Melville, NY, USA), equipped with a Perfect Focus System and a Photometrics CoolSnap HQ2 charge-coupled device camera (Photometrics, Tucson, AZ, USA). Ca^{2+} levels were determined ratiometrically using Fura-2 AM fluorescence (emission 510 nm), following alternate excitation at 340 and 380 nm. This was acquired at a frequency of 0.25 Hz, and the relative 340nm/380nm fluorescence ratio further plotted as a function of time. Images were acquired/analyzed using NIS-Elements AR software (Nikon, Inc.).

Measurement of membrane potential

The relative changes in membrane potential of single neurons were evaluated using bis-(l,3-dibutylbarbituric acid) trimethine oxonol, $DiBAC_4(3)$ (Invitrogen), a slow response voltage-sensitive dye, as previously described [15, 16]. Upon membrane hyperpolarization, the dye concentrates in the cell membrane, leading to a decrease in fluorescence intensity, while

depolarization induces the sequestration of the dye into the cytosol, resulting in an increase of the fluorescence intensity. RBMVEC were incubated for 30 min in HBSS containing 0.5 mM $DiBAC_4(3)$ and the fluorescence monitored at 0.17 Hz, excitation/emission: 480 nm/540 nm. Calibration of $DiBAC_4(3)$ fluorescence following background subtraction was performed using the Na^+-K^+ ionophore gramicidin in Na^+-free physiological solution and various concentrations of K^+ (to alter membrane potential) and N-methylglucamine (to maintain osmolarity). Under these conditions, the membrane potential was approximately equal to the K^+ equilibrium potential determined by the Nernst equation. The intracellular K^+ and Na^+ concentration were assumed to be 130 mM and 10 mM, respectively.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). One-way ANOVA followed by post hoc analysis using Bonferroni and Tukey tests were used to evaluate significant differences between groups; $P < 0.05$ was considered statistically significant.

Results and discussion

To determine the effect of cocaine on stored-operated Ca^{2+} entry, we used a classical protocol to induce SOCE. Briefly, ER Ca^{2+} stores were depleted in Fura-2-loaded cells using the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase inhibitor thapsigargin (1 μM) in the absence of extracellular Ca^{2+} (Fig 1A). No differences in the resultant rise in cytosolic Ca^{2+} were observed in the presence or absence of cocaine (3 μM, 15 min) indicating no effect of cocaine on ER Ca^{2+} content. SOCE was then stimulated by the addition of Ca^{2+} (1 mM). Surprisingly, a ~50% decrease in SOCE was observed in cocaine-pretreated cells (Fig 1A, B).

Since cocaine is a Sig-1R agonist [12], we assessed the possibility that Sig-1R mediates cocaine-induced SOCE inhibition. Pretreatment with the selective Sig-1R antagonists BD-1063 (10 μM, 20 min) [17] or NE-100 (3 μM, 20 min) [18, 19], completely eliminated cocaine-induced SOCE inhibition (Fig 1A, B).

To exclude any bias due to potential effects of cocaine on membrane potential, we examined the effect of cocaine on membrane potential in RBMVEC loaded with a voltage-sensitive dye, $DiBAC_4(3)$ [15]. We observed no changes in membrane potential in response to cocaine application (Fig. 1C).

Finally, RBMVECs were transfected with four different Sig-1R shRNA constructs. Western analysis revealed that Sig-1R expression was decreased by 22.5 ± 2.5% (n = 3 independent experiments) after transfection with Sig-1R shRNA (B) (ATCATCTCTGGCACTTTCCACCAGTGGAG) and 28 ± 4.3% (n = 3) after transfection with Sig-1R shRNA (D) (TTGCACGCCTCGCTGTCTGAGTACGTGCT). Sig-1R shRNA (C) was less efficacious in knocking down Sig-1R, and (A) was ineffective; these constructs were not used in the subsequent functional studies. Fluorescence microscopic analysis of RFP expression revealed ~60% transfection efficiency. Since the 22.5 ± 2.5% and 28 ± 4.3% knockdowns observed by Western analysis were based on whole populations, the actual knockdowns in transfected cells (selectively measured in SOCE assay) were 45.6%

for Sig-1R shRNA (B) and 56.8% for Sig-1R shRNA (D). As depicted in Fig 2B and 2C, under these conditions, cocaine again failed to inhibit SOCE. Considered collectively, these findings reveal that cocaine inhibits SOCE in cerebral microvascular endothelial cells via Sig-1R activation.

