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Circadian variations of prostaglandin E2 and F2 α release in the golden hamster retina

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Abstract

Circadian variations of prostaglandin E2 and F2α release were examined in the golden hamster retina. Both parameters showed significant diurnal variations with maximal values at midnight. When hamsters were placed under constant darkness for 48 h, the differences in prostaglandin release between subjective mid-day and subjective midnight persisted. Western blot analysis showed that cyclooxygenase (COX)-1 levels were significantly higher at midnight than at mid-day, and at subjective midnight than at subjective mid-day, whereas no changes in COX-2 levels were observed among these time points. Immunohistochemical studies indicated the presence of COX-1 and COX-2 in the inner (but not outer) retina. Circadian variations of retinal prostaglandin release were also assessed in suprachiasmatic nuclei (SCN)-lesioned animals. Significant differences in retinal prostaglandin release between subjective mid-day and subjective midnight were observed in SCN-lesioned animals. These results indicate that hamster retinal prostaglandin release is regulated by a retinal circadian clock independent from the SCN. Thus, the present results suggest that the prostaglandin/COX-1 system could be a retinal clock output or part of the retinal clock mechanism.

Keywords: circadian rhythms, cyclooxygenases, prostaglandins, retina.

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Prostaglandins (PGs) are biologically active arachidonic acid-derived lipids of the eicosanoid family, together with leukotrienes and thromboxanes. PGs are ubiquitously synthesized and participate in the regulation of a wide variety of physiological systems, including the central nervous system, the cardiovascular system and the immune system. Through paracrine or autocrine mechanisms, PGs regulate autonomic activity, algesia and fever, as well as the cross-talk between glia and neurons, among many other processes (Friedman *et al.* 1978; Bilak *et al.* 2004). In the eye, PGE2 causes miosis, vasodilatation, disruption of the blood–aqueous barrier (Bhattacherjee and Hammond 1975; Sternschantz *et al.* 1989) and, depending on the dose, it raises or lowers intraocular pressure (Bito 1989; Alm and Villumsen 1989). PGF2α reduces intraocular pressure, possibly by increasing the uveoscleral outflow (Alm and Villumsen 1989). PGs synthesis from arachidonic acid is catalyzed by cyclooxygenases (COX). Two microsomal isoenzymes (COX-1 and COX-2) differing in their structure and regulatory mechanisms have been described. COX-1 is constitutive, ubiquitous and responsible for the synthesis of PGs that participate in cellular homeostasis control. COX-2 is almost undetectable in basal conditions, but it is induced by a wide range of stimuli, mostly inflammatory and oxidative stress (Morita 2002). However, more recent studies indicate that both COX isoforms could be constitutively expressed, particularly in the central nervous system (Maihöfner *et al.* 2000).

The mammalian retina contains relatively large amounts of arachidonic acid esterified to membrane phospholipids. The

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Abbreviations used: COX, cyclooxygenases; GCL, ganglion cell layer; INL, inner nuclear layer; PGs, Prostaglandins; SCN, suprachiasmatic nuclei.

release of arachidonic acid occurs in the retina under various experimental conditions, e.g. anoxia, high K^+ depolarization (Birkle and Bazan 1984a) and light exposure (Dentchev *et al.* 2007). COX activity is present in the retina (reviewed by Bazan 1989), and the human and bovine retina can synthesize prostaglandins E2, F2α, I2, D2 and thromboxane A2 from [^{14}C]-arachidonic acid (Kulkarni 1991). However, the available information regarding these autacoids at retinal level is still fragmentary and more pharmacologically than physiologically oriented. In that sense, the involvement of PGs in cystoid macular edema (Milch and Yannuzzi 1987) and diabetic retinopathy (Du *et al.* 2004) has been postulated. In physiological conditions, local PGs can act as modulators of synaptic responses, regulating the release of dopamine (al-Zadjali *et al.* 1994) and aspartate (LeDay *et al.* 2004), as well as the electroretinographic response (Siminoff and Bito 1981–1982).

