Upregulation of P2X7 Receptor Currents in Müller Glial Cells during Proliferative Vitreoretinopathy

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PURPOSE. Müller glial cells from the human retina express purinergic P2X7 receptors. Because extracellular adenosine triphosphate (ATP) is assumed to be a mediator of the induction or maintenance of gliosis, this study was undertaken to determine whether the expression of these receptors is different in human Müller cells obtained from retinas of healthy donors and of patients with choroidal melanoma and proliferative vitreoretinopathy (PVR).

METHODS. Human Müller cells were enzymatically isolated from donor retinas, and whole-cell patch-clamp recordings were made to characterize the density of the P2X7 currents and the activation of currents through Ca2+-activated K+ channels of big conductance (I_BK) that reflects the increase of the intracellular Ca2+ concentration.

RESULTS. Stimulation by external ATP or by benzoylbenzoyl ATP (BzATP) evoked both release of Ca2+ from thapsigargin-sensitive intracellular stores and opening of Ca2+-permeable P2X7 channels. These responses caused transient and sustained increases in I_BK. In Müller cells from patients with PVR, the mean density of the BzATP-evoked cation currents was significantly greater compared with cells from healthy donors. As a consequence, such cells displayed an enlarged I_BK during application of purinergic agonists. ATP and BzATP increased the DNA synthesis rate of cultured cells. This effect could be reversed by blocking the I_BK.

CONCLUSIONS. The increased density of P2X7 receptor channels may permit a higher level of entry of extracellular Ca2+ into cells from patients with PVR. Enhanced Ca2+ entry and the subsequent stronger activation of I_BK may contribute to the induction or maintenance of proliferative activity in gliotic Müller cells during PVR. (Invest Ophthalmol Vis Sci. 2001;42:860–867)

Müller (radial glial) cells are the main type of macrogial cells within the vertebrate retina. Although the plasma membrane permeability of Müller cells is predominated by inwardly rectifying K+ currents (I_K),1 the cells also express various distinct types of depolarization-activated ion channels—among them, Ca2+-activated K+ channels of big conductance (BK).2,5 The activity of BK channels has been implicated in the regulation of cultured Müller cell proliferation,2,4 and has been found to be elevated in human Müller cells from patients with proliferative vitreoretinopathy (PVR) compared with cells from control retinas.5

Müller glial cells express a diversity of receptors for neurotransmitters and other biologically active substances6 that may modulate the membrane conductances of the cells.7 Adenosine triphosphate (ATP) is an important transmitter in the retina,8 being crucially involved in early retinal development9,10 and in the neuronal information processing of the mature retina.11 Müller cells may express different types of purinergic P2 receptors. In isolated salamander Müller cells and in rat Müller cells in situ, activation of P2Y receptors by extracellular ATP stimulates the release of Ca2+ from internal stores.11,12,13 Activation of P2 receptors inhibits the uptake of γ-amino butyric acid (GABA) by rat Müller cells.11 Recently, the presence of ionotropic P2X receptors was described in Müller cells freshly isolated from the human retina.15 In human Müller cells, extracellular ATP and 2',3'-O-(4-benzoylbenzoyl) ATP (BzATP), a more specific agonist of P2X7 receptors,16 open nonselective cation channels that may be permeable for Ca2+ ions.15 The nonactivating current kinetics, as well as single-cell reverse transcription-polymerase chain reaction (RT-PCR) and immunocytochemical evidence, indicate the expression of P2X7 receptors in these cells.15

Because the activation of purinergic receptors is thought to be a mediator of the induction of reactive gliosis,17–19 we wanted to investigate whether the expression of P2 receptors by human Müller cells is altered under pathologic conditions. For this purpose, the density of BzATP-evoked currents in cells from patients with PVR and those with choroidal melanoma was compared with that in cells from healthy donors. Alterations of cell membrane conductances during activation of P2 receptors were investigated electrophysiologically in two ways: Either the P2X7 receptor-mediated cation conductance or the stimulation of the Ca2+-activated K+ currents were recorded. An amplitude increase of the Ca2+-activated K+ currents reflects the increase of the intracellular Ca2+ concentration induced by activation of the purinergic receptors.

