Characterization of Maxi-K-Channels in Bovine Trabecular Meshwork and Their Activation by Cyclic Guanosine Monophosphate

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Purpose. Electrophysiological characterization of trabecular meshwork cells and investigation of their response to elevation of cytosolic cyclic guanosine monophosphate (cGMP).

Methods. Bovine trabecular meshwork cells were cultured according to established methods and were studied, using the whole-cell and single-channel configurations of the patch-clamp technique.

Results. In single-channel experiments, cells expressed a channel with characteristics typical of maxi-K-channels. The channel was densely distributed in the membrane and had a high conductance of 326 ± 4 pS (Pico Siemens) (symmetrical 150 mmol/l KCl; 37°C) for potassium and negligible conductance for sodium (0.9 ± 1 pS). The open probability could be elevated by depolarization, increasing cytosolic calcium, or adding adenosine triphosphate (1 mmol/l). The channel could be blocked by external charybdotoxin (10^{-8} mol/l), external TEA^{+} tetraethyl ammonium chloride (1 mmol/l) and by internal Ba^{2+} (10 mmol/l), whereas external Ba^{2+} and internal TEA^{+} (10 mmol/l) had no effect. In whole-cell experiments, trabecular meshwork cells displayed a strong outward conductance. Part of this conductance (35 ± 5%) could be blocked by charybdotoxin and stimulated by ionomycin (10^{-5} mol/l). Addition of 8-bromo-cGMP (10^{-3} mol/l) stimulated the current to 290 ± 57% (n = 4) of the original level, charybdotoxin led to a reduction of this current to 156 ± 28% of the initial value.

Conclusions. Trabecular meshwork cells express maxi-K-channels. These channels can be stimulated by raising internal cGMP levels and are known for their importance in smooth muscle relaxation. The results in this study supply further evidence that trabecular meshwork displays smooth muscle-like properties and contributes to the clarification of the mechanism leading to the relaxation of trabecular meshwork by nitrate and nonnitrate vasodilators. Invest Ophthalmol Vis Sci. 1997;38:1883-1892.

To date, clinical management of glaucoma centers around the normalization of intraocular pressure. Despite the controversial discussion concerning the underlying pathophysiological mechanisms of vision loss in glaucoma,1 the detrimental contribution of elevated intraocular pressure has not been refuted.2,3 Thus, the search for new drugs to reduce intraocular pressure with fewer ophthalmologic and systemic side effects is justified. In particular, an ideal pressure-reducing drug should not have vasoconstrictive properties that potentially interfere with the microcirculation of the retina and of the optic nerve.4

Classically, the ocular outflow pathway is treated as a passive filter with functional pores that can be extended by the ciliary muscle. However, considerable evidence has shown that trabecular meshwork displays smooth-muscle-like properties and is actively involved in aqueous humor dynamics.5-8 In particular, it has been shown that the tonus of trabecular meshwork can be influenced by a variety of drugs,6 and that substances that relax trabecular meshwork increase ocular outflow in the model of the perfused anterior segment with ciliary muscle detachment.8 However, the search for a more specific drug with minimal ac-
tion on the ciliary muscle continues; and for this, more detailed information concerning the specific physiological properties of trabecular meshwork is needed.

Despite a number of studies that have used various investigative methods, including extensive morphologic and histologic studies, recording of membrane voltage, and direct contractility measurements, so far no attempt has been made to characterize trabecular meshwork cells in more detail, using the patch-clamp technique. Smooth muscle cells from various tissues have been investigated using this method, however; and it has helped to identify some mechanisms of smooth muscle contractility.13-16

One of the channels that has received increasing attention is the maxi-K-channel (BKca), or calcium-dependent potassium channel.13-15 Recent evidence suggests that the maxi-K-channel, distributed densely in the cell membrane of smooth muscle of various tissues, is important for the regulation of smooth muscle tone and is a target protein mediating the action of various smooth-muscle-relaxant substances.

The results of our previous study enabled us to show that vasodilatators that activate the nitric oxide-cyclic guanosine monophosphate (cGMP) system relax trabecular meshwork strips.16 Using the same bovine model in the current study, we were able to observe maxi-K-channels directly in trabecular meshwork cells, and we present evidence for their activation by elevation of intracellular levels of cGMP.

