NITRIC OXIDE AND CYCLIC GMP MODULATE PHOTORECEPTOR CELL RESPONSES IN THE VISUAL SYSTEM OF THE LOCUST

OLIVER SCHMACHTENBERG AND GERD BICKER*

School of Veterinary Medicine Hannover, Cell Biology, Bünteweg 17d, D-30559 Hannover, Germany

*Author for correspondence and present address: Tierärztliche Hochschule Hannover, Institut für Tierökologie und Zellbiologie, Bünteweg 17d, D-30559 Hannover, Germany (e-mail: gbicker@zellbiologie.tiho-hannover.de)

Summary

Nitric oxide (NO) is a membrane-permeant messenger molecule which activates the cyclic GMP (cGMP)-synthesizing enzyme soluble guanylyl cyclase. Using cytochemical techniques, we recently reported NO-induced cGMP immunoreactivity in the photoreceptor cells of the compound eye of the locust *Schistocerca gregaria* and also detected NADPH diaphorase staining, a marker of NO synthase, in a subset of the monopolar cells of the lamina. By recording the corneal electroretinogram (ERG), we found that the application of neurochemicals that raise NO/cGMP levels in the optic lobe increased the ERG amplitude, whereas the experimental reduction of NO levels caused a decrease in the response to light. An increase in the light response was also found in intracellular recordings after application of a NO donor, suggesting that the NO-induced changes in the ERG are not caused by changes in the resistive isolation of the retina. Our cytochemical and electrophysiological data are both consistent with the hypothesis that NO synthesized in monopolar cells is a retrograde messenger to the presynaptic photoreceptor neurones.

Key words: nitric oxide synthase, soluble guanylyl cyclase, phototransduction, monopolar cell, electroretinogram, retrograde messenger, locust, *Schistocerca gregaria*, *Locusta migratoria*.

Introduction

Nitric oxide (NO) is a membrane-permeant messenger activating the cGMP-synthesizing enzyme soluble guanylyl cyclase (sGC) (for reviews, see Bredt and Snyder, 1992; Garthwaite and Boulton, 1995). In the nervous system, NO is produced in an activity-dependent process by Ca^{2+}/calmodulin-stimulated NO synthases (NOSs). Since NO is thought to diffuse from its site of production through cell membranes to its target receptor, it functions as a rather unconventional transmitter in the vertebrate brain. NO-mediated signalling is also found in many invertebrates (Elofsson et al., 1993), for example in insect nervous systems (Elphick et al., 1993; Müller and Buchner, 1993; Müller, 1994). In *Drosophila melanogaster*, the genes encoding a Ca^{2+}/calmodulin-dependent NOS (Regulski and Tully, 1995) and the two subunits of sGC have been cloned (Liu et al., 1995; Shah and Hyde, 1995). The anatomical distributions and biochemical properties of NOS and NOS-related markers of the insect nervous system have recently been reviewed (Müller, 1997).

Locusts are especially useful as neurobiological preparations for studies of information processing in invertebrate nervous systems at the cellular and systems levels (for a review, see Burrows, 1996). The presence of NO/cGMP signalling is well established in the olfactory system of the locust (Müller and Bicker, 1994; Bicker and Hähnlein, 1995; Elphick et al., 1995;

Bicker et al., 1996), and there is growing cytochemical evidence that locusts also employ NO signalling in their visual system. Using NADPH diaphorase staining as a marker for NOS in fixed tissue, the distribution of potential NO-synthesizing cells has been mapped in the optic lobe (Elphick et al., 1996; Bicker and Schmachtenberg, 1997; Seidel and Bicker, 1997). Staining locust nervous tissue for NADPH diaphorase provides a more detailed histological resolution in the neuropile than immunocytochemistry using a polyclonal antiserum against rat cerebellar NOS (Elphick et al., 1995). In the visual system, prominent NADPH diaphorase staining was found in a subset of monopolar cells, the first-order interneurones of the visual system. Using double staining for NADPH diaphorase and NO-induced cGMP immunoreactivity (cGMP-IR), the accumulation of cGMP could be demonstrated in the presynaptic photoreceptor cells but not in other cellular targets of the NADPH-diaphorase-expressing monopolar cells in the medulla (Bicker and Schmachtenberg, 1997). These cytochemical experiments suggested that NO serves as a retrograde signal at the photoreceptor-to-monopolar cell synapse.

To emphasize the presence of NO-induced cGMP-IR in the presynaptic photoreceptors but its absence in the postsynaptic monopolar cells, the immunocytochemical data in this paper are presented without counterstaining for NADPH diaphorase.

The cytochemical staining resolves immunoreactivity along the entire length of both the short and long visual fibres, but not in the monopolar cells. To demonstrate that photoreceptor neurones are potential targets of an endogenous NO release system, we have investigated the effects of neurochemicals that affect NO/cGMP signalling on both intracellular recordings from the photoreceptors and the corneal electroretinogram (ERG).