METHODS

Human tissue was used in accordance with applicable laws and with the Declaration of Helsinki, after approval by the ethics committee of the Leipzig University Medical School. Eyes obtained at autopsy from organ donors with no reported history of eye disease (referred to in the text as healthy donors) were supplied within 12 and 24 hours after death. Retinal tissue from patients with PVR was obtained from vitreoretinal surgery 1 to 3 hours after the tissue was removed. Retinal tissue from patients with choroidal melanoma was obtained 1 to 3 hours after enucleation. Müller cells were isolated from retinal areas far away from the regions where the melanoma cells were located. Müller cells were isolated using papain- and DNase I-containing solutions, as described previously.3,20 The cell suspensions were stored at 4°C (up to 10 hours) before use.

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Electrophysiological Recordings

Records were made in the whole-cell or in the excised patch configuration of the patch-clamp technique. To create outside-out patches, the whole-cell configuration was established, and thereafter the pipette was drawn back to excise a membrane patch. Voltage-clamp records were performed at room temperature (22°C–25°C) using an amplifier (EPC 7; List, Darmstadt, Germany) and a computer program (Tidast ver. 5.72, Heka Elektronik, Lambrecht, Germany). The signals were low-pass filtered at 4 kHz (three-pole Bessel filter) at a sampling rate of 15 kHz. The series resistance (10–18 MΩ) was compensated by 30% to 50%. Patch pipettes were pulled from thick-walled borosilicate glass (WPI, Sarasota, FL) and had resistances between 3 and 5 MΩ when K+-containing bath and pipette solutions were used. To investigate ATP-evoked responses, the whole-cell currents were elicited by a standard step protocol (holding potential, V₀ = −80 mV; depolarizing and hyperpolarizing voltage steps of 250-msec duration with an increment of 20 mV) or by continuous recording at a V₀ of −60 mV with voltage steps of 50 msec duration to +120 mV and to −100 mV at a frequency of 2.5 Hz. The traces were not leak subtracted. Data were not corrected for liquid junction potentials, because these did not exceed 3 mV. The membrane capacitance of the cells was measured by the integral of the uncompensated capacitive artifact evoked by a hyperpolarizing voltage step from −80 to −90 mV when K⁺ ions (1 mM) were present in the bath solution to block the K⁺ conductance. For recording of the capacitive artifact, the sampling rate was 50 kHz, and the frequencies above 10 kHz were cut off.

Solutions

The recording chamber was continuously perfused with bath solution. Test substances were added by fast (<15 seconds) changes of the perfusate. For recording the effects of purinergic agonists on the whole-cell currents and on the BK channel activity in excised membrane patches, a low-divalent cation bath solution was used composed of (mM) 110 NaCl, 3 KCl, 0.5 CaCl₂, 10 HEPES, and 11 glucose with pH adjusted to 7.4 with Tris. The pipette solution was made of (mM) 10 NaCl, 130 KCl, 5 MgCl₂, 0.1 EGTA, and 10 HEPES with pH adjusted to 7.2 with Tris. When the BzATP-induced cation currents were recorded in K⁺-free conditions, the bath solution consisted of (mM) 116 NaCl, 1 Na₂HPO₄, 25 NaHCO₃, 11 glucose, 10 HEPES (pH 7.4), and was gassed with 95% O₂/5% CO₂. The pipette solution contained (mM) 10 NaCl, 130 CsCl, 1 CaCl₂, 2 MgCl₂, 10 EGTA, 10 HEPES (pH 7.1). Iberiotoxin was obtained from Alomone Laboratories (Jerusalem, Israel) and papain from Boehringer-Mannheim (Mannheim, Germany). All other substances were from Sigma (Deisenhofen, Germany).

Cell Culture

Primary cultures of Müller cells were obtained from retinas of healthy donors. The excised retinas were dispersed in Ca²⁺ free phosphate buffer supplemented with nagarse (1 mg/ml) for 30 minutes at 37°C. After they were washed in phosphate buffer containing DNase I (200 U/ml), the dissociated cells were seeded on coverslips (100 μl cell suspension per coverslip; the retinal cells from two eyes were distributed on 54 coverslips) and cultured at 37°C in a gas mixture of 95% air-5% CO2. The minimum essential medium was supplemented with 10% fetal calf serum. The medium was exchanged twice a week. After 3 weeks in culture, the test substances were added to the culture medium 16 hours before the cultures were fixed. During this latter period, substances were tested in serum-free medium. Lipophilic substances were dissolved in dimethyl sulfoxide (DMSO). Vehicle alone did not affect the DNA synthesis rate.

