Dark-dependent soluble guanylyl cyclase activity in locust photoreceptor cells

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The enzyme soluble guanylyl cyclase (SGC) mediates physiological effects of the gaseous signalling molecule nitric oxide by generating the second messenger molecule cyclic-GMP (cGMP). Here we have demonstrated that SGC is expressed in photoreceptor cells of locust compound eyes. However, stimulation of SGC activity in the eyes was observed only in the dark, indicating that light may cause inhibition of SGC activity in locust photoreceptor cells. Because light causes elevation of cytosolic Ca^{2+} in insect photoreceptor cells, we investigated the involvement of Ca^{2+} in mediating the inhibitory effect of light on SGC activity in the locust eye. Light-adapted locust eyes incubated with Ca^{2+} -free physiological saline displayed a similar level of stimulated SGC activity to that normally seen only in dark-adapted eyes. These data indicate for the first time that Ca^{2+} may regulate SGC activity in cells. Moreover, the dark dependence of SGC activity in the locust eye suggests that SGC and cGMP may participate in dark-adaptation mechanisms in insect photoreceptor cells.

Keywords: soluble guanylyl cyclase; nitric oxide; insect; *Schistocerca gregaria*; cyclic-GMP; phototransduction

1. INTRODUCTION

The enzyme soluble guanylyl cyclase (SGC) is a heterodimeric protein comprising an α (SGC α) and a β (SGC β) subunit (Yuen & Garbers 1992). SGC is activated by the gaseous signalling molecule nitric oxide (NO), producing an increase in cyclic-GMP (cGMP) in cells expressing SGC (Garthwaite 1991; Elphick *et al.* 1993). Analysis of the physiological roles of this NO–cGMP signalling pathway in mammals has revealed diverse actions, including modulation of synaptic transmission (Schuman & Madison 1994) and regulation of smooth muscle tone (Palmer *et al.* 1987; Bult *et al.* 1990). In invertebrates, there is evidence that the NO–cGMP pathway participates in, for example, olfactory processing mechanisms (Gelperin 1994; Müller & Hildebrandt 1995), regulation of feeding behaviour (Elphick *et al.* 1995a; Colasanti *et al.* 1997) and learning (Robertson *et al.* 1996).

Analysis of NO function in insects has been facilitated by anatomical studies showing the distribution of NO synthase (NOS) in the nervous system using NADPH diaphorase histochemistry (Müller & Bicker 1994; Elphick *et al.* 1995b, 1996; Ott & Burrows 1998). Although NADPH diaphorase activity is only an indirect measure of NOS localization, it does correspond with the distribution of NOS in the nervous systems of both mammals (Matsumoto *et al.* 1993) and insects (Müller & Bicker 1994; Elphick *et al.* 1995b). Moreover, in locusts, NADPH diaphorase-positive neurons are particularly abundant in the antennal and optic lobes of the brain, indicating that NO may participate in sensory processing mechanisms.

Recently, we have investigated the distribution of SGC in the locust brain and eyes using an antiserum to an evolutionarily conserved region of the *Drosophila* SGC α subunit (Elphick & Jones 1998). SGC α immunoreactivity was detected in a distinct population of neurons in the locust brain which included olfactory interneurons and large ocellar interneurons. In addition, however, SGC α immunoreactivity was also detected in photoreceptor cells of locust compound eyes and simple eyes (ocelli). Previous studies have shown that SGC is expressed in the retina of *Drosophila* compound eyes but the precise cellular and subcellular location of the enzyme was not investigated (Shah & Hyde 1995).