Materials and methods

cGMP immunocytochemistry on frozen sections

Experiments were performed on adult *Schistocerca gregaria* and *Locusta migratoria*, which were either reared in a crowded colony or were obtained commercially from Blades Biological (Edenbridge, UK). All reagents, salts and solvents were of analytical grade and were obtained from Sigma, Gibco or Boehringer unless stated otherwise. Locusts were chilled on ice, and the cerebral ganglion was dissected out of the head capsule. Several cerebral ganglia were pooled in ice-cold Leibovitz L-15 medium (Gibco), and the ganglionic sheath was removed completely with fine forceps. The ganglia were then incubated for 30 min (at 4 °C) in L-15 containing 1 mmol l^{-1} 3-isobutyl-1-methylxanthine (IBMX) to block the activity of phosphodiesterases. After the addition of sodium nitroprusside (SNP) to a final concentration of 100 µmol l^{-1}, the ganglia were incubated for 15 min at room temperature (20–22 °C). The tissues were fixed in 4 % carbonate-buffered formaldehyde solution (pH 6.9) for 2 h at room temperature, postfixed at 4 °C overnight, and stored in phosphate-buffered saline (PBS) containing 30 % sucrose for 20 h. The tissue was immersed in Tissue-Tek II (Miles), frozen and cut in a cryostat (18 µm). The plane of the sections was based on the body axis. The sections were mounted on poly-D-lysine-coated slides and incubated for 30 min in the blocking solution of the Vectastain ABC kit (Vector Laboratories), diluted in PBS (pH 7.4). The primary cGMP antiserum was applied at 4 °C overnight at a dilution of 1:1000 in PBS with 0.1 % Triton X-100. To visualize cGMP immunoreactivity (cGMP-IR), we used the ABC kit (Vector Laboratories) according to the instructions of the manufacturer, diluting in PBS buffer. After the development of staining with diaminobenzidine, sections were washed, dehydrated in an ethanol series, cleared in xylene and mounted in Entellan (Merck). Sections were viewed and photographed through a Polyvar microscope (Reichert-Jung).

Specificity of staining

The primary antiserum was raised in rabbits against cGMP coupled to thyroglobulin using formaldehyde. The antiserum has previously been used to detect the cellular localization of cGMP on sections of the mammalian brain (De Vente et al., 1987) and on whole-mounts of *Manduca sexta* ganglia (Ewer et al., 1994). Details of the generation and tests for specificity of the anti-cGMP serum employed have been given elsewhere (De Vente et al., 1987; De Vente and Steinbusch, 1993). Briefly, the affinity of the antiserum for the protein carrier/cGMP conjugate and its lack of cross-reactivity with GMP and cyclic AMP (cAMP) have been demonstrated using a gelatin model system. Control sections of locust nervous tissue, incubated for immunocytochemistry but in the absence of primary antiserum, showed no immunoreactivity. Preincubation of the antiserum with 10 µmol l^{-1} cGMP for 2 h at room temperature completely blocked immunoreactivity on frozen sections, whereas preincubation with 100 µmol l^{-1} cAMP or GMP did not affect immunoreactivity.

Extracellular recordings from the locust eye

After the removal of the antennae and forelegs, locusts were immobilized in a plastic tube. The head and thorax were fixed in position with beeswax. A drop of electrode gel (Parker) was applied to one compound eye, and a glass electrode, filled with 3 mol l^{-1} KCl and with a resistance of approximately 1 MΩ, was used for recordings. Silver wire, inserted through a hole into the head capsule, served as the ground electrode. Signals were fed to a conventional direct current amplifier for intracellular recordings and were stored on an electronic chart recorder (MacLab). Three green high-intensity light-emitting diodes (LEDs) were used for light stimulation of the locust eye from a distance of 1 cm. They were modulated by square pulses of variable voltage and duration generated by the stimulator of the MacLab chart recorder. Maximum light intensity was 250 mcd at 566 nm (half-width 40 nm). Before the start of electroretinogram (ERG) recordings, locusts were dark-adapted for 1 h. Dim red light was used as background illumination. As determined by the ERG experiments, light intensities using LEDs were chosen to yield a half-maximal photoreceptor response.

The compound eye was stimulated with a constant series of light pulses (0.5 Hz, 200–300 ms duration). Membrane-permeant cGMP analogues, sodium nitroprusside (SNP) as a NO donor, the NO scavengers haemoglobin and carboxy-PTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) (Akaike et al., 1993) and the NOS inhibitor ETU (2-ethyl-2-thiopseudourea) (Garvey et al., 1994) were applied during light stimulation. GMP served as a control for cGMP, and methaemoglobin served as a control for haemoglobin. All reagents were diluted in locust Ringer (NaCl, 140 mmol l^{-1}; KCl, 10 mmol l^{-1}; CaCl$_2$, 2 mmol l^{-1}; Hepes, 10 mmol l^{-1}; pH 6.9). The following protocol was used for drug application: locust Ringer, 8-Br-cGMP (10 mmol l^{-1}), 8-Br-GMP (10 mmol l^{-1}), dibutyryl-cGMP (10 mmol l^{-1}), SNP (10 mmol l^{-1}, freshly prepared and degassed for 48 h as control), haemoglobin (100 µmol l^{-1}), methaemoglobin (100 µmol l^{-1}) and carboxy-PTIO (100 µmol l^{-1}; Alexis Biochemicals) were injected through a glass pipette into the retina using a picopump (WPI). The injected volume was 0.2 µl, leading eventually to an estimated elevation of the whole-body concentration to 1 µmol l^{-1} (8-Br-cGMP, 8-Br-GMP, dibutyryl-cGMP and SNP) or 10 nmol l^{-1} for haemoglobin, methaemoglobin and carboxy-PTIO. Calibration was achieved by ejecting the drops into oil and measuring their diameter. A maximum of five injections were given, spaced at intervals of 5 min.

To analyse the effects of the injections on the ERG amplitude, the three ERGs preceding and following the injection were averaged and normalized with respect to the three ERGs before injection (100 %). The six ERGs of each experiment were compared using analysis of variance (ANOVA) for repeated measures. Prior to injection, ERG amplitudes never displayed any significant differences. Groups of ten ERGs, before and 10 min after injection, were averaged and compared using Student's t-test for paired samples.