Circadian clocks are self-sustaining genetically based molecular clocks that impose approximately 24-h rhythmicity on physiology and behavior, synchronizing biological functions with the day/night cycle. Although the master circadian pacemaker is located in the suprachiasmatic nuclei (SCN), many peripheral tissues also contain independent or quasi-independent circadian oscillators. Several lines of evidence strongly support the presence of an autonomous circadian clock in the retina (Tosini and Menaker 1996; Iuvone *et al.* 2005), which regulates a variety of cellular, biochemical and physiological processes, such as expression of immediate-early genes, activities of enzymes involved in signal transduction pathways, rod outer segment disc shedding, phagocytosis by retinal pigment epithelium and biosynthesis of melatonin (reviewed by Tosini and Fukuhara 2002). Retinal circadian clocks are also involved in daily modulations of visual sensitivity and the electroretinographic response (Lu *et al.* 1995). However, the localization of the intraretinal clock(s) as well as *in vivo* or *in vitro* clock-resetting factors that synchronize retinal oscillators have not been fully clarified.

Period 1 (Per 1) is a core molecular component of circadian clocks and its expression is necessary for circadian rhythmicity (for a review see Reppert and Weaver 2002). Recently, it has been demonstrated that PGE2 induces the expression of Per 1 mRNA and the subsequent oscillation of clock genes in NIH3T3 fibroblast cells (Nakahata *et al.* 2006), and that intraperitoneal injections of PGE2 trigger a phase shift of Per 1 expression in mouse liver, kidney, and heart (Tsuchiya *et al.* 2005).

In the retina, PGs participate in darkness-related induction of cone and retinal pigment epithelial movements, while COX inhibitors decrease dark-induced cone elongation and pigment aggregation in cultured sunfish retinas (Cavallaro and Burnside 1988). These results suggest a role for PGs in retinal circadian physiology as well as in the retinal processing of photic information. In this context, the aim of the present work was to analyze the hamster retinal biosynthesis of PGs from a chronobiological standpoint.

Materials and methods

Reagents and drugs

PGE2 and PGF2α were obtained from Sigma Chemical Co. (St Louis, MO, USA). [^{3}H]-PGE2 and [^{3}H]-PGF2α (specific activity 160 and 169.7 Ci/mmol, respectively, purity greater than 97%) were purchased from New England Nuclear Corp. (Boston, MA, USA), while anti-COX-1 and anti-COX-2 antibodies were obtained from Cayman Chemical (Ann Arbor, MI, USA).

Animals

Male golden hamsters (*Mesocricetus auratus*, average weight 120 ± 20 g), derived from a stock supplied by Charles River Breeding Laboratories (Wilmington, MA, USA), were purchased from a local dealer. Hamsters were kept under a photoperiod of 14 h of light–10 h of darkness (L : D, lights on at 6:00 h), with free access to food and water. In some experiments, animals were kept in constant darkness (D : D) for 48 h before killing. Animals were killed by decapitation at 4:00, 8:00, 12:00, 16:00, 20:00 and 24:00 h. The average circadian period for our hamsters is 24.1 h, so in 48 h under constant conditions, the circadian times would shift, maximally, 6–12 min. Thus, we assumed that the circadian times would be approximately equal to their previous Zeitgeber times. Dark-exposed hamsters were killed under dim red light (< 1 lux) and incubations were performed under darkness. After killing, eyeballs were quickly enucleated and the retinas removed and processed as described bellow for each assay.

All animal use procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals.

Assessment of retinal PG release

Individual retinas were incubated in 150 µL Krebs-Ringer bicarbonate solution at 37°C for 1 h under a 95% O_2–5% CO_2 atmosphere. At the end of the incubation period, tissues were removed and samples from the incubation media were used for PGE2 and PGF2α levels determination, which was performed by radioimmunoassay, as previously described (Ribeiro *et al.* 2003). Briefly, aliquots (100 µL for PGE2 and 20 µL for PGF2α) of the incubation media were pre-incubated with a specific antiserum (1 : 20 for both anti-PGE2 and anti-PGF2α) for 45 min at 4°C. Then, [^{3}H]-PGE2 or [^{3}H]-PGF2α (20 000 dpm), were added and incubated for 1 h at 4°C. PG levels were obtained from a standard curve, with an assay limit sensitivity of 1 pg per tube. The intraperitoneal injection of indomethacin (10 mg/kg), 3 h before killing, caused a highly significant reduction in retinal PGE2 and PGF2α release (~85%).