Detailed analysis of locust compound eyes revealed that SGC α immunoreactivity is associated with the rhabdomeric compartment of the photoreceptor cells (Elphick & Jones 1998). This is interesting because it is in the rhabdoms of insect eyes that phototransduction occurs. The detection of SGC α immunoreactivity in locust rhabdoms suggests that SGC-derived cGMP may participate in the regulation of phototransduction. However, although cGMP plays a key role in vertebrate phototransduction, its function in insect photoreceptors is not known (Ranganathan *et al.* 1995). In insects, light causes depolarization of photoreceptor cells but the biochemical pathway from the activation of rhodopsin molecules in the rhabdom to the opening of light-activated ion channels has yet to be fully characterized. A cGMP-gated channel which is expressed in *Drosophila* eyes has been identified (Baumann *et al.* 1994) but its role in phototransduction is unknown. Therefore, the discovery that SGC α is expressed in locust photoreceptor cells (Elphick & Jones 1998) has provided a new impetus for investigating the function of cGMP in these cells.

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Here we have examined the expression of SGC in the locust visual system using affinity-purified antibodies to the *Drosophila* SGC α subunit, extending our previous analysis (Elphick & Jones 1998) in which polyclonal antisera were used. Moreover, we have investigated the functional properties of SGC enzyme activity in locust compound eyes by measuring the cGMP content of eyes under a variety of experimental conditions.

2. MATERIALS, METHODS AND RESULTS

(a) Materials

Adult *Schistocerca gregaria* were obtained from Blades Biological, Edenbridge, Kent, UK. The locusts were kept under a 12 L:12 D regime (lights on at 08.00 and off at 20.00) for at least seven days prior to use. 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was purchased from Tocris Cookson, Bristol, UK and *S*-nitroso-*N*-acetylpenicillamine (SNAP) was provided by Wellcome Research Laboratories, Beckenham, UK. All other chemicals used were purchased from Sigma, Poole, UK, unless stated otherwise.

(b) Analysis of SGC expression in locust eyes using affinity-purified antibodies to the *Drosophila* SGC α subunit

Antisera (termed XA-4, XB-3 and XC-3) were raised in rabbits to an evolutionarily conserved region (X) of the *Drosophila* SGC α subunit as described by Elphick & Jones (1998). Antibodies that bind to the X-peptide sequence were affinity purified from one of these antisera (XA-4), using a novel method devised in conjunction with Martin Hudson at the University of Sussex, UK.

First, the X-peptide was N-terminal thiolated through Traut's reaction. For this, 1 mg X-peptide was dissolved in 100 μ l of 50 mM triethanolamine-HCl (TEA) buffer containing 100 mM NaCl and 1 mM ethylenediaminetetracetic acid (EDTA). Next, 8.5 μ l of Traut's reagent (2-iminothiolane-HCl dissolved in 500 mM TEA buffer at 1 mg per 20 μ l) was added and the mixture left to react for 1 h at room temperature on a rotator. The reaction was stopped by adding 20 μ l of 2.2 M glycine in 10 mM phosphate-buffered saline (PBS), pH 7.4.

Second, the modified peptide was conjugated to maleimide-activated keyhole limpet haemocyanin (ImjectTM Kit, Pierce, Chester, UK) according to the manufacturers instructions and the reaction mixture passed through a chromatography column, provided in the kit, to purify the conjugate. The conjugate was next bound to 100 μ l (packed volume) of tosyl-activated DynabeadsTM (Dynal, Bromborough, UK), according to the manufacturers instructions.

Finally, the X-peptide-bound Dynabeads were used to affinity purify aliquots of the XA-4 antisera using a method adapted from Campbell *et al.* (1951). Briefly, 100 μ l of XA-4 was diluted 1:10 in 10 mM Tris, pH 7.5 and centrifuged immediately using a microcentrifuge to remove any precipitate. The diluted antiserum was added to the DynabeadsTM and gently mixed for 2 h at room temperature on a rotator. The beads were then washed with 20 volumes of 10 mM Tris, pH 7.5 and then 20 volumes of 10 mM Tris, pH 7.5 containing 500 mM NaCl. Bound antibodies were eluted by washing the beads with ten volumes of 100 mM glycine, pH 2.5, followed by ten volumes of 100 mM triethylamine, pH 11.5. The eluates were immediately added to one volume of 1 M Tris, pH 8.0 and dialysed against 4 l PBS containing 0.02% sodium azide overnight at 4 °C.

