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Protein synthesis inhibition promotes nitric oxide generation and activation of CGKII-dependent downstream signaling pathways in the retina

Marcelo Cossenza[a,c,*,1], Renato Socodato[a,g,1], Telmo A. Mejía-García[a,1], Ivan Domith[a], Camila C. Portugal[a,g], Luis F.H. Gladulich[a], Aline T. Duarte-Silva[b], Latika Khatri[d], Shannon Antoine[e], Franz Hofmann[f], Edward B. Ziff[d], Roberto Paes-de-Carvalho[a,b,**]

[a] Program of Neurosciences, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil
[b] Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói, RJ, Brazil
[c] Department of Physiology and Pharmacology, Biomedical Institute, Fluminense Federal University, Niterói, RJ, Brazil
[d] Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, United States
[e] Graduate Program in Neuroscience & Physiology, New York University School of Medicine, New York, NY, United States
[f] Institut für Pharmakologie und Toxikologie der TU-München, Munich, Germany
[g] Instituto de Investigação e Inovação em Saúde (i3S) and Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal

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ABSTRACT

Nitric oxide is an important neuromodulator in the CNS, and its production within neurons is modulated by NMDA receptors and requires a fine-tuned availability of L-arginine. We have previously shown that globally inhibiting protein synthesis mobilizes intracellular L-arginine "pools" in retinal neurons, which concomitantly enhances neuronal nitric oxide synthase-mediated nitric oxide production. Activation of NMDA receptors also induces local inhibition of protein synthesis and L-arginine intracellular accumulation through calcium influx and stimulation of eucariotic elongation factor type 2 kinase. We hypothesized that protein synthesis inhibition might also increase intracellular L-arginine availability to induce nitric oxide-dependent activation of downstream signaling pathways. Here we show that nitric oxide produced by inhibiting protein synthesis (using cycloheximide or anisomycin) is readily coupled to AKT activation in a soluble guanylyl cyclase and cGKII-dependent manner. Knockdown of cGKII prevents cycloheximide or anisomycin-induced AKT activation and its nuclear accumulation. Moreover, in retinas from cGKII knockout mice, cycloheximide was unable to enhance AKT phosphorylation. Indeed, cycloheximide also produces an increase of ERK phosphorylation which is abrogated by a nitric oxide synthase inhibitor. In summary, we show that inhibition of protein synthesis is a previously unanticipated driving force for nitric oxide generation and activation of downstream signaling pathways including AKT and ERK in cultured retinal cells. These results may be important for the regulation of synaptic signaling and neuronal development by NMDA receptors as well as for solving conflicting data observed when using protein synthesis inhibitors for studying neuronal survival during development as well in behavior and memory studies.

Abbreviations: nNOS, neuronal nitric oxide synthase; eEF2K, eukaryotic elongation factor 2 kinase; eEF2, eukaryotic elongation factor; CHX, cycloheximide; aniso, anisomycin; AKT/PKB, Protein kinase B; ERK, extracellular signal regulated kinases; MAP kinase, Mitogen Activated Protein Kinase; cGKII, cyclic GMP-dependent kinase type II; FBS, fetal bovine serum; MEM, minimum essential medium; DAF-FM-DA, 4-Amino-5-Methylamino-2′,7′-Difluorofluorescein Diacetate; DAPI, 4′,6-Diamidine-2′-phenylindole dihydrochloride; KT5823, Methyl (15*S*,16*R*,18*R*)-6-methoxy-4,15-dimethyl-3-oxo-28-oxa-4,14,19triazaoctacyclo [12.11.2.115,18.02,6.07,27.08,13.019,26.020,25]octacosa-1,6,8,10,12,20,22,24,26-nonaene-16-carboxylate; 7-NI, 7-Nitroindazole; NMDA, N-Methyl-D-aspartic acid; SNAP, S-Nitroso-*N*-acetyl-DL-penicillamine; ODQ, 1H-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one; L-NAME, Nω-Nitro-L-arginine methyl ester hydrochloridester; PVDF, polyvinylidene difluoride;ECL Enhanced chemiluminescence; D-AP5, (2*R*)-amino-5-phosphonovaleric acid; CREB, cAMP response element-binding protein; LTP, Long Term Potentiation; L-NNA, N^G-nitro-L-Arginine; PTIO, 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl 3-oxide

* Correspondence to: M. Cossenza, Departamento de Fisiologia e Farmacologia, Instituto Biomédico, Universidade Federal Fluminense, Instituto Biomédico, bloco E (prédio novo) - R. São João Batista, 187, 4° andar, Laboratório de Farmacologia Molecular - Centro, Niterói, RJ 24020-005, Brazil.
** Correspondence to: R. Paes-de-Carvalho, Departamento de Neurobiologia, Instituto de Biologia, Universidade Federal Fluminense, Caixa Postal 100180, Niterói, RJ 240120-971, Brazil.
E-mail addresses: mcossenza@gmail.com (M. Cossenza), robpaesuff@gmail.com (R. Paes-de-Carvalho).
[1] These authors equally contributed to this work.

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