Western blotting

Retinas were homogenized in 200 µL of 10 mM HEPES; pH 7.9, 1 mM EDTA, 1 mM EGTA, 10 mM KCl, 1 mM dithiothreitol, 0.5 mM phenylmethyl sulfonyl fluoride, 40 µg/mL leupeptin, 2 µg/mL pepstatin A, 0.5 µg/mL aprotinin and Triton was added to reach 0.5% (v/v). After 15 min at 4°C, the homogenates were gently vortexed for 15 s and centrifuged at 8000 *g* for 15 min. Supernatants were used to determine protein concentration. Proteins

(100 μg/sample) were separated in sodium dodecyl sulfate, 10% polyacrylamide gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes for 60 min at 15 V in a Bio-Rad Trans-Blot SD system (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked in 5% non-fat dry milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween 20 for 60 min at room temperature and then incubated overnight at 4°C in a 1 : 500 dilution of polyclonal rabbit anti-COX-1 or anti-COX-2 antibody. Membranes were washed with Tris-buffered saline with Tween and then incubated for 1 h with a 1 : 3000 dilution of horseradish peroxidase-conjugated secondary antibody. Bands were visualized by enhanced chemiluminescence (Western Blotting Analysis System; Amersham Biosciences, Buenos Aires, Argentina) and β-actin was used as an internal load control. Developed membranes were scanned and the intensity of bands was determined by using the ImageJ program (National Institutes of Health, Bethesda). Values were expressed as arbitrary units of COX/β-actin.

Immunocytochemistry

Eyes were enucleated at mid-day or midnight, and immediately immersed for 24 h in a fixative containing 4% formaldehyde in 0.1 M phosphate buffer (pH 7.2). Eyecups were then dehydrated, embedded in paraffin and sectioned with a microtome at 5-μm thickness along the vertical meridian through the optic nerve head. Antigen retrieval was performed by heating (90°C) for 30 min unstained sections immersed in citrate buffer (pH 6.3) and endogenous peroxidase activity was blocked with 0.3% H_2O_2 for 20 min. Then, sections were pre-incubated with normal horse serum for 1 h. For the detection of COX-1 and COX-2, sections were incubated overnight at 4°C with the same antibodies used for western blotting experiments, at a dilution of 1 : 500. A labeled streptavidin-biotin immunohystochemical staining was performed using the LSAB2® System HRP Dakocytomation, according to manufacturer's instructions. Some sections were treated without the primary antibodies to confirm the specificity of immunoreactivity. Microscopic images were digitally captured with a Nikon Eclipse E400 microscope (illumination: 6-V halogen lamp, 20 W, equipped with a stabilized light source) via a Nikon Coolpix s10 camera.

SCN lesion

Animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine hydrochloride (2 mg/kg) administered intraperitoneally and placed in a stereotaxic apparatus. Electrolytic lesions of the SCN were made using the following coordinates (from bregma): +0.6 mm rostral, −8.2 mm ventral and 0.0 in the medial line (with the stereotaxic toothbar set at −2.0 mm). Continuous current (2.5 mA) was applied for 20 s. In sham-operated animals, the procedure was similar but no current was applied. After the surgery, animals were placed under a 14 h of light/10 h dark photoperiod for 2 weeks and then animals were kept under D : D for 2 days before killing. SCN lesion was verified by the loss of locomotor activity circadian rhythm under constant darkness conditions as well as by post-mortem histology of brain coronal sections by the Kluver-Barrera staining.

Actogram and periodogram assessment

Animals were kept individually in cages and daily locomotor activity was monitored with passive infrared detectors mounted over each cage. The passive IR proximity sensor works by emitting pulses of IR light and then measuring the distance to objects from the flight time of the reflected signal. Whenever the distance changes, the detector opens or closes a switch. All detectors were tested to ensure response uniformity. Recorded activity was analyzed using data acquisition software (ElTemps, Barcelona, Spain).

Protein content was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as the standard.

Statistical analysis of results was made by a Student's *t*-test or by a two-way analysis of variance (ANOVA) followed by Tukey's test, as stated.