Intracellular recordings from photoreceptors

Locusts were dark-adapted for 1 h, decapitated, and their antennae were removed. The heads were fixed with needles on a Sylgard-coated Petri dish and covered with aerated locust Ringer (see above). All preparations were observed under a stereo microscope using dim red light as illumination. A notch approximately 1.5 mm long was cut into the anterior part of an eye, exposing the array of photoreceptors. A large-tipped (50–70 µm) injection pipette and connecting tubing were filled and inserted into the notch. During experiments, a freshly prepared and light-protected 1 mmol l^{-1} solution of SNP was used while Ringer served as a control. A green high-intensity LED was used for light stimulation of the locust eye from a distance of 1 mm. It was modulated by square pulses of variable voltage and duration, generated by the stimulator of the MacLab chart recorder. Maximum light intensity was 250 mcd at 566 nm (half-width 40 nm). The resistance of the recording electrode, which was filled with 3 mol l^{-1} KCl, was typically 75 MΩ. The LED, the injection pipette and the recording electrode were carefully positioned so that they were aimed at the same spatial region of the eye. To prevent premature leakage of SNP from the pipette, a small amount of Ringer was sucked up before the onset of experiments. Injection volume (0.2 ml) and time were controlled using a manual microinjector (Leitz). Injections were repeated up to four times at intervals of 3 min, but only one photoreceptor was recorded per head. Intracellular recordings were fed *via* a conventional direct current amplifier into a MacLab chart recorder system. A silver wire coated with silver chloride was inserted as ground electrode into the Ringer bath.

Results

cGMP immunocytochemistry

After incubation of whole excised locust brains in sodium nitroprusside (SNP), a strong cGMP immunoreaction could be observed in all parts of the photoreceptor cells (Fig. 1). Staining was intense in the photoreceptor cell bodies and their retinular segments and could easily be traced over the entire length of the axons, terminating in the lamina or medulla. Of
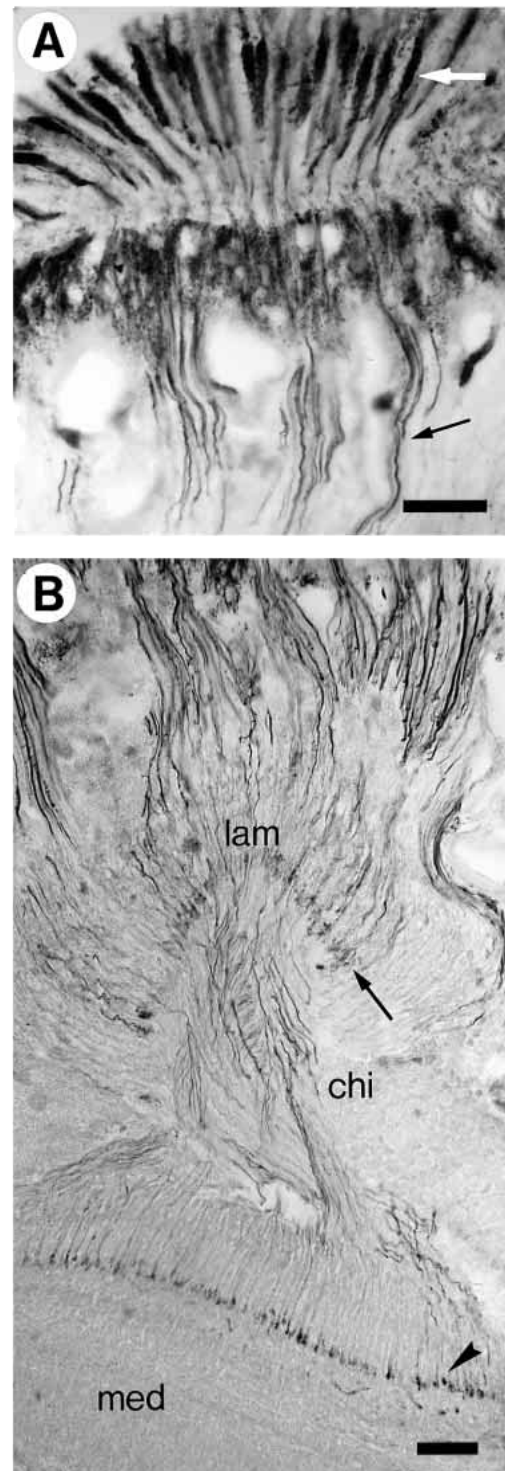

Fig. 1. NO-induced cGMP immunoreactivity in horizontal sections through the optic lobe. (A) A section through the distal part of the visual system demonstrates strong immunoreactivity in the cell body segments of the photoreceptors (white arrow). Immunoreactive axons penetrate the basal lamina and wind in bundles through the fenestrated layer before reaching the lamina (black arrow). The dark staining basal to the retina does not represent immunoreactivity but is pigmentation. Distal orientation is up. (B) A section showing the distribution of photoreceptor axons in the lamina (lam) and medulla (med) of the optic lobe. The black arrow marks the photoreceptor terminals in the lamina. Some axons pass through the lamina and enter the medulla *via* the first optic chiasma (chi). Their synaptic endings can be seen in a distinct layer of the medullary neuropile (arrowhead). The monopolar cells of the lamina are not stained. Distal orientation is up. Scale bars, 50 µm.

the eight photoreceptor neurones of a single ommatidium, six send their axons into the first optic neuropile, the lamina, where they terminate and form synapses with the first-order interneurones, the monopolar cells (Shaw, 1968; Horridge and Meinertzhagen, 1970; Nowel and Shelton, 1981). Two photoreceptor cells send long axons through the lamina into the second optic neuropile, the medulla. Our cytochemical staining protocol revealed immunoreactivity in both types of photoreceptor. Fig. 1A shows the retinular segments of the photoreceptors and their axons as they pass through the basal lamina and the proximal pigment layer before crossing the trachea-rich fenestrated layer in bundles. Fig. 1B shows the remainder of the axonal distribution, displaying the termination of the short visual fibres in a distinct region of the lamina called external plexiform layer II (Nowel and Shelton, 1981) and following the path of the long visual fibres through the first optic chiasma to their synaptic terminals in the medulla. It is important to note that cGMP-IR could not be detected in the monopolar cells (Fig. 1B). Thus, the NADPH-diaphorase-positive population of monopolar cells (Elphick et al., 1996) can be considered as a likely source, but not as a target, of NO (Bicker and Schmachtenberg, 1997). The presence of immunocytochemical staining was entirely dependent upon the presence of exogenous NO before fixation and on the inhibition of phosphodiesterases by IBMX. Omission of either SNP or IBMX abolished the pattern of immunoreactivity. In this paper, we focused exclusively on NO-induced cGMP-IR. We did not investigate how illumination conditions might affect cGMP levels.

