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Phil. Trans. R. Soc. Lond. B 2000 **355**, 1199-1203
doi: 10.1098/rstb.2000.0667

THE ROYAL SOCIETY

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Light-adaptive role of nitric oxide in the outer retina of lower vertebrates: a brief review

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The role of nitric oxide (NO) as a novel neurochemical mechanism controlling light adaptation of the outer retina is discussed by considering mainly published results. The emphasis is on the retinae of fishes and amphibia, but some data from the mammalian (rabbit) retinae have also been included for completeness. In the fish retina, application of NO donors in the dark caused light-adaptive photomechanical movements of cones. The normal effect of light adaptation in inducing cone contractions was suppressed by pretreatment of retinae with an NO scavenger. NO donors modulated horizontal cell activity by uncoupling the cells' lateral gap junctional interconnections and enhancing negative feedback to cones, again consistent with a light-adaptive role of NO. Direct evidence for light adaptation-induced release of NO has been obtained in fish (carp) and rabbit retinae. The results strongly suggest that control of retinal light adaptation is under multiple neurochemical control, with NO and dopamine having an interactive role.

Keywords: nitric oxide; retina; light adaptation; photoreceptor; horizontal cell

1. INTRODUCTION

Light adaptation of the retina, which contributes to the ability of the vertebrate visual system to respond to light intensities that may vary by $\geqslant 10$ orders of magnitude, occurs in several stages (Dowling 1987). A part of this mechanism operates in the outer retina and involves photoreceptors' (PCs') intrinsic activity and interactions with second-order neurons in the outer plexiform layer. Synaptic interactions (feedforward and feedback) between PCs and horizontal cells (HCs) are also modulated by light or dark adaptation.

A considerable body of evidence suggests that dopamine (DA) is released in vertebrate retinae during light adaptation and mimics many of the light-adaptive changes seen in PCs and HCs (Djamgoz & Wagner 1992). However, in several instances, DA-independent effects of light adaptation have also been found (e.g. Djamgoz *et al.* 1996*b*). This has raised the possibility that retinal light adaptation may involve additional modulators.

This paper briefly reviews evidence showing (i) that nitric oxide (NO) is a novel neuromodulator in the outer retina, and (ii) that the effects of NO are consistent with it being involved in the control of several aspects of the light-adaptation process.

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2. LOCALIZATION OF NITROGEN OXIDE-SYNTHESIZING CELLS IN FISHES AND AMPHIBIAN RETINAE

A number of studies on fish and amphibian retinae have shown that NO can potentially be synthesized by various types of retinal neuron. NO-synthesizing neurons can be visualized either by histochemistry of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase or by immunocytochemistry of NO synthase (NOS), which catalyses the synthesis of NO from the substrate L-arginine. The two methods have given broadly consistent results; typical localization of NADPH-diaphorase in the carp retina is illustrated in figure 1. Clearly, a wide variety of retinal neurons are potentially capable of synthesizing (and presumably releasing) NO, including HCs, bipolar, amacrine, Müller and ganglion cells, with intense staining also occurring in PC ellipsoid regions (Djamgoz *et al.* 1996*a*). A similar extensive distribution of NADPH-diaphorase-positive and/or NOS-immunoreactive cells has also been found in goldfish and catfish retinae. A broad pattern of potentially NO-synthesizing cells would be indicative of NO having a diverse functional role in the teleost retina. On the other hand, such a wide distribution is rather surprising since the 'sphere of influence' of any NO released is thought to be some hundreds of microns, comparable with the whole thickness of a neural retina. This situation