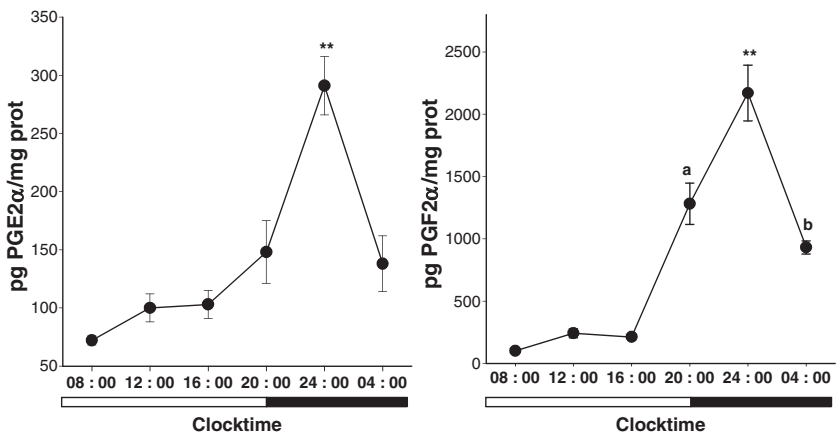

Fig. 1 Prostaglandin (PG) release in retinas from hamsters kept under L : D and killed at the times indicated. Retinas were incubated and PG levels were assessed as described in Materials and methods. PGE2 and F2α release showed significant diurnal variations ($p < 0.01$ by ANOVA). The release of both PGs at 24:00 h was significantly higher than that observed at all the other intervals (**$p < 0.01$, by Tukey's test). PGF2α release at 20:00 h significantly differed (a: $p < 0.01$) from all the other intervals, except for 4:00 h, and values at 4:00 h differed from all the other intervals, except for 20:00 h (b: $p < 0.01$). Data are mean ± SEM values ($n = 10$ animals per group).

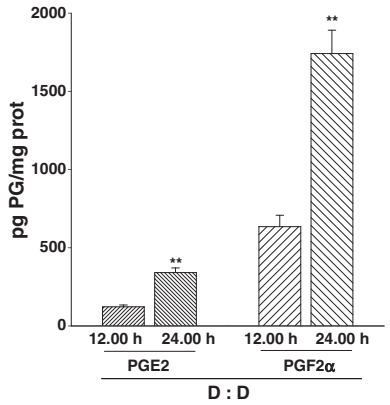

Fig. 2 Prostaglandin (PG) release in the retina of hamsters kept under D : D and killed at the times indicated. Retinas were incubated under darkness and PG levels were assessed as described in Materials and methods. The release of PGE2 and PGF2α was significantly higher at subjective midnight than at subjective mid-day. Data are mean ± SEM (n = 10 animals per group). $^{**}p < 0.01$ as compared with subjective mid-day values, by Student's t-test.

Results

Figure 1 depicts the diurnal changes of PGE2 and PGF2α release by golden hamster retinas from animals killed at different intervals through a 24-h cycle. Both parameters exhibited significant diurnal variations, peaking at 24:00 h. When hamsters were placed under constant darkness (dark/dark, D : D) for 48 h before the experiment and killed at time points representing subjective mid-day and subjective midnight, the release of both PGs was significantly higher at subjective midnight than at subjective mid-day (Fig. 2).

Figure 3 shows representative western blots for COX-1 and COX-2 in retinas excised at mid-day or midnight, as well as at subjective mid-day or subjective midnight. Bands of 72 and 70 kDa were identified in all samples, in agreement with the molecular mass reported for COX-1 and COX-2 respectively, in other tissues. Scanning densitometry analysis of the bands (shown in the right panel of the Figure) revealed significant differences for COX-1 levels between mid-day and midnight as well as between subjective mid-day and subjective midnight. Moreover, COX-1 levels were higher at subjective mid-day (D : D) than at mid-day (L : D), and at subjective midnight (D : D) than at midnight (L : D). Under L : D or D : D conditions, no significant variations in COX-2 levels were observed among groups.

An immunohistochemical analysis was performed to determine COX-1 and COX-2 localization. In normal hamster retinas from animals killed at mid-day, COX-1 immunoreactivity was present in neurons of the ganglion cell layer (GCL) and in cells of the inner nuclear layer (INL) (Fig. 4a). A punctate staining in the innermost sublaminae of