Cocaine is well defined as a modulator of endothelial function and has been shown to increase blood-brain barrier permeability [13, 20] and facilitate transmigration of inflammatory leukocytes into the brain [21–23]. Involvement of Sig-1R in this effect has also been established [13, 14]. What was not previously recognized, however, is the possibility that these effects of cocaine and Sig-1R on endothelial permeability and activation could be Ca^{2+}-dependent. However, changes in Ca^{2+} concentration are well known to modulate a wide variety of endothelial functions [24] including barrier permeability [25], endothelial proliferation [9, 26] and expression of adhesion molecules responsible for inflammatory cell recruitment [27]. Hence, the current investigation provides a new role for SOCE modulation in cocaine- and Sig-1R-induced changes in endothelial function.

Based on the present findings, Sig-1Rs can be added to the growing list of endogenous molecules with the capacity to modulate SOCE [28]. Importantly, Sig-1Rs appear to mediate SOCE termination, the mechanisms of which have remained elusive.

Although we have not defined this mechanism in the current study, there are several possibilities ranging from interfering with STIM-Orai interaction to kinase modulation. Indeed, Sig-1R has been shown to modulate the functions of p38 MAPK [29], ERK1/2 [30] and calcineurin [31], all of which regulate STIM1 phosphorylation [32–34]. Moreover, both STIM1 [33, 35] and Orai1 [36, 37] are known to be negatively regulated by phosphorylation. Future investigations may provide further insight into the novel biochemical mechanism.

Acknowledgments

Funding information: This work was supported by the NIH grants DA035926 (to MEA), P30DA013429 (to EMU).

Abbreviations

ER	endoplasmic reticulum
RBMVEC	rat brain microvascular endothelial cells
RFP	red fluorescent protein
Sig-1R	sigma-1 receptor
SOCE	store-operated calcium entry
STIM1	stromal interaction molecule 1