Figure 1. Detection of an *ca.* 65 kDa SGC α subunit in locust eye. Western blot showing an *ca.* 65 kDa protein in locust eye homogenates which is immunoreactive with affinity-purified antibodies (XA-4) to a conserved region (X) of the *Drosophila* SGC α subunit. Pre-absorption of antibodies with the X-peptide antigen (XA-4 + X peptide) abolishes immunostaining of the *ca.* 65 kDa protein.

Following dialysis, the affinity-purified antibodies were stored at 4 °C until used.

Affinity-purified XA-4 antibodies were used to examine SGC α expression in the locust eye by Western blotting methods and immunocytochemistry. For Western blot analysis, the methods used were same as those described by Elphick & Jones (1998) except for the use of affinity-purified antibodies instead of X-peptide antisera and the use of an enhanced chemiluminescence (ECL) revealing system (Amersham) instead of an alkaline phosphatase-based revealing system. The affinity-purified XA-4 antibodies were used at a dilution of 1:50 (in PBS) of the antibody solution collected following the affinity-purification procedure described above. A single band with a molecular mass of *ca.* 65 kDa in locust eye homogenates was immunoreactive with XA-4 antibodies and detection of this band was abolished when the antibody solution was pre-incubated with X-peptide antigen (20 μ M) (figure 1).

Immunocytochemical analysis of the locust visual system was performed using the same methods as those described by Elphick & Jones (1998) except for the use of an affinity-purified XA-4 antibody solution diluted 1:20 instead of XA-4 antiserum. The pattern of pre-absorbable staining observed with affinity-purified XA-4 antibodies was the same as seen with the XA-4 antiserum (Elphick & Jones 1998). However, the use of affinity-purified XA-4 antibodies resulted in lower levels of background staining than with the antiserum and this enabled a more detailed analysis of the cellular and subcellular distribution of SGC α in the locust visual system.

Photoreceptor cells are the only cell type in the locust eye that exhibit SGC α immunoreactivity (figure 2a). Thus, the cone apparatus that focuses light onto the rhabdom is devoid of SGC α immunoreactivity (figure 2a). Similarly, the pigment cells that surround the cone apparatus and photoreceptor cells (see Wilson *et al.* (1978) for diagram) are also devoid of SGC α immunoreactivity (figure 2a). Within photoreceptor cells, SGC α immunoreactivity is clearly associated with the rhabdomeric compartment (figure 2a), as described earlier (Elphick & Jones 1998), but SGC α does not appear to be confined to the rhabdom. For example, in the distal portion of photoreceptor cell bodies below the cone cell layer, SGC α immunoreactivity appears to extend beyond the rhabdom into the surrounding cytoplasm (figure 2a). At the base of the retina where the

Figure 2. Cellular and subcellular localization of SGC α in the locust visual system. (a) The distal portion of the locust retina. SGC α immunoreactivity is expressed by photoreceptor cell bodies and is localized in and around their central rhabdomeric compartments (open arrows). SGC α immunoreactivity also extends into apical processes of the photoreceptor cells (small black arrows) which surround the cone apparatus (c). The cone cells which form the cone apparatus are not immunoreactive. The pigment cells (p) which surround each cone apparatus are also devoid of immunoreactivity. Note also the unstained cone cell processes (small white arrow) which penetrate the distal core of each rhabdom. Refer to Wilson *et al.* (1978) for a detailed description of the ultrastructure of the locust eye. (b) The proximal portion of the locust retina. SGC α immunoreactivity is concentrated in and around the central rhabdom (open arrows) formed by the photoreceptor cells of each ommatidium. The photoreceptor cell axons which emanate at the base of the photoreceptor cell bodies do not exhibit observable immunoreactivity (filled black arrows). (c) The lamina. The lamina monopolar cells (LMCs), which are postsynaptic to the axons of photoreceptor cells R1–R6, do not exhibit SGC α immunoreactivity in their somata (open arrows). Very weak immunoreactivity is evident in the photoreceptor axons as they enter the lamina (filled black arrows). The lamina neuropile (La), where the synapses

