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Research Report

Localization of protein kinase C isoforms in the optic pathway of mouse embryos and their role in axon routing at the optic chiasm

Liqing Wang[a,b], *Joyce Shi-Ying Lam*[a], *Hui Zhao*[a], *Jun Wang*[c], *Sun-On Chan*[a,*]

[a]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China*
[b]*Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China*
[c]*Department of Anatomy and Embryology, School of Basic Medical Sciences, Peking University, Beijing, China*

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ABSTRACT

Protein kinase C (PKC) plays a key role in many receptor-mediated signaling pathways that regulate cell growth and development. However, its roles in guiding axon growth and guidance in developing neural pathways are largely unknown. To investigate possible functions of PKC in the growth and guidance of axons in the optic chiasm, we first determined the localization of major PKC isoforms in the retinofugal pathway of mouse embryos, at the stage when axons navigate through the midline. Results showed that PKC was expressed in isoform specific patterns in the pathway. PKC-α immunoreactivity was detected in the chiasm and the optic tract. PKC-βII was strong in the optic stalk but was attenuated on axons in the diencephalon. Immunostaining for PKC-ε showed a colocalization in the chiasmatic neurons that express a surface antigen stage specific embryonic antigen-1 (SSEA-1). These chiasmatic neurons straddled the midline of the optic chiasm, and have been shown in earlier studies a role in regulation of axon growth and guidance. Expression levels of PKC-βI, -δ and -γ were barely detectable in the pathway. Blocking of PKC signaling with Ro-32-0432, an inhibitor specific for PKC-α and -β at nanomolar concentration, produced a dramatic reduction of ipsilateral axons from both nasal retina and temporal crescent. We conclude from these studies that PKC-α and -βII are the predominant forms in the developing optic pathway, whereas PKC-ε is the major form in the chiasmatic neurons. Furthermore, PKC-α and -βII are likely involved in signaling pathways triggered by inhibitory molecules at the midline that guide optic axons to the uncrossed pathway.

1. Introduction

Mechanisms that guide retinal ganglion cell (RGC) axons to distal targets in the diencephalon and the midbrain have long been a central issue in understanding development of the visual pathway. In mouse embryos, RGC axons navigate through the optic stalk and the chiasm at ventral midline of the diencephalon. Within this structure, axons from

*Corresponding author. Fax: +852 39436898.
E-mail addresses: merrysums@gmail.com (L. Wang), joyce.lam@cuhk.edu.hk (J.-Y. Lam), zhaohui@cuhk.edu.hk (H. Zhao), wangjun74008@bjmu.edu.cn (J. Wang), sunonchan@cuhk.edu.hk (S.-O. Chan).

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peripheral parts of the ventrotemporal retina (or temporal crescent) are deflected from the midline and grow into the optic tract on the same side, whereas axons from the rest (or nasal) of the retina decussate to the contralateral optic tract (Colello and Guillery, 1990; Godement et al., 1987, 1990) (Fig. 1), establishing a bilaterally projecting pathway that is essential for binocular vision in mammals.

Previous findings in the mouse have shown that this axon routing pattern is controlled by inhibitory molecules expressed on some early generated neurons and radial glial cells in the optic chiasm. One example is chondroitin sulfate proteoglycans (Chung et al., 2000a, 2000b), which is expressed on the chiasmatic neurons at the midline and in regions caudal to the chiasm. These neurons are identified by the antiserum against the stage specific embryonic antigen-1 (SSEA-1) (Lin et al., 2005; Marcus and Mason, 1995). Another molecule is ephrin-B2. It is expressed on the radial glia at the chiasmatic midline and interacts with its receptor EphB1 on the axons originating from the ventrotemporal retina (Williams et al., 2003b). In a recent report we have shown that an axon growth inhibiting molecule, Nogo, is also expressed on the radial glia in the chiasm, which may affect bilateral routing of axons through regulated expression of its receptor on the optic axons (Wang et al., 2008a, 2008b).

How these inhibitory molecules affect growth cone behaviors at the midline are unclear. Studies in rat cerebellar granule neurons have shown that neurite outgrowth inhibition induced by chondroitin sulfate and Nogo is mediated by an enzyme protein kinase C (PKC) (Sivasankaran et al., 2004), suggesting a potential role in routing processes at the chiasmatic midline. PKC is a heterogeneous family of phospholipid-dependent serine/threonine protein kinases that are widely expressed in many tissues (Battaini, 2001; Jaken, 1996). At least 11 isoforms of PKC were described, based on their structure, cofactor and substrate requirements (Ohno and Nishizuka, 2002; Powell et al., 2001). The conventional or classical group (α, βI, βII, γ) is functionally regulated by calcium and requires the second messenger diacylglycerol and phorbol ester as cofactors. Functions of the novel class (δ,ε,η,θ,μ) are not mediated by calcium binding and need only diacylglycerol and phorbol ester for activation. The atypical group (ζ,λ) is insensitive to calcium, phorbol ester or diacylglycerol (Jaken, 1996; Powell et al., 2001).