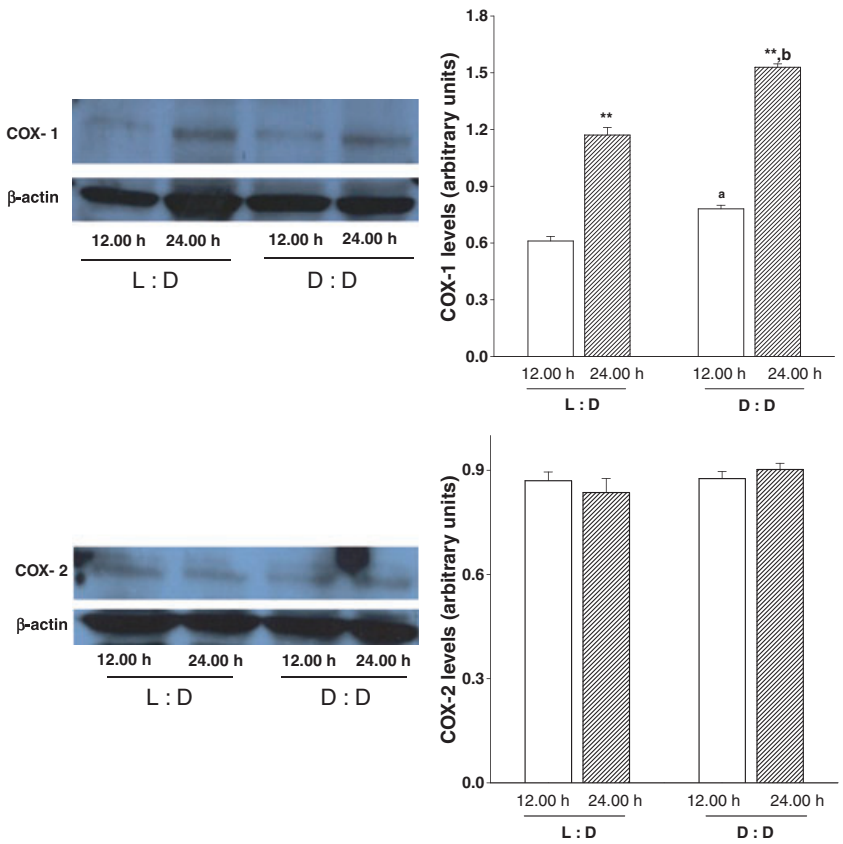

Fig. 3 Upper panel (left): representative gel for retinal cyclooxygenase (COX)-1 from hamsters kept under L : D or D : D and killed at the indicated time point. A band of ~72 kDa was detected in all the samples. Upper panel (right): densitometric analysis revealed higher levels of COX-1 at midnight than at mid-day (L : D) and at subjective midnight than at subjective mid-day (D : D). $^{**}p < 0.01$ as compared with mid-day or subjective mid-day values, (a) $p < 0.01$ vs. mid-day (L : D), (b) $p < 0.01$ vs. midnight (L : D), ANOVA, Tukey's test. Lower panel (left): representative gel for retinal COX-2 from hamsters kept under L : D or D : D and killed at the indicated time point. A band of ~70 kDa was detected in all samples. Lower panel (right): densitometric analysis of the samples for COX-2 revealed no significant differences between mid-day and midnight (L : D) or between subjective mid-day and subjective midnight (D : D; for both isoenzymes, each lane was loaded with 80 μg of proteins; data are mean ± SEM (n = 8 animals per group).

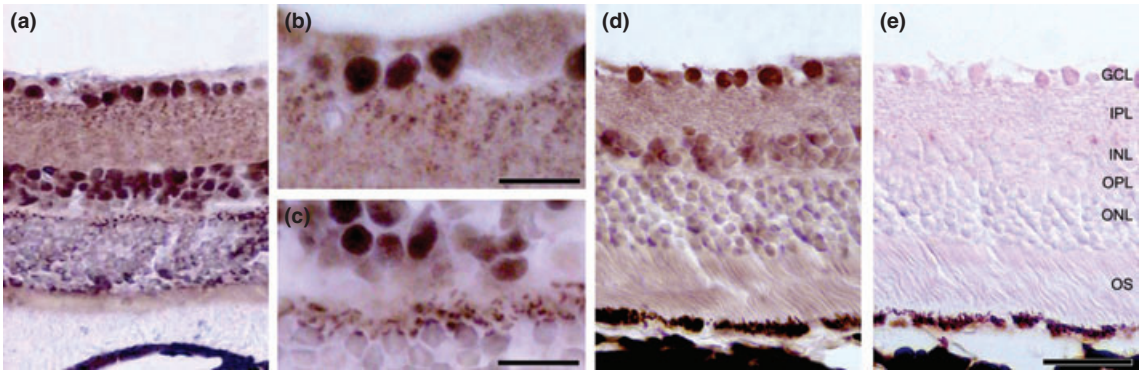

Fig. 4 Immunohistochemical analysis of retinal cyclooxygenase (COX)-1 and COX-2 localization in the golden hamster retina. A strong immunostaining for COX-1 was detected in GCL and INL cells (a), and an intense punctate staining was found in the IPL (b) and OPL (c). Immunostaining for COX-2 is shown in (d). A strong staining was confined to CGL cells, and a weak staining was also observed in the INL. Negative control without primary antibody (e). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segment of photoreceptors. Scale bars: (a, d, e) 50 μm; (b, d) 25 μm.