MATERIALS AND METHODS

Tissue Culture

Primary bovine trabecular meshwork cell cultures were prepared as described previously.5,12,17,18 In summary, freshly enucleated bovine eyes were obtained from a local slaughterhouse. All animals were handled in accordance to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Small pieces of trabecular meshwork were obtained from the eyes, placed in a sterile Petri dish under a glass coverslip, and fed twice weekly with Dulbecco’s modified Eagle’s minimum essential medium (DMEM) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (all cell culture material from Biochrom, Berlin, Germany). Cultures were maintained in a 95% air–5% CO2 atmosphere at 37°C. The cultures were passaged, using the trypsin–EDTA method. Only cells from the third passage were used for experiments. A few experiments were performed on freshly isolated cells obtained by digestion of trabecular meshwork strips, using 0.4% collagenase (type IX, Sigma, Deisenhofen, Germany). In these experiments, cells were used directly after settling without further treatment. No significant differences were observed in the electrophysiological properties of these cells and cultured cells.

Patch-Clamp Experiments

To obtain single cells from confluent cell layers, cells were dispersed by enzymatic treatment with trypsin for 3 minutes, suspended in cell culture medium, and allowed to settle on glass coverslips for 0.5 hour at 37°C. The coverslips were introduced into a perfusion chamber on the stage of an inverted microscope (Axiovert 35, Zeiss, Oberkochen, Germany) and were superfused with Ringer’s solution. The chamber itself was heated (LN Temperature Controller, Luigs and Neumann, Ratingen, Germany) and the perfusate prewarmed. All experiments were performed at 37°C. Fluid exchange was complete within less than 5 seconds. Standard patch-clamp techniques were used.19 Borosilicate glass-patch pipettes (wall thickness 0.3 mm; Clark Electromedical Instruments, Reading, UK) were pulled and polished with a DMZ Universal Puller (Zeitz, Augsburg, Germany). The input resistance of the pipettes was 4 to 5 MΩ for experiments in the whole-cell configuration and 8 to 9 MΩ for single-channel experiments. Potentials were referenced to the bath, using an Ag–AgCl electrode connected to the bath solution by an agar bridge electrode, so that a negative potential corresponded to a negative pipette potential. Liquid junction potentials were determined according to the method described by Neher.20 Unless indicated otherwise, positive ions flowing into the pipette correspond to a negative current and are depicted in figures as going downward, whereas positive ions flowing out of the pipette are designated by a positive current in the upward direction. For inside-out patches, the pipette potential corresponds to the negative membrane potential.

Currents were recorded by an EPC 9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Pulse generation, data collection, and data analysis were performed with TIDA for Windows Software (HEKA) and filtered with a 2.9 kHz Bessel filter. Records were corrected for capacitance. Whole-cell measurements were not leak-subtracted, because it was difficult to distinguish between a nonspecific leak and a physiological nonvoltage-dependent current. Access resistance was below 8 MΩ and was corrected with TIDA software. Several different types of pulse protocols were used. For single channel experiments, voltage was automatically stepped to various levels. Data acquisition started 0.5 seconds after the voltage step at a sampling rate of 10 kHz for a duration of 6 seconds before the next voltage step. In whole-cell experiments, two types of protocols were used. Changes in external solution were monitored by a protocol that generated steps of 100-msec duration to various voltages. After each individual step, the voltage returned...
to a holding potential of -40 mV for 100 msec. Currents were continuously sampled at 100 Hz throughout this protocol. After completion of the solution changes and a stabilization of current levels, a second, conventional pulse protocol was used to record currents. In this protocol, data were first acquired at the holding potential for 20 msec and the potential was stepped to the potential to be tested for 100 msec, followed by a 50-msec return to the holding potential. Throughout this recording, data were sampled at 5 kHz.

Solutions and Chemicals

Control Ringer’s solution contained the following ion concentrations (in millimoles per liter): 151 NaCl, 4 KCl, 1.7 CaCl₂, 1 KH₂PO₄, 0.9 MgSO₄, 10 HEPES, and 5 glucose adjusted to pH 7.4 (NaOH). The standard intracellular solution (pipette solution) for whole-cell experiments contained (in millimoles per liter): 119 K-glutamate, 10 NaCl, 1 KH₂PO₄, 0.9 MgSO₄, 3.5 EGTA, 6.6 CaEGTA, and 10 HEPES adjusted to pH 7.2 (NaOH). This solution corresponds to an internal calcium concentration of 2.7 × 10⁻⁷ mol/l and will be referred to as K-glutamate solution. For single-channel experiments, the standard bath solution contained (in millimoles per liter): 134 KCl, 11 NaCl, 1 KH₂PO₄, 0.9 MgCl₂, 1 CaCl₂, and 10 HEPES adjusted to pH 7.4 (NaOH). For different activities of free calcium, the solutions were prepared according to established methods that use EGTA and nitrilotriacetic acid (NTA) as calcium buffers. Potassium concentrations were varied by substituting equimolar amounts of NaCl for KCl. All chemicals were of the highest available grade of purity. Charybdotoxin (ChTX, Bachem, Heidelberg, Germany) was dissolved in phosphate-buffered saline (PBS, Biochrom) before addition to the solution. Tetraethylammonium chloride (TEACl) was from Merck (Darmstadt, Germany).

