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BRAIN RESEARCH

Research Report

Altered pupillary light reflex in PACAP receptor 1-deficient mice

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ABSTRACT

The pupillary light reflex (PLR) is regulated by the classical photoreceptors, rods and cones, and by intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin. IpRGCs receive input from rods and cones and project to the olivary pretectal nucleus (OPN), which is the primary visual center involved in PLR. Mice lacking either the classical photoreceptors or melanopsin exhibit some changes in PLR, whereas the reflex is completely lost in mice deficient of all three photoreceptors. The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is co-stored with melanopsin in ipRGCs and mediates light signaling to the brain via the specific PACAP receptor 1 (PAC1R). Here, we examined the occurrence of PACAP and PAC1R in the mouse OPN, and studied if lack of PAC1R affected the PLR. PACAP-immunoreactive nerve fibers were shown in the mouse OPN, and by *in situ* hybridization histochemistry, we demonstrated the presence of PAC1R mRNA. Mice lacking PAC1R exhibited a significantly attenuated PLR compared to wild type mice upon light stimulation, and the difference became more pronounced as light intensity was increased. Our findings accord well with observations of the PLR in the melanopsin-deficient mouse. We conclude that PACAP/PAC1R signaling is involved in the sustained phase of the PLR at high irradiances.

1. Introduction

A subgroup of ganglion cells of the inner retina is photosensitive due to their expression of the photopigment melanopsin (Berson et al., 2002; Hattar et al., 2002). These intrinsically photosensitive retinal ganglion cells (ipRGCs) are involved in the regulation of a number of so called non-image-forming (NIF) functions including light entrainment of circadian rhythms,

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Abbreviations: 3V, 3rd ventricle; APT, anterior pretectal nucleus; AUC, area under curve; BW, body weight; CG, ciliary ganglion; CtB, choleratoxin subunit B; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; EW, edinger westphal nucleus; FITC, fluorescence isothiocyanate; GCL, ganglion cell layer; IGL, intergeniculate leaflet; INL, inner nuclear layer; IPL, inner plexiform layer; ipRGC, intrinsically photosensitive retinal ganglion cells; -ir, -immunoreactive; KO, knock-out; NIF, non-image-forming; ON, over night; ONL, outer nuclear layer; OPL, outer plexiform layer; OPN, olivary pretectal nucleus; PACAP, pituitary adenylate cyclase-activating polypeptide; PAC1R, PACAP receptor 1; PBS, phosphate buffered saline; pc, posterior commissure; PLR, pupillary light reflex; PPT, posterior pretectal nucleus; rd/rd cl, rodless coneless; RHT, retinohypothalamic tract; RPE, retinal pigment epithelium; SCN, suprachiasmatic nucleus; SSC, saline sodium citrate; VLG, ventral lateral geniculate nucleus

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negative masking in nocturnal rodents and the pupillary light reflex (PLR; Hattar et al., 2003; Lucas et al., 2003; Panda et al., 2003). IpRGCs send monosynaptic projections known as the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN), the intergeniculate leaflet (IGL) and the olivary pretectal nucleus (OPN), brain sites that process these ocular, yet non-visual, light responses (Gooley et al., 2003; Hannibal and Fahrenkrug, 2004; Hattar et al., 2006).

Transgenic mice lacking melanopsin and the classical photoreceptors, rods and cones, exhibit a complete loss of circadian rhythms, negative masking behavior and PLR, suggesting that the three photoreceptors together account for all NIF functions (Hattar et al., 2003; Panda et al., 2003). Recently, it has been demonstrated that rods and cones act solely through ipRGCs, as mice degenerate of ipRGCs also suffer from a complete loss of NIF functions (Goz et al., 2008; Guler et al., 2008; Hatori et al., 2008). In mice deficient in either melanopsin or rods and cones, small changes in phase delay, negative masking and PLR have been reported, but overlapping roles of the photoreceptors seem to exist (Foster et al., 1991; Lucas et al., 2001, 2003; Panda et al., 2002; Ruby et al., 2002; Semo et al., 2003; Yoshimura et al., 1994).

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is exclusively co-stored with melanopsin in ipRGCs (Hannibal et al., 2002) and localized in retinal nerve terminals in many brain areas including the SCN, IGL and OPN (Hannibal and Fahrenkrug, 2004). PACAP together with glutamate mediates light signaling from ipRGCs to the brain (Hannibal, 2002a) in a complex and not completely understood interplay involving glutamate receptors and the specific PACAP receptor 1 (PAC1R; Chen et al., 1999; Engelund et al., 2010; Hannibal, 2006; Hannibal et al., 2000). Studies on the SCN in brain slice cultures have demonstrated how PACAP modulates glutamatergic signaling in a time- and concentration-dependent manner (Chen et al., 1999; Harrington et al., 1999). Further, transgenic mice lacking PACAP or PAC1R exhibit impairment in their ability to phase shift and mask, and demonstrate a shorter endogenous rhythm (tau) in dark/dark cycles (Colwell et al., 2004; Hannibal et al., 2001, 2008; Kawaguchi et al., 2003, 2010). In the present study, we used PAC1R-deficient mice to test the hypothesis that PACAP signaling is involved in the PLR in mice as has been demonstrated in other NIF functions.