rhabdom terminates and the photoreceptor cell axons emanate the intensity of SGC α immunoreactivity fades (figure 2b). In all preparations the intensity of staining in photoreceptor axons was much fainter than in the cell body and in some preparations was not discernible above the background (figure 2b and 2c). Similarly, in the first optic neuropile (lamina) weak staining or no staining (figure 2c) was observed, indicating that the level of SGC α expression in photoreceptor axon terminals is much lower than in the cell bodies.

(c) Analysis of the effect of the NO donor SNAP on the cGMP content of locust eyes

The expression of functional SGC enzyme activity in locust photoreceptor cells was investigated by testing the effect of an NO-releasing compound, SNAP, on the cGMP content of locust eyes. A scalpel blade was used to remove eyes from the head such that only the eye cuticle and underlying retinal tissue was collected. With this procedure the photoreceptor cells were severed basally but this ensured that little or no tissue from the optic lobes of the brain was included. Eyes were then placed individually in 100 μ l of locust physiological saline (5 mM HEPES, pH 7.2, containing 140 mM NaCl, 5 mM KCl, 5 mM CaCl₂, 1 mM MgCl₂, 100 mM sucrose and 20 mM glucose) in separate wells of a 96-well microtitre plate. After 15 min, 100 μ l of physiological saline containing 2 mM SNAP was added to each well. Then, after a further 15 min the eyes were transferred individually to 1.5 ml Eppendorf tubes containing 300 μ l of 50 mM sodium acetate, pH 4.75 and boiled for 5 min. Following boiling, the eyes were homogenized separately in glass homogenizers and the resulting homogenates centrifuged at 13 000 r.p.m. in a microcentrifuge for 5 min. The supernatants were collected and stored at 20 °C until analysed for cGMP content using the radioimmunoassay method described by Elphick *et al.* (1993).

Eyes incubated in locust physiological saline instead of 2 mM SNAP were examined to determine the basal cGMP content of locust eyes. Additional negative control experiments were performed using 2 mM *N*-acetylpenicillamine (NAP) instead of 2 mM SNAP. NAP is chemically identical to SNAP except for the absence of an NO moiety. Therefore, any effects which are observed with SNAP but not with NAP can be attributed specifically to SNAP's ability to release NO in solution.

First, experiments were performed on light-adapted eyes collected at 18.00 ('daytime'). The basal cGMP content of the locust eyes was 10–15 pmol per eye but surprisingly SNAP did not cause a significant increase in cGMP above this level (figure 3a). Experiments were then performed on dark-adapted eyes collected at 06.00 ('night-time'). Again the basal cGMP content of the locust eyes was found to be 10–15 pmol per eye (figure 3b). Moreover, SNAP caused a significant increase in the cGMP of the eyes, doubling the cGMP content above basal levels to 20–25 pmol per eye (figure 3b). Importantly, NAP had no effect on the cGMP content of the eyes, therefore demonstrating that the effect of SNAP was due to its ability to release NO.

Having identified this day:night-dependent difference in the ability of SNAP to activate cGMP production in locust eyes, we then investigated whether this difference in responsiveness

Figure 2. (Cont.) between photoreceptor cells and LMCs are located, exhibits little or no SGC α immunoreactivity. These data indicate that SGC is not expressed or is expressed at a low level in photoreceptor cell axons and their terminals. (Magnification in (a), (b) and (c): $\times 384$.)