References

1. Hayashi T, Rizzuto R, Hajnoczky G, Su TP. MAM: more than just a housekeeper. Trends Cell Biol. 2009; 19:81–88. [PubMed: 19144519]
2. Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell. 2007; 131:596–610. [PubMed: 17981125]
3. Su TP, Hayashi T, Maurice T, Buch S, Ruoho AE. The sigma-1 receptor chaperone as an inter-organelle signaling modulator. Trends Pharmacol Sci. 2010; 31:557–566. [PubMed: 20869780]
4. Hayashi T, Su TP. Intracellular dynamics of sigma-1 receptors (sigma(1) binding sites) in NG108-15 cells. J Pharmacol Exp Ther. 2003; 306:726–733. [PubMed: 12730356]
5. Kourrich S, Su TP, Fujimoto M, Bonci A. The sigma-1 receptor: roles in neuronal plasticity and disease. Trends Neurosci. 2012; 35:762–771. [PubMed: 23102998]
6. Tsai SY, Hayashi T, Mori T, Su TP. Sigma-1 receptor chaperones and diseases. Cent Nerv Syst Agents Med Chem. 2009; 9:184–189. [PubMed: 20021352]
7. Hogan PG, Rao A. Store-operated calcium entry: Mechanisms and modulation. Biochem Biophys Res Commun. 2015; 460:40–49. [PubMed: 25998732]
8. Redondo PC, Rosado JA. Store-operated calcium entry: unveiling the calcium handling signalplex. Int Rev Cell Mol Biol. 2015; 316:183–226. [PubMed: 25805125]
9. Abdullaev IF, Bisaillon JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. Circ Res. 2008; 103:1289–1299. [PubMed: 18845811]
10. Smyth JT, Beg AM, Wu S, Putney JW Jr, Rusan NM. Phosphoregulation of STIM1 leads to exclusion of the endoplasmic reticulum from the mitotic spindle. Curr Biol. 2012; 22:1487–1493. [PubMed: 22748319]
11. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. J Cell Biol. 2006; 174:815–825. [PubMed: 16966423]
12. Sharkey J, Glen KA, Wolfe S, Kuhar MJ. Cocaine binding at sigma receptors. Eur J Pharmacol. 1988; 149:171–174. [PubMed: 2840298]
13. Yao H, Duan M, Buch S. Cocaine-mediated induction of platelet-derived growth factor: implication for increased vascular permeability. Blood. 2011; 117:2538–2547. [PubMed: 21148086]
14. Yao H, Kim K, Duan M, Hayashi T, Guo M, Morgello S, Prat A, Wang J, Su TP, Buch S. Cocaine hijacks sigma1 receptor to initiate induction of activated leukocyte cell adhesion molecule: implication for increased monocyte adhesion and migration in the CNS. J Neurosci. 2011; 31:5942–5955. [PubMed: 21508219]
15. Altmann JB, Yan G, Meeks JF, Abood ME, Brailoiu E, Brailoiu GC. G protein-coupled estrogen receptor-mediated effects on cytosolic calcium and nanomechanics in brain microvascular endothelial cells. J Neurochem. 2015; 133:629–639. [PubMed: 25703621]
16. Barr JL, Deliu E, Brailoiu GC, Zhao P, Yan G, Abood ME, Unterwald EM, Brailoiu E. Mechanisms of activation of nucleus accumbens neurons by cocaine via sigma-1 receptor-inositol 1,4,5-trisphosphate-transient receptor potential canonical channel pathways. Cell Calcium. 2015
17. Matsumoto RR, Bowen WD, Tom MA, Vo VN, Truong DD, De Costa BR. Characterization of two novel sigma receptor ligands: antidystonic effects in rats suggest sigma receptor antagonism. Eur J Pharmacol. 1995; 280:301–310. [PubMed: 8566098]
18. Berardi F, Ferorelli S, Colabufo NA, Leopoldo M, Perrone R, Tortorella V. A multireceptorial binding reinvestigation on an extended class of sigma ligands: N-[omega-(indan-1-yl and tetralin-1-yl)alkyl] derivatives of 3,3-dimethylpiperidine reveal high affinities towards sigma1 and EBP sites. Bioorg Med Chem. 2001; 9:1325–1335. [PubMed: 11377189]
19. Chaki S, Okuyama S, Ogawa S, Tanaka M, Muramatsu M, Nakazato A, Tomisawa K. Solubilization and characterization of binding sites for [3H]NE-100, a novel and potent sigma 1 ligand, from guinea pig brain. Life Sci. 1996; 59:1331–1340. [PubMed: 8876662]
20. Dhillon NK, Peng F, Bokhari S, Callen S, Shin SH, Zhu X, Kim KJ, Buch SJ. Cocaine-mediated alteration in tight junction protein expression and modulation of CCL2/CCR2 axis across the

blood-brain barrier: implications for HIV-dementia. J Neuroimmune Pharmacol. 2008; 3:52–56. [PubMed: 18046654]