Determination of the DNA Synthesis Rate

The DNA synthesis rate was determined by measuring the bromodeoxyuridine (BrdU) incorporation. BrdU (10 μM) was added 16 hours before fixation with 4% paraformaldehyde. BrdU incorporation into nuclei of mitotically active cells was revealed by a murine anti-BrdU IgG-antibody (Bu 33; Sigma) and Cy3-tagged secondary antibodies. Counter-labeling of all cell nuclei was performed with acridine orange or Hoechst 33258. In the peripheral (i.e., nonconfluent) regions of the cultures, six distinct areas of each coverslip (each approximately 60,000 μm², resulting in a total area of 0.42 mm² per coverslip) were studied by means of a semiautomatic image analysis system (SIS; Soft Imaging Systems, Münster, Germany). The results from three coverslips per culture were summarized. The experiments involved four independent cultures. The ratio of BrdU immunoreactive versus total cell nuclei was taken as marker for the DNA synthesis rate.

Data Analysis

The steady state whole-cell currents were measured at the end of 250-msec voltage steps. To determine disease-related changes of currents, 6 to 13 cells per donor were recorded; in most of the further statistical analysis, only the mean values of the cells from each donor were used. Statistical analysis (Mann-Whitney test, two-tailed; non-parametric regression analysis) and curve fits were made by computer (Pricem; GraphPad, San Diego, CA). Data are expressed as means ± SD (electrophysiological data) or as means ± SEM (proliferation experiment).

RESULTS

Effect of ATP on the Whole-Cell Currents

Freshly isolated human Müller cells from patients with various eye diseases do not have the Kᵢₑᵣ normally expressed by human Müller cells from healthy retinas. Therefore, in these cells, hyperpolarizing voltage steps evoked only a small “leak” current, whereas depolarizing voltage steps activated delayed rectifying K⁺ currents and Ca²⁺-activated K⁺ currents (Fig. 1A). Extracellular application of Na-ATP (1 mM) reversely increased the amplitudes of both the inward and the outward currents. In particular, the currents at strongly depolarized potentials were elevated by ATP (the uppermost noisy current traces in Fig. 1A). A similar strong activation of outwardly directed K⁺ currents was observed when cells from healthy donors were exposed to BzATP (50 μM; Fig. 1B). Figure 1C illustrates the mean steady state currents of four cells from patients with PVR before (control) and during external exposure of Na-ATP (1 mM), and after washout of the drug. ATP increased the inwardly directed currents at negative membrane potentials and the outwardly directed current at positive potentials. During ATP exposure, the Müller cells depolarized, as indicated by the shift of the zero current potential of the whole-cell currents by 17.0 ± 5.3 mV toward more positive voltages (P < 0.01; inset in Fig. 1C).

To isolate the currents that were evoked by ATP-induced Ca²⁺ entry from the extracellular space, the whole-cell currents were recorded in two different bath solutions: one containing 0.5 mM Ca²⁺ and the other nominally Ca²⁺ and Mg²⁺ free and containing 1 mM EGTA. Figure 1D illustrates the Na-ATP (1 mM)–evoked currents that were recorded under the two conditions in cells from patients with PVR (the ATP-evoked currents were calculated by subtraction of the control currents from the currents recorded during exposure of ATP). The inwardly directed current (downward) was only slightly modulated by extracellular Ca²⁺. The density of the ATP-induced inward current was 5.32 ± 1.12 pA/pF with 0.5 mM Ca²⁺ in the bath solution (measured at the voltage step to −160 mV; n = 6), whereas in the Ca²⁺-free solution, it was 4.78 ± 1.53 pA/pF (n = 4; not significant). Thus, the inwardly directed current represents mainly a nonelective cation conductance, as also indicated by its reversal potential near 0 mV (Fig. 1D). In contrast, the outwardly directed currents (upward) were strongly depressed after omitting the Ca²⁺ ions from the bath solution. In the Ca²⁺-containing bath solution,
that a large portion of the BzATP-induced outward current was significantly different amplitudes when the drug was tested in Ca\(^{2+}\)-containing or in Ca\(^{2+}\)-free bath solution. In Ca\(^{2+}\)-containing bath solution, the BzATP-induced current had a mean density of 17.3 ± 0.1 pA/pF (n = 7; measured at the voltage step to +140 mV), whereas in Ca\(^{2+}\)-free solution the current density was only 3.3 ± 4.2 pA/pF (n = 8, P < 0.01). It is concluded that a large portion of the BzATP-induced outward current was evoked by Ca\(^{2+}\) entry from the extracellular space and may represent the activation of Ca\(^{2+}\)-activated K\(^+\) currents. A similar increase of depolarization-evoked, Ca\(^{2+}\)-activated K\(^+\) currents was observed in cells from healthy donors (Fig. 3).