Statistical Analysis

Single-channel current–voltage relationships were fitted, using the Goldman–Hodgkin–Katz equation. Because it was not possible to determine the exact number of channels per patch, channel activity was estimated from the product N Pₒ, in which N is the number of channels and Pₒ is single-channel open probability. The time integral of current divided by the product of mean single-channel current and time of recording is equal to N Pₒ. Relationships between voltage and channel activity were fitted, using the Boltzmann equation. In other cases, simple spline fits were used. All fits were carried out with commercially available software (Sigma Plot Scientific Graph System, Version 1.02, Jandel, San Rafael, CA, USA). Data are given as mean values ± standard error of the mean (SEM) and tested for significance, using the paired Student’s t-test. The number (n) refers to the number of experiments. Each experiment was performed on one cell. Care was taken to ensure that these cells were not from the same explant.

RESULTS

Excised Inside-Out Patches

In previous studies on human and bovine trabecular meshwork cells, by employing conventional measurements of membrane voltage, we found indirect evidence of the presence of maxi-K-channels in trabecular meshwork cells. We used the single-channel configuration of the patch-clamp technique to demonstrate their existence in these cells, first using the inside-out configuration to allow manipulation of cytosolic content.

In inside-out patches, a channel with high conductance could be observed (Fig. 1A). Channel density was greater than six channels per patch with 8-MΩ patch pipettes; individual patches sometimes possessed so many channels that individual openings could not be resolved. The channel was blocked by adding 10 mmol/l Ba²⁺ to the cytosolic surface, whereas intracellular 10 mmol/l TEACl had no effect (both n = 3). This is a typical property of maxi-K-channels.

To determine whether these large channels were potassium channels, we measured conductance, using symmetrical and asymmetrical KCl–NaCl solutions to assess the relative contributions of sodium and potassium to the entire conductance. In experiments with 50 mmol/l or 135 mmol/l potassium chloride in the patch pipette, reducing bath potassium from 135 mmol/l to 50 mmol/l by isoosmotic replacement with sodium resulted in a dramatic decrease of unitary current (Figs. 1A, 1B). The current–voltage relationships that could be observed in these experiments were fitted, using the Goldman–Hodgkin–Katz equation (Fig. 1C). The permeability coefficient that can be derived from these fits for the two ions is independent of the ion concentrations used. For a symmetrical concentration of 150 mmol/l, an average conductance of 326 ± 4 pS (n = 10) for potassium and 0.9 ± 1 pS (n = 10) for sodium (Fig. 1C) was calculated. For a symmetrical concentration of 135 mmol/l KCl, this conductance level corresponds to 293 pS. This is the upper range of conductance levels reported for mammalian cells by Hille.

Open probability was dependent on the voltage applied (Fig. 2A). Note that a few openings can be seen at positive pipette potentials, corresponding to negative, physiological membrane potentials. However, at these potential levels, openings are very rare and short. The long intervals between the short openings are not shown in the figure.
To determine whether the channel was calcium dependent, we performed experiments in which cytosolic calcium levels were increased. Although conductance levels were not affected by this maneuver, channel activity increased dramatically with the level of calcium on the cytosolic surface of the patch (Fig. 2B). In Figure 3, data from five different patches were averaged and fitted, using the Boltzmann equation. Each of the patches was exposed to varying concentrations of cytosolic calcium, starting with low calcium levels and ending with the higher levels to eliminate possible artifacts caused by rundown of channel activity. For concentrations in the range of $10^{-7}$ to $10^{-6}$ mol/l calcium, activity decreased with pipette potential (corresponding to an increase with membrane potential levels; Fig. 3A). However, no significant difference between these two calcium concentrations could be observed. Activity increased greatly when calcium was raised from $10^{-6}$ to $10^{-5}$ mol/l calcium (Fig. 3B). Voltage dependence and open probability changed significantly when calcium was raised from $10^{-5}$ to $10^{-4}$ mol/l (Fig. 3C).

