Bernd Walz · Kyrill Ukhanov · Bernhard Zimmermann

Actions of neomycin on electrical light responses, Ca\(^{2+}\) release, and intracellular Ca\(^{2+}\) changes in photoreceptors of the honeybee drone

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Abstract Neomycin, known to inhibit phospholipase C-mediated IP\(_3\) formation, was applied in the bath or injected into cells and its effects on electrical light responses were analyzed. Neomycin effects on inositol 1,4,5-trisphosphate- and Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the endoplasmic reticulum and/or the light-induced Ca\(^{2+}\) elevation were also studied. Neomycin (0.5 mmol l\(^{-1}\)) blocked inositol 1,4,5-trisphosphate-, caffeine-, and Ca\(^{2+}\)-induced Ca\(^{2+}\) release. Bath application of neomycin decreased the sensitivity to 20-ms light flashes by a factor of up to 100 and slowed the kinetics of dim flash responses. Intracellularly injected neomycin desensitized the photoreceptors more than 1 log unit, increased the latency, and slowed the rate of rise of the light response. Neomycin (0.5 mmol l\(^{-1}\)) in the bath delayed and reduced the transient component of responses to 1-s steps of light at intermediate intensities. It also decreased and slowed the light-induced, and it blocked the caffeine-induced intracellular Ca\(^{2+}\) elevation. The combined pharmacological effects of neomycin are suggested to decrease the Ca\(^{2+}\)-mediated amplification of the phototransduction cascade and the Ca\(^{2+}\)-mediated acceleration of processes determining the kinetics of light responses.

Key words Photoreceptors · Phototransduction · Neomycin · Calcium · Phosphoinositide cascade

Introduction

Microvillar photoreceptors in invertebrates respond to light stimulation with a depolarizing receptor potential caused by the opening of cation channels in their microvillar membrane. The mechanism and excitatory transmitter responsible for the opening of the light-sensitive channels remain enigmatic (for reviews see Hardie 1993; O’Day et al. 1997; Montell 1999). It is well established that light stimulation activates, via a G protein, the phosphoinositide cascade. Indeed, the key enzyme of the phosphoinositide cascade, phospholipase C (PLC), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP\(_3\)), is considered to be essential in the cascade of events between the light absorption by rhodopsin and the generation of the electrical light response. Key observations are that the norp A mutant of Drosophila lacks functional PLC and is unexcitable by light (Bloomquist et al. 1988), but that it can be rescued by expression of norp A protein (McKay et al. 1995). In addition, injections of IP\(_3\) or Ca\(^{2+}\) intoLimulus ventral nerve photoreceptors mimic the effects of light stimulation and produce bursts of inward current having the same reversal potential as the light-induced current (Brown et al. 1984; Fein et al. 1984; Payne et al. 1985, 1986). Recently IP\(_3\) has been shown to activate an inward ionic current in isolated rhabdometric photoreceptors in the mollusc Lima (Gomez and Nasi 1998), although by as yet unknown mechanisms. Nevertheless, an involvement of IP\(_3\)-induced Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores seems not to be an obligatory step in phototransduction in every invertebrate microvillar photoreceptor. In Drosophila photoreceptors, which are considered as a prototype of microvillar visual cells, Ca\(^{2+}\) elevation alone seems to be insufficient to cause excitation, because photolytic release of Ca\(^{2+}\) from caged Ca\(^{2+}\) does not induce excitation (Hardie 1995) but affects only the gain and kinetics of the light responses. The most serious challenge to the importance of
IP$_3$-induced Ca$^{2+}$ release for *Drosophila* phototransduction has come from a recent molecular genetic study showing that a null mutation for the IP$_3$ receptor does not eliminate electrical light responses (Acharya et al. 1997). Alternatively, a recent study by Chyb et al. (1999) has provided evidence for the involvement of the DAG branch of the phosphoinositide pathway for excitation in *Drosophila* photoreceptors.

In this study, we have sought to assess the importance of the phosphoinositide signaling pathway for excitation and adaptation in honeybee drone photoreceptors by pharmacological experiments. In this species we have previously shown that light releases Ca$^{2+}$ from the submicrovillar endoplasmic reticulum (ER) Ca$^{2+}$ stores (Baumann et al. 1991), and that these stores have both an IP$_3$-induced and a Ca$^{2+}$-induced Ca$^{2+}$ release mechanism (Baumann and Walz 1989; Walz et al. 1995). Neomycin is known to bind to PIP$_2$ and to reduce or block PLC-mediated IP$_3$ formation (e.g., Carney et al. 1985; Gaber et al. 1989; Schacht 1976, 1978; Schibeci and Schacht 1977; Wojcikiewitz and Fain 1988). It also acts in some other systems in a nonspecific manner and has been shown to exert a number of pharmacological effects, such as the inhibition of Ca$^{2+}$ currents in *Paramecium* and clonal GH3 pituitary cells (Suarez-Kurtz and Reuben 1987; Gustin and Hennessey 1988) and the blocking of ATP-induced inward currents in outer hair cells (Lin et al. 1993). The direct inhibition of Ca$^{2+}$ release from internal stores has been reported for cells of vertebrate origin (Prentki et al. 1986; Palace 1987; Sayers and Michelangeli 1993). To substantiate our neomycin experiments, we have therefore tested whether this compound also directly affects Ca$^{2+}$ handling (ATP-driven Ca$^{2+}$ uptake, IP$_3$-induced and Ca$^{2+}$-induced Ca$^{2+}$ release) by the submicrovillar ER in bee photoreceptors.