Figure 2A illustrates the time course of the whole-cell current changes induced by BzATP (50 µM) exposure. Examples of records from four cells are shown that were made in Ca\(^{2+}\) (0.5 mM)-containing (Fig. 2A, left) or in Ca\(^{2+}\)-free (Fig. 2A, right) bath solution. In Ca\(^{2+}\)-containing bath solution, the cells showed a biphasic elevation of the amplitude of the outward currents at +120 mV. Just after beginning of drug exposure, there was a transient elevation of the current amplitude (asterisks) that thereafter switched into a sustained elevation (Fig. 2A, left). This biphasic response mimicked the ATP-induced biphasic increase of the intracellular Ca\(^{2+}\) concentration described previously in human Müller cells.\(^{15}\) Therefore, it is assumed that the transient response was caused by release of Ca\(^{2+}\) ions from intracellular stores (through metabotropic ATP receptors), whereas the sustained response was mainly caused by a Ca\(^{2+}\) entry through an ATP-induced cation conductance (i.e., ionotropic ATP receptors). To test this assumption, cells were recorded in Ca\(^{2+}\)-free extracellular solution (Fig. 2A, right). Indeed, under these conditions the majority of cells responded to BzATP with a large transient elevation of the outward currents (asterisks), whereas the sustained response was greatly reduced from 303.4% ± 124.1% to 124.3% ± 14.2% (as compared with the control currents set at 100%; P < 0.01; Fig. 2B). The transient elevation of the mean outward current amplitude was virtually independent of extracellular Ca\(^{2+}\) (Fig. 2B) which strongly supports the assumption that this response is caused by Ca\(^{2+}\) release from intracellular stores. The release of intracellular Ca\(^{2+}\) was relatively fast and independent of the activation of the inwardly directed cation currents and could occur before, during, or after the onset of inward currents (Fig. 2A). By contrast, the inward currents developed very slowly, and full activation of these currents was observed 1 to 2 minutes after the beginning of drug exposure.
Exposure of the cells to thapsigargin (1 μM, 3 minutes) in Ca\(^{2+}\)-free bath solution did not alter the sustained BzATP responses at +120 mV (amplitude 116.0% ± 17.2% compared with the value before drug exposure [100%], n = 5, not significant). Thapsigargin itself did not induce a transient activation of the outward currents (not shown). However, pretreatment with thapsigargin resulted in a significantly decreased amplitude of the BzATP-induced initial transient response (decrease to 51.1% ± 21.1% compared with five cells without pretreatment, P < 0.05), which is consistent with the assumption that a large portion of the transient activation of the outward currents by BzATP was caused by release of Ca\(^{2+}\) from intracellular stores. The specific types of P2Y receptors involved in the Ca\(^{2+}\) release from internal stores remain to be determined in future experiments.

To determine whether the outward current elevated by BzATP represents a BK-channel–mediated current (I\(_{\text{hk}}\)), the effect of ibeiriotoxin was tested. Figure 3A illustrates an example of current records in one Müller cell from a healthy donor. Extracellular application of BzATP (50 μM) induced a strong increase of the outwardly directed currents that was blocked by simultaneous exposure to ibeiriotoxin (100 nM). The time course of the drug’s effect is shown in Figure 3B for one cell. Iberiotoxin fully reversed the BzATP-induced increase of the outward currents at +120 mV but had no effect on the inward currents at −100 mV. The voltage-dependence of the whole-cell currents reveals that BzATP induced a negative shift of the activation of I\(_{\text{hk}}\); under control conditions, the activation threshold of the I\(_{\text{hk}}\) was at +120 mV, and during BzATP exposure, the I\(_{\text{hk}}\) was evoked at potentials positive to 0 mV.