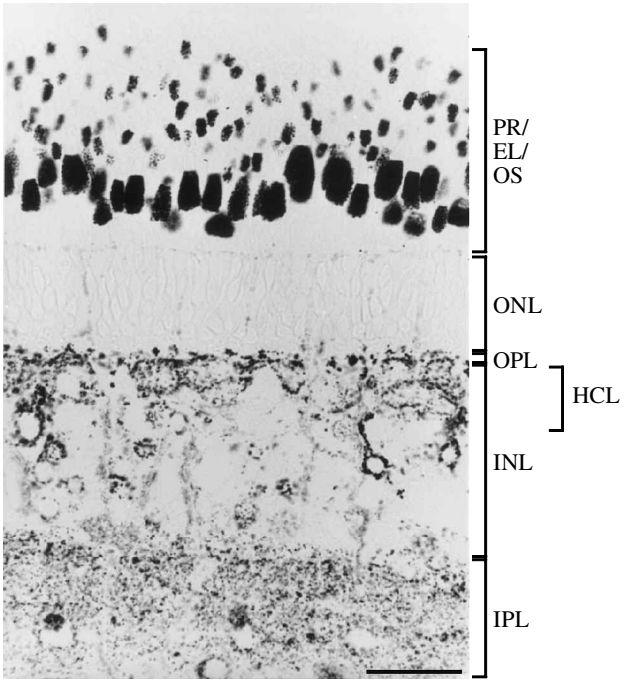

Figure 1. An overview of the staining of cellular layers within the carp retina by histochemistry of NADPH-diaphorase. PR/EL/OS, photoreceptor ellipsoid and outer segment region. ONL, outer nuclear layer. OPL, outer plexiform layer. HCL, horizontal cell layers. INL, inner nuclear layer. IPL, inner plexiform layer. Scale bar, 26 μm. Modified from Djamgoz *et al.* (1996*a*).

may have important implications for the mode of action of retinal NO.

3. CELLULAR EFFECTS OF NITRIC OXIDE IN THE OUTER RETINA

The putative effects of NO could be studied by treating the retina with NO donor compounds such as sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP), S-nitrosoglutathione (SNOG) or S-nitrosocysteine (SNC). As regards the possible role of endogenous NO, some NO 'scavenger' compounds have been used, including haemoglobin and 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide potassium (cPTIO).

(a) *Photoreceptor cells*

Light and dark adaptation of PCs incorporates both structural/biochemical and physiological changes in which NO has been shown to have a putative role.

(i) *Electrophysiological aspects*

An integral component of the phototransduction pathway in PCs is the second messenger cyclic guanosine 3′,5′-monophosphate (cGMP), which gates cation channels in the plasma membrane. The identification in PCs of both soluble guanylate cyclase (sGC; an enzyme responsible for cGMP synthesis and a primary target of NO) and NOS would suggest that NO could act as a modulator of PC activity. In fact, application of SNP to isolated rods of *Rana esculenta* was found to accelerate the recovery phase of the light-evoked response (Schmidt *et al.* 1992). This was suggested to be due to an increased rate in cGMP turnover induced by NO enhancement of sGC activity. Conversely, suppressing endogenous NO production by inhibiting NOS with N^{ω}-monomethyl-L-arginine slowed down the recovery phase of the PC response to light. Importantly, this occurred even though solitary PCs (dissociated from the retina) were used (see § 3(a)(ii)).

(ii) *Photomechanical movements*

PC cells of 'lower' vertebrates (fishes and amphibia), which have no or only a weak pupillary response, possess the ability to undergo photomechanical movements (PMMs). Thus, during light adaptation, cones (and melanin granules of pigment epithelial cells) move towards the outer limiting membrane while rods move away from it.

Greenstreet & Djamgoz (1994) originally and Haamedi (1999) subsequently showed that application of a variety of NO donors (SNP, SNAP or SNOG) to dark-adapted retinae of cyprinid fishes (roach and carp) produced light-adaptive cone contractions. Pretreatment of the retinae with cPTIO largely blocked the effect of test light adaptation, consistent with light-evoked release of endogenous NO.

At present, the cellular origin(s) of NO modulating the light-evoked PC responses, including cone PMMs, is not clear. It is generally thought that NO acts upon cells other than those that produce it, due to the conflicting Ca^{2+} requirements of sGC and NOS, i.e. the intracellular Ca^{2+} levels required to activate NOS should inhibit sGC activity. However, the electrophysiological data obtained from the solitary rods would suggest that the unique compartmentalized structure of the PC could permit NO production in one region and action at a different intracellular site. Further work is required to investigate the effects of NO on both electrophysiological and contractile activities of PCs, and to substantiate the evidence that PCs may indeed respond to NO that the cells themselves could produce.