At resting-potential levels and levels of internal calcium ($10^{-7}$ mol/l), channel openings were extremely rare. Addition of adenosine triphosphate (1 mmol/l) to the cytosolic side greatly increased the activity of the channels and shifted the voltage dependence of open probability toward more negative potential levels (Fig. 4).
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Excised Outside-Out Patches

In excised outside-out patches, which allow an exchange of the solution facing the external side of the cell membrane, the same high-conductance potassium channel could be observed. Adding charybdotoxin \(10^{-8}\) mol/l, a specific maxi-K-blocker, greatly and reversibly reduced the open probability, reflecting blockage of a number of channels in the patch \((n = 3, \text{Fig. 5})\). The same could be observed after addition of 1 mmol/l TEACl \((n = 2)\). Adding 10 mmol/l Ba\(^{2+}\) to the exterior surface of the same patch did not significantly block currents \((n = 3)\).

Cell-Attached Recordings

To determine whether the channel is active under physiological conditions, cell-attached recordings were performed with a low-chloride solution in the pipette \((n = 5, \text{data not shown})\). In this configuration, the cytosolic compartment remains intact. A high conductance channel opened at physiological voltage levels (pipette potential: 0 mV). In the cell-attached mode, this means that the cell’s own, physiological membrane potential is applied to the patch. The low amount of chloride in the patch pipette excludes chloride as a charge carrier for this channel. Addition of ionomycin \(10^{-5}\) mol/l enhanced its open probability. The voltage dependence corresponded to that observed for maxi-K-channels.

Whole-Cell Experiments

Measuring in the whole-cell configuration makes it possible to assess the relative contribution of channels to the entire ionic current flowing in and out of the cell membrane. In the absence of stimulation, cul-

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933425/)  
**FIGURE 3.** Dependence of open probability (NP\(\_p\)) on voltage and cytosolic calcium. Five inside-out patches were successively exposed to various concentrations of calcium. (A) Calcium levels \(10^{-7}\) and \(10^{-6}\) mol/l. (B) Calcium levels \(10^{-6}\) and \(10^{-5}\) mol/l. (C) Calcium levels \(10^{-5}, 10^{-4}, \text{and} 10^{-3}\) mol/l.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933425/)  
**FIGURE 4.** Application of adenosine triphosphate \(1\) mmol/l to the cytosolic side of the membrane patch increases the open probability of the maxi-K-channel. (A) Traces from one patch before and after application of adenosine triphosphate. (B) Summary of the data.
tured bovine trabecular meshwork cells had a reversal potential of 42 ± 4 mV (n = 22) shortly after breaking into the whole-cell configuration. The average capacitance was 47 ± 4 pF. Under voltage-clamp conditions, a strong outward current could be observed at high voltages (pipette: K-glutamate solution, bath: Ringer’s solution; Fig. 6). Addition of the specific blocker for maxi-K-channels, charybdotoxin (10⁻⁷ mol/l), to the external solution led to a reduction of this outward current by 35 ± 5% (P (paired) < 0.005, n = 5, V = 80 mV).

To verify the calcium dependence of the outward current, cytosolic calcium was elevated. Addition of the calcium ionophore ionomycin (10⁻⁵ mol/l) led to a dramatic increase of the total outward current to 645 ± 45% (P (paired) < 0.005, n = 3, V = 80 mV) of the original value. Charybdotoxin blocked 37 ± 6% (P (paired) < 0.001, n = 3, V = 80 mV) of this current (Fig. 7). No attempt was made to isolate this current pharmacologically. Parts of it may well be attributable to other conductances. Typically, external TEACl (10 mmol/l, n = 3 and 1 mmol/l, n = 3) also dose-dependently blocked outward current.

**Experiments With 8-Bromo-Cyclic Guanosine Monophosphate**

Knowing that previous experiments have shown that application of vasodilators that elevate cytosolic cGMP levels relaxes bovine trabecular meshwork strips precontracted by carbachol, we studied the response of trabecular meshwork cells after an exposure to 8-bromo-cGMP. This lipid-diffusible cGMP analogue is able to cross the cell membrane and enter the cytosolic compartment. In these experiments, cells were filled with K-glutamate solution (with calcium levels of 2.7 • 10⁻⁷, comparable to those reported for trabecular meshwork cells stimulated with carbachol). To mimic physiological intracellular solutions, I mmol/l adenosine triphosphate was added.