Figure 3. Effect of the NO donor SNAP on the cGMP content of locust eyes compared with NAP (negative control). The basal cGMP content of locust eyes is seen in eyes incubated with physiological saline (PS). For each treatment, the graphs show the mean cGMP content of eyes from four individuals (i.e. $n=8$) with error bars showing standard errors. Experimental analysis was carried out under four different conditions: (a) under light conditions at 18.00 (day), (b) under dark conditions at 06.00 (night), (c) at 18.00 (day) but after 1 h of dark adaptation, and (d) at 06.00 (night) but after 1 h of light adaptation. No significant difference in the cGMP content of eyes was observed between the three treatments of light-adapted eyes ((a) and (d) $p>0.05$, t -test). In dark-adapted eyes ((b) and (c)) there was a significant increase in the cGMP content of SNAP-treated eyes compared with eyes incubated with NAP or physiological saline ($p<0.01$, t -test). These data demonstrate that stimulation of SGC activity in locust eyes is dark dependent.

reflects circadian changes in the biochemistry of the eyes or reflects differences between dark- and light-adapted eyes. To do this we performed experiments on eyes subjected to 1 h of dark adaptation (17.00–18.00) during the day and on eyes subjected to 1 h of light adaptation during the night (05.00–06.00). In each case, eyes were then removed and incubated with SNAP, NAP or physiological saline as described above. Eyes which were subjected to 1 h of dark adaptation during the day (figure 3c) displayed the same responsiveness to SNAP as seen previously with dark-adapted eyes tested during the night (figure 3b). Likewise, eyes which were subjected to 1 h of light adaptation during the night (figure 3d) were not responsive to SNAP treatment as seen previously with light-adapted eyes tested during the day (figure 3a). These experiments indicate that SGC is not under circadian control but that stimulation of SGC activity in the locust eye is dark dependent with light having an inhibitory effect on SGC activity.

The data shown in figure 3, however, do not indicate that the basal cGMP content of eyes is influenced either by light or by circadian time. To address this issue, we carried out further analysis of the basal cGMP content of locust eyes over a 24-h period (12 h light and 12 h dark) in which eyes ($n=8$) were sampled every 2 h (data not shown). No clear trend was

apparent but when the pooled daytime data were compared with the pooled night-time data, the mean cGMP content of eyes in the night (dark) was slightly (16%) but significantly ($p=0.038$, t -test; $n=48$) higher than in the day (light). It remains to be established, however, whether this slight difference is of any functional significance.

(d) Analysis of the effect of SGC inhibitors on the cGMP content of locust eyes

Having established that SGC activity can be detected in the locust eye, albeit only under dark conditions, we then tested the effect of SGC inhibitors on the cGMP content of locust eyes. The objective here was, first, to establish whether SNAP-induced elevation of cGMP in dark-adapted locust eyes is blocked when eyes are pre-treated with SGC inhibitors. Second, these experiments were performed to determine whether SGC activity may contribute to basal cGMP production in locust eyes. Two SGC inhibitors were tested: methylene blue, which is a non-selective inhibitor of SGC (Miki *et al.* 1987; Mayer *et al.* 1993) and ODQ which reportedly is a selective inhibitor of SGC (Garthwaite *et al.* 1995).

For methylene blue experiments, locust eyes were removed from dark-adapted locusts at 05.00 and incubated in microtitre plate wells for 15 min in 100 μ l of physiological saline containing 100 μ M methylene blue. Control eyes were incubated for the same length of time in 100 μ l of physiological saline. For methylene blue-treated eyes, 100 μ l of physiological saline containing 100 μ M methylene blue plus 2 mM SNAP or 2 mM NAP was then added to the well for a further 15 min. For control eyes, 100 μ l of physiological saline containing 2 mM SNAP or 2 mM NAP was added to the well for a further 15 min. The cGMP content of eyes was then determined as described above. Experiments with ODQ were carried out in the same way as for methylene blue except for preparation of the drug in ethanol due to its low solubility in water. ODQ was tested at a concentration of 10 μ M in 0.1% ethanol–physiological saline and control eyes were incubated with 0.1% ethanol–physiological saline.

