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Somatostatin - Nitric Oxide Interactions in the Mouse Retina

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S u m m a r y. Previous studies in our laboratory have established the ability of somatostatin to influence the nitric oxide system in rat retina (1,2). In the present study, we examined the presence of NADPH-diaphorase (the marker of nitric oxide synthase-NOS), neuronal NOS and the possible colocalization of NADPH-diaphorase with somatostatin receptor (sst_{2A}) in mouse retina. In addition, we examined NADPH-diaphorase histostain and sst_{2A}-immunoreactivity in somatostatin deficient mice.

INTRODUCTION

The neuropeptide somatostatin is one of many neuroactive substances that influence retinal physiology by activating a number of somatostatin receptor subtypes, namely sst_1, sst_2, and sst_4 (for a review, see Thermos, 2003 (3). While the role of each sst is not known, studies in our laboratory have shown that sst_2 activation may influence the regulation of nitric oxide. Nitric oxide is believed to play a variety of roles in ocular physiology, including the maintenance of tight junction integrity, the development of immune and inflammatory responses and blood flow 4).

METHODS

Male C57BL/6J mice (wildtype, heterozygotes and SRIF-deficient mice[5] were employed. PCR studies were performed to determine the homozygosity of the SRIF -/- mice, using DNA tail biopsies and the following primers:

w/t fragment- 5'AGTTTCTGCAGAAGTCTCTGGC3',
KOfragments-5'CATAAGCGCATGCTCCAGAC3' and
common primer-5'CAGGATGTGAATGTCTTCCAG3'.

The animals (90 days old) were killed with ether inhalation and the eyecups removed. Histo- and immunohistochemical studies were performed according to (1). Rabbit polyclonal antibodies against sst_{2A} (1/1000) and bNOs (1/3000), mouse monoclonal antibodies against PKC (1/250) and MAP-1A (1/250) and rat monoclonal antibody against SRIF (1/50) were employed.

RESULTS

NADPH-diaphorase staining was localized in the photoreceptors, amacrine cells in the inner nuclear layer (INL) and displaced amacrine cells in the ganglion cell layer (GCL). Extensive NADPH-diaphorase reactivity was found in the outer nuclear layer (ONL). Colocalization of NADPH-diaphorase staining with known markers, such as protein kinase C (PKC) and microtubules associated protein-1A (MAP-1A) confirmed the presence of NADPH-diaphorase in rod bipolar and ganglion cells, respectively. In parallel, neuronal NOS immunoreactivity was examined and found in two populations of amacrine cells, type I and type II, as well as in displaced amacrine cells. NADPH-diaphorase was found to be colocalized with the sst_{2A} receptor in rod bipolar cells. SRIF immunoreactivity (I-R) was detected in amacrine cell bodies in the INL and cell processes in the inner plexiform layer (IPL) of retinas of (+/-) mice, but not in retinas of SRIF (-/) mice. NADPH-diaphorase staining was assessed in both SRIF (+/-) and (-/-) mice, but no significant differences were observed, as was the case for sst_{2A} immunoreactivity.

CONCLUSIONS

NADPH-diaphorase is localized in different retinal neurons such as the photoreceptors, rod bipolar, amacrine and ganglion cells. However, the neuronal NOS is localized only in amacrine

cells and processes of the IPL, suggesting the presence of other NOS in mouse retina. NADPH-diaphorase is colocalized with the sst_{2A} receptor suggesting that somatostatin may regulate nitric oxide function in mouse retina. The use of retinas of SRIF (-/-) mice did not afford any changes in NADPH-diaphorase staining. Possible changes may be age dependent and experiments are in progress examining NADPH-diaphorase staining during different developmental stages. No differences were observed in sst_{2A} immunoreactivity in the retinas of SRIF (-/-) mice, yet very preliminary data from radioligand autoradiography experiments support an upregulation of the sst_1 and sst_2 receptors, a finding that we have to further substantiate.

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