2. Results

2.1. *Retinal morphology*

We examined whether there were any changes in the retinal morphology of PAC1R-deficient mice. Hematoxylin stained retinas from PAC1R$^{+/+}$ and PAC1R$^{-/-}$ revealed that the mor-

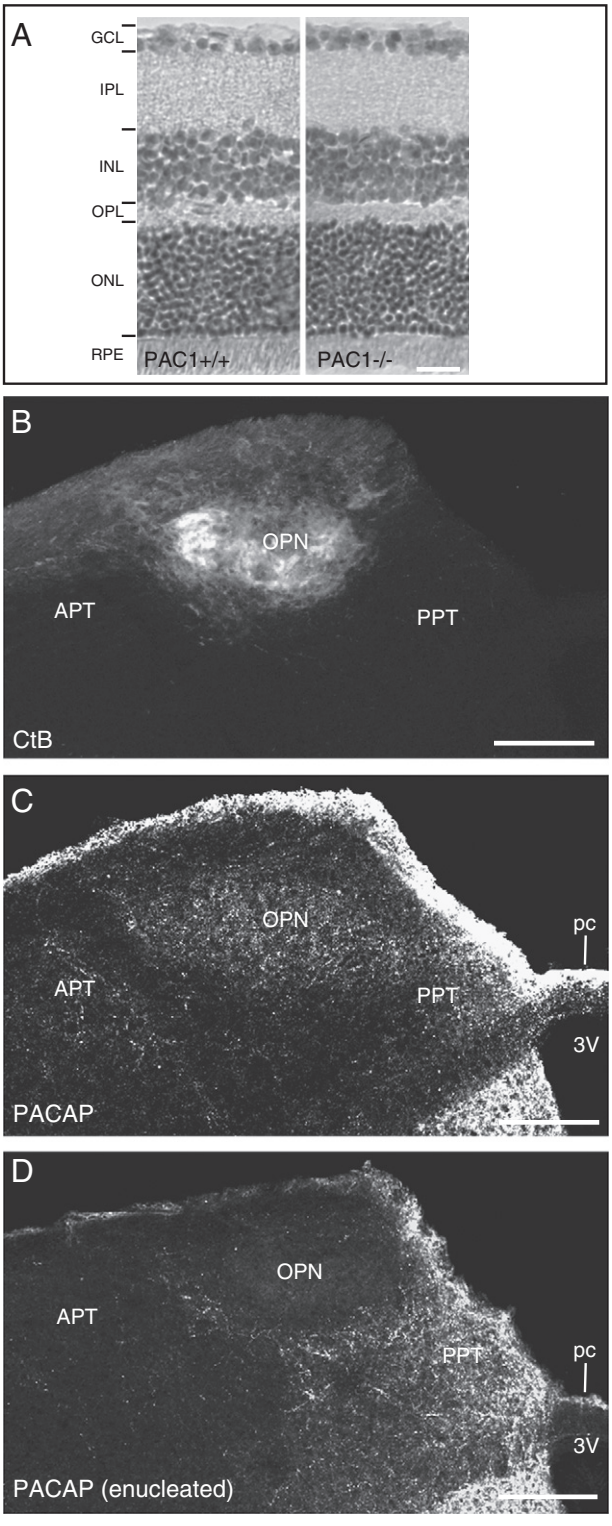

Fig. 1 – A: Retinal morphology of PAC1R$^{-/-}$ and PAC1R$^{+/+}$ mice visualized by hematoxylin staining. All retinal layers were identical in the two genotypes. B–D: Confocal photomicrographs of coronal sections through the mediorostral part of the OPN. (B) Visualization of the OPN in wild type mouse by the use of bilateral injections with the anterograde tracer CtB conjugated to Alexa Fluor 594. (C) Immunostaining of the same sections for PACAP. Note the PACAP-positive fibers evenly distributed in the core and shell of the OPN. (D) Immunostaining for PACAP using brain from bilaterally eye enucleated wild type mouse. Note the complete absence of PACAP-ir fibers in the OPN. For abbreviations, see list. *Scale bars:* A; 25 μM,B–D; 200 μM.