FIGURE 2. Time-dependent changes of the membrane conductance of human Müller cells in response to extracellular application of BzATP (50 μM). The records were made in cells from patients with PVR. (A) Examples of records in four cells that showed transient elevations of the outward currents after the beginning of drug exposure (>). The records were made in bath solutions containing 0.5 mM Ca\(^{2+}\) (left) or in Ca\(^{2+}\)-free bath solutions containing 1 mM EGTA (right). Voltage steps were applied at a frequency of 2.5 Hz from a holding potential of −60 mV (middle traces) to +120 mV (upper traces) and to −100 mV (lower traces; inset). Dashed lines: Zero current levels. The amplitudes were measured at the end of each of the 50-msec voltage steps. Arrows: Beginning of drug exposure; horizontal bars: 10 seconds; vertical bars: 250 pA. (B) Mean amplitudes of the transient and sustained outward currents activated by BzATP. The cells were examined in Ca\(^{2+}\)-free (n = 6) or in Ca\(^{2+}\)-containing bath solution (n = 7). The amplitudes of the transient currents and of the sustained currents were measured at the end of 50-msec voltage steps to +120 mV from a holding potential of −60 mV and are calculated as the percentage of the control currents that were measured 20 seconds before drug application (100%). Significant differences of P < 0.05 (●) and of P < 0.01 (●●), n.s., not significant.

FIGURE 3. BzATP increased the amplitude of ibeiriotoxin-sensitive outward currents in human Müller cells. (A) Example of current records in a cell derived from a healthy human donor. For current activation, the membrane was stepped to increasing depolarizing and hyperpolarizing potentials between −160 and +200 mV (250 msec, 20-mV increments, holding potential −80 mV). Cells were exposed to BzATP (50 μM) in the absence and thereafter in the presence of ibeiriotoxin (100 nM). (B) Time course of whole-cell current changes in one cell. Bars: Application of BzATP (50 μM) and ibeiriotoxin (100 nM). Currents were measured at +120 mV (upper trace), at −60 mV (middle trace), and at −100 mV (lower trace). Dashed line: Zero current level. For voltage step protocol, see inset in Figure 2A. (C) Mean steady state current versus voltage relationships of three cells. Currents were recorded before (control) and during BzATP (50 μM) exposure and during simultaneous exposure to BzATP and ibeiriotoxin (100 nM). Currents were evoked using the voltage step protocol shown in (A). (D) Mean amplitudes of the sustained outward currents at +120 mV (left) and of the inwardly directed currents at −100 mV (right) in 12 cells from patients with PVR. The currents were measured during BzATP (50 μM) exposure, in the absence and in the presence of ibeiriotoxin (100 nM). Significant differences of P < 0.05 (●) and of P < 0.001 (●●●). The currents were evoked using the continuous step protocol shown in the inset of Figure 2A.
positive membrane potentials. In three patches, BzATP in-
membrane increased the open probability of BK channels at
(Fig. 3C). Iberiotoxin blocked the BzATP-activated
the extracellular side of the membrane. 

A cell from a healthy donor was reversibly inhibited by iberiotoxin to
channels in a patch that was excised from the end foot membrane of
FIGURE 4.

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The expression of P2X<sub>7</sub> receptors was electrophysiologi-
cally investigated in freshly isolated Müller cells from various species (human [n = 318 cells], pig [n = 14], rat [n = 8],
mouse [n = 9], guinea pig [n = 6], and rabbit [n = 18]), by
using extracellular application of BzATP (50 μM). It was found,
however, that BzATP induced a cation conductance only in the
case of human Müller cells, whereas metabotropic P2Y receptors
seem to be present also in Müller cells from other species
(data not shown). Therefore, the expression of P2X<sub>7</sub> receptors
by Müller cells may be a specific phenomenon of the human
retina.

Taken together, the results indicate that extracellular ATP
may have three effects on human Müller cells: (1) Through
activation of P2X<sub>7</sub> receptors, ATP evokes the opening of a
nonselective cation conductance which mediates a Ca<sup>2+</sup> entry
from the extracellular space and which depolarizes the cells;
(2) Ca<sup>2+</sup> ions are released from intracellular stores, probably
through an activation of P2Y receptors; and (3) the depolar-
ization and the elevation of the intracellular Ca<sup>2+</sup> concentra-
tion together increase I<sub>hk</sub>.