the inner plexiform layer (Fig. 4b) and a strong punctate staining throughout the outer plexiform layer (Fig. 4c) was also observed. COX-2 immunoreactivity was found in the inner retina, mainly in the GCL. A weak COX-2 immunoreactivity was observed in the INL (Fig. 4d). No immunolabelling for either isoenzyme was observed in the outer retina. No significant differences in COX-1 and COX-2 localization were observed between animals sacrificed at mid-day or midnight (data not shown).

To analyze the influence of the SCN on the circadian variations in retinal PG release, hamsters were submitted to a bilateral lesion of the SCN. One week before and 25 days

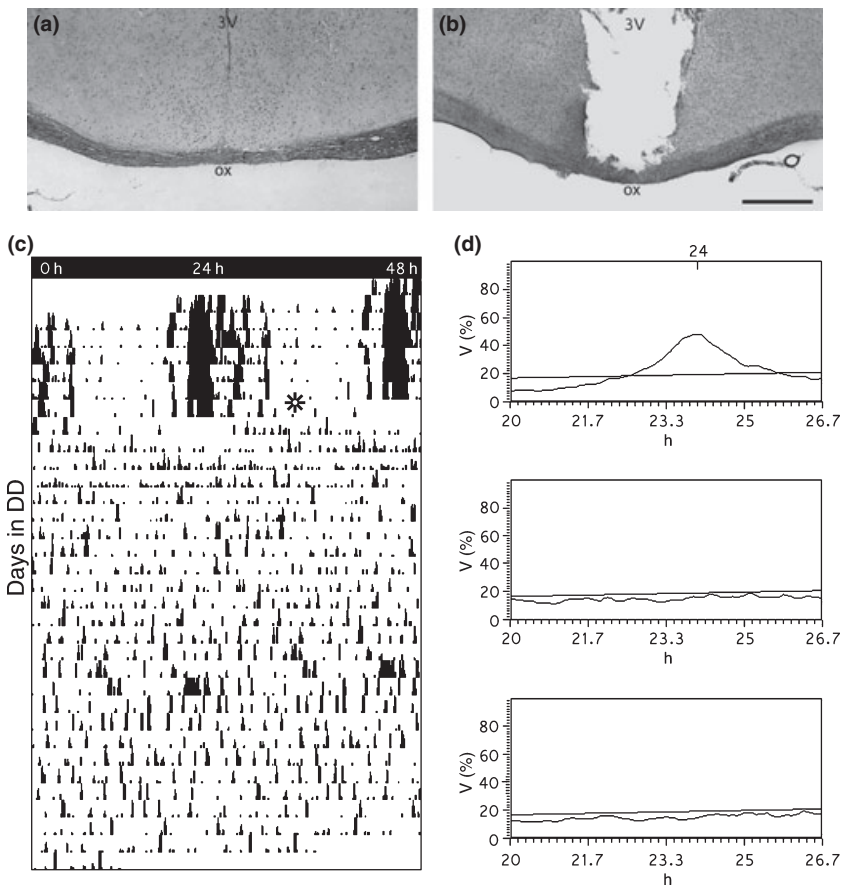

Fig. 5 Rhythm of locomotor activity and post-mortem morphological analysis from suprachiasmatic nuclei (SCN)-lesioned animals. Representative photomicrographs of Kluver Barrera-stained coronal section at the level of the SCN from sham-operated (a) and lesioned animal (b) ($n = 7$ animals/group). In control animals, intact bilateral nuclei above the optic chiasm were evident, whereas in lesioned animals, an absence of the SCN without affecting the integrity of the optic chiasm was observed. (c) Locomotor activity of a representative SCN-lesioned animal registered under constant darkness. Free-running circadian rhythm was observed during 1 week before surgery, whereas the SCN-lesion (indicated by an asterisk) provoked lost of rhythmicity. (d) Periodogram of an SCN-lesioned animal. Upper panel shows the percentage of variance related to the period of the animal before the lesion. Middle panel shows the lost of periodicity after the surgery which was sustained for several weeks (lower panel). Scale bar 500 μm. Ox, optic chiasm; 3V, third ventricle.