Electroretinogram recordings from the locust eye

To collect further evidence for an involvement of cGMP in insect vision, we used the electroretinogram to monitor of the light sensitivity of the photoreceptors. Compared with intracellular recordings, it was technically easier to obtain a stable baseline in ERG recordings while performing drug injections. Thus, the majority of our electrophysiological experiments relied on the corneal ERG to investigate the effects of drugs interfering with NO/cGMP signalling. Initially, we injected membrane-permeant cGMP analogues (dibutyryl-cGMP and 8-Br-cGMP) into the retina while recording the ERG from the locust eye. Injection of saline into the retina yielded a slight reduction in ERG amplitude of approximately 10 %. Amplitudes returned to their initial values over the course of a few seconds. In contrast, injection of cGMP analogues led to a sudden conspicuous increase in retinogram amplitude (Fig. 2A). This increase was approximately equal for 8-Br-cGMP and dibutyryl-cGMP and was significantly different from the effects of the Ringer ($P<0.001$) or 8-Br-GMP ($P<0.01$) as controls (Fig. 3). The drug-induced changes in ERG amplitude were usually completely reversed after 10 s. Since cGMP immunoreactivity had been shown to depend on the application of exogenous NO before fixation, we now injected the NO donor SNP into the retina using SNP that had been degassed for 48 h as a control. We observed a significant ($P<0.001$) increase in ERG
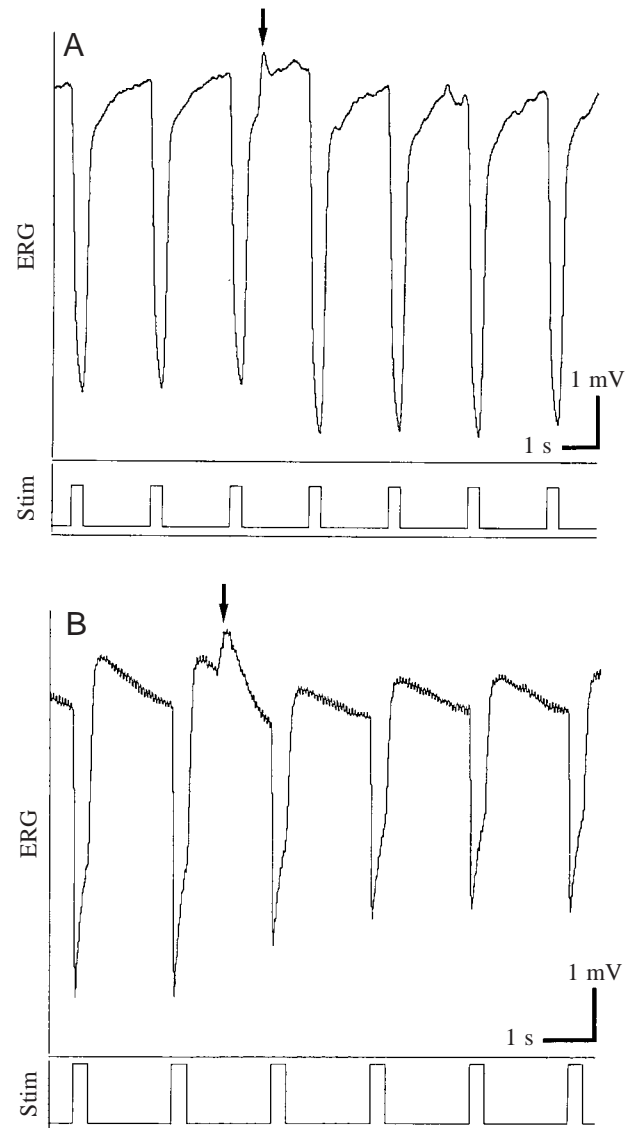

Fig. 2. Recordings of the corneal electroretinogram (ERG) showing sensitivity to cGMP and haemoglobin. (A) Injection of 0.2 μl of a 10 mmol l^{-1} solution of 8-Br-cGMP into the retina leads to a marked increase in ERG amplitude. (B) Injection of 0.2 μl of a 100 μmol l^{-1} solution of haemoglobin causes a pronounced decrease in ERG amplitude. The recording is a.c.-coupled. Stim, light stimulus. Arrows indicate the time of injection.

amplitude that was comparable to the rise that followed cGMP injection (Fig. 4). To test whether endogenous NO release affects ERG amplitude, we injected haemoglobin and carboxy-PTIO, both potent NO scavengers, in an effort to block extracellular diffusion of NO to the photoreceptor neurones. As shown in Fig. 2B, injection of haemoglobin decreased the retinogram amplitude. Similar effects were observed after injection of carboxy-PTIO. The averaged ERG amplitudes are presented in Fig. 4. The effects of both haemoglobin and carboxy-PTIO were significantly different ($P<0.01$) from the effects of methaemoglobin and Ringer as controls.
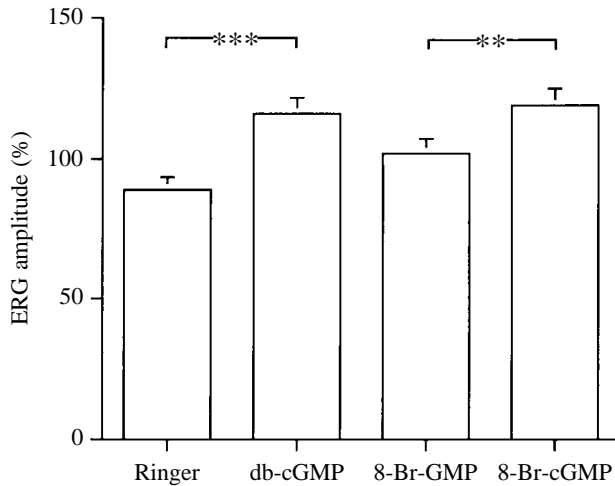
In addition, we applied arginine analogues as NOS