Methylene blue inhibited the effect of SNAP on locust eyes, reducing the cGMP content of SNAP-treated eyes to basal levels (figure 4a). In control eyes treated with NAP, methylene blue did not have any effect on the basal cGMP content (figure 4a). Likewise, ODQ inhibited the effect of SNAP on locust eyes, reducing the cGMP content of SNAP-treated eyes to basal levels (figure 4b). In control eyes treated with NAP, ODQ caused a slight but non-significant ($p>0.05$, t -test) reduction in the basal cGMP content (figure 4b). These data demonstrate that the stimulatory effect of SNAP on cGMP production in locust eyes is specifically attributable to activation of SGC. The observation that SGC inhibitors do not affect the basal cGMP content of eyes indicates that stimulation of SGC by NO is not required to maintain the basal cGMP content of eyes.

(e) Analysis of the role of Ca^{2+} in light-induced inhibition of SGC activity in locust eyes

The observation that SNAP causes cGMP elevation in dark-adapted locust eyes but not in light-adapted eyes (figure 3) indicates that light causes inhibition of SGC activity in photoreceptor cells. It seemed unlikely, however, that photons could have a direct inhibitory effect on SGC and, therefore, we investigated the role of light-regulated biochemical mediators. Photon-induced activation of rhodopsin molecules in the rhabdomeric microvilli of insect photoreceptor cells results in

Figure 4. Effect of the SGC inhibitors methylene blue and ODQ on the cGMP content of SNAP-treated and NAP-treated (control), dark-adapted locust eyes. For each treatment, the graphs show the mean cGMP content of eyes from four individuals (i.e. $n=8$) with error bars showing standard errors. (a) Methylene blue does not have a significant effect on the mean basal cGMP content of locust eyes as seen in NAP-treated controls ($p>0.1$, t -test). However, methylene blue causes a significant reduction in the mean cGMP content of SNAP-treated eyes when compared with control eyes incubated with physiological saline instead of methylene blue ($p<0.01$, t -test). (b) ODQ causes a slight but non-significant ($p>0.05$, t -test) reduction in the mean basal cGMP content of locust eyes as seen in NAP-treated controls. However, ODQ causes a significant reduction in the mean cGMP content of SNAP-treated eyes when compared with control eyes incubated with 0.1% ethanol/physiological saline (vehicle) instead of ODQ ($p<0.01$, t -test). In fact, the mean cGMP content of ODQ-treated eyes is not significantly different from basal levels seen in NAP-treated eyes ($p>0.1$, t -test). These data demonstrate that the stimulatory effect of SNAP on cGMP production in locusts eyes is specifically mediated by NO-dependent activation of SGC.

activation of phospholipase C (PLC) via G protein-mediated mechanisms (Ranganathan *et al.* 1995). PLC then produces the second messenger molecule inositol 1,4,5-trisphosphate (IP_3) which stimulates release of Ca^{2+} from intracellular Ca^{2+} stores and/or influx of extracellular Ca^{2+} (Ranganathan *et al.* 1995). Thus, light causes an increase in the cytosolic Ca^{2+} concentration of photoreceptor cells. Here we have investigated the role of Ca^{2+} in mediating light-induced inhibition of SGC activity in locust eyes.

Figure 5. Effect of Ca^{2+} -free physiological saline on light-dependent inhibition of SGC activity in locust eyes. For each treatment, the graphs show the mean cGMP content of light-adapted eyes from four individuals (i.e. $n=8$) with error bars showing standard errors. The basal cGMP content is seen in the NAP-treated controls where there is no significant difference between eyes incubated with physiological saline and eyes incubated with Ca^{2+} -free saline ($p>0.1$, t -test). Treatment of eyes incubated in normal physiological saline with SNAP does not cause a significant increase ($p>0.1$, t -test) in the mean cGMP content above basal levels (as also seen in figure 3a and 3d). However, eyes incubated with Ca^{2+} -free saline (plus 1 mM EGTA) display a significant doubling in cGMP content above basal levels when treated with SNAP ($p<0.01$, t -test). These data indicate that light causes Ca^{2+} -dependent inhibition of SGC activity in locust eyes.