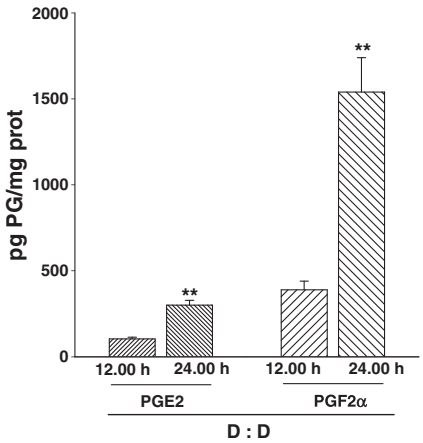

Fig. 6 Prostaglandin (PG) release from retinas of suprachiasmatic nuclei-lesioned hamsters kept under D : D and killed at the times indicated. Retinas were incubated under darkness and PG levels were assessed as described in Materials and methods. The release of PGE2 and PGF2α was significantly higher at subjective midnight than at subjective mid-day. Data are mean ± SEM ($n = 7$ animals per group). $^{**}p < 0.01$ as compared with subjective mid-day values, by Student's *t*-test.

after surgery, hamster's locomotor activity under constant darkness was recorded in IR sensor equipped cages to verify the loss of their rhythmic locomotor activity, as shown in Fig. 5. The post-mortem histology showing that the SCN was completely removed is also shown. SCN-lesioned animals were killed at subjective mid-day or subjective midnight and the release of both PGs was analyzed. PGE2 and PGF2α levels were significantly higher at subjective midnight than at subjective mid-day in SCN-lesioned animals, as shown in Fig. 6.

Discussion

The present results indicate that the release of PGE2 and PGF2α in the hamster retina is regulated by a retinal circadian clock independent from the SCN. Bovine and rat retinas can generate PGE2 and PGF2α from radiolabeled arachidonic acid (Birkle and Bazan 1984b, 1986), and rabbit retinas are able to release PGE2 and PGF2α from endogenous arachidonic acid (Preud'homme *et al.* 1985), PGE2 being the predominant eicosanoid formed. Our findings indicate that the hamster retina is able to spontaneously release PGs from endogenous sources, although higher levels of PGF2α than of PGE2 were observed. Although there is no ready explanation for this discrepancy, it seems likely that species-dependent factors could account for it.

Significant daily variations in the release of PGE2 and PGF2α were observed under L : D conditions, peaking at midnight. A daily retinal rhythm may be controlled by a circadian oscillator, by direct response to environmental lighting levels, or by a combination of these mechanisms. As PGs are highly photosensitive compounds, it is possible that light during the incubation period could have a deleterious effect on these molecules. In fact, a significant increase in both PG levels was observed under darkness than under light conditions. However, the observation that subjective mid-day/subjective midnight variations persisted under constant darkness supports the idea of a clock-controlled function. Thus, although the possibility that light may influence retinal PG biosynthesis is not excluded, the light/dark cycle seems not to be necessary for the generation of its rhythm. Circadian variations of PG levels were described in cerebrospinal fluid (Pandey *et al.* 1995) and bone/marrow diaphyseal organ from rat (Yosipovitch *et al.* 1995), and rabbit aqueous humor (Liu 2000). In the diaphyseal bone, PG levels are elevated during the evening and dark periods and reduced during the morning, whereas in the cerebrospinal fluid and rabbit aqueous humor, an opposite peak phase in PG production was demonstrated. Therefore, it is possible that the temporal coordination of PG release is exerted at a tissular/cellular level, which would make sense when taking into account that these molecules usually represent a local regulatory mechanism.

Higher levels of COX-1 (but not COX-2) were observed at midnight than at mid-day and at subjective midnight than at subjective mid-day, which supports a correlation between this isoenzyme levels and PG release. Therefore, the present results suggest that retinal COX-1 expression is regulated by an endogenous circadian clock. Although COX-1 was originally considered a constitutive isoform, altered COX-1 expression in the retina was observed in several experimental conditions (Fang *et al.* 1997).

