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Modulation of the neuronal response to ischaemia by somatostatin analogues in wild-type and knock-out mouse retinas

Davide Cervia,*,† Davide Martini,*,† Chiara Ristori,† Elisabetta Catalani,* Anna Maria Timperio,* Paola Bagnoli† and Giovanni Casini*

*Department of Environmental Sciences, University of Tuscia, Largo dell'Università snc, Viterbo, Italy

†Department of Biology, Unit of General Physiology, University of Pisa, via San Zeno, Pisa, Italy

Abstract

Somatostatin acts at five G protein-coupled receptors, sst_1-sst_5. In mouse ischaemic retinas, the over-expression of sst_2 (as in sst_1 knock-out mice) results in the reduction of cell death and glutamate release. In this study, we reported that, in wild-type retinas, somatostatin, the multireceptor ligand pasireotide and the sst_2 agonist octreotide decreased ischaemia-induced cell death and that octreotide also decreased glutamate release. In contrast, cell death was increased by blocking sst_2 with cyanamide. In sst_2 over-expressing ischaemic retinas, somatostatin analogues increased cell death, and octreotide also increased glutamate release. To explain this reversal of the anti-ischaemic effect of somatostatin agonists in the presence of sst_2 over-expression, we tested sst_2 desensitisation because of internalisation or altered receptor function. We observed that (i) sst_2 was not internalised, (ii) among G protein-coupled receptor kinases (GRKs) and regulators of G protein signalling (RGSs), GRK1 and RGS1 expression increased following ischaemia, (iii) both GRK1 and RGS1 were down-regulated by octreotide in wild-type ischaemic retinas, (iv) octreotide down-regulated GRK1 but not RGS1 in sst_2 over-expressing ischaemic retinas. These results demonstrate that sst_2 activation protects against retinal ischaemia. However, in the presence of sst_2 over-expression sst_2 is functionally desensitised by agonists, possibly because of sustained RGS1 levels.

Keywords: cell death, G protein-coupled receptor kinases, glutamate release, regulators of G protein signalling, somatostatin receptors.

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The primary cause of neuronal death in retinal diseases is ischaemia, a condition that can be considered a final common pathway for injury in different retinal pathologies (Quigley *et al.* 1985, 1995; Levin and Louhab 1996; Osborne *et al.* 1999, 2004; Barber 2003). Glutamate excitotoxicity, occurring during retinal ischaemia (Osborne *et al.* 2004), is likely to be a major cause of neuronal damage following an ischaemic insult (Lipton 1999; Won *et al.* 2002; Arundine and Tymianski 2003; Camacho and Massieu 2006). The peptide somatostatin (somatotropin release-inhibiting factor, SRIF) plays important physiological roles, mostly inhibitory, which have formed the basis for the clinical use of SRIF compounds (Weckbecker *et al.* 2003; Cervia and Bagnoli 2007), and it may protect the retina against ischaemia in a variety of retinal diseases (Cervia *et al.* 2008). SRIF binds to its 5 G protein-coupled receptors (GPCRs) which have been named sst_1-sst_5 (Hoyer *et al.* 1995) and are expressed in the retina (Bagnoli *et al.* 2003; Thermos 2003; Casini *et al.* 2005; Cervia *et al.* 2008). SRIF has a protective role in the retina, as shown in studies in guinea pigs and rats where sst_2 agonists protect, to a certain extent, retinal cells from the

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Address correspondence and reprint requests to Davide Cervia, Department of Environmental Sciences, University of Tuscia, Largo dell'Università snc, blocco D, 01100 Viterbo, Italy.
E-mail: d.cervia@unitus.it

DC and DM equally contributed to the work and are listed in alphabetical order.

Abbreviations used: CT, cycle threshold; DAPI, 4′-6-diamidino-2-phenylindole; ESI-MS, electrospray tandem mass spectrometry; GCL, ganglion cell layer; GPCRs, G protein-coupled receptors; GRKs, GPCR kinases; INL, the inner nuclear layer; KO, knock-out; ONL, outer nuclear layer; PB, phosphate buffer; QPCR, real-time RT-PCR; RGSs, regulators of G protein signalling; RT, reverse transcriptase; SRIF, somatotropin release-inhibiting factor/somatostatin; sst_{1-5}, SRIF receptor 1 through 5; WT, wild-type.