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Effects of Nitric Oxide on the Horizontal Cell Network and Dopamine Release in the Carp Retina

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In the teleost retina the intercellular messenger nitric oxide can be synthesized by several cell types including cone photoreceptors and H1 horizontal cells, indicating a modulatory role within the outer plexiform layer, the first stage of the visual information processing. Therefore, the aim of this study was to elucidate the effects of nitric oxide on the physiology of cone horizontal cells in the intact retina. The nitric oxide donor sodium nitroprusside (0.5–2.5 mM) enhanced the light responsiveness of cone horizontal cells and reduced the degree of electrical coupling in the network. Furthermore, the spread of intracellularly injected Lucifer Yellow was restricted. The effects on light responsiveness and electrical coupling were qualitatively mimicked by 8-bromo-cGMP (0.5 mM) and could not be achieved by ferrocyanide (1 mM), the byproduct of nitric oxide liberation from nitroprusside. The effects of NO on the responsiveness of horizontal cells may be due to an action on green- and red-sensitive cones. Nitroprusside (0.1 mM) diminished the K^+-stimulated release of endogenous dopamine by 50%, whereas the basal dopamine release was not affected, indicating that the effects on electrotonic horizontal cell coupling were not elicited by an NO-induced release of dopamine. With respect to the morphologic plasticity of the cone–horizontal cell synapse the inhibitor of endogenous nitric oxide synthesis L-nitroarginine (0.1 mM) had no influence on the formation or retraction of spinules. These results show that NO affects the responsiveness and coupling of the horizontal cell network in a dopamine-independent way.

Synaptic plasticity Gap junctions Spinules cGMP Electrical coupling

INTRODUCTION

Since its discovery as a biological messenger nitric oxide (NO) has been found to be involved in a variety of both neuronal and non-neuronal functions. In the vertebrate nervous system distinct neuronal populations are known to contain the NO synthesizing enzyme, termed neuronal nitric oxide synthase (nNOS). This enzyme needs Ca^{2+}-bound calmodulin as a cofactor for activation, and once activated produces NO in the picomolar range (Schuman & Madison, 1994). Nitric oxide does not act like a classical neurotransmitter, since it is not stored in vesicles and is not released by an exocytotic mechanism. Instead its synthesis takes place upon demand and is located in the cytosol. Furthermore, the action of this gaseous messenger is not restricted to morphologically specialized synaptic sites as it readily diffuses out of its originating cell to interact with ubiquitous receptor molecules inside all cells within its diffusional reach. Yet, the diffusional spread is limited by the short lifetime of this highly reactive molecule. To exert its communicational functions NO triggers the synthesis of the second messenger cGMP, which then interacts with ion channels, protein kinase, or phosphodiesterase to elicit the target cell's physiological response. Besides this, direct effects of NO on target proteins like the NMDA receptor or the enzyme ADP-ribosyltransferase (Brune & Lapetina, 1989) are also known.

In the vertebrate retina, nNOS localization is predominantly found among cells of the proximal inner nuclear layer, but substantial nNOS activity is also known from the ganglion cell layer, photoreceptors, and glial cells (for a review see Vincent, 1994). Striking evidence for the presence of nNOS in horizontal cells comes from the teleost retina (Baldridge *et al.*, 1993; Weiler & Kewitz, 1993; Liepe *et al.*, 1994; Östholm *et al.*, 1994), and there are hints that the distribution of

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nNOS among the horizontal cells is restricted to the somata of the H1 subtype (Weiler & Kewitz, 1993).

Horizontal cells of the vertebrate retina show an intense coupling between cells of the same subtype, which is mediated by gap junctions. This allows for a rapid spread of electrical signals throughout the whole horizontal cell network, and represents the basis for the creation of the antagonistic surround component in the receptive fields of bipolar cells. Electrical coupling between horizontal cells is highly plastic, and there is ample evidence for a dopaminergic decoupling of the network in the teleost retina (Negishi & Drujan, 1979; Teranishi *et al.*, 1983, 1984; Mangel & Dowling, 1985, 1987; Shigematsu & Yamada, 1988; Tornqvist *et al.*, 1988; Baldridge & Ball, 1991; Harsanyi & Mangel, 1992), reflecting an adaptation of visual acuity to the ambient light conditions. Furthermore, modulation of the gap junction conductance between horizontal cells can be achieved by other factors which may not be related to the dopaminergic pathway, such as protons (Negishi *et al.*, 1985; DeVries & Schwartz, 1989) or cGMP (DeVries & Schwartz, 1989, 1992; McMahon, 1994). Besides that, the common NO donor nitroprusside causes a decrease in junctional conductivity between pairs of isolated horizontal cells in the catfish (DeVries & Schwartz, 1989) and zebrafish retina (McMahon, 1994). Further evidence for a modulatory role of NO with respect to horizontal cell coupling comes from the turtle retina, where injections of nitroprusside or L-arginine, the biological substrate of NO formation, into H1 horizontal cells lead to electrical decoupling (Miyachi *et al.*, 1990). In the rabbit retina lateral spread of Lucifer Yellow or Neurobiotin among A-type horizontal cells can be limited by intracellular injections of L-arginine (Mills & Massey, 1993).

In this study we examined the effects of NO on horizontal cells in the intact carp retina. The main interest was given to the modulation of the spatial organization of the receptive fields using the amplitude ratio of the cell's responses to annular and small spot illumination as a qualitative measure, and the two-dimensional length constant of the network as a quantitative approach. By studying chromaticity-type as well as H1 horizontal cells we tested whether the influence of NO is restricted to the population of cells which generate NO. Since NO is reported to increase the release of dopamine in some neuronal tissue (Hanbauer *et al.*, 1992; Zhu & Luo, 1992; Lonart *et al.*, 1993) we also tested whether the modulatory action of NO on horizontal cell coupling was related to dopamine release. This was done by examining the direct influence of NO on endogenous retinal dopamine release monitored by HPLC techniques. Finally, we checked for the involvement of NO in the dynamics of horizontal cell spinule formation inside cone pedicles.

MATERIALS AND METHODS

All experiments were performed on carps (*Cyprinus carpio*) of 15–25 cm body length which were kept in an aerated tank under a 12 hr light–dark cycle.

Electrophysiology

Intracellular recordings of light-evoked responses were made from cone horizontal cells using the everted eyecup preparation under constant superfusion. An oxygenated physiological solution (102 mM NaCl, 2.6 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM D-glucose, and 28 mM $NaHCO_3$, bubbled with 95% O_2/5% CO_2, pH 7.4) was delivered with a flow rate of 0.8 ml/min on the top of the everted eyecup and was allowed to run over the preparation into a chamber from which it was removed by means of a vacuum pump. Change of superfusion media was enabled by a system of three-way stopcocks and manifold. Test drugs were dissolved directly in the superfusion solution. Nitric oxide (NO) was generated by sodium nitroprusside (SNP) added to the superfusate immediately before the application. Concentrations of 0.5–2.5 mM SNP were used, providing an estimated NO tissue concentration of approximately 50–250 nM (Southam & Garthwaite, 1991). Effects of ferrocyanide, the byproduct of NO liberation from SNP, were studied by storing the SNP solution for 3 days in the light in order to exhaust NO production (Lei *et al.*, 1992). Dopamine was applied at concentrations of 20–75 μM in Ringer to which 0.1 mM ascorbic acid was added. The membrane-permeant cGMP analogue 8-bromo-cGMP was used at a concentration of 0.5 mM.

Dissection of the eye was done in normal room light, and the preparation was dark-adapted for 20 min in the Faraday cage before the onset of the experiments.

Membrane potentials were recorded using 3 M KCl filled microelectrodes which showed resistances of 80–140 MΩ. A chlorided silver wire which was connected to the superfusion chamber via a bridge of 3 M KCl in 1% agar served as reference electrode. The signal from the microelectrode was amplified, displayed on an oscilloscope screen, monitored on a paper recorder and taped with an FM tape recorder for off-line analysis.

Penetration of a cell was achieved by gentle taps on the recording bench or by briefly overcompensating the capacitance of the electrode. The type of the impaled cell was determined by the shape of the photoresponse and the size of the receptive field. Subtypes of horizontal cells were easily distinguished by their spectral response characteristics.