21. Fiala M, Eshleman AJ, Cashman J, Lin J, Lossinsky AS, Suarez V, Yang W, Zhang J, Popik W, Singer E, Chiappelli F, Carro E, Weinand M, Witte M, Arthos J. Cocaine increases human immunodeficiency virus type 1 neuroinvasion through remodeling brain microvascular endothelial cells. J Neurovirol. 2005; 11:281–291. [PubMed: 16036808]
22. Fiala M, Gan XH, Zhang L, House SD, Newton T, Graves MC, Shapshak P, Stins M, Kim KS, Witte M, Chang SL. Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? Adv Exp Med Biol. 1998; 437:199–205. [PubMed: 9666272]
23. Gan X, Zhang L, Berger O, Stins MF, Way D, Taub DD, Chang SL, Kim KS, House SD, Weinand M, Witte M, Graves MC, Fiala M. Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. Clin Immunol. 1999; 91:68–76. [PubMed: 10219256]
24. Tran QK, Watanabe H. Calcium signalling in the endothelium. Handb Exp Pharmacol. 2006:145–187. [PubMed: 16999219]
25. Wu S, Cioffi EA, Alvarez D, Sayner SL, Chen H, Cioffi DL, King J, Creighton JR, Townsley M, Goodman SR, Stevens T. Essential role of a Ca2+-selective, store-operated current (ISOC) in endothelial cell permeability: determinants of the vascular leak site. Circ Res. 2005; 96:856–863. [PubMed: 15790951]
26. Li J, Cubbon RM, Wilson LA, Amer MS, McKeown L, Hou B, Majeed Y, Tumova S, Seymour VA, Taylor H, Stacey M, O'Regan D, Foster R, Porter KE, Kearney MT, Beech DJ. Orai1 and CRAC channel dependence of VEGF-activated Ca2+ entry and endothelial tube formation. Circ Res. 2011; 108:1190–1198. [PubMed: 21441136]
27. Gandhirajan RK, Meng S, Chandramoorthy HC, Mallilankaraman K, Mancarella S, Gao H, Razmpour R, Yang XF, Houser SR, Chen J, Koch WJ, Wang H, Soboloff J, Gill DL, Madesh M. Blockade of N0X2 and STIM1 signaling limits lipopolysaccharide-induced vascular inflammation. J Clin Invest. 2013; 123:887–902. [PubMed: 23348743]
28. Soboloff J, Rothberg BS, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol. 2012; 13:549–565. [PubMed: 22914293]
29. Moon JY, Roh DH, Yoon SY, Kang SY, Choi SR, Kwon SG, Choi HS, Han HJ, Beitz AJ, Lee JH. Sigma-1 receptor-mediated increase in spinal p38 MAPK phosphorylation leads to the induction of mechanical allodynia in mice and neuropathic rats. Exp Neurol. 2013; 247:383–391. [PubMed: 23333567]
30. Mueller BH 2nd, Park Y, Ma HY, Dibas A, Ellis DZ, Clark AF, Yorio T. Sigma-1 receptor stimulation protects retinal ganglion cells from ischemia-like insult through the activation of extracellular-signal-regulated kinases 1/2. Exp Eye Res. 2014; 128:156–169. [PubMed: 25305575]
31. Mari Y, Katnik C, Cuevas J. sigma-1 Receptor Inhibition of ASIC1a Channels is Dependent on a Pertussis Toxin-Sensitive G-Protein and an AKAP150/Calcineurin Complex. Neurochem Res. 2014
32. Pozo-Guisado E, Casas-Rua V, Tomas-Martin P, Lopez-Guerrero AM, Alvarez-Barrientos A, Martin-Romero FJ. Phosphorylation of STIM1 at ERK1/2 target sites regulates interaction with the microtubule plus-end binding protein EB1. J Cell Sci. 2013; 126:3170–3180. [PubMed: 23687376]
33. Sundivakkam PC, Natarajan V, Malik AB, Tiruppathi C. Store-operated Ca2+ entry (SOCE) induced by protease-activated receptor-1 mediates STIM1 protein phosphorylation to inhibit SOCE in endothelial cells through AMP-activated protein kinase and p38beta mitogen-activated protein kinase. J Biol Chem. 2013; 288:17030–17041. [PubMed: 23625915]
34. Vasauskas AA, Chen H, Wu S, Cioffi DL. The senne-threonine phosphatase calcineurin is a regulator of endothelial store-operated calcium entry. Pulm Circ. 2014; 4:116–127. [PubMed: 25006427]
35. Smyth JT, Petranka JG, Boyles RR, DeHaven WI, Fukushima M, Johnson KL, Williams JG, Putney JW Jr. Phosphorylation of STIM1 underlies suppression of store-operated calcium entry during mitosis. Nat Cell Biol. 2009; 11:1465–1472. [PubMed: 19881501]

36. Kawasaki T, Ueyama T, Lange I, Feske S, Saito N. Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca2+ level via the store-operated Ca2+ channel. J Biol Chem. 2010; 285:25720–25730. [PubMed: 20534587]

37. Hooper R, Zhang X, Webster M, Go C, Kedra J, Marchbank K, Gill DL, Weeraratna AT, Trebak M, Soboloff J. Novel PKC-mediated Control of Orai1 function in Invasive Melanoma. Mol Cell Biol. 2015

Summary statement

We provide evidence that cocaine induces sigma-1 receptor-mediated inhibition of store-operated calcium entry (SOCE) in rat brain microvascular endothelial cells. Thus, we reveal sigma-1 receptors as SOCE blockers, adding novel insight regarding endothelial effects of cocaine and endogenous SOCE modulation.

