Day/night variations of dopamine ocular content during Xenopus laevis ontogeny


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Abstract

Concentration of dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid is quantified by high-pressure liquid chromatography with a coulometric detection system in the eye of Xenopus laevis through ontogeny and in adults at two times during photocycle (midday and midnight). Ocular dopaminergic activity remains low during pre- and prometamorphosis and significantly rises in postmetamorphic froglets. This increase is more pronounced at midnight than at midday. The dualism of DA content versus DA release in Xenopus ocular tissue is studied in an eyecup culture system. On a 24-h cycle of DA release from adult Xenopus eyecups the highest DA release by eyecups is produced during daytime, and significantly decreases in darkness. From these results it can be concluded that in spite of the early development of the retinal dopaminergic system in the ontogeny of Xenopus, the final maturation must occur during the metamorphic climax. Endogenous DA release is significantly inhibited by light offset, which explains the higher ocular DA content found at midnight as compared to midday in postmetamorphic froglets and adults. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Dopamine (DA) is the main catecholamine in the vertebrate retina where, in addition to its usual role as a synaptic transmitter, it acts as a neuromodulator for light adaptation. Thus, DA induces retinomotor movements in cones and retinal pigment epithelium [12], reduces horizontal cell coupling [18] and regulates melatonin synthesis [7]. Previous reports suggest that DA synthesis in the retina is regulated by the lighting cycle. Light onset enhances the activity of the tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, in the retina of several vertebrates [19]. In amphibians, light exposure stimulates TH activity and DA synthesis [6] and DA release and turnover from both eyecup preparations [2] and retina [20] of adult Xenopus. However, day/night changes in ocular DA content in vivo has not yet been investigated.

Neurohistological techniques have been used to study developmental aspects of the dopaminergic system in the vertebrate retina. The retinal differentiation of dopaminergic cells in amphibians has been reported using immunohistochemical techniques with antibodies against TH and DA in the tiger salamander [15], Bufo marinus [21], Xenopus laevis [4,5] and Rana pipiens [8]. Autoradiographic studies have shown the time of DA synthesis and uptake during retinal histogenesis in Xenopus [13], but a quantitative analysis of ocular DA content has not been conducted through amphibian ontogeny.

The present study investigates the ocular content of DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) through Xenopus ontogeny and in comparison to adults. Moreover, the dualism of DA content versus DA release in Xenopus ocular tissue is studied by means of an eyecups culture system, where a 24-h cycle of DA release has been analysed.

Adult Xenopus laevis breeding pairs were induced to mate, and tadpoles obtained in the laboratory were housed in aquaria with dechlorinated water on a 12:12 h light/dark photoperiod at constant water temperature of 20 ± 1°C. Tadpoles were fed boiled spinach and postmetamorphic froglets and adult frogs were fed chicken liver. Tadpoles were staged according to Nieuwkoop and Faber [11]. Animals were decapitated at midday and midnight (n = 5–8/stage of development) during the 12L:12D photoperiod.
cycle. The eyes were pooled due to the very small size of tadpoles (4–25 mg bw). Thus, 10–12 eyes/sample were used during premetamorphosis (stages 44 and 49), 3–4 eyes/samp ple during prometamorphosis (stages 53 and 59), 2 eyes/sample for postmetamorphic froglets (stage 66) and 2 retinas/sample for adult frogs. Eyes were removed and immediately stored frozen at −80°C until DA and DOPAC quantification. The catecholamine content was quantified by high-pressure liquid chromatography (HPLC) with coulometric detection as previously described [3]. Perchloric acid (PCA)-precipitated pellets were used for protein determination by the Lowry method [10].

For the eyecup culture the eyes from adult Xenopus (n = 7) were removed, the cornea and the lens separated and the eyecups with attached retinas cultured as previously described [14]. Culture time was 24 h, and the culture medium was collected and replaced by fresh medium at 3 h intervals. Samples were stored frozen until DA and DOPAC quantification.

Data were expressed as mean ± SEM and statistical analysis were carried out by using an analysis of variance (ANOVA) followed by the Duncan range test. Differences were considered significant at P < 0.05.

Fig. 1 shows the ocular content of DA, DOPAC, and the DOPAC/DA ratio throughout Xenopus ontogeny and in adults. Ocular DA throughout pre- and prometamorphosis ranged between 3 and 5 pmol/mg prot, and there were no significant differences among different stages or between the two phases of photocycle. The significant increase in ocular DA content in postmetamorphic froglets and in adults, in comparison to previous developmental stages, was much more pronounced at midnight than at midday (P < 0.01). A day/night difference was observed in ocular DOPAC content during premetamorphosis and in adults, with the nocturnal values being significantly higher than diurnal ones. The DOPAC/DA ratio was also significantly higher at midnight than at midday during premetamorphosis and in adult frogs. The highest DA turnover was observed in adults, where both diurnal and nocturnal values showed around a four-fold increase in relation to the respective values during ontogeny. Fig. 2 represents a 24-h cycle of DA release from adult Xenopus eyecups. Darkness exposure of Xenopus eyecups evoked a significant reduction in DA

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**Fig. 1.** Day/night variation of DA (upper) and DOPAC (middle) contents, and DOPAC/DA ratio (bottom) in the Xenopus laevis retina throughout ontogeny and in adults. Data are expressed as mean ± SEM. *P < 0.05, **P < 0.01 differences between midday and midnight values, *P < 0.05, **P < 0.01 differences among stages of development.