Disease-Related Changes of BzATP-Evoked
Currents

To determine whether P2 receptors of the Müller cells are
implicated in transdifferentiation processes accompanying gli-
osis in cases of human retinal disease, two kinds of retinal
diseases were investigated: choroidal melanoma and PVR. Müller
cell gliosis is characterized by different features, including
upregulation of the immunoreactivity of intermediate filaments
and by cell hypertrophy. In the case of PVR, Müller cells may
also become proliferative, whereas in other types of gliosis
(e.g., during choroidal melanoma), Müller cells do not prolif-
erate. Müller cells from patients with PVR displayed a signifi-
cantly greater cell membrane capacitance (81.5 ± 22.4 pF, n =
19 patients) compared with cells from healthy retinas (54.3 ±
10.9 pF, n = 13 donors, P < 0.001) indicating hypertrophy of
Müller cells in PVR retinas. Similarly, Müller cells from three
patients with melanoma displayed hypertrophy (mean mem-
brane capacitance, 93.0 ± 12.3 pF, n = 3).

Whole-cell currents were recorded before and during expo-
sure to BzATP (50 μM) in K<sup>-</sup>-containing or K<sup>-</sup>-free solutions.
Figure 5A shows the mean steady state current density–voltage
relations of BzATP-evoked currents measured in K<sup>-</sup>-containing
solutions after the transient stimulations of I<sub>hk</sub> had ceased (in
most cells, 2 to 3 minutes after the beginning of drug exposure;
currents were calculated by subtraction of the control currents
from the drug-induced currents). Mean curves from three
donor groups are shown: healthy donors, patients with PVR, and
patients with melanoma. The BzATP-evoked currents consist of
two components: At negative membrane potentials, the in-
wardly (downwardly) directed currents reflect the activation of
the BzATP-induced cation conductance.

In excised outside–out patches, BzATP reversibly increased the
activity of iberiotoxin-sensitive (Fig. 4A) K<sup>+</sup> channels of
large conductance (134.3 ± 16.6 pS). As shown in Figure 4B,
exposure to BzATP (50 μM) of the extracellular side of the
membrane increased the open probability of BK channels at
positive membrane potentials. In three patches, BzATP in-
creased the mean channel-open probability at +80 mV from
0.04 ± 0.05 to 0.14 ± 0.10. After washout of the drug, the
value returned to 0.04 ± 0.04.
The induced decrease of $I_{\text{K7}}$, the BzATP-evoked cation conductance was measured under K$^+$-free conditions. In fact, the density of the BzATP-evoked inward currents at a holding potential of $-80$ mV was significantly greater in cells from patients with PVR (3.6 ± 3.0 pA/pF, $n=58$ cells) than in cells from healthy donors (2.1 ± 1.4 pA/pF, $n=70$ cells, $P<0.001$).

Figure 5B shows mean values of the amplitudes of the transient and sustained $I_{\text{BK}}$ activated by BzATP (50 µM). Maximal amplitudes were measured at the voltage step from $-60$ to $+120$ mV. Donor numbers in parentheses. (A, B) Significant differences between cells from PVR retinas and cells from healthy donors of $P<0.01$ (●) and $P<0.001$ (●●) respectively. n.s., not significant. Dependences of the voltage, at which the sustained $I_{\text{BK}}$ was half-maximally activated (C) and of the density of the maximally activated sustained $I_{\text{BK}}$ (D), from the density of the BzATP-evoked inward currents. The BzATP-evoked inward currents were measured at the voltage step from $-100$ mV to $-140$ mV. (C, D) Each symbol represents the mean of all investigated cells from one donor.

Modulation of the DNA Synthesis Rate by Extracellular ATP

Both extracellular Na-ATP (500 µM) and BzATP (20 µM) increased the DNA synthesis rate of cultured human Müller cells (Fig. 6A). Simultaneous exposure to iberiotoxin (70 nM) decreased the effects of the purinergic agonists indicating an
The involvement of BK channel activity in mediating the proliferation-stimulating effect of extracellular ATP.