We show that neomycin directly inhibits both IP$_3$-induced and Ca$^{2+}$-induced Ca$^{2+}$ release and, thus, can be expected to interfere at several stages with the phosphoinositide signaling cascade. Our finding that neomycin reduces the gain and speed of the transduction process strengthens previous proposals that these are the critical parameters affected by the phosphoinositide pathway.

**Materials and methods**

**Animals, preparation, solutions**

Honey bee drones (*Apis mellifera*) were obtained from a local hive or from Dr. J. Kefuss, Toulouse, France. The drones were kept together with worker bees and fed a 50% sugar solution.

Recordings were made from 400-800-µm-thick slices of the head of each drone. The slices were cut as previously described (Coles and Orkand 1983), mounted in a glass-bottomed recording chamber, and continuously superfused with oxygenated physiological saline of the following composition (mmol l$^{-1}$): NaCl 270, KCl 10, MgCl$_2$ 10, CaCl$_2$ 1.6, TRIS 10, pH 7.4. During the preparation and mounting of the slices, they were only illuminated with red light (filter: RG 610) to avoid exciting the photoreceptor cells (Bertrand et al. 1979).

**Light stimulation**

Light from a 100-W halogen lamp was passed through an electromechanical shutter, a heat absorbing filter (KG1), and neutral-density filters and illuminated the retinal slice from below. The intensity of the unattenuated beam was about 1 mW mm$^{-2}$ in the specimen plane. However, the absolute light intensity at the recording site within the retinal slice was not known, because the eye contains screening pigments and the slices vary in thickness. Therefore, only the relative light intensities are given as log$_{10}$ I/I$_0$, where I$_0$ is the unattenuated light intensity.

**Electrical recording**

Intracellular recordings were made by standard techniques. Conventional microelectrodes were filled with 3 mol l$^{-1}$ KCl and had resistances between 50 MΩ and 100 MΩ. Data were only accepted from cells having resting membrane potentials greater than −50 mV.

For pressure injections of neomycin, the electrodes contained 500 mmol l$^{-1}$ neomycin in a carrier solution of the following composition (mmol l$^{-1}$): K-aspartate 125, HEPES 10, pH 7.0. Most of these electrodes were slightly broken by touching them against the brain before impalement in order to facilitate injections.

Nevertheless, a rather high neomycin concentration (500 mmol l$^{-1}$) was necessary in the pipettes in order to load the cells with pharmacologically active neomycin concentrations.

The membrane potential was continuously recorded on a pen recorder. Responses to test flashes were digitized and stored on floppy disk. A/D conversion, data display, and output were controlled by the software EASYEST (Axyst Software Technologies, Rochester, N.Y.) or TestPoint (Keithley, Germering, Germany).

**Microphotometric recordings of Ca$^{2+}$ uptake into and Ca$^{2+}$ release from the ER**

The rates of Ca$^{2+}$ fluxes across the ER membrane were measured microphotometrically in situ in permeabilized tissue slices by means of the oxalate method described in detail previously (Walz 1982a, 1982b; Baumann and Walz 1989; Walz and Baumann 1989; Walz et al. 1995). The retinal tissue slices were first permeabilized for ~20 min at room temperature with 200 µg ml$^{-1}$ saponin in 200 mmol l$^{-1}$ potassium aspartate, 5 mmol l$^{-1}$ MgCl$_2$, 2 mmol l$^{-1}$ K$_2$EGTA, 5 mmol l$^{-1}$ Na$_2$ATP, 20 mmol l$^{-1}$ HEPES, pH 7.0. The slices were then incubated in standard accumulation medium (AM) containing 180 mmol l$^{-1}$ potassium aspartate, 20 mmol l$^{-1}$ potassium oxalate, 5 mmol l$^{-1}$ MgCl$_2$ 2 mmol l$^{-1}$ K$_2$EGTA, 3 mmol l$^{-1}$ CaEGTA, 5 mmol l$^{-1}$ Na$_2$ATP, 20 mmol l$^{-1}$ HEPES, pH 7.0. The free Ca$^{2+}$ concentration of the standard AM was 0.5 µmol l$^{-1}$ when measured fluorometrically with fluo-3 (Walz et al. 1994). Modifications of the AM, such as its free Ca$^{2+}$ concentration, additions of neomycin, IP$_3$ etc., are specified in the figure legends.

Incubation of permeabilized tissue slices in AM stimulates Ca$^{2+}$-uptake by the ER. When the Ca-oxalate solubility product is exceeded in the ER lumen because of Ca$^{2+}$ uptake (the ER membrane is permeable to the oxalate anion), Ca-oxalate precipitates within the ER (Walz 1982a, 1982b; Baumann and Walz 1989; Walz and Baumann 1989). Ca-oxalate is birefringent; therefore, the rate of Ca-oxalate formation can be measured microphotometrically by using a polarization microscope. The rate of change in the optical signal can be used as a reliable and linear measure of the rate of Ca$^{2+}$ uptake or release, because (1) the size of the optical signal is proportional to the rate of Ca-oxalate in the field of measurement (in the preparation), and (2) a linear function exists between the rate of the rise in the optical signal and the various rates of Ca$^{2+}$ accumulation into the compartment in which its precipitation as Ca-oxalate occurs (for experimental tests of the linearity of the method, see Walz 1982b; Walz et al. 1995).

For the microphotometric measurements, the recording chamber was placed on the stage of a Zeiss UEM microscope equipped...