Fig. 3. Averaged electroretinogram (ERG) amplitudes after drug injection. The histogram displays the effects of dibutyryl-cGMP (db-cGMP) and 8-Br-cGMP compared with the effects of 8-Br-GMP and Ringer as controls. Values are normalized to levels before injection (100%). Both dibutyryl-cGMP and 8-Br-cGMP lead to a significant increase of ERG amplitude in the range 20–25%. $N=10$ experiments. Each column shows the mean + S.E.M. **$P<0.01$; ***$P<0.001$.

inhibitors. Injection of such compounds also reduced the ERG amplitude (data not shown). However, a significant reduction in the ERG amplitude required estimated whole-body concentrations higher than $20\,\text{mmol}\,l^{-1}$, presumably because of competition with a large pool of endogenous L-arginine. In contrast, the NOS inhibitor ETU reduced the peak ERG deflection at a whole-body concentration of $100\,\mu\text{mol}\,l^{-1}$.

Intracellular recordings from photoreceptor cells

The pharmacological experiments described above provide compelling evidence that the extracellularly recorded photocurrent is modulated by drugs affecting NO/cGMP levels. To investigate the possibility that NO-dependent changes in the ERG are caused by changes in the resistive isolation of the retina from the haemolymph, we monitored the effects of SNP on intracellular recordings from the

photoreceptors. We used a preparation in which a notch cut into the retina facilitated the access of the NO donor to the photoreceptor cells. In this preparation, the pressure wave that accompanied drug application was reduced to a minimum. Care was taken to record from photoreceptor cells at the border between the notch and the intact eye. In all the intracellular recordings taken from this region that had stable resting potentials in the range -40 to $-55\,\text{mV}$, lasting for longer than 10 min, application of SNP caused an increase in the amplitude of the light response (Fig. 5). The increase in response amplitude was reversible and returned within 3 min to the levels prior to drug injection (Fig. 5). This effect on the sensitivity of the photoreceptor was never seen in Ringer controls.

Injection of SNP increased the amplitude of the receptor potential from $100\pm9\%$ (mean ± S.E.M.) in the pretest to $116\pm5.3\%$ ($P<0.05$; t-test for paired variables, $N=10$). Moreover, the difference between injections of SNP and Ringer was also significant ($P<0.05$, t-test for unpaired variables, $N=10$; 5). Injection of Ringer did not cause any significant alteration in response amplitude, yielding a slight reduction from $100\pm9\%$ in the pretest to $95\pm9.3\%$ ($P>0.05$; t-test for paired variables, $N=5$). Thus, we have to assume that NO can directly affect the sensitivity of the photoreceptor cells.

Discussion

This study was motivated by the finding that SNP induces cGMP-IR in the photoreceptor neurones but not in the postsynaptic monopolar cells (Fig. 1). To detect NO/cGMP-induced changes in photoreceptor responses, we recorded the corneal ERG. However, the ERG comprises current flow both from the retina and from the underlying optic ganglia. In contrast to that of the fly (Zimmermann, 1978), the locust corneal ERG is monophasic, lacking ON- and OFF-transients (Fig. 2). Both tracer studies and electrical measurements indicate the presence of a significant barrier to diffusion and current flow between the retina and lamina (Shaw, 1975, 1978a). This barrier probably results from the presence of an extensive capillary bed proximal to the basement membrane,
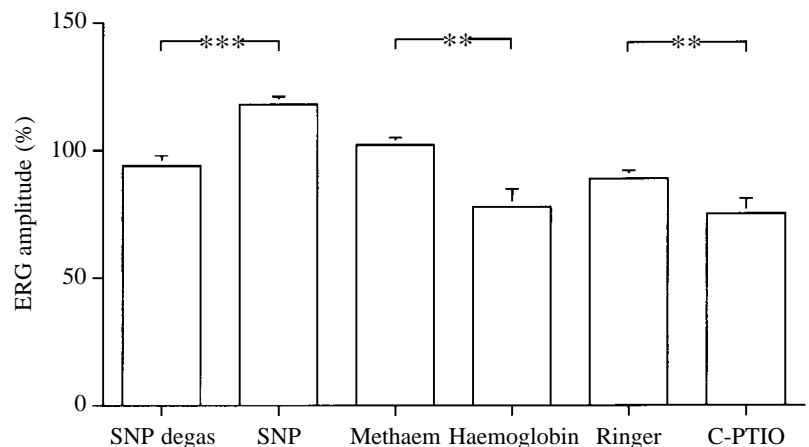

Fig. 4. Averaged electroretinogram (ERG) amplitudes after injection of sodium nitroprusside (SNP), haemoglobin and carboxy-PTIO (C-PTIO) compared with the effects of degassed SNP (degas SNP), methaemoglobin (Methaem) and Ringer, respectively, as controls. 100% is the value before injection. Whereas SNP leads to a significant increase in ERG amplitude, haemoglobin and carboxy-PTIO cause a marked reduction compared with respective control levels. $N=10$ experiments. Each column shows the mean + S.E.M. **$P<0.01$; ***$P<0.001$.
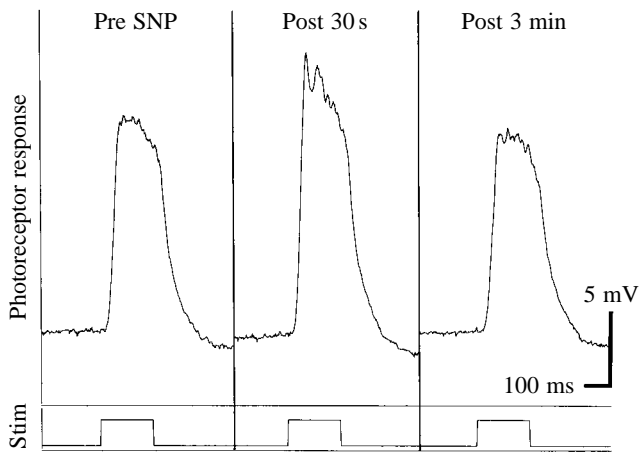
Fig. 5. Intracellular recordings from a photoreceptor neurone. The traces show the light-induced photoreceptor responses before (Pre), 30 s after (Post 30 s) and 3 min after (Post 3 min) the injection of 1 mmol l^{-1} sodium nitroprusside (SNP) into a notch cut into the retina. The traces are representative of 10 successful experiments. Stim, 200 ms light stimulus.