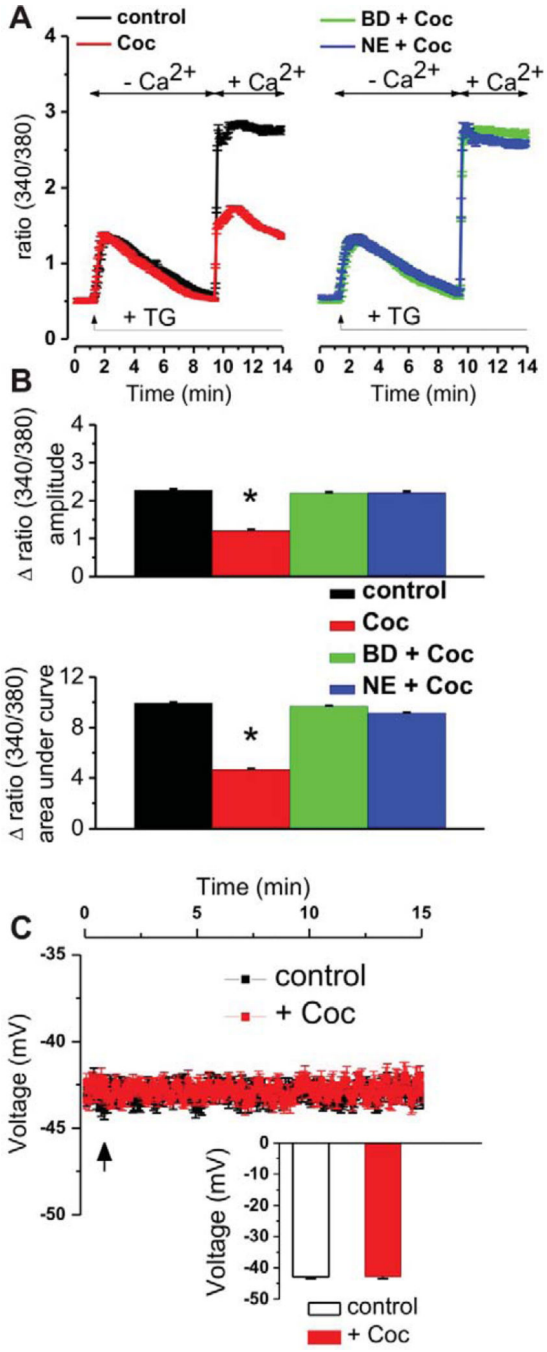

Figure 1. Cocaine inhibits SOCE in brain microvascular endothelial cells: pharmacological evidence of Sig-1R involvement

A, Averaged traces of fura-2 fluorescence ratio (F340/F380) indicating the modulation of store-operated Ca^{2+} entry by pretreatment with cocaine (Coc, 3 μM, 15 min, n = 64 cells) alone, cocaine in the presence of Sig-R antagonist BD-1063 (BD, 10 μM, 20 min, n = 68 cells) or NE-100 (NE, 3 μM, 20 min, n = 57 cells) in RBMVEC cells. Cocaine reduced the SOCE amplitude as compared with control RBMVEC (n = 61 cells); the reduction was prevented by treatment with Sig-1R antagonists. **B,** Comparison of the amplitudes and areas

under curve of SOCE elicited by cocaine in each of the conditions mentioned before. *P < 0.05 as compared with control. **C,** Cocaine does not change the resting membrane potential (RMP) of RBMVEC: averaged traces and comparison of medium RMP of cocaine-treated (n = 63 cells) and control, untreated cells (n = 47 cells); the arrow indicates the moment of cocaine application.