Light-adapted eyes collected at 18.00 were incubated in microtitre plate wells for 15 min in 100 μ l of Ca^{2+} -free physiological saline containing 1 mM ethylene glycol-bis(β -aminoethyl ether) N,N,N' -tetracetic acid (EGTA), a Ca^{2+} chelator. Then 100 μ l of Ca^{2+} -free saline (plus 1 mM EGTA) containing either 2 mM SNAP or 2 mM NAP was added to each well for a further 15 min. The cGMP content of eyes was then determined as described above. Control eyes were treated in the same way but with normal physiological saline.

Light-adapted control eyes displayed the same responses as seen before in figure 3, with SNAP not causing an increase in cGMP above the basal levels observed with NAP (figure 5). However, in light-adapted eyes incubated in Ca^{2+} -free saline containing 1 mM EGTA, SNAP caused a twofold increase in cGMP above basal levels (figure 5). Thus, light-adapted eyes incubated with Ca^{2+} -free saline displayed the same responsiveness to SNAP as seen in dark-adapted eyes. These data indicate that light causes Ca^{2+} -dependent inhibition of SGC activity in locust eyes.

3. DISCUSSION

Using affinity-purified antibodies to the *Drosophila* SGC α subunit, we have demonstrated that an *ca.* 65 kDa SGC α subunit is expressed in photoreceptor cells of locust compound eyes. Immunocytochemical analysis using affinity-purified SGC α antibodies revealed that SGC α is expressed specifically in photoreceptors cells and not in other cell types of compound eyes (i.e. cone cells and pigment cells). This is an important observation because it suggests that all SGC enzyme activity detected in the

locust eye is derived from photoreceptor cells. This interpretation is also supported by the observations of Bicker & Schmachtenberg (1997) who showed that the NO donor sodium nitroprusside induces cGMP immunoreactivity in locust photoreceptor cells. The locust eye is therefore a preparation in which it may be possible to examine the biochemical properties and physiological roles of SGC in a cell population with a well-characterized function in the behaviourally important process of insect vision.

Here we have begun to investigate the properties of SGC in photoreceptor cells of locust compound eyes by measuring the cGMP content of eyes under a variety of experimental conditions. We have found that the basal cGMP content of locust eyes is 10–15 pmol per eye but maintenance of this basal cGMP content of the eyes is unaffected by SGC inhibitors, at least for the duration of our experiments. There are a number of possible interpretations of these results. The SGC inhibitor ODQ acts by preventing interaction of NO with a haem moiety which is associated with SGC in its heterodimeric form (Schrammel *et al.* 1996). Therefore, the basal cGMP content of the eye could reflect ongoing low-level activity of SGC in the absence of NO. Moreover, if cGMP hydrolysis in the eye occurs at a low rate, basal cGMP may reflect SGC activity that occurred in the eye before the experiment. Alternatively, it is possible that the basal cGMP content of the eye is due to the activity of other isoforms of guanylyl cyclase present in the eye. For example, in addition to SGCs that are activated by NO, there are a family of membrane-associated guanylyl cyclases that are activated by peptide ligands (Yuen & Garbers 1992). However, it remains to be determined what other guanylyl cyclases apart from SGC are expressed in locust eyes and whether these are expressed in photoreceptor cells or in other cell types of the retina.