which shunts extracellular current. In this regard, the locust is a particularly well-suited animal to reveal changes in photoreceptor sensitivity by simple ERG recordings because the corneal ERG reflects, to a large extent, the contribution of photoreceptor neurones. Furthermore, application of a NO donor while recording the intracellular light response (Fig. 5) produced an effect consistent with that observed in the ERG. This experiment provides evidence that alterations in ERG amplitude can be interpreted as changes in the sensitivity of the photoreceptors as opposed to effects on retinal resistance barriers.

Our results show that the application of cGMP, but not of GMP analogues, increases the ERG amplitude (Fig. 3), supporting the hypothesis that cGMP modulates photoreceptor responses. Moreover, application of a NO donor has exactly the same effect, demonstrating the responsiveness of photoreceptor cells to NO.

Remarkably, in the vertebrate retina, NO/cGMP signalling also modulates ion channel functions of photoreceptor cells (Kurenny et al., 1994; Noll et al., 1994). Cyclic-nucleotide-gated channels mediate synaptic feedback between cone photoreceptors and horizontal cells by endogenous NO release (Savchenko et al., 1997). In the locust, a reduction in ERG amplitude caused by the NO scavengers carboxy-PTIO and haemoglobin suggests the presence of endogenous NO diffusing to the photoreceptors (Figs 2B, 4).

The temporal characteristics of the putative NO release by the monopolar cells remains an intriguing question. It has been shown in the fly (*Calliphora vicina*) that photoreceptor transmitter release is tonic, even in darkness (Uusitalo et al., 1995). Postsynaptic monopolar cells show phasic hyperpolarizing membrane responses to light pulses (Laughlin, 1994; Shaw, 1968, 1978b; Juusola et al., 1996). Thus, Ca^{2+}/calmodulin-stimulated NO release should be reduced

during the LED flashes. Surprisingly, NO scavengers are effective in decreasing the light-induced peak amplitude of the ERG (Fig. 2B). Since monopolar cells have a low resting potential, from which they hyperpolarize in response to an increase and depolarize in response to a decrease in light intensity (Laughlin, 1994; Shaw, 1968, 1978b; Juusola et al., 1996), the results would argue for tonic release of NO at ambient light levels.

It is premature to assign any physiological functions to NO/cGMP signalling in insect visual mechanisms. There are, however, several cellular processes that are candidate targets for NO/cGMP modulation. The depolarizing light response of the rhabdomeric receptor cells of arthropods is thought to involve activation of the phosphoinositide cascade which, through the release of Ca^{2+} from internal stores, opens plasma membrane channels (for reviews, see Hardie and Minke, 1993; Ranganathan et al., 1995; Zuker, 1996). The signal transduction steps causing the release of Ca^{2+} from internal stores are not yet understood (Acharya et al., 1997). However, unlike the ciliated photoreceptors of vertebrates, in which cGMP gates light-activated cation channels (for a review, see Baylor, 1996), cGMP is not generally considered to be an integral part of the phototransduction process in rhabdomeric photoreceptors. Nonetheless, electrophysiological and molecular biological approaches have also provided evidence for the expression of cGMP-gated channels in arthropod photoreceptors. In excised *Limulus polyphemus* photoreceptor membranes, light-activated ion channels could be opened by the action of cGMP (Bacigalupo et al., 1991). The compound eye of *Drosophila melanogaster* expresses homologues of cGMP-regulated vertebrate ion channels (Baumann et al., 1994) and of sGC (Shah and Hyde, 1995). Moreover, cGMP amplifies light-induced depolarisations in *D. melanogaster* photoreceptors and even induces photocurrents in the absence of a light stimulus (Bacigalupo et al., 1995). Thus, the sensitivity of the locust ERG to cGMP (Fig. 3) is not without precedents. In fly photoreceptors, cGMP is one of the activators of pigment granule migration, a process that is triggered separately from phototransduction (Hanyu and Franceschini, 1993). Cyclic-GMP-mediated pigment granule migration may thus contribute to the regulation of light flux in rhabdomeric photoreceptors.

The NO-induced cGMP-IR in the photoreceptor cells of the locust (Fig. 1) and the electrophysiological sensitivity to NO donors and scavengers (Fig. 4) suggest the presence of a transcellular NO/cGMP signalling system with the NADPH-diaphorase-positive monopolar cells as probable sources of NO. Such a retrogradely acting NO/cGMP signal transmission system may function during adaptation. For example, a dimming of ambient light levels causes depolarizing membrane responses in monopolar cells (Laughlin, 1994; Shaw, 1968, 1978b; Juusola et al., 1996) resulting eventually in enhanced NO release, which serves as a retrogradely acting message. This, in turn, would activate sGC in the photoreceptor cells to enhance sensitivity *via* cGMP production. An enhancement of sensitivity during dark

adaptation would be reflected in an increase in ERG amplitude. Unfortunately, the recording of the extracellular current flow of the corneal ERG does not allow any changes in synaptic gain that may accompany the adaptation process at the synaptic level to be determined.