Photostimulation was achieved by two beams, each originating from a 100 W tungsten–halogen source. Sets of neutral density (50%, 12%, 3% transmission; Zeiss) and narrow-band interference filters (DIL, 11.5 nm full width at half-maximum; Schott) served to control the intensity and spectral content of the beams. The spatial pattern of the stimulus was controlled by sets of circular and annular apertures of different dimensions. Light flashes had a duration of 500 msec and were delivered every 2–4 sec.

Following impalement of a horizontal cell, its light responsiveness was determined by applying full-field

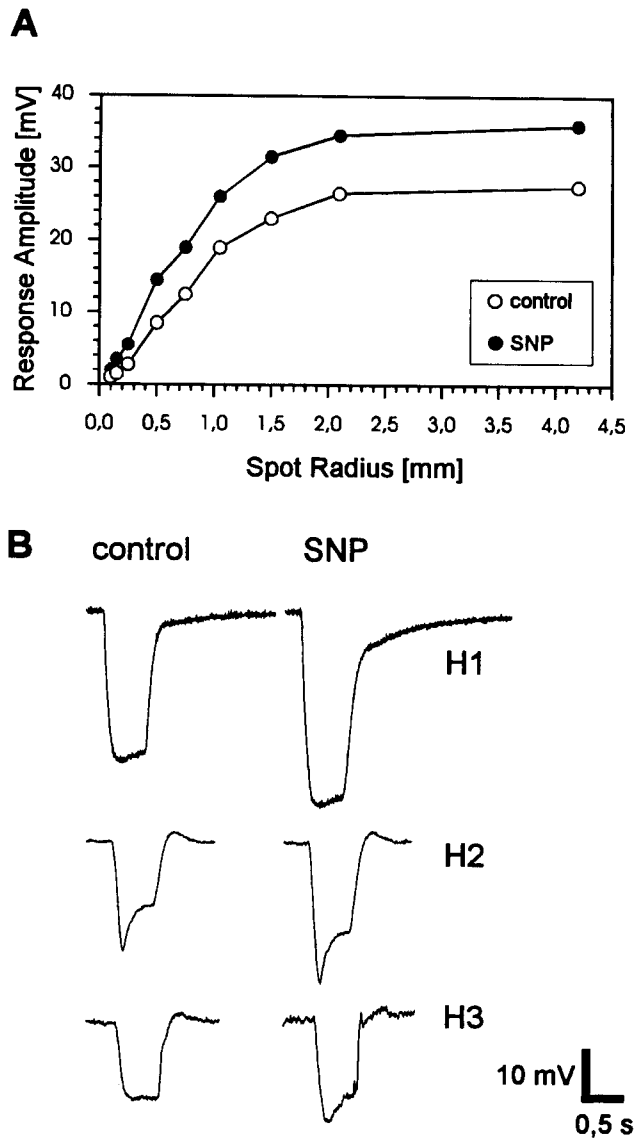

FIGURE 1. Modulation of cone horizontal cell light responses by sodium nitroprusside (SNP). (A) Alterations of response amplitudes of an H1 horizontal cell to spot illumination of different radii resulting from an application of 0.5 mM SNP for 4 min. Traces in (B) showing comparison of full-field responses before and after SNP application for all subtypes of cone horizontal cells. SNP applications were 0.5 mM/4 min for the H1 and 1 mM/5 min in the case of the H2 and H3 subtype. Full-field response amplitudes were enlarged by 31, 28 and 35%, respectively.

stimuli of different intensities. Only cells with a response amplitude of $\geq$30 mV to white light full-field stimuli of supersaturating intensity were used in this study. Furthermore, the cell's responses (V_r) to circular light stimuli of different radii (r) were determined for several intensities of white light, and the length constant (λ) of the horizontal cell network was calculated for each light intensity by fitting these data to an exponential function (Owen & Hare, 1989) using a root mean square minimizing routine:

$$V_r = V_{ff}[1 - (1 + r/\lambda)\exp(-r/\lambda)] \quad (1)$$

V_{ff} corresponds to the cell's response to full-field illumination of a given intensity and was set to unity. The length constant is defined by

$$\lambda = (R_m/R_c)^{\frac{1}{2}} \quad (2)$$

where R_m is the membrane resistance (Ωcm^2) of each cell and R_c is the coupling resistance (Ω) between adjacent horizontal cells (Lamb, 1976).

Additionally, changes in the spatial organization of the receptive field during the drug application were monitored by observing the ratio of responses to annular (0.6 mm inner diameter, 2.2 mm outer diameter) and small spot (0.5 mm diameter) illumination (A/S ratio). For that purpose white light stimuli of either spatial pattern were given intermittently, and the light intensity of both beams was adjusted to generate non-saturating full-field responses of equal amplitudes resulting in an equal light density for both stimuli. A decrease in the A/S ratio indicates a reduction in the receptive field size of the observed horizontal cell.

The effects of NO on the amount of dye-coupling were studied using 4% Lucifer Yellow CH in the recording electrode. Following 20 min of dark adaptation, horizontal cells were impaled and Lucifer Yellow was injected for 3 min by means of rectangular current pulses of -5 nA amplitude and 600 msec duration given every 4 sec. Injections were performed during normal Ringer superfusion and following an application of 1 mM SNP during the last 5 min of the dark adaptation period. After the end of the injection under SNP treatment the eyecups were fixed using 4% paraformaldehyde and 3% sucrose in 0.1 M phosphate buffer pH 7.4 for 1 hr at room temperature and subsequently rinsed in 0.1 M phosphate buffer pH 7.4. Retinas were removed from the eyecups, whole-mounted with the vitreous side up on a glass slide, and examined for injected cells under an epifluorescence microscope.

Dopamine release

Experiments were performed around noon. After 90 min of dark adaptation, the retina was dissected under dim red light and sandwiched between two polymer grids. The sandwich was placed, receptor side up, in a superfusion chamber with a volume of about 1 ml. The two retinas of an animal were used in parallel. One retina was the control and the NO donor was omitted from the superfusion medium. The flow direction of the superfusion media was from the bottom of the chamber to the top and the flow rate was kept constant at 1 ml/min with a peristaltic pump. The chamber was kept in a dark box throughout the experiment. The superfusion media were (i) control Ringer (same as for electrophysiology); (ii) control Ringer with 0.1 mM SNP (freshly prepared for each experiment); (iii) high potassium Ringer (25 mM KCl); (iv) high potassium Ringer with 0.1 mM SNP. In addition, the dopamine uptake blocker benztropine (10 μM) and the antioxidant ascorbic acid (0.1 mM) were added to all the superfusion media. Fractions of the superfusate (500 μl) were collected every 10 min into vials containing 0.1 M EDTA, 0.04 M glutathione, and

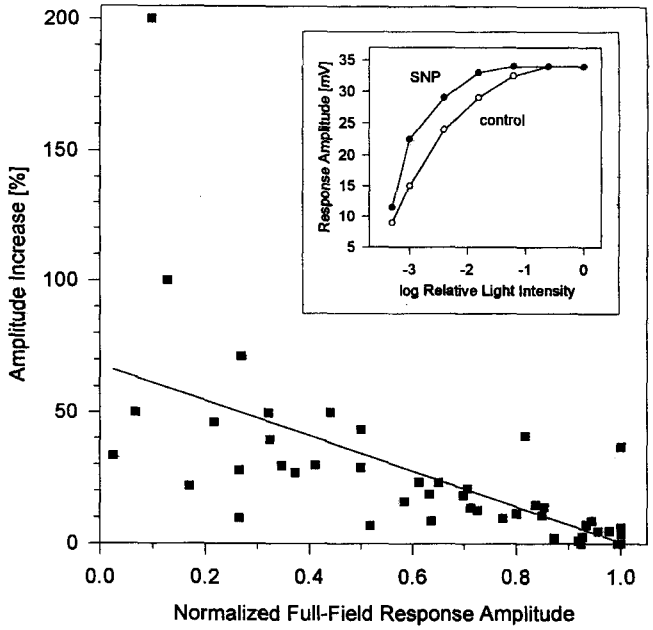

FIGURE 2. Dependence of the SNP-induced increase of full-field response amplitudes on the stimulus intensity. For 10 horizontal cells the per cent increase in amplitude due to SNP treatment (1 mM for 4 min) is plotted against the full-field response amplitudes before the SNP application. Full-field responses were normalized for each cell by its maximal response amplitude. The line through the data points was obtained by linear regression analysis ($r=-0.65$). Inset shows the relationship between the full-field response amplitude and the stimulus light intensity for a H1 horizontal cell before and after SNP treatment.