The localization of COX-1 and COX-2 has been previously examined in the retina from several species such as mouse, rat and human (Ju and Neufeld 2002). In the mouse retina, COX-1 is localized in the outer segments of photoreceptor cells, horizontal cells, microglia, retinal ganglion cells and displaced amacrine cells, whereas in rat and human retina, it is mainly present in retinal ganglion cells and displaced amacrine cells. COX-2 immunoreactivity in the mouse and rat retina is present in processes of the outer plexiform layer and in certain amacrine cells and retinal ganglion cells, while in the human retina, COX-2 immunoreactivity is only present in processes of the outer plexiform layer. In the hamster retina, COX-1 and COX-2 were localized in the inner (but not outer) retina, mostly in the GCL and INL, indicating that the retinal localization of both COX isoenzymes is a species-dependent phenomenon.

Many relevant retinal parameters such as GABA (Jaliffa *et al.* 2001), dopamine (Jaliffa *et al.* 2000) and melatonin levels (Faillace *et al.* 1994; Tosini and Menaker 1996) among many others, significantly fluctuate according the time of the day, even under constant lighting conditions, supporting the existence of a retinal circadian clock.

However, the cellular location (or possibly multiple locations) of the retinal circadian clock(s) has not been fully identified yet. Several lines of evidence support that the retinal circadian clock is located in photoreceptors (Tosini *et al.* 2007). In that sense, multiple clock components were exclusively located in *Xenopus* photoreceptors (reviewed by Anderson and Green 2000). We have recently shown that cultures of chick embryonic retinal ganglion cells show self-sustained rhythms in arylalkylamine *N*-acetyltransferase mRNA expression and melatonin biosynthesis during at least three cycles with a period near 24 h (Garbarino-Pico *et al.* 2004), suggesting that chick retinal ganglion cells may function as autonomous circadian oscillators synthesizing melatonin during the day. Based on the present results, it seems possible that mammalian retinal ganglion cells may function as circadian oscillators synthesizing PGs. Other studies have also shown the presence of a circadian clock in the inner retina. Circadian rhythms in the dopaminergic and melatonergic systems occur in rats with dystrophic retina (Sakamoto *et al.* 2004), and it has been shown that mouse inner retinal neurons co-express mRNA for several clock genes (Witkovsky *et al.* 2003; Ruan *et al.* 2006). Moreover, a circadian rhythm in the retina of Period 2-luciferase expression in animals with almost no photoreceptors was demonstrated (Ruan *et al.* 2006). Taken together, these results suggest the presence of at least two different circadian clock systems in the mammalian retina.

Retinal circadian clocks presumably have two main coordinating functions (Witkovsky *et al.* 2003). The first is intrinsic to the retina and is evidenced by the findings that many functional molecules and many retinal activities have a circadian rhythm of expression (for a review see Tosini and Fukuhara 2002). In many of the studies, the eyes were not separated from the animal, permitting a possible centrifugal influence of the SCN on the retina. However, it was shown that cultured retinas clearly retain their circadian rhythm in melatonin production (Cahill and Besharse 1993; Tosini and Menaker 1996). Moreover, Terman *et al.* (1993) showed that lesions of the SCN did not affect the rod disk shedding rhythm. As shown herein, the retinal rhythm of PG release persisted in SCN-lesioned hamsters, suggesting that this rhythm is generated in the retina itself. Retinal PGs were associated to several darkness-linked phenomena such as the inhibition of dopamine release in the rabbit retina (al-Zadjali *et al.* 1994). A second potential coordinating role for retinal clocks is in relation to the SCN, which the retina entrains to the daily rhythm of light and darkness (Lee *et al.* 2003). A specialized subset of retinal ganglion cells provides photic input to mammalian endogenous clock in the SCN. Thus, the fact that COX-1 and COX-2 were localized in the inner nuclear and GCLs, suggests that PGs could be involved in both coordinating functions of the retinal circadian clock(s).

Recently, it has been shown a hitherto unidentified function of PGE2, that is, resetting of the rhythm-generating core feedback loops of circadian gene expression in peripheral clocks (Tsuchiya *et al.* 2005). Experiments are planned to address this role of PGE2 on the retinal circadian clock.

In conclusion, the present results indicate a robust circadian rhythm in retinal PG release, presumably associated to COX-1, suggesting that the PGs/COX-1 system could be a retinal clock output or part of the retinal clock mechanism.

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