(b) *Horizontal cells*

(i) *Cone–HC feedforward synaptic transmission*

The neurotransmitter glutamate released from PCs acts upon HCs at both ionotropic and metabotropic receptors. NO affects both sets of synaptic transmission.

Ionotropic glutamate receptors

Application of SNP to dissociated hybrid bass HCs had two modulatory effects on the glutamate-evoked currents (McMahon & Schmidt 1999). First, the affinity of the synaptic receptors for glutamate was decreased. This effect, also seen with 8-Br-cGMP (a membrane-permeable analogue of cGMP), was probably mediated by a pathway involving sGC, cGMP and a cGMP-dependent protein kinase (PKG). Second, the maximal current was increased. This was probably due to receptor desensitization being blocked, since the effect was not seen with the non-desensitizing agonist kainate and was suppressed by the glutamate receptor desensitization blocker cyclothiazide. Accordingly, in the dark (when the glutamate release would be high), NO donors would depolarize

the resting membrane potential of HCs and such an effect has indeed been recorded in the isolated retina of carp (M. B. A. Djamgoz, unpublished data). However, any possible presynaptic effect of NO on glutamate release itself was not studied.

Metabotropic glutamate receptors

It is becoming increasingly apparent that H1 type HC responses generated by long wavelength (LW) versus short wavelength (SW) stimuli are processed differently in the cyprinid fish (carp) retina (Djamgoz *et al.* 1996*b*, 1998; Furukawa *et al.* 1997). In particular, SW-driven synaptic input to H1 HCs may involve a 2-amino-4-phosphonobutyrate (APB)-sensitive metabotropic glutamate receptor mechanism negatively coupled to a cGMP-dependent pathway (as in the case of centre-depolarizing bipolar cells). Thus, during light stimulation, cGMP production in the H1 HC is increased and this activates a depolarizing conductance. A role for NO in the enhancement of the SW-transmission pathway has been established. Furukawa *et al.* (1997) measured the change in input resistance of carp H1 HCs during LW- or SW-light stimulation. Light adaptation induced a chromatic difference in the light-evoked change in equal-voltage input resistance, thereby suggesting the possible presence of at least two different receptor or channel mechanisms in the H1 HCs. Application of SNP to dark-adapted retina mimicked the effect of light adaptation while APB eliminated the chromatic difference in input resistance change. Furthermore, light adaptation sharpened the spectral sensitivity profile of the H1 HC in the red region of the spectrum (figure 2*a*) and it was proposed that this was due to potentiation of the depolarizing SW component (Djamgoz *et al.* 1996*b*; Yamada *et al.* 1999). An effect similar to that of light adaptation was produced by application of NO donors to dark-adapted retinae (figure 2*b*). Thus, it would seem that NO enhances the SW transmission to H1 HCs and sharpens their spectral response peak, mimicking the effect of light adaptation (Yamada *et al.* 1999). Indeed, pretreatment of the retinas with haemoglobin blocked the spectral effect of light adaptation, consistent with release of endogenous NO (figure 2*c*).