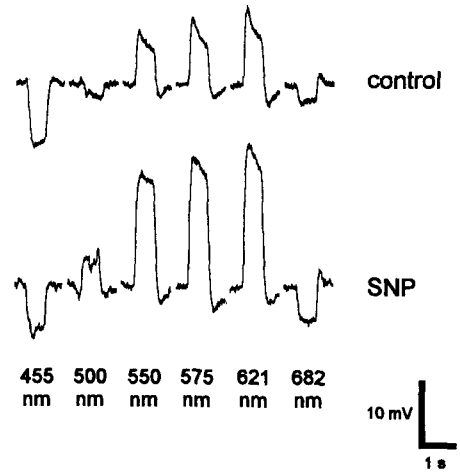

FIGURE 3. Effect of SNP on the spectral responses of H3 horizontal cells. Response amplitudes to full-field stimulation with light above 500 nm wavelength were enlarged following application of 1 mM SNP for 5 min. No increase of the response amplitude to a 455 nm stimulus was observed, whereas the response to a 500 nm stimulus changed polarity.

500 pg of the internal standard 3,4-dihydroxybenzylamine hydrochloride per 500 μl 1 M Tris buffer, pH 8.6. The first fraction was collected after 15 min of equilibration. At the end of the experiment the retina was homogenized and the total dopamine content was determined. Dopamine was analysed using HPLC techniques as described previously (Kolbinger & Weiler, 1993).

Electron microscopy

The formation of spinules was analysed using conventional electron microscopical techniques as described previously (Weiler *et al.*, 1991). Nitroarginine was injected in 10 μl solvent into the vitreous of the right eye of an animal in order to obtain concentrations of 50–500 μM at the retinal level. The left eye received an injection of only the solvent and served as a control. Injections were made into the eyes of dark or light adapted animals. After an initial 15–90 min the animals were kept for an additional 45 min, either in their original state of adaptation or under the opposite condition of adaptation. After this, retinas were quickly dissected and put into the fixative.

RESULTS

The effects of NO were studied in 32 H1, 9 H2 and 2 H3 horizontal cells. The dark membrane potential of these cells was -33.43 ± 1.04 mV (mean $\pm$ SEM; $n = 43$), and application of 1 mM SNP for 4 min caused a slight depolarization of 1.91 ± 0.52 mV ($n = 22$). Recovery of the original potential was achieved within 1–3 min after stopping the SNP application.

Light responsiveness

In all of the cone horizontal cells studied the generation of NO by SNP resulted in a reversible augmentation of the light responsiveness. Figure 1(A) shows the effect of 0.5 mM SNP on the responses of an H1 horizontal cell to spot stimuli of increasing radius. The amplitudes of all photoresponses, including the response to full-field illumination, were increased. The light responsiveness increased for all types of cone horizontal cells, and in a given preparation the average increase was about the same for each type of horizontal cell. For the examples shown in Fig. 1(B) the light responsiveness increased by about 31% for H1, H2, and H3. The augmentation of the light responsiveness could already be observed within a short time span after the onset of the SNP administration. For example, application of 1 mM led to an increase of an H2 horizontal cell's full-field response from 15 to 20 mV within 30 sec.

The increase of the full-field response amplitude was inversely proportional to the original amplitude before the drug application, as shown in Fig. 2. Whereas full-field responses induced by lower light intensities showed an SNP-dependent amplitude increase of about 70% on average, larger response amplitudes could only be minimally increased. This suggests that the reversal potential of the current generated from the photoreceptor input is not affected by SNP.

The above results were obtained using white light as a stimulus, after the cells had been initially characterized as H1, H2 and H3 using red, green and blue light stimuli. Comparing the effects of SNP on the spectral response profile revealed an interesting aspect. Whereas the responsiveness to stimuli of 455–700 nm was more or

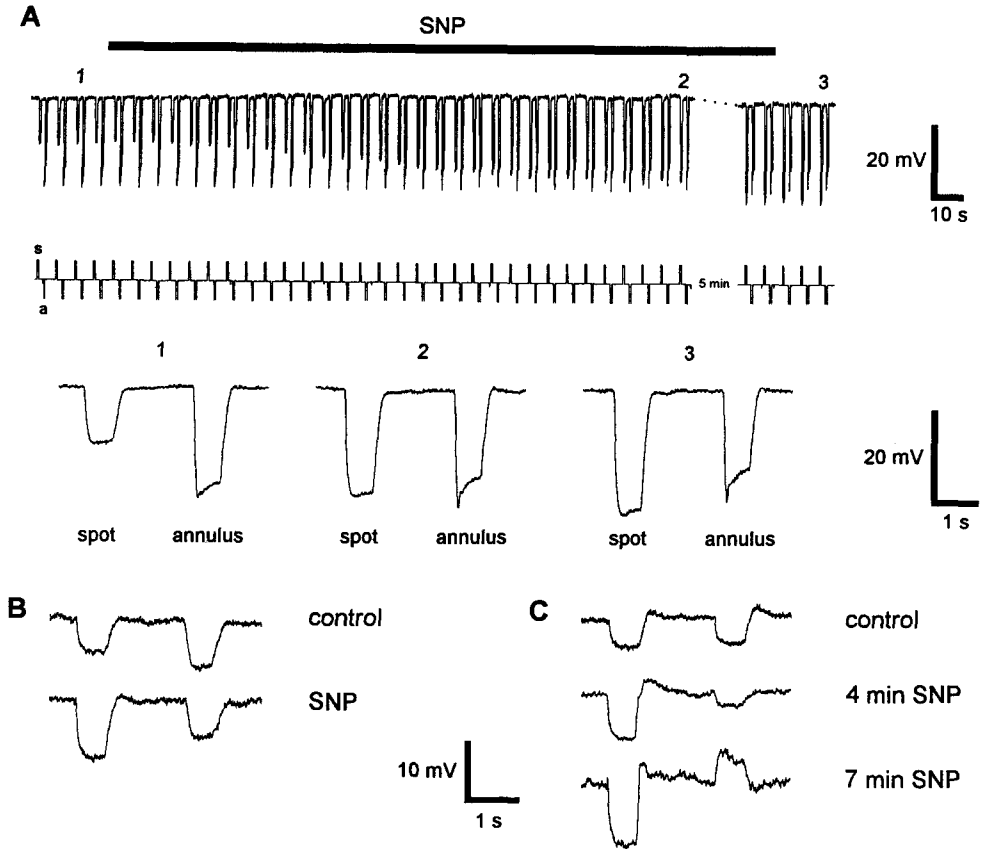

FIGURE 4. Modulation of cone horizontal cell responses to central and peripheral light stimulation. (A) Response amplitudes to small centred spot illumination (s) increased in an H1 horizontal cell during application of SNP (1 mM for 3 min), whereas the response to annular stimulation (a) remained unchanged in amplitude. Following long-term application of SNP the spot response exceeded the annulus response in amplitude. A/S ratio was reduced from 2.0 (1) via 1.09 (2) to 0.88 (3). Similar effects were observed in (B) H2 (0.8 mM for 4 min) and (C) H3 horizontal cells (1 mM for 3 min), where the A/S ratio decreased from 1.29 to 0.55 and from 0.98 to 0.31, respectively. In the H3 horizontal cell long-term application of SNP even changed the polarity of the response to the peripheral stimulus.

less uniformly enhanced in H1 and H2 horizontal cells (not shown), we observed a wavelength-dependent effect on the responsiveness in H3 horizontal cells. As is shown in Fig. 3, the full-field response of an H3 cell to a 455 nm stimulus showed a slight amplitude decrease following SNP treatment. In contrast, depolarizing responses to green (550, 575 nm) and red stimuli (621 nm) as well as the hyperpolarizing response to a 682 nm stimulus were doubled in amplitude. Moreover, the response to a 500 nm full-field stimulus (Fig. 3) reversed polarity, from a hyperpolarization to a depolarization.