To prove the existence of retrograde synaptic transmission is a technically demanding problem, requiring extra- and intracellular application of NO scavengers and NOS inhibitors. For example, pharmacological studies have suggested that long-term potentiation in the CA1 area of the hippocampus requires NO as a retrograde messenger (for a review, see Garthwaite and Boulton, 1995). For reasons of methodology, this view has been rather controversial (e.g. Williams et al., 1993). Meanwhile, results from the application of a variety of modern cell biological techniques, such as the genetic knock-out of NOS enzymes, membrane targeting of a specific NOS isoform and the experimental manipulation of NO signalling at isolated synapses in primary culture, support the idea that NO serves as a retrograde messenger during long-term potentiation (Arancio et al., 1996; Kantor et al., 1996; Son et al., 1996).

Although final proof of the retrograde action of NO in the locust visual system is still lacking, the photoreceptor output synapse may provide a novel opportunity for investigating NO/cGMP signalling at tonically active synapses. Signal transfer between photoreceptors and monopolar cells operates by graded-potential transmission through continuous neurotransmitter release (Laughlin, 1994; Uusitalo et al., 1995; Juusola et al., 1996). The anterograde transmitter of the photoreceptor output synapse has been identified as histamine (Hardie, 1987, 1989). A specific contribution of retrograde NO signalling in the early stages of visual information processing has yet to be elucidated.

References

Acharya, J. K., Jalink, K., Hardy, R. W., Hartenstein, V. and Zuker, C. S. (1997). InsP3 receptor is essential for growth and differentiation but not for vision in *Drosophila. Neuron* **18**, 881–887.

Akaike, T., Yoshida, M., Miyamoto, Y., Sato, K., Kohno, M., Sasamoto, K., Miyazaki, K., Ueda, S. and Maeda, H. (1993). Antagonistic action of imidazolineoxyl N-oxides against endothelium-derived relaxing factor/NO through a radical reaction. *Biochemistry* **32**, 827–832.

Arancio, O., Kiebler, M., Lee, C. J., Lev-ram, V., Tsien, R. Y., Kandel, E. R. and Hawkins, R. D. (1996). Nitric oxide acts directly in the presynaptic neuron to produce long-term potentiation in cultured hippocampal neurons. *Cell* **87**, 1025–1035.

Bacigalupo, J., Bautista, D. M., Brink, D. L., Hetzer, J. F. and O'Day, P. M. (1995). Cyclic-GMP enhances light-induced excitation and induces membrane currents in *Drosophila* retinal photoreceptors. *J. Neurosci.* **15**, 7196–7200.

Bacigalupo, J., Johnson, E. C., Vergara, C. and Lisman, J. E. (1991). Light-dependent channels of excised patches of *Limulus* ventral photoreceptors are opened by cGMP. *Proc. Natl. Acad. Sci. USA* **88**, 7938–7942.

Baumann, A., Frings, S., Godde, M., Seifert, R. and Kaupp, U. B. (1994). Primary structure and functional expression of a *Drosophila* cyclic nucleotide-gated channel present in eyes and antennae. *EMBO J.* **13**, 5040–5050.

Baylor, D. (1996). How photons start vision. *Proc. Natl. Acad. Sci. USA* **93**, 560–565.

Bicker, G. and Hähnlein, I. (1995). NADPH-diaphorase expression in neurones and glial cells of the locust brain. *Neuro Report* **6**, 325–328.

Bicker, G. and Schmachtenberg, O. (1997). Cytochemical evidence for nitric oxide/cyclic GMP signal transmission in the visual system of the locust. *Eur. J. Neurosci.* **9**, 189–193.

Bicker, G., Schmachtenberg, O. and De Vente, J. (1996). The nitric oxide/cyclic GMP messenger system in olfactory pathways of the locust brain. *Eur. J. Neurosci.* **8**, 2635–2643.

Bredt, D. S. and Snyder, S. H. (1992). Nitric oxide, a novel neuronal messenger. *Neuron* **8**, 3–11.

Burrows, M. (1996). *The Neurobiology of an Insect Brain*. Oxford, New York, Tokyo: Oxford University Press.

De Vente, J. and Steinbusch, H. W. M. (1993). Immunocytochemistry of second messenger molecules: the study of formaldehyde-fixed cyclic GMP. In *Immunohistochemistry* (ed. A. C. Cuello), pp. 409–427. New York: John Wiley & Sons.

De Vente, J., Steinbusch, H. W. M. and Schipper, J. (1987). A new approach to immunocytochemistry of 3′,5′-cyclic guanosine monophosphate: preparation, specificity and initial application of a new antiserum against formaldehyde-fixed 3′,5′-cyclic guanosine monophosphate. *Neuroscience* **22**, 361–373.

Elofsson, R., Carlberg, M., Moroz, L., Nezlin, L. and Sakharow, D. (1993). Is nitric oxide (NO) produced by invertebrate neurones? *Neuroreport* **4**, 279–282.

Elphick, M. R., Green, I. C. and O'Shea, M. (1993). Nitric oxide synthesis and action in an invertebrate brain. *Brain Res.* **619**, 344–346.

Elphick, M. R., Rayne, R. C., Riveros-Moreno, V., Moncada, S. and O'Shea, M. (1995). Nitric oxide synthesis in locust olfactory interneurones. *J. Exp. Biol.* **198**, 821–829.

Elphick, M. R., Williams, L. and O'Shea, M. (1996). New features of the locust optic lobe: Evidence of a role for nitric oxide in insect vision. *J. Exp. Biol.* **199**, 2395–2407.

Ewer, J., De Vente, J. and Truman, J. W. (1994). Neuropeptide induction of cyclic GMP increases in the insect CNS: resolution at the level of single identifiable neurons. *J. Neurosci.* **14**, 7704–7712.

Garthwaite, J. and Boulton, C. L. (1995). Nitric oxide signaling in the central nervous system. *Annu. Rev. Physiol.* **57**, 683–706.

Garvey, E. P., Oplinger, J. A., Tanoury, G. J., Sherman, P. A., Fowler, M., Marshall, S., Harmon, M. F., Paith, J. E. and Furfine, E. S. (1994). Potent and selective inhibition of human nitric oxide synthases. Inhibition by non-amino acid isothioureas. *J. Biol. Chem.* **269**, 26669–26676.