Addition of 8-bromo-cGMP (10⁻³ mol/l) to the external solution induced a prolonged, large, notably variable increase in outward current (290 ± 57%, n = 4, P < 0.05; Fig. 8). Addition of the specific maxi-K-channel blocker charybdotoxin (10⁻⁷ mol/l) led to a reduction of this current to 156 ± 28% of the initial current level (n = 4, P < 0.05). In two of the cells investigated, the response was biphasic. The outward current was preceded by a short inward current. The ionic conductance underlying this current has yet to be determined.

**DISCUSSION**

The channel we report here is a channel known for its importance for smooth muscle contractility. Indirect
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Evidence for the presence of maxi-K-channels in human and bovine trabecular meshwork cells was presented by our group from classic measurements of membrane voltage. No significant differences between the electrophysiological properties of bovine cells and human cells were shown in the results of these studies. Unlike human eyes, bovine eyes allow macroscopic separation of trabecular meshwork and ciliary muscle, making it possible to measure directly the tonus of trabecular meshwork strips in response to various substances. In addition, it is possible to measure the effect on outflow in the model of the perfused anterior segment with ciliary muscle detachment. In this study, we wanted to obtain more information on the underlying cellular mechanisms of these responses, using the patch-clamp technique. For better comparability, we used cells derived from the same preparation as was used for the contractility experiments.

Initial patch-clamp experiments revealed a strikingly high density of maxi-K-channels in the cell membrane of all cells investigated (it was difficult to obtain a single patch without at least one active channel in it). Another striking feature of this channel is its dependence on intracellular calcium levels and on membrane voltage, making it an obvious focusing point for an investigation centered around mechanisms of contraction and relaxation in such contractile tissue as trabecular meshwork. Thus, evidence for the presence of other ionic channels in trabecular meshwork, which can be seen in the experimental data presented here, will not be discussed in this report.

As described by those investigating other cells, maxi-K-channels in trabecular meshwork are highly selective for potassium. The single-channel conductance was in the upper range of levels reported for other mammalian cells but was not unique. Thus, in rabbit intestinal smooth muscle maxi-K-chan-

**FIGURE 7.** Ionomycin (10^{-5} mol/l) led to an increase of the total outward current. Charybdotoxin (10^{-7}mol/l) blocked part of this current. (A) Original trace from whole-cell recording. (B) Effect of ionomycin on three individual cells. Each symbol represents a different cell.

**FIGURE 8.** Application of 8-Bromo-cyclic guanosine monophosphate led to a large increase of current levels. Charybdotoxin (10^{-7} mol/l) blocked part of this current. (A) Original trace from whole-cell recording. (B) Effect of 8-bromo-cyclic guanosine monophosphate on four individual cells. Each symbol represents a different cell.
nals have been found with a conductance of 230 pS in 100 mmol/1 symmetric KCl solution, corresponding to a conductance of 345 pS in 150 mmol/1 KCl solution, according to the Goldman–Hodgkin–Katz theory. In canine coronary artery smooth muscle cells, conductances of 274 pS in 140 mmol/1 KCl solution (at 24°C) were reported (corresponding to 293 pS in 150 mmol/KCl). Bolotina et al. report conductances of up to 300 pS for cells derived from rabbit aorta.

The open probability of the channel described here was significantly reduced by external application of charybdotoxin, a specific blocker of maxi-K-channels with a half-maximal inhibitory concentration of $10^{-8}$ mol/l and total blockage at approximately $10^{-7}$ mol/l. External TEACl (1 mmol/l) and internal barium (10 mmol/l) blocked the channel completely. This is a common feature of maxi-K-channels reported for nearly all tissues. Internal TEACl (10 mmol/l) and external Ba$^{2+}$ (10 mmol/l) had no effect on outward potassium current through the channel. High thresholds for block by internal TEACl have been reported for rat myotubes, rabbit T-tubules, and bovine chromaffin cells. The failure of external barium to block potassium currents out of the cell has been reported for various cells, including rabbit smooth muscle cells, and may be explained by the model of binding within the pore, so that the flow of ions through the pore is blocked in one direction but not in the other.

In whole-cell experiments, outward current was greatly enhanced by external application of the ionophore ionomycin in the presence of $10^{-3}$ mol/l CaCl$_2$. Only part of this current could be blocked by application of charybdotoxin. Apparently, the maxi-K-channel is only one of the channels stimulated by increasing intracellular calcium.