Spatial properties

Small spots and annuli were used to analyse the spatial properties. The horizontal cells showed a small hyperpolarization in response to a small centred spot (0.2 mm^2) of white light, whereas annular illumination elicited usually a two- to three-fold larger response, reflecting the larger area of illumination (3.5 mm^2). Following application of SNP a decrease in the ratio of the response amplitudes induced by the two stimulus patterns (A/S) was observed in 16 out of 32 H1 [Fig. 4(A)], six of nine H2 [Fig. 4(B)], and both H3 horizontal cells tested [Fig. 4(C)]. Administration of 1 mM SNP for 4 min resulted in a reduction of the A/S ratio from 2.11 ± 0.22 (mean $\pm$ SEM; $n = 20$) down to 1.58 ± 0.20, which is highly significant ($P < 0.0001$; paired t-test). This decrease in the A/S ratio resulted from a drastic increase of the spot response amplitude in parallel with a reduction of the annulus response amplitude. However, in a substantial number of cells we observed no change in the annular response amplitude or even a slight increase, although the A/S ratio was markedly diminished. Following exposure to SNP the annular response of H3 horizontal cells was initially attenuated and then turned from a hyperpolarization to a depolarization of increasing amplitude [Fig. 4(C)].

The time course of the effect on H1 and H2 cells showed that the duration of the SNP application had to last 2 min in order to obtain a stable effect. Reversibility of the A/S ratio's decrease was unequivocal among the studied cells, but with varied time course (2 to >10 min).

As is already known for the fish and turtle retina, the length constant of the horizontal cell network depends strongly on the intensity of the applied light stimulus (Lamb, 1976; Perlman *et al.*, 1985; Shigematsu & Yamada, 1988; Perlman & Ammermüller, 1994). Probably due to a light-dependent increase of R_m (Perlman &

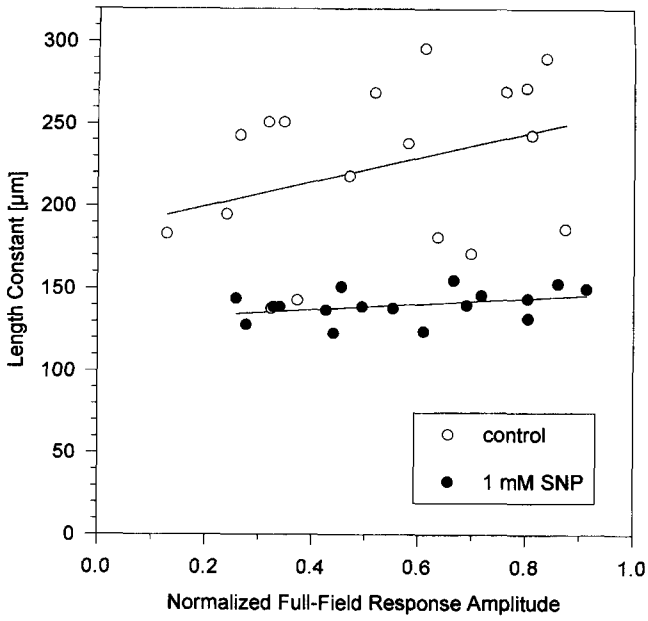

FIGURE 5. Effect of SNP on the two-dimensional length constants of the H1 horizontal cell network. For each cell the length constants were calculated from Eq. (1) for several light intensities. The diagram shows the length constants of five cells in relation to the full-field response amplitudes which were elicited by the different light intensities. Full-field responses were normalized for each cell by its maximal response amplitude. SNP (1 mM for 4 min) substantially decreased the slope of the regression line through the data points.

Ammermüller, 1994), the length constant increases with increasing full-field response amplitude of the horizontal cell used for its determination. Figure 5 shows the relationship between the length constants and the normalized full-field response amplitudes for five H1 horizontal cells of this study. The slope of the regression line through the data points was 73 μm and the extrapolated intercept was 185 μm. Application of 1 mM SNP for 4 min caused a substantial reduction of the length constants leading to a decrease of the regression line's extrapolated intercept down to 130 μm. In addition, the slope of the regression line was flattened to a value of 17 μm. When the effects of SNP on the slope and intercept were tested for five horizontal cells using a paired t-test, only the reduction of the slope was found to be significant ($P < 0.025$).

When Lucifer Yellow was injected into a single H1 horizontal cell soma, the dye could afterwards normally be detected in between six and twelve neighbouring somata of the same morphology (8.43 ± 1.09; $n = 7$). Following an application of 1 mM SNP the spread of dye was restricted to solely the injected cell (1.2 ± 0.2, $n = 5$; Fig. 6), indicating a significant reduction in gap junction permeability ($P < 0.0003$; unpaired t-test).

Horizontal cells which showed no apparent change in receptive field organization following SNP application could, however, be affected by dopamine. As shown in Fig. 7, the A/S ratio of an H1 horizontal cell was not affected by an application of 2.5 mM SNP for 3 min, but decreased by 43% in response to a 2 min administration

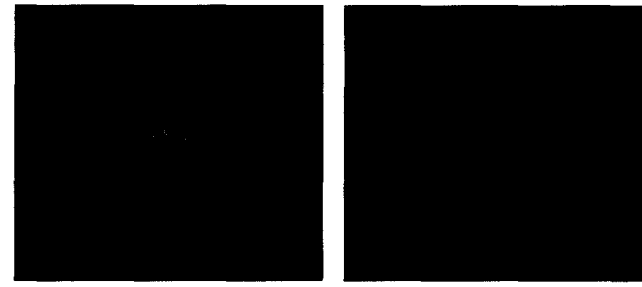

FIGURE 6. Alteration of Lucifer Yellow coupling between horizontal cells by SNP. Lucifer Yellow injected for 3 min in an H1 horizontal cell revealed labelling of several nearby horizontal cell somata (A). The spread of dye was absent when the retina was previously superfused with 1 mM SNP for 5 min (B). Scale bar 20 μm.

of 50 μM dopamine. Dopamine always affected the spot response but not in all instances the annulus response.

Dissociation of SNP in an aqueous solution not only gives rise to NO, but also to ferrocyanide, which is known to inhibit the NMDA receptor channel (East *et al.*, 1991; Kiedrowski *et al.*, 1992; Manzoni *et al.*, 1992a). Therefore, we applied SNP solutions (1 mM) with exhausted NO production in order to check for effects of ferrocyanide. In the cells studied ($n = 8$) we observed no apparent change in the spatial organization of the receptive fields following ferrocyanide administration with respect to both length constants and A/S ratio. Furthermore, no enhancement of the cell's light responsiveness could be detected. Instead, application of ferrocyanide tended to diminish the response amplitude to full-field illumination in all types of cone horizontal cells.

Effects of cGMP

Since NO exerts many of its biological functions by activating the soluble guanylyl cyclase, we studied the effects of the membrane-permeant cGMP analogue 8-bromo-cGMP. Following application of 0.5 mM 8-bromo-cGMP we observed an increase in the H1 horizontal cell's response to a full-field stimulus of about 26% [Fig. 8(A)]. Additionally, the A/S ratio was reduced by about 32% [Fig. 8(B)].

Dopamine release

The effect of SNP on dopamine release was monitored using a superfused retina preparation. Basal dopamine release increased about seven times following depolarization with high potassium Ringer [Fig. 9(A)]. Dopamine release is given as percentage release of the total dopamine content present at the beginning of the corresponding fraction. The values of two successive fractions for each superfusion medium were pooled for Fig. 9. The potassium-induced increase of dopamine release was approx. halved if SNP was added to the superfusion medium. No decrease in dopamine release was ever seen in the control eye [Fig. 9(B)]. The striking decrease in dopamine release in SNP was not due to any detrimental effect of SNP on the retina or on dopaminergic neurons, since high potassium Ringer caused a