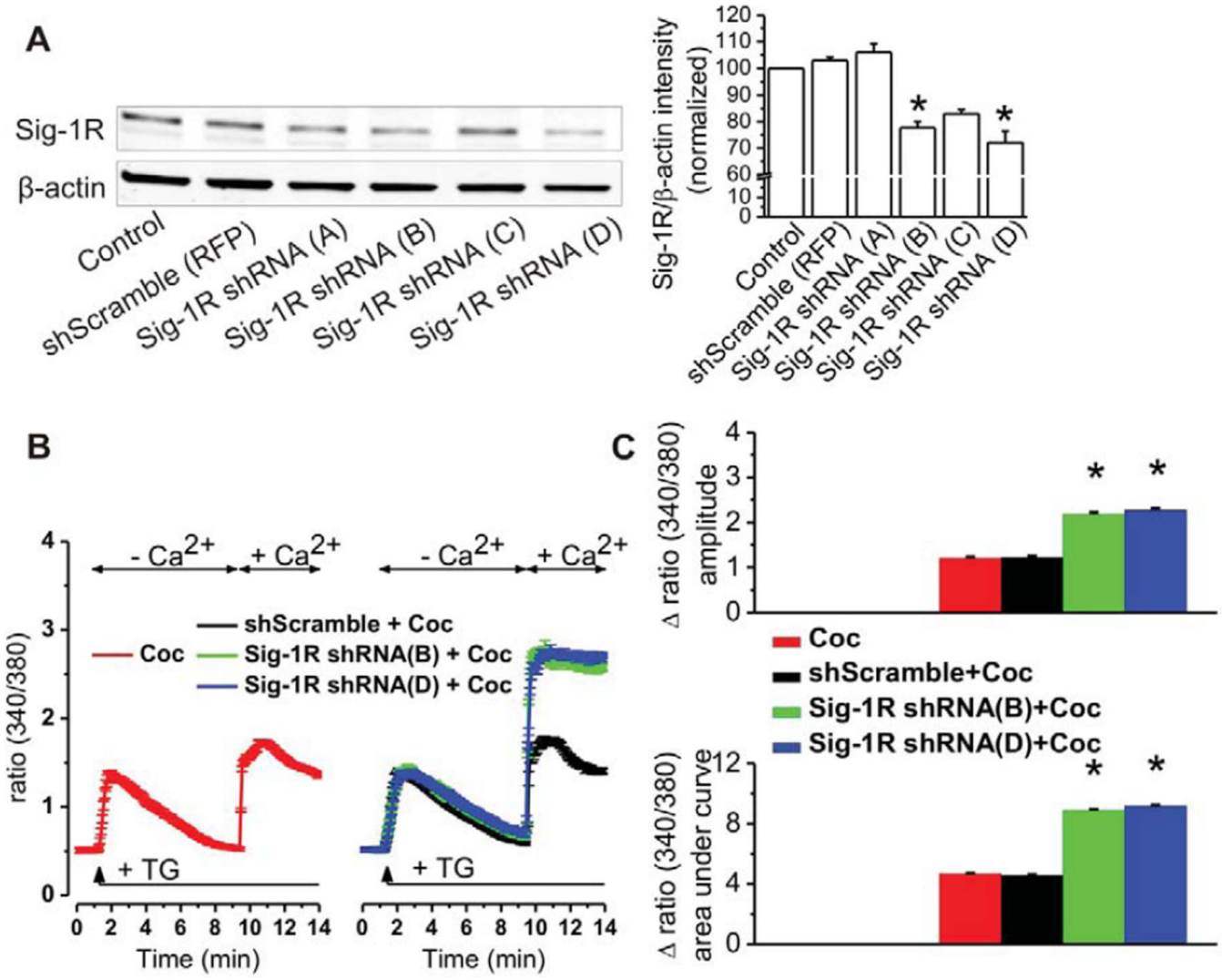

Figure 2. Knockdown of Sig-1Rs prevents cocaine-induced SOCE inhibition

A, RBMVEC express Sig-1R protein; Sig-1R protein expression is significantly reduced by transfection with Sig-1R shRNA constructs B and D, but not altered by transfection with scrambled negative control non-effective shRNA-RFP (**P*<0.05 as compared with control untransfected and with scrambled shRNA transfected conditions),. Immunoblot on the left is representative for three independent experiments; quantifications are indicated on the right. **B,** Averaged traces of fura-2 fluorescence ratio (F340/F380) revealing that SOCE inhibition by cocaine is reversed by Sig-1R knockdown using shRNA constructs B and D, but not by scrambled shRNA. **C,** Comparison of the amplitudes and areas under curve of SOCE elicited by cocaine in naive RBMVEC (n = 64 cells) or RBMVEC transfected with scrambled shRNA (n = 54 cells), Sig-1R shRNA (B) (n = 59 cells) or Sig-1R shRNA (D) (n = 62 cells); **P* < 0.05 as compared with cocaine.