Using a bovine model, we were able to show in a previous report that the application of 8-bromo-cGMP, nitro-, and non-nitro-vasodilators to precontracted trabecular meshwork strips induced relaxation in a dose-dependent manner. In the current report, cells from the same tissue were exposed to 8-bromo-cGMP in a dosage comparable to that used by other investigators in patch-clamp studies ($10^{-8}$ mol/l). After stimulation of a short inward current, an outward current could be observed, the greater part of which could be blocked by charybdotoxin, a specific blocker of the maxi-K-channel. The initial inward peak is an interesting phenomenon for future studies.

When employing the whole-cell technique in excised inside-out patches, the open probability of the maxi-K-channel was typically very small in the physiological range of membrane voltage and intracellular calcium. This observation may raise the question of the physiological significance of this channel. However, we observed a higher open probability in cell-attached patches, and the open probability could be enhanced by adding adenosine triphosphate. Apparently, the channel is more active in its native state than it is under the artificial circumstances employed in experiments designed to establish defined potassium gradients and membrane voltage for an exact calculation of channel conductance. However, in excised patches also, individual channel openings could be observed at any potential level, depending on the observation time and the number of channels in the patch. It is a well-known fact that very few channel openings are required to change membrane voltage significantly.

Physiologically, potassium leaves the cell through this channel, leading to hyperpolarization. Hyperpolarization can lead to several different effects, depending on what channels are prevalent in the tissue investigated. In nonexcitable tissues and at voltages well below the activation threshold of voltage-operated calcium channels, hyperpolarization enhances calcium influx through "leak" pathways that include nonselective divalent cation channels and nonvoltage-gated calcium-selective channels (I$_{\text{Ca,L}}$). In excitable cells, conversely, depolarization stimulates and repolarization limits calcium uptake through voltage-operated calcium channels. Thus, a prediction concerning the effect of stimulating maxi-K-channels on cytosolic calcium levels and smooth muscle tone has to be made with some care, depending on the importance of the leak pathway, the voltage-dependent calcium entry mechanism in the tissue investigated, and the cell potential at which stimulation occurs.

In trabecular meshwork, measurements of membrane voltage and contractility have provided conclusive evidence for the existence of voltage-operated calcium channels. Thus, trabecular meshwork seems to follow the pattern generally observed in smooth muscle cells: Contracting agents lead to a depolarization of membrane voltage, stimulating voltage-operated calcium channels and leading to an influx of calcium. Depolarization and higher intracellular calcium levels stimulate the maxi-K-channel, causing potassium to leave the cell and leading to repolarization. Thus, the maxi-K-channel serves as a very effective feedback mechanism, allowing for regulation of contractility.

Interestingly, recent evidence suggests that the maxi-K-channel may be involved in the effects of a variety of compounds known to relax smooth muscle. Thus, $\beta$-adrenergic substances have been reported to have a stimulating effect on maxi-K-channels. It appears that nitric oxide (NO) has a direct effect on maxi-K-channels and an effect mediated by cGMP.

In the latter pathway, NO stimulates guanylate cyclase, leading to an increase in the intracellular level
of cGMP. This, in turn, activates a cGMP-dependent protein kinase, which phosphorylates maxi-K-channels, increasing their open probability. As a result, potassium efflux increases, leading to repolarization, closing of voltage-activated calcium channels, and relaxation.

It has become increasingly obvious that NO is a major molecule regulating smooth muscle contractility. In vivo, NO is generated from L-arginine by nitric oxide synthase NOS. Interestingly, NOS has been found in various parts of the eye, including the trabecular meshwork, and a physiological role for NO in the regulation of intraocular pressure has been postulated.

The powerful potential of compounds that elevate intracellular cGMP for relaxing smooth muscle is very well documented. We were able to demonstrate this in measurements of contractility of isolated strips of trabecular meshwork and ciliary muscle. It is of special interest that the relaxation induced by cGMP, nitrovasodilators and nonnitrovasodilators was more pronounced in trabecular meshwork than in ciliary muscle. The clarification of the mechanism of action of this class of smooth muscle relaxants on trabecular meshwork thus seems of considerable interest. In patch-clamp experiments performed on cells derived from the same preparation of bovine trabecular meshwork used in the contractility measurements cited above, we were able to demonstrate a high density of maxi-K-channels and a stimulation of these channels by elevation of cytosolic cGMP, implying an involvement of the maxi-K-channel in the relaxation of this tissue. Thus, the data presented here support the model that trabecular meshwork has smooth-muscle-like properties and plays an active role in the dynamics of aqueous humor outflow.

Key Words

cyclic guanosine monophosphate (cGMP), glaucoma, maxi-K-channel, smooth muscle cells, trabecular meshwork

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