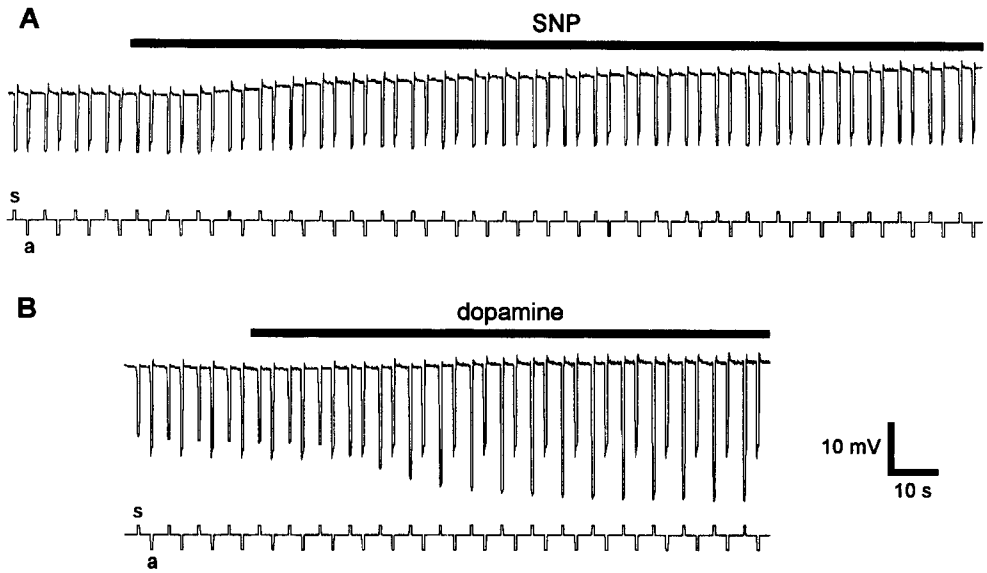

FIGURE 7. Differential effects of SNP and dopamine on central and peripheral illumination. (A) The H1 horizontal cell showed an enhancement of response amplitudes to stimulation with a centred spot (s) and an annulus (a) during administration of 2.5 mM SNP (3 min) but experienced no alteration of the A/S ratio. (B) 50 μM dopamine decreased the A/S ratio from 1.2 to 0.73.

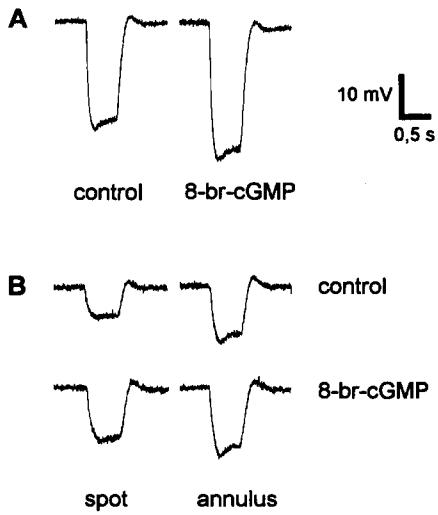

FIGURE 8. Alteration of horizontal cell light responses by 8-bromo-cGMP. Light responses of an H1 horizontal cell to full-field stimulation (A) and to centred spot and annular illumination (B) increased following application of 0.5 mM 8-bromo-cGMP for 7 min. A/S ratio decreased from 1.91 to 1.3.

greater increase in dopamine release after SNP was washed out. The recovery was not complete and the standard deviations of the following fractions increased, most likely due to variations in the wash-out of SNP. The basal release of dopamine was not significantly affected by NO [Fig. 9(C)]. In general there was a slight but insignificant increase. The effects of SNP were only present with freshly prepared solutions.

Spinule dynamics

Spinules are dynamic morphological alterations within the cone–horizontal cell synaptic complex that relate to physiological changes of the horizontal cell light responses (Weiler & Wagner, 1984). The number of spinules per synaptic ribbon within a cone pedicle, which is a convenient parameter for spinule expression (Weiler & Wagner, 1984) remained unaffected by inhibition of endogenous NO synthesis by nitroarginine in the *in vivo* experiment. Nitroarginine (100 μM) did not affect the SPR value of light and dark adapted retinas. When injected 15 or 90 min prior to a change of the adaptation situation, it was also without effect on the SPR value (Fig. 10), making it unlikely that NO is involved in the formation or retraction of spinules.

DISCUSSION

The presence of nNOS in H1 horizontal cells is known from the carp retina (Weiler & Kewitz, 1993). Therefore, we examined the physiological implications of NO in this tissue with respect to the horizontal cells. Two apparent effects of exogenous NO have been described in this study, that is an enhancement of the horizontal cell's light responsiveness and an uncoupling of the horizontal cell network. These effects were not only observed in H1 horizontal cells, but in all types of cone horizontal cells, and could be mimicked by an analogue of cGMP.

Light responsiveness

The light responsiveness of the horizontal cells was measured by illuminating the whole retina. This leads to an equal hyperpolarization of all cells in the network. For that reason, the coupling state of the network does not interfere with the cell's light response, since no ionic leakage takes place through the gap junctions (Usui *et al.*, 1983). The observed increase of the light responsiveness by NO reflects changes in the process of synaptic transmission from the photoreceptors to the horizontal cells, which involves glutamate receptors and/or a

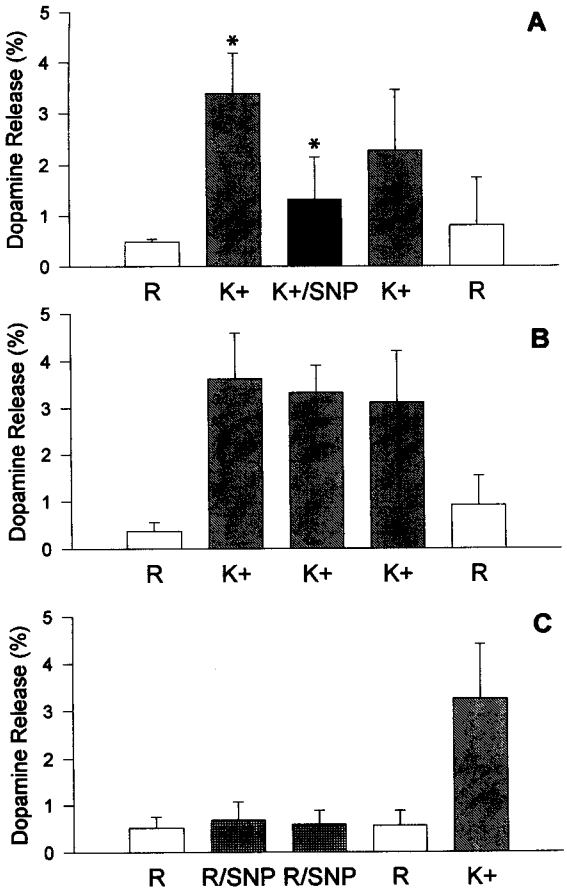

FIGURE 9. Effect of SNP on the retinal dopamine release. (A) K^+-stimulated dopamine release was significantly (*: $P < 0.005$, paired t-test) reduced following application of 0.1 mM SNP (K^+/SNP), whereas no time-correlated decrease in release could be observed under control conditions (B). (C) Basal dopamine release was not significantly affected by 0.1 mM SNP (R/SNP). For each experiment three retinas were used.

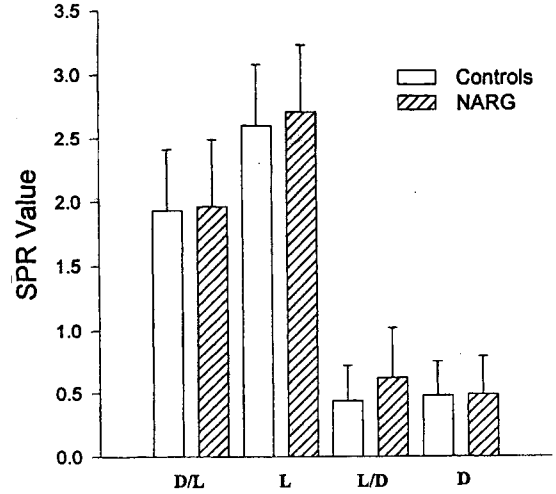

FIGURE 10. Nitric oxide does not affect spinule dynamics within the cone–horizontal cell synaptic complex. The inhibition of endogenous NO synthesis by nitroarginine (NARG, 0.1 mM) had no effect on the number of spinules per ribbon under constant light conditions (L: light, D: dark) and during adaptation from dark to light (D/L) or light to dark (L/D). Each column includes data from three retinas.