Hanyu, Y.. and Franceschini, N. (1993). Pigment granule migration and phototransduction are triggered by separate pathways in fly photoreceptor cells. *Neuro Report* **4**, 215–218.

Hardie, R. C. (1987). Is histamine a neurotransmitter in insect photoreceptors ? *J. Comp. Physiol.* **161**, 201–213.

Hardie, R. C. (1989). A histamine-activated chloride channel

involved in neurotransmission at a photoreceptor synapse. *Nature* **339**, 704–706.

Hardie, R. C. and Minke, B. (1993). Novel Ca^{2+} channels underlying transduction in *Drosophila* photoreceptors: implications for phosphoinositide-mediated Ca^{2+} mobilization. *Trends Neurosci.* **16**, 371–376.

Horridge, G. A. and Meinertzhagen, I. A. (1970). The exact neural projection of the visual fields upon the first and second ganglia of the insect eye. *Z. Vergl. Physiol.* **66**, 369–378.

Juusola, M., French, A. S., Uusitalo, R. O. and Weckström, M. (1996). Information processing by graded-potential transmission through tonically active synapses. *Trends Neurosci.* **19**, 292–297.

Kantor, D. B., Lanzrein, M., Stary, S. J., Sandoval, G. M., Smith, W. B., Sullivan, B. M., Davidson, N. and Schuman, E. M. (1996). A role for endothelial NO synthase in LTP revealed by adenovirus-mediated inhibition and rescue. *Science* **274**, 1744–1748.

Kurenny, D. E., Moroz, L. L., Turner, R. W., Sharkey, K. A. and Barnes, S. (1994). Modulation of ion channels in rod photoreceptors by nitric oxide. *Neuron* **13**, 315–324.

Laughlin, S. B. (1994). Matching coding, circuits, cells and molecules to signals: general principles of retinal design in the fly's eye. *Prog. Retinal Eye Res.* **13**, 165–196.

Liu, W., Yoon, J., Burg, M., Chen, L. and Pak, W. L. (1995). Molecular characterization of two *Drosophila* guanylate cyclases expressed in the nervous system. *J. Biol. Chem.* **270**, 12418–12427.

Müller, U. (1994). Ca^{2+}/calmodulin dependent nitric oxide synthase in *Apis mellifera* and *Drosophila melanogaster*. *Eur. J. Neurosci.* **6**, 1362–1370.

Müller, U. (1997). The nitric oxide system in insects. *Prog. Neurobiol.* **51**, 363–381.

Müller, U. and Bicker, G. (1994). Calcium activated release of nitric oxide and cellular distribution of nitric oxide synthesizing neurons in the nervous system of the locust. *J. Neurosci.* **14**, 7521–7528.

Müller, U. and Buchner, E. (1993). Histochemical localization of NADPH-diaphorase in adult *Drosophila* brains: Is nitric oxide a neuronal messenger also in insects? *Naturwissenschaften* **80**, 524–526.

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

Nowel, M. S. and Shelton, P. M. J. (1981). A Golgi-electron-microscopical study of the structure and development of the lamina ganglionaris of the locust optic lobe. *Cell Tissue Res.* **216**, 377–401.

Ranganathan, R., Malicki, D. M. and Zucker, C. S. (1995). Signal transduction in *Drosophila* photoreceptors. *Annu. Rev. Neurosci.* **18**, 283–317.

Regulski, M. and Tully, T. (1995). Molecular and biochemical characterization of *dNOS*: a *Drosophila* Ca^{2+}/calmodulin dependent nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* **92**, 9072–9076.

Savchenko, A., Barnes, S. and Kramer, R. H. (1997). Cyclic-nucleotide-gated channels mediate synaptic feedback by nitric oxide. *Nature* **390**, 694–698.

Seidel, C. and Bicker, G. (1997). Colocalization of NADPH-diaphorase and GABA-immunoreactivity in the olfactory and visual system of the locust. *Brain Res.* **769**, 273–280.

Shah, S. and Hyde, D. R. (1995). Two *Drosophila* genes that encode the α and β-subunits of the brain soluble guanylyl cyclases. *J. Biol. Chem.* **270**, 15368–15376.

Shaw, S. R. (1968). Organization of the locust retina. *Symp. Zool. Soc. Lond.* **23**, 135–163.

Shaw, S. R. (1975). Retinal resistance barriers and electrical lateral inhibition. *Nature* **255**, 480–483.

Shaw, S. R. (1978a). The extracellular space and blood–eye barrier in an insect retina: an ultrastructural study. *Cell Tiss. Res.* **188**, 35–61.

Shaw, S. R. (1978b). Signal transmission by graded slow potentials in the arthropod peripheral visual system. In *The Neurosciences. Fourth Study Program* (ed. F. O. Schmitt and F. G. Worden), pp. 275–295. Cambridge, MA: MIT Press.

Son, H., Hawkins, R. D., Martin, K., Kiebler, M., Huang, P. L., Fishman, M. C. and Kandel, E. R. (1996). Long-term potentiation is reduced in mice that are doubly mutant in endothelial and neuronal nitric oxide synthase. *Cell* **87**, 1015–1023.

Uusitalo, R. O., Juusola, M., Kouvalainen, E. and Weckstrom, M. (1995). Tonic transmitter release in a graded potential synapse. *J. Neurophysiol.* **74**, 470–473.

Williams, J. H., Li, Y.-G., Nayak, A., Errington, M. L., Murphy, K. P. S. J. and Bliss, T. V. P. (1993). The suppression of long-term potentiation in rat hippocampus by inhibitors of nitric oxide synthase is temperature and age dependent. *Neuron* **11**, 877–884.

Zimmermann, R. P. (1978). Field potential analysis and the physiology of second-order neurons in the visual system of the fly. *J. Comp. Physiol.* **126**, 297–316.

Zuker, C. H. (1996). The biology of vision in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **93**, 571–576.