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L-DOPA supply to the neuro retina activates dopaminergic communication at the early stages of embryonic development

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Abstract

DOPA decarboxylase (DDC; aromatic-L-amino acid decarboxylase; EC 4.1.1.28) is absent in retinas from 6-day-old chicken embryos (E6) but is expressed in retina of E8 embryos, in the presumptive outer plexiform layer. Thereafter, DDC appears in cell bodies of presumptive amacrine cells. The dopamine (DA) content of E9/10 and E15/16 retinas, pre-incubated with L-DOPA for 1 h, increased 250- and 600-fold, respectively, showing that DDC is active since early in development. Intercellular communication, measured by endogenous cyclic AMP accumulation, was observed when retinas from E9/10 to E15/16 were pre-incubated for 1 h with 1 mM L-DOPA, washed and followed by incubation in the presence of 0.5 mM 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor. Cyclic AMP accumulation was prevented when pre-incubation with L-DOPA was carried out in the presence of carbidopa. Moreover, the accumulation of cyclic AMP was inhibited by SCH 23390 (2 μM). The incubation of retinas in medium previously conditioned by retina-pigmented epithelium (RPE) also increased its cyclic AMP content with the characteristics described for L-DOPA. Our results show that dopaminergic communication takes place in the embryonic retina, before tyrosine hydroxylase expression, provided L-DOPA is supplied to the tissue. It also shows that RPE is a potential source of L-DOPA early in development.

Keywords: DOPA decarboxylase, dopamine communication, embryonic retina.

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Dopamine (DA) is the main catecholamine in the retina of most species and mediates its action via the activation of D_1- and D_2-like receptors (Schorderet and Nowak 1990). In the embryonic retina these receptors seem to modulate embryological functions associated with several stages of the tissue differentiation. Cultured retina neurons respond to DA by reducing filopodia movements followed by neurite retraction (Lankford *et al*. 1988; Lankford *et al*. 1987). The regulation of dendrite growth is mediated by increased cAMP production via the activation of D_{1A} receptors, the predominant form of the receptor expressed early in development (Soares *et al*. 2000). Similarly, early postnatal rat retina display cAMP accumulation in response to DA. In this tissue, several dopaminergic agonists of the D_1-like receptors inhibit apoptosis in the neuroblastic layer of the undifferentiated tissue (Varella *et al*. 1999). Recently, we have also shown that DA limits the number, as well as the extent of neurite complexity of dopaminergic amacrine cells in the developing retina (Guimarães *et al*. 2001).

In the embryonic chick retina, the capacity of DA to stimulate cAMP production is already detected in the tissue obtained from embryos on the seventh day of incubation (E7) (de Mello 1978), far before the onset of dopaminergic synapses and the expression of tyrosine hydroxylase (TH), the rate limiting enzyme for DA synthesis, that occurs only after E12 (Gardino *et al.* 1993). Therefore, although

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Abbreviations used: BME, basal medium of Eagle; BSA, bovine serum albumin; CM, conditioned medium; DA, dopamine; DAT, dopamine transporter; DCC, DOPA decarboxylase; HBH, *m*-hydoxybenzylhydrazine·HCl; HRP, horseradish peroxidase; IBMX, 3-isobutyl-1-methylxanthine; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RPE, retina-pigmented epithelium; TCA, trichloroacetic acid; TH, tyrosine hydroxylase.