We have demonstrated that SGC activity is present in the locust eye with the observation that the NO donor SNAP causes an increase in the cGMP content of dark-adapted locust eyes twofold above basal levels. Moreover, this effect of SNAP is inhibited by the SGC inhibitors methylene blue and ODQ. SGC α immunoreactivity is only observed in photoreceptor cells and, therefore, we conclude that the 10–15 pmol increase in cGMP observed in SNAP-treated eyes occurs specifically in the population of photoreceptor cells and not in other cell types of the locust eye. However, without knowing which cell types the basal cGMP content of the eye is derived from we are unable to determine the magnitude of the increase in cGMP concentration that occurs in photoreceptor cells when SGC is activated. A twofold increase in cGMP in response to SNAP is quite small compared to results from other tissues. For example, in the locust brain SNAP caused a four- to fivefold increase in cGMP above basal levels (Elphick *et al.* 1993). Nevertheless, if a proportion of the basal cGMP content of the eye is derived from cells apart from photoreceptor cells then the magnitude of the increase in cGMP in photoreceptor cells upon activation of SGC is likely to be in excess of twofold. Such changes in cGMP concentration may have important physiological consequences for photoreceptor cell function.

Importantly, we have found that SGC activity in the locust eye is inhibited by light in a Ca²⁺-dependent

manner. This observation is intriguing because it suggests that SGC and cGMP may participate in mechanisms of light–dark adaptation in photoreceptor cells. Our data indicate that, when SGC is activated endogenously, cGMP elevation would occur in photoreceptor cells under dark conditions but not in the light. What might be the physiological consequences of SGC activation in dark-adapted photoreceptor cells? Schmachtenberg & Bicker (1997) recently reported preliminary experimental findings which may begin to address this question. These authors examined the effects of the NO donor sodium nitroprusside and a membrane-permeable analogue of cGMP on retinograms recorded from dark-adapted locust eyes stimulated with green-light flashes. Both sodium nitroprusside and cGMP caused an increase in the size of retinograms whereas NO scavengers and a NO synthase inhibitor caused a reduction in retinogram size. These observations indicate that activation of SGC in locust photoreceptor cells may increase sensitivity to light under dark conditions. Importantly, this conclusion is consistent with our observation that stimulation of SGC activity in photoreceptor cells is dark dependent.

A number of important questions arise from these findings. It remains to be determined what causes activation of SGC *in vivo*. In our *in vitro* experiments an NO-donor was used to stimulate SGC but this does not prove that NO is necessarily responsible for activation of SGC *in vivo*. NO is not the only molecule that functions as an endogenous activating ligand for SGC; the gas carbon monoxide (CO) synthesized by haem oxygenase is also involved in regulation of SGC *in vivo* (Dawson & Snyder 1994). Assessment of NO and CO as potential activating ligands for SGC in photoreceptor cells requires analysis of the expression in the locust visual system of the enzymes responsible for their synthesis, NO synthases and haem oxygenases, respectively. The distribution of NO synthase in the locust visual system has already been examined using NADPH diaphorase histochemistry (Elphick *et al.* 1996). Importantly, NADPH diaphorase activity is not detected in photoreceptor cells or in other cell types of the locust eye. The nearest NADPH diaphorase-positive cells to photoreceptor cells are a subpopulation of the lamina monopolar cells which are postsynaptic to photoreceptor cells R1–R6 (Elphick *et al.* 1996). It remains to be determined, however, whether NO production by these cells is responsible for regulation of SGC in photoreceptor cells. Moreover, the absence of NO synthase in cells of the retina raises the possibility that CO and not NO may be responsible for regulation of SGC activity in the rhabdomic compartment of photoreceptor cell bodies where SGC α immunoreactivity is concentrated.

The observation that SGC activity in photoreceptor cells is inhibited by light in a Ca²⁺-dependent manner is intriguing. One possibility is that this is due to activation of Ca²⁺-dependent phosphodiesterases which hydrolyse SGC-derived cGMP. If this were true, we would also expect basal cGMP levels to be negatively affected by light. The experiments shown in figure 3 do not show a decrease in basal cGMP in the light and when we analysed eyes over a 24 hour period we observed only a slight decrease in the light compared to in the dark. Therefore, although the involvement of phosphodiesterases cannot be excluded, the alternative explanation is

that light causes Ca^{2+} -dependent inhibition of SGC. There is no evidence of such Ca^{2+} -dependent regulation of SGC in mammalian cells and this may therefore represent a fundamental difference in the properties of SGC in insects and mammals.

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