modification of non-synaptic currents in the horizontal cell itself. However, it is not clear whether the NO-elicited increase in the horizontal cell responsiveness is due to an effect on glutamate receptors. Direct NO-induced inhibition of NMDA receptors has been described in brain tissue (Hoyt *et al.*, 1992; Lei *et al.*, 1992; Manzoni *et al.*, 1992b; Manzoni & Bockaert, 1993) and cultivated neurons of the rat retina (Ujihara *et al.*, 1993), but there is no evidence for the presence of NMDA receptors in carp horizontal cells (Lasater & Dowling, 1982; Lasater, 1991). Of the glutamate receptor types which are located in carp horizontal cells, only the AMPA-receptor is reported to be desensitized by NO. This effect is mediated via cGMP and takes place in cerebral Purkinje cells, where it may play a role with respect to long-term depression (Ito & Karachot, 1990). However, a desensitization of the horizontal cell's receptor molecules must lead to a tonic hyperpolarization of the dark membrane potential in these cells, which was not the case in our experiments. Further action of NO on ion currents is known in the case of the Ca^{2+}-gated K^+ channel which can be directly activated by NO (Bolotina *et al.*, 1994). Since such a current was not detected in isolated horizontal cells of the goldfish retina (Tachibana, 1983), it is unlikely that such an NO effect can explain the present results. Instead, the enhancement of the horizontal cell's light responsiveness may reflect an action of the administered NO on the photoreceptors. In fact, nitroprusside is reported to increase cGMP concentration in rods isolated from the frog retina, leading to a slight depolarization of the dark membrane potential and an acceleration of the recovery from light responses (Schmidt *et al.*, 1992). The opposite effects are obtained by an application of NOS inhibitors (Nöll *et al.*, 1994). However, these effects may not account for an increase of the horizontal cell's light responsiveness and moreover have not been seen in cone photoreceptors. Thus, enhancing the light responsiveness of cone horizontal cells without causing a tonic hyperpolarization may only be achieved by an enhancement of one of the steps leading from phototransduction to the regulation of transmitter release. Curiously, NO was able to depolarize the dark membrane potential in some of the horizontal cells, in addition to augmenting the light responsiveness. Whereas these two effects are contrary to each other, a plausible explanation for the tonic change arises from the findings of Rieke and Schwartz (1994), who discovered a cGMP-activated Ca^{2+}-channel in the pedicles and inner segments of the cones. Current through this channel is related to transmitter release and can be enhanced by nitroprusside. Therefore, application of NO may cause an increase in intracellular concentration of cGMP sufficient to cause an increase in transmitter release in the dark, which could then result in a depolarization of horizontal cells.

Further evidence for the notion of an NO action on photoreceptors arises from the spectral responses of H3 horizontal cells. In these cells the light responsiveness was enhanced for green and red illumination, but slightly

decreased for blue stimulus light. Since the responses to all colour stimuli were comparably modulated in H1 and H2 horizontal cells, which receive no input from blue-sensitive cones, we suggest a differential effect of NO on cone photoreceptors. Whereas red- and green-sensitive cones are affected by SNP, blue-sensitive cones are not. Blue-sensitive cones are reported to differ from the other cone subtypes relative to their biochemical machinery and share many features with the rod photoreceptors (Müller *et al.*, 1989; Hamilton & Hurley, 1990; Nork *et al.*, 1990; Petry & Murphy, 1995). This is consistent with our preliminary observation that SNP caused a slight diminution of the light responsiveness of rod horizontal cells (not shown).

Spatial properties

In this study most of the cells showed an increase in the amplitudes of responses to both peripheral and central illumination, which points to an NO-induced enhancement of the light responsiveness. Nevertheless, due to an overproportional increase of the small spot response amplitude, half of these horizontal cells showed a reduction in the A/S ratio of up to about 30%.

Nitroprusside affected the relationship between the length constants and the full-field response amplitudes by reducing the slope of the regression line, which is determined exclusively by the coupling conductance (Perlman & Ammermüller, 1994). Therefore, our results indicate an NO-induced decrease in the conductance of the horizontal cell gap junctions. This idea is supported by our findings of an NO-induced reduction in the amount of dye-coupling.

The uncoupling of the horizontal cell syncytium by NO observed in this study is consistent with previous reports. Thus, the junctional conductance is substantially decreased by nitroprusside in isolated horizontal cell pairs from the catfish (DeVries & Schwartz, 1989) and zebrafish retina (McMahon, 1994). Likewise, the conductivity of gap junction hemi-channels is reduced (DeVries & Schwartz, 1992). In zebrafish horizontal cells this conductance decrease is achieved by a reduction in the frequency of the hemi-channel openings (McMahon, 1994). Reduction of gap junction conductance is also reported in H1 horizontal cells in the intact turtle retina using intracellular injections of nitroprusside or L-arginine (Miyachi *et al.*, 1990). Since these effects can be mimicked by cGMP or its unhydrolysable analogues (DeVries & Schwartz, 1989; Miyachi *et al.*, 1990; McMahon, 1994), activation of soluble guanylyl cyclase may be responsible for the mediation of the NO function. Following application of 8-bromo-cGMP, we also observed changes in the receptive field size of carp H1 horizontal cells, providing further evidence for that idea. A direct connection between NO and cGMP is known in the rabbit retina, where incubation with nitroprusside leads to an increased content of cGMP in both plexiform layers, cone ON-bipolar cells, and A-type horizontal cells detected by immunocytochemistry (Massey *et al.*, 1993). In addition, these horizontal cells show a restriction in dye coupling following an intracellular injection of L-arginine (Mills & Massey, 1993). Hence, an NO-action via the cGMP-pathway in horizontal cells is very likely.

Apparent uncoupling was only found in half of the cells in this study, although an enhancement of the light responsiveness could be observed in all cells, indicating a contact with NO. Since regulation of gap junction properties in horizontal cell axon terminals seems to be different from that of the horizontal cell somata (Hida *et al.*, 1984; Baldridge *et al.*, 1987), cells with an NO-insensitive receptive field size may be axon terminals, which probably lack guanylyl cyclase.

Previous studies have demonstrated the potency of the common NO-donor nitroprusside in reducing the conductance of horizontal cell gap junctions (DeVries & Schwartz, 1989; McMahon, 1994). However, no attention was given to the possible effects of ferrocyanide, the byproduct of NO liberation from nitroprusside. Since ferrocyanide is reported to partially mediate the effects of nitroprusside on NMDA receptors (East *et al.*, 1991; Kiedrowski *et al.*, 1992; Manzoni *et al.*, 1992a), examination of its role in retinal physiology was performed in the present study by using a solution of nitroprusside with exhausted NO production. Our finding that ferrocyanide did not affect the receptive field size of horizontal cells provides a parallel to the fact that stimulation of endogenous NO synthesis via application of L-arginine results in a diminished gap junctional conductance (Miyachi *et al.*, 1990). Furthermore, 8-bromo-cGMP was able to mimic the effects of SNP in our study, providing more evidence for the notion that the effects of SNP were not due to the liberation of ferrocyanide. According to our observations ferrocyanide seems to decrease the light responsiveness of horizontal cells. This could, for instance, be performed by a direct interaction with a glutamate receptor. At any rate, this can be considered merely a weak effect, since it was readily overcompensated by the NO-action when a fresh solution of nitroprusside was used.

Dopamine release

Dopamine is the major uncoupling agent for the horizontal cell network in the vertebrate retina (Dowling, 1991; Witkovsky & Dearry, 1991). Since NO also modulates the coupling state of the horizontal cell syncytium and additionally stimulates the release and inhibits the uptake of dopamine in the corpus striatum (Hanbauer *et al.*, 1992; Lonart *et al.*, 1993; Zhu & Luo, 1992; Pogun *et al.*, 1994), the observed uncoupling effect of NO might arise from a modulation of retinal dopamine release. However, the increase in light responsiveness of cone horizontal cells is not consistent with a sensitization of glutamate receptors by dopamine (Knapp & Dowling, 1987; Knapp *et al.*, 1990; Schmidt *et al.*, 1994). Additionally, we observed a dopamine-induced uncoupling of some horizontal cells, in cases where NO did not influence gap junction conductance. Previous reports also suggest an independence of dopamine from NO effects. In preparations like isolated horizontal cell pairs, where

effects of endogenous dopamine are excluded, nitroprusside increases the coupling resistance (DeVries & Schwartz, 1989; McMahon, 1994). Single channel recordings from zebrafish horizontal cells reveal an NO-induced reduction only in the frequency of gap junction channel openings, whereas dopamine is known to decrease the average channel open time as well (McMahon, 1994).

The findings from the dopamine release experiments almost exclude the possibility that the effects of NO on the coupling between horizontal cells are mediated through dopamine. Our results demonstrate that NO does not increase dopamine release in the carp retina. On the contrary, potassium-induced release was significantly decreased and basal release was not significantly affected. These observations are in agreement with two recent reports showing that NO decreases potassium-induced release in the bovine and rabbit retina (Bugnon *et al.*, 1994; Djamgoz *et al.*, 1995).