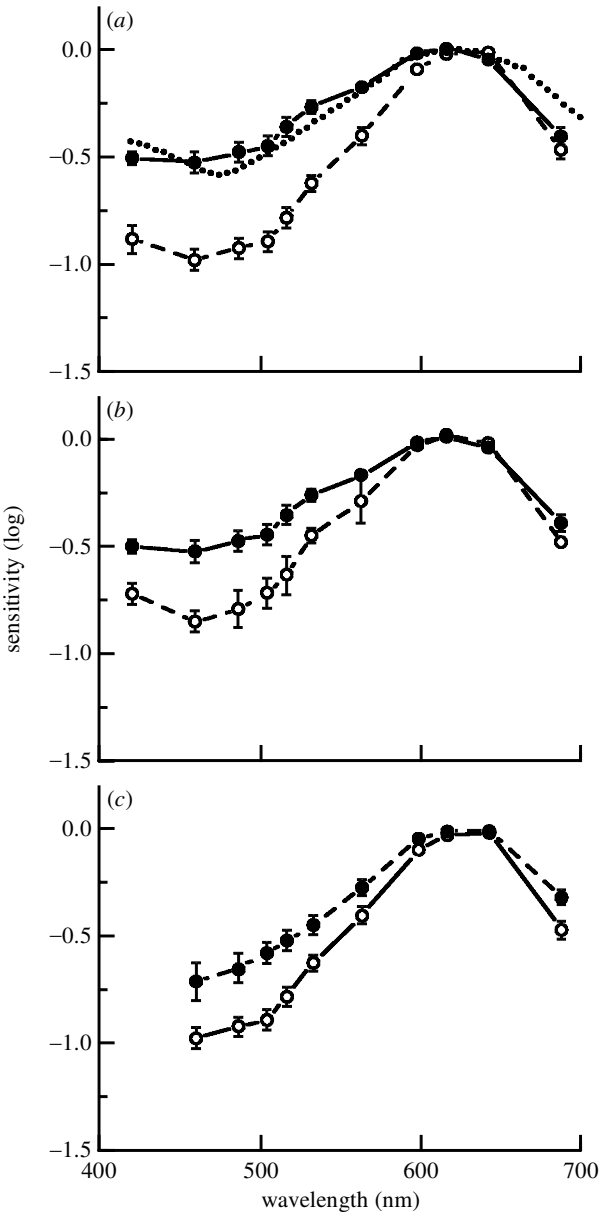

Figure 2. Spectral profiles of H1 HCs in carp retinae under different light- and dark-adaptive experimental conditions. Data are shown as means ± s.e. ($n = 5$–14 cells). (*a*) Dark- and light-adapted control retinae (filled and open circles, respectively). The dotted curve represents the absorption spectrum of the cyprinid red-sensitive cone obtained by microspectrophotometry. (*b*) Effect of 0.25 mM SNOG on the spectral sensitivity in dark-adapted retinae (open circles). The data obtained with normal Ringer solution are also shown (filled circles). (*c*) Effects of 10 μM haemoglobin on the spectral sensitivity in light-adapted retinae (filled circles). The data obtained with normal Ringer solution are also shown (open circles). Data modified from Yamada *et al.* (1999).

(ii) *HC–cone feedback*

The negative feedback pathway between cones and HCs subserves several important functions in visual processing. Both electrophysiological mechanisms and morphological aspects of this interaction have been studied.

Electrophysiological aspects

In isolated cone terminals of the tiger salamander retina, Savchenko *et al.* (1997) identified clusters of cGMP-gated (CNG) channels which could be activated by SNC. In order to determine whether NO enhancement of the CNG conductance could affect synaptic transmission from the cone terminal, dissociated catfish HCs were used as 'biosensors' to monitor glutamate release from the terminals. Application of SNC or a membrane permeable analogue of cGMP to the PC–HC pair induced an increase in the current variance of the HC consistent with NO increasing neurotransmitter release. It was suggested that the NO-induced enhancement of the mainly voltage-independent CNG channel in the cone terminal could be responsible for neurotransmitter release at the hyperpolarized potentials encountered during retinal light adaptation.

Morphological aspects

In the teleost retina, HC–cone feedback has been suggested to occur via HC spinules, finger-like extensions

of HC dendrites positioned laterally at synaptic ribbons within cone pedicles (Wagner & Djamgoz 1993). Application of NO donors (SNP, SNAP or SNOG) to dark-adapted cyprinid fish (roach and carp) retinae produced HC spinules, mimicking the effect of normal light adaptation (Greenstreet & Djamgoz 1994; Haamedi 1999). These effects were concentration dependent and were completed within 10–20 min following a given treatment. Pretreatment with cPTIO largely (but not completely) blocked the normal light adaptation-induced formation of spinules, consistent with (i) endogenous NO being produced during light adaptation, and (ii) HC spinules being under NO control.