**Fig. 2.** A 24-h pattern of dopamine release into culture medium by adult Xenopus laevis eyecups. Black bar indicate the culture time in darkness. Data are expressed as mean ± SEM. *P < 0.05 differences versus light-exposed retinas and the first time interval of dark-exposure.
release from eyecups, reaching the lowest values at midnight.

The present quantitative analysis of ocular DA and DOPAC content contributes to establish the activity of the dopaminergic system during ontogeny. Our data confirm previous neurohistological reports on the early development of the dopaminergic system in the *Xenopus* retina [4]. Dopaminergic cells in the clawed frog retina have been described by autoradiography as early as stage 35/36 [13]. In recent studies, dopaminergic amacrine cells were detected in the *Xenopus* retina from stage 41 [5]. The first stage of development at which we have quantified ocular DA content is stage 44, coincident with the first appearance of DA uptake mechanisms in the *Xenopus* retina [13]. In spite of the fact that the number of immunoreactive amacrine cells increases from stage 53 to adult, the rise in the retinal area is much more pronounced through this period, and thus, cell density in the *Xenopus* retina decreases in adults with respect to tadpoles [21]. In our study DA and DOPAC ocular content remain unchanged during pre- and prometamorphosis, but dopaminergic activity exhibits a notable increase after metamorphic climax and in adults. These results agree with the immunocytochemical studies showing a very similar basic pattern of ocular dopaminergic organisation in juvenile stages and adults. Moreover, our data suggest that some additional process must be occurring during climax in order to reach the final maturation of dopaminergic system in the juvenile retina.

The lack of day/night variation in ocular DA content during pre- and prometamorphosis would not support the role of DA as a light-adaptive neuromodulator in the retina of embryos and tadpoles. This could be due to an incomplete differentiation of light-evoked regulation of DA synthesis and release, which would be developed during the metamorphic climax. In fact, the occurrence of cellular types and/or the synthesis of neuromodulators are not necessarily correlated to the onset of the function. To date, there are no data related to the emergence of DA functions in the amphibian ontogeny. Sarthy and co-workers [13] reported that synthesis, uptake and release mechanisms for DA during retinal differentiation in *Xenopus* emerge in an orderly sequence, synthesis appears to precede the development of the high-affinity uptake system which is followed later by the appearance of the DA release mechanism. This report supports our results on basal levels in ocular DA content during pre- and prometamorphosis until the complete maturation of the DA release mechanism at the metamorphic climax.

It has been extensively reported that the lighting cycle regulates ocular DA production [19], although some controversy regarding the retinal elements involved remains. Light increases DA synthesis and release in *Xenopus* retina [2,18]. Moreover, it appears that DA synthesis and turnover may be separately regulated [20]. Some reports have suggested that it is the transition from dark to light, rather than steady light, which stimulates DA release [9,17]. The onset of darkness also decreases the overflow of DA from carp retina [16]. From our results it is clear that endogenous DA release is significantly inhibited by light offset, which supports the higher DA accumulation in the retina found at midnight in postmetamorphic froglets and adults. The apparent time lag observed in DA release inhibition by light offset (Fig. 2) is explained by two facts: the inertia of this static culture system versus a superfusion system and the very slow turnover of DA in the eye of *Xenopus* [20]. Thus, we suggest that over a 24-h period of DA synthesis in the retina, the catecholamine would be preferentially stored during the scotophase and the light onset would stimulate DA release, which would explain lower ocular DA content at midday as compared to midnight in frogs. In effect, previous studies from Boatright and co-workers [2] show that although light exposure stimulates ocular DA release, this does not significantly change ocular DA concentrations. We suggest that the high DOPAC content at midnight could be explained by a switch on DA destination, the release during daytime versus transformation into DOPAC during night-time. This hypothesis is supported by the inhibition of DA release during darkness (present results), which would produce the replenishment of DA intracellular stores. In fact, very early data by Baker and co-workers [1] described higher ocular monoamine oxidase activity in embryos of *Xenopus* reared in light-surfaced aquaria. Considering that the transformation of DA to DOPAC occurs mainly in dopaminergic amacrine cells [20], the lower DOPAC/DA ratio found in the present study at midday could also be explained by DA diffusion away from release (and reuptake) sites during the daytime. Finally, it cannot be ruled out that the DOPAC increase may result from increased presynaptic DA reuptake, secondary to DA release, which would allow more DA to be oxidised to DOPAC. The cellular events underlying the effects of light on the differential control of DA synthesis and release in the retina are currently being investigated in our laboratory.

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