Spinule dynamics

The presence of nNOS in the dendritic terminals of teleost horizontal cells (Östholm *et al.*, 1994) proposes a significance of NO for the photoreceptor to horizontal cell synapse. Spinules are prominent ultrastructural features within the synaptic complex between cones and horizontal cells that are governed by the ambient light conditions and correlate with physiological parameters of the horizontal cell light responses (Weiler & Wagner, 1984). Their formation is mediated by activation of protein kinase C and their retraction is mediated by glutamate and subsequent activation of CaMKII (Weiler *et al.*, 1991; Weiler & Schultz, 1993; Weiler *et al.*, 1995). Since spinules are plastic and affect the light responses of horizontal cells, it was of interest to analyse a possible effect of NO on spinules. The findings of the present study demonstrate that in the *in vivo* situation, NO does not affect the persistence, formation or retraction of spinules. This result is partially at variance with a recent finding in the roach retina, where NO induced the formation of spinules in the *in vitro* situation (Greenstreet & Djamgoz, 1994). In those experiments isolated retinas from animals under mesopic conditions were placed on a drop of Ringer solution containing SNP and an increase of spinules per synaptic ribbon was noticed. A similar discrepancy between the *in vivo* and *in vitro* situation was found for the action of cAMP. Whereas cAMP slightly increases the number of spinules in an isolated retina (Behrens *et al.*, 1992) it does not have this effect in the *in vivo* preparation (Kohler & Weiler, 1990). Spinule dynamics are under efferent control (Behrens *et al.*, 1993) and it appears that loss of this control favours the formation of spinules following drug treatment. In addition, the stimulating effect of NO on cGMP is Ca^{2+}/CaMKII-dependent and it has recently been shown that activation of CaMKII triggers the retraction of spinules (Weiler *et al.*, 1995), making it further unlikely that NO is involved in the formation of spinules.

REFERENCES

Baldridge, W. H. & Ball, A. K. (1991). Background illumination reduces horizontal cell receptive-field size in both normal and 6-hydroxydopamine-lesioned goldfish retinas. *Visual Neuroscience, 7*, 441–450.

Baldridge, W. H., Ball, A. K. & Miller, R. G. (1987). Dopaminergic regulation of horizontal cell gap junction particle density in goldfish retina. *Journal of Comparative Neurology, 265*, 428–436.

Baldridge, W. H., Jamieson, M. S. & Ball, A. K. (1993). Role of nitric oxide in the control of horizontal cell receptive-field size. *Investigative Ophthalmology and Visual Science, 34* Suppl., 1382.

Behrens, U. D., Douglas, R. H. & Wagner, H.-J. (1993). Gonadotropin-releasing hormone, a neuropeptide of efferent projections to the teleost retina induces light-adaptive spinule formation on horizontal cell dendrites in dark-adapted preparations kept *in vitro*. *Neuroscience Letters, 164*, 59–62.

Behrens, U. D., Wagner, H.-J. & Kirsch, M. (1992). cAMP-Mediated second messenger mechanisms are involved in spinule formation in teleost cone horizontal cells. *Neuroscience Letters, 147*, 93–96.

Bolotina, V. M., Najibi, S., Palacino, J. J., Pagano, P. J. & Cohen, A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature, 368*, 850–853.

Brune, B. & Lapetina, E. G. (1989). Activation of cytosolic ADP-ribosyltransferase by nitric oxide generating agents. *Journal of Biological Chemistry, 264*, 8455–8458.

Bugnon, O., Schaad, N. C. & Schorderet, M. (1994). Nitric oxide modulates endogenous dopamine release in bovine retina. *NeuroReport, 5*, 401–404.

DeVries, S. H. & Schwartz, E. A. (1989). Modulation of an electrical synapse between solitary pairs of catfish horizontal cells by dopamine and second messengers. *Journal of Physiology, 414*, 351–375.

DeVries, S. H. & Schwartz, E. A. (1992). Hemi-gap-junction channels in solitary horizontal cells of the catfish retina. *Journal of Physiology, 445*, 201–230.

Djamgoz, M. B. A., Cunningham, J. R., Davenport, S. L. & Neal, M. J. (1995). Nitric oxide inhibits depolarization-induced release of endogenous dopamine in the rabbit retina. *Neuroscience Letters, 198*, 33–36.

Dowling, J. E. (1991). Retinal neuromodulation: the role of dopamine. *Visual Neuroscience, 7*, 87–97.

East, S. J., Batchelor, A. M. & Garthwaite, J. (1991). Selective blockade of NMDA receptor function by the nitric oxide donor nitroprusside. *European Journal of Pharmacology, 209*, 119–121.

Greenstreet, E. H. & Djamgoz, M. B. A. (1994). Nitric oxide induces light-adaptive morphological changes in retinal neurones. *NeuroReport, 6*, 109–112.

Hamilton, S. E. & Hurley, J. B. (1990). A phosphodiesterase inhibitor specific to a subset of bovine retinal cones. *Journal of Biological Chemistry, 265*, 11259–11264.

Hanbauer, I., Wink, D., Osawa, Y., Edelman, G. M. & Gally, J. A. (1992). Role of nitric oxide in NMDA-evoked release of tritiated dopamine from striatal slices. *NeuroReport, 3*, 409–412.

Harsanyi, K. & Mangel, S. C. (1992). Activation of a D_2 receptor increases electrical coupling between retinal horizontal cells by inhibiting dopamine release. *Proceedings of the National Academy of Sciences USA, 89*, 9220–9224.

Hida, E., Negishi, K. & Naka, K.-I. (1984). Effects of dopamine on photopic L-type S-potentials in the catfish retina. *Journal of Neuroscience Research, 11*, 373–382.

Hoyt, K. R., Liang-Hong, T., Aizenman, E. & Reynolds, I. J. (1992). Nitric oxide modulates NMDA-induced increases in intracellular Ca^{2+} in cultured rat forebrain neurons. *Brain Research, 592*, 310–316.

Ito, M. & Karachot, L. (1990). Messengers mediating long-term desensitization in cerebellar Purkinje cells. *NeuroReport, 1*, 129–132.

Kiedrowski, L., Costa, E. & Wroblewski, T. (1992). Sodium nitroprusside inhibits NMDA-evoked calcium influx via nitric oxide- and cGMP-independent mechanism. *Molecular Pharmacology, 41*, 779–784.

Knapp, A. G. & Dowling, J. E. (1987). Dopamine enhances excitatory amino acid-gated conductances in cultured retinal horizontal cells. *Nature, 325*, 437–439.

Knapp, A. G., Schmidt, K.-F. & Dowling, J. E. (1990). Dopamine modulates the kinetics of ion channels gated by excitatory amino acids in retinal horizontal cells. *Proceedings of the National Academy of Sciences USA, 87*, 767–771.

Kohler, K. & Weiler, R. (1990). Dopaminergic modulation of transient neurite outgrowth from horizontal cells of the fish retina is not mediated by cAMP. *European Journal of Neuroscience, 2*, 788–794.

Kolbinger, W. & Weiler, R. (1993). Modulation of endogenous dopamine release in the turtle retina. Effects of light, calcium and neurotransmitters. *Visual Neuroscience, 10*, 1035–1041.

Lamb, T. D. (1976). Spatial properties of horizontal cell responses in the turtle retina. *Journal of Physiology, 263*, 239–255.

Lasater, E. M. (1991). Membrane properties of distal retinal neurons. *Progress in Retinal Research, 11*, 215–246.

Lasater, E. M. & Dowling, J. E. (1982). Carp horizontal cells in culture respond selectively to L-glutamate and its agonists. *Proceedings of the National Academy of Sciences USA, 79*, 936–940.

Lei, S. Z., Pan, Z.-H., Aggarwal, S. K., Chen, H.-S. V., Hartmann, J., Sucher, N. J., Lipton, S. A. (1992). Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron, 8*, 1087–1099.

Liepe, B. A., Stone, C., Koistinaho, J. & Copenhagen, D. R. (1994). Nitric oxide synthase in Müller cells and neurons of salamander and fish retina. *Journal of Neuroscience, 14*, 7641–7654.