(iii) *Electronic coupling of HCs*

HCs of the same subtype form gap junction-coupled syncytia imparting upon these cells wide receptive fields. In teleosts, HC coupling is dynamically regulated by the adaptational state of the retina. Light adaptation decreases coupling resulting in an increase in the spatial resolution, whereas in the dark the HCs are strongly coupled so as to increase the absolute sensitivity of the system. DA was found initially to decrease HC coupling, mimicking the effects of light adaptation (Djamgoz & Wagner 1992); NO has been shown subsequently also to have a similar role (e.g. Lu & McMahon 1997). Application of SNP or 8-Br-cGMP to pairs of cultured hybrid bass HCs decreased the amplitude of the junctional coupling current (Lu & McMahon 1997). The effects of SNP could be blocked by the application of LY-83583, an inhibitor of sGC, or intracellular injection of RKRARKE, a PKG inhibitor. It was proposed that NO modulation of HC electrical coupling occurs via PKG phosphorylation of the gap junctions.

Interestingly, a chromatic difference in the receptive field size of carp H1 HCs has been observed (Djamgoz *et al.* 1998). In the light-adapted retina, the receptive field of H1 HCs was found to be smaller for SW- in comparison with LW-light stimulation. APB application suppressed the chromatic difference whereas intracellular cGMP injection increased it. The differential effects of the spectral stimulation on the receptive field size was suggested to be due to the APB-sensitive, conductance-decreasing SW input to the H1 HC, as described above. NO has been linked to the enhancement of the SW-transmission pathway and so it is possible that NO could also be involved in the modulation of the chromatic difference in the H1 HC receptive field size during light adaptation.

4. LIGHT-EVOKED RETINAL PRODUCTION OF NITRIC OXIDE

Recent evidence suggests that endogenous NO production does indeed occur during light adaptation in both fish and mammalian retinae. Neal *et al.* (1998) used a method based upon nitrate reductase activity and electrochemical detection to assay NO in perfusates of rabbit retinae treated experimentally *in vivo* in various different ways. This method gave more consistent data, compared with attempts to measure NO production directly. Thus, both steady and flickering light adaptation led to production of NO. Interestingly, these effects were blocked by *cis*-2,3-piperidinedicarboxylic acid and APB, indicating that the corresponding synaptic control occurred through amacrine and centre-depolarizing bipolar cells, respectively.

Sekaran *et al.* (1999) applied this technique to the isolated retina of carp and also found light-adaptation-induced synthesis of NO. An interesting aspect of NO production in the carp retina was that it was associated with a very narrow operating range ($\leqslant$ 1 log unit). It followed, therefore, that NO could have a 'switching' (rather than a graded) role in the light-adaptation process. We have also recently shown that both steady and flicker light enhanced NO release in the carp retina, flicker stimulus being relatively more effective. The cellular origin of the light-evoked NO release was investigated. 6-Cyano-7-nitroquinoxaline-2,3-dione had no effect on the level of NO, but APB blocked both steady and flicker light adaptation-induced NO production, suggesting that the centre-depolarizing bipolar cell pathway plays an important role in the light-evoked release of NO (Sekaran *et al.* 2000).

5. CONCLUDING REMARKS: MULTIPLICITY OF NEUROCHEMICAL CONTROL OF RETINAL LIGHT ADAPTATION

The available evidence taken together suggests strongly that NO is a novel signal of light adaptation in the outer retina and may account for some of the interesting properties of this process, such as its spread. Since DA is another well-established light-adaptative modulator, it follows that retinal light adaptation is under multiple neurochemical control. It is likely that this multiplicity is interactive rather than independent. In fact, both antagonistic and synergistic modes of NO–DA interaction have been found (e.g. Djamgoz *et al.* 1995; McMahon & Schmidt 1999). Such multiplicity and potential intricacy of signalling would imply that the neurochemical control of retinal light adaptation is even more complex than previously thought. In the case of aquatic or semi-aquatic vertebrates, such control probably reflects the dynamic nature of the visual environment under water.

We are aware that regretfully we could not include all the published evidence concerning the role of NO in vertebrate retinae due to space limitations. Our work is supported by the UK Biotechnology and Biological Sciences Research Council (special studentship to S.S.), grants from the European Union (STRIDE Programme) and the Sardinian Regional Government (A.R.A., S.V. and J.H.), and The British Council, Tokyo (M.B.A.D. and M.Y).

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