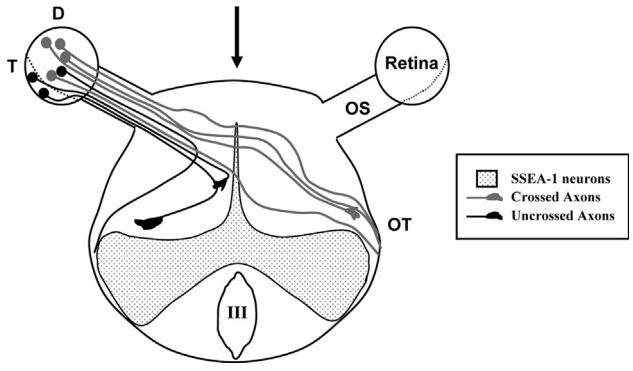

Fig. 1 – Diagram showing the routing of retinal axons in the mouse embryos. The anterior was the top and the midline was indicated by the arrow. Axons from the ganglion cells in the central or nasal parts of the retina crossed the ventral midline and formed contralateral projections; whereas uncrossed axons came largely from cells in the ventral temporal crescent (central border marked by the dotted line in the retina) were deviated from the chiasm and formed ipsilateral projections. The midline raphe (dotted area) served as a barrier for the growth of uncrossed axons. D, dorsal; T, temporal; OS, optic stalk; OT, optic tract; III, the third ventricle.

In the brain, activation of PKC is related to the control of many long term and short term functions, for example, ion channel regulation, receptor modulation, neurotransmitter release and synaptic potentiation and depression (Battaini, 2001). Suppression of its activity also affects neurite growth and regeneration (Bixby, 1989; Heacock and Agranoff, 1997; Sivasankaran et al., 2004). In explant culture of the retina, we have characterized the types of PKC isoforms in retinal growth cones and shown that suppression of PKC activity can abolish the chondroitin sulfate induced inhibition to neurite growth from E14 mouse retina (Lam et al., 2008). However, the pattern of PKC expression and the role in mediating axon routing in the optic pathway are undetermined. To further investigate possible functions of PKC in axon growth, we characterized the expression pattern of six PKC isoforms (α, βI, βII, δ, ε and γ) in the optic pathway of E14 mouse embryos, when retinal axons are navigating through the chiasm and the optic tract. These isoforms have been shown to express in rodent optic pathways (Kosaka et al., 1998; Wood and Osborne, 1997). Furthermore, the effect of blocking PKC function on development of uncrossed pathway in E16 embryos was investigated, at the time when the major population of uncrossed axons from peripheral regions of the ventral temporal retina navigates the chiasm and optic tract.

2. Results

2.1. *Expression pattern of PKCs in the retina and the optic stalk*

At E14 when most RGC axons have already grown into the chiasm and the optic tract (Chan and Chung, 1999; Colello and Guillery, 1990; Godement et al., 1987), immunohistochemistry revealed prominent expression of α ($n=6$ embryos), βI ($n=2$), βII ($n=4$) and ε ($n=4$) isoforms of PKC in the retina (Fig. 2A–G). Staining for γ ($n=3$, Fig. 2H) and δ ($n=2$) isoforms (not shown) was barely detectable. Strong labeling was also detected in the lens. Within the retina, the PKC-like immunoreactivity was most prominent in the inner regions corresponding to the ganglion cell layer. This pattern was particularly prominent for the PKC-α, -βI and -ε (Fig. 2A, C, and F), but less obvious for the -βII isoform (Fig. 2E). Detailed analyses of the staining showed a localization of βI in the ganglion cells and their axons (Fig. 2D); whereas the labels for PKC-ε were confined to the cells in the ganglion cell layer and were weak in the optic fiber layer (Fig. 2 G). Staining for PKC-α was largely found in cells that appear to migrate from the ventricular to the ganglion cell layer (Fig. 2B), and was also strong in the optic fiber layer and the optic disc.

In the optic stalk, intense staining was observed for PKC-α, -βI and -βII (Fig. 3 A-C). Among these PKC-βII appears to have an