Lonart, G., Cassels, K. L. & Johnson, K. M. (1993). Nitric oxide induces calcium-dependent [^{3}H]dopamine release from striatal slices. *Journal of Neuroscience Research, 35*, 192–198.

Mangel, S. C. & Dowling, J. E. (1985). Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. *Science, 229*, 1107–1109.

Mangel, S. C. & Dowling, J. E. (1987). The interplexiform-horizontal cell system of the fish retina: effects of dopamine, light stimulation and time in the dark. *Proceedings of the Royal Society London, B231*, 91–121.

Manzoni, O. & Bockaert, J. (1993). Nitric oxide synthase activity endogenously modulates NMDA receptors. *Journal of Neurochemistry, 61*, 368–370.

Manzoni, O., Prezeau, L., Desagher, S., Sahuquet, A., Sladeczek, F., Bockaert, J. & Fagni, L. (1992a) Sodium nitroprusside blocks NMDA receptors via formation of ferrocyanide ions. *NeuroReport, 3*, 77–80.

Manzoni, O., Prezeau, L., Marin, P., Deshager, S., Bockaert, J. & Fagni, L. (1992b) Nitric oxide-induced blockade of NMDA receptors. *Neuron, 8*, 653–662.

Massey, S. C., Mills, S. L. & de Vente, J. (1993). Nitric oxide: identification of target cells in the rabbit retina. *Investigative Ophthalmology and Visual Science, 34* Suppl., 1382.

McMahon, D. G. (1994). Modulation of zebrafish horizontal cell electrical synapses by nitroprusside and cGMP. *Investigative Ophthalmology and Visual Science, 35* Suppl., 1821.

Mills, S. L. & Massey, S. C. (1993). L-Arginine uncouples A-type horizontal cells in rabbit retina. *Investigative Ophthalmology and Visual Science, 34* Suppl., 1382.

Miyachi, E.-I., Murakami, M. & Nakaki, T. (1990). Arginine blocks gap junctions between retinal horizontal cells. *NeuroReport, 1*, 107–110.

Müller, B., Peichl, L., De Grip, W., Gery, I. & Korf, H.-W. (1989). Opsin- and S-antigen-like immunoreactions in photoreceptors of the tree shrew retina. *Investigative Ophthalmology and Visual Science, 30*, 530–535.

Negishi, K. & Drujan, B. D. (1979). Reciprocal changes in center and surround S-potentials of fish retina in response to dopamine. *Neurochemical Research, 4*, 313–318.

Negishi, K., Teranishi, T. & Kato, S. (1985). Opposite effects of ammonia and carbon dioxide on dye coupling between horizontal cells in the carp retina. *Brain Research, 342*, 330–339.

Nöll, G. N., Billek, M., Pietruck, C. & Schmidt, K.-F. (1994). Inhibition of nitric oxide synthase alters light responses and dark voltage of amphibian photoreceptors. *Neuropharmacology, 33*, 1407–1412.

Nork, T. M., McCormick, S. A., Chao, G.-M. & Odom, J. V. (1990). Distribution of carbonic anhydrase among human photoreceptors. *Investigative Ophthalmology and Visual Science, 31*, 1451–1458.

Östholm, T., Holmqvist, B. I., Alm, P. & Ekström, P. (1994). Nitric oxide synthase in the CNS of the Atlantic salmon. *Neuroscience Letters, 168*, 233–237.

Owen, W. G. & Hare, W. (1989). Signal transfer from photoreceptors to bipolar cells in the retina of the tiger salamander. *Neuroscience Research (Suppl.), 10*, 77–88.

Perlman, I. & Ammermüller, J. (1994). Receptive-field size of L1 horizontal cells in the turtle retina: effects of dopamine and background light. *Journal of Neurophysiology, 72*, 2786–2795.

Perlman, I., Normann, R. A., Itzhaki, A. & Daly, S. J. (1985). Chromatic and spatial information processing by red cones and L-type horizontal cells in the turtle retina. *Vision Research, 25*, 543–549.

Petry, H. M. & Murphy, H. A. (1995). Differentiation of short-wavelength-sensitive cones by NADPH diaphorase histochemistry. *Proceedings of the National Academy of Sciences USA, 92*, 5121–5123.

Pogun, S., Baumann, M. H. & Kuhar, M. J. (1994). Nitric oxide inhibits [^{3}H]dopamine uptake. *Brain Research, 641*, 83–91.

Rieke, F. & Schwartz, E. A. (1994). A cGMP gated curent can control exocytosis at cone synapses. *Neuron, 13*, 863–873.

Schmidt, K.-F., Kruse, M. & Hatt, H. (1994). Dopamine alters glutamate receptor desensitization in retinal horizontal cells of the perch (*Perca fluviatilis*). *Proceedings of the National Academy of Sciences USA, 91*, 8288–8291.

Schmidt, K.-F., Nöll, G. N. & Yamamoto, Y. (1992). Sodium nitroprusside alters dark voltage and light responses in isolated retinal rods during the whole-cell recording. *Visual Neuroscience, 9*, 205–209.

Schuman, E. M. & Madison, D. V. (1994). Nitric oxide and synaptic function. *Annual Review of Neuroscience, 17*, 153–183.

Shigematsu, Y. & Yamada, M. (1988). Effects of dopamine on spatial properties of horizontal cell responses in the carp retina. *Neuroscience Research (Suppl.), 8*, 69–80.

Southam, E. & Garthwaite, J. (1991). Comparative effects of some nitric oxide donors on cyclic GMP levels in rat cerebellar slices. *Neuroscience Letters, 130*, 107–111.

Tachibana, M. (1983). Ionic currents of solitary horizontal cells isolated from goldfish retina. *Journal of Physiology, 345*, 329–351.

Teranishi, T., Negishi, K. & Kato, S. (1983). Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina. *Nature, 301*, 243–246.

Teranishi, T., Negishi, K. & Kato, S. (1984). Regulatory effect of dopamine on spatial properties of horizontal cells in carp retina. *Journal of Neuroscience, 4*, 1271–1280.

Tornqvist, K., Yang, Y.-L. & Dowling, J. E. (1988). Modulation of cone horizontal cell activity in the teleost fish retina. III. Effects of prolonged darkness and dopamine on electrical coupling between horizontal cells. *Journal of Neuroscience, 8*, 2279–2288.

Ujihara, H., Akaike, A., Tamura, Y., Yokota, T., Sasa, M., Kashii, S. & Honda, Y. (1993). Blockade of retinal NMDA receptors by sodium nitroprusside is probably due to nitric oxide formation. *Japanese Journal of Pharmacology, 61*, 375–377.

Usui, S., Mitarai, G. & Sakakibara, M. (1983). Discrete nonlinear reduction model for horizontal cell response in the carp retina. *Vision Research, 23*, 413–420.

Vincent, S. R. (1994). Nitric oxide: a radical neurotransmitter in the central nervous system. *Progress in Neurobiology, 42*, 129–160.

Weiler, R. & Kewitz, B. (1993). The marker for nitric oxide synthase, NADPH-diaphorase, co-localizes with GABA in horizontal cells and cells of the inner retina in the carp retina. *Neuroscience Letters, 158*, 151–154.

Weiler, R., Kohler, K. & Janssen, U. (1991). Protein kinase C mediates transient spinule-type neurite outgrowth in the retina during light adaptation. *Proceedings of the National Academy of Sciences USA, 88*, 3603–3607.

Weiler, R. & Schultz, K. (1993). Ionotropic non-N-methyl-D-aspartate agonists induce retraction of dendritic spinules from retinal horizontal cells. *Proceedings of the National Academy of Sciences USA, 90*, 6533–6537.

Weiler, R., Schultz, K. & Janssen-Bienhold, U. (1995). Retraction of spinule-type neurites from carp retinal horizontal cell dendrites during dark adaptation involves the activation of Ca^{2+}/calmodulin-dependent protein kinase II. *European Journal of Neuroscience, 7*, 1914–1919.

Weiler, R. & Wagner, H.-J. (1984). Light-dependent change of cone–horizontal cell interactions in carp retina. *Brain Research, 298*, 1–9.

Witkovsky, P. & Dearry, A. (1991). Functional roles of dopamine in the vertebrate retina. *Progress in Retinal Research, 11*, 247–292.

Zhu, X.-Z. & Luo, L.-G. (1992). Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. *Journal of Neurochemistry, 59*, 932–935.

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