**DISCUSSION**

**I\textsubscript{BK} of Human Müller Cells**

In the whole-cell records (e.g., Figs. 1 and 3), I\textsubscript{BK} activated at very positive membrane potentials (positive to +100 mV; Fig. 1C). Application of extracellular ATP induced a shift of the I\textsubscript{BK} activation threshold toward more positive potentials (Figs. 1C, 3C). The I\textsubscript{BK} activation at positive membrane potentials was previously described using whole-cell records in Müller cells of several mammalian species\textsuperscript{22,23} including humans.\textsuperscript{3} However, this activation at positive potentials does not reflect the real activation state of BK channels in Müller cells, because in cell-attached records, single BK channel activity can be observed at the native resting membrane potential and at slightly depolarized potentials.\textsuperscript{5,22-24} In Müller cells from patients with PVR, the single BK channel activity at the resting membrane potential was even found to be significantly increased if compared with cells from healthy donors.\textsuperscript{5} Very probably, the strong positive shift of the I\textsubscript{BK} activation in whole-cell records was mainly caused by the fact that the artificial pipette solution (replacing the normal intracellular milieu in the whole-cell configuration) did not contain essential cytoplasmic components that may have coactivated BK channels, such as activators of protein kinase A.\textsuperscript{3,24}

**Purinergic Receptors of Müller Cells**

In human Müller cells, both ATP and BzATP activate a nonselective, noninactivating cation current,\textsuperscript{15} consistent with the involvement of P2X\textsubscript{7} receptors. The nonselective cation channels allow for an entry of Ca\textsuperscript{2+} ions from the extracellular space\textsuperscript{15} that causes a sustained increase of I\textsubscript{BK} (Fig. 1D). ATP and BzATP caused also transient elevations of I\textsubscript{BK} that persisted in Ca\textsuperscript{2+}-free extracellular solution (Fig. 2A), indicating that they induced a release of Ca\textsuperscript{2+} from thapsigargin-sensitive intracellular stores. Although BzATP is assumed to preferentially activate ionotropic P2X\textsubscript{7} receptors, the present results indicate that BzATP also stimulates internal Ca\textsuperscript{2+} release, as previously described for other cell types.\textsuperscript{25} The mechanism of the BzATP-induced release of internal Ca\textsuperscript{2+} remains to be identified. This may be a secondary step after activation of P2X\textsubscript{7} receptors or a direct (additional) activation of P2Y receptors by BzATP, as previously shown for P2Y\textsubscript{2} receptors.\textsuperscript{26} The presence of P2Y receptors in human Müller cells was also indicated by preliminary experiments that showed that, in addition to ATP, extracellular uridine triphosphate (UTP) and guanosine triphosphate (GTP) evoke a transient activation of I\textsubscript{BK} (not shown).

**Involvement of Purinergic Receptors in Müller Cell Gliosis**

The involvement of purinergic receptors in induction or maintenance of gliosis in vivo has been previously discussed.\textsuperscript{17,19} In the brain, activation of P2 receptors may induce astrogliosis, leading to hypertrophy and proliferation of astrocytes.\textsuperscript{18,27} In the current study, we showed for the first time that gliosis in vivo may be connected with an upregulation of a distinct type of purinergic receptor. Although the currents through P2X\textsubscript{7} receptor channels were upregulated in Müller cells from patients with PVR compared with healthy donors, the release of intracellular Ca\textsuperscript{2+} that is probably mediated by P2Y receptors was unchanged in these cells. However, further investigations are necessary to provide evidence for a causal relationship between the expression of P2X\textsubscript{7} receptor-mediated currents and the induction or maintenance of Müller cell gliosis.

Although gliosis was present in eyes with choroidal melanoma, evidenced by a hypertrophy of the cells, all other investigated membrane conductances, including the density of P2X\textsubscript{7} receptor currents, were in the range of the cells from healthy retinas. By contrast, gliosis accompanying PVR was characterized by hypertrophy, by a strong downregulation of I\textsubscript{Kir} and a less negative resting membrane potential,\textsuperscript{5,20} by an increased expression of voltage-gated Na\textsuperscript{+} channels,\textsuperscript{26} and by a decrease of currents through voltage-gated Ca\textsuperscript{2+} channels,\textsuperscript{29} whereas the density of P2X\textsubscript{7} receptor currents was upregulated. When data from all donor groups used in the present study were considered, an increase of the density of sustained BzATP-evoked inward currents was correlated with a decrease of the I\textsubscript{Kir} density ($r = -0.574, n = 31$ donors, $P < 0.001$), a depolarization of the membrane ($r = 0.596, P < 0.001$), a decrease of the peak currents through high-voltage-activated Ca\textsuperscript{2+} channels ($r = -0.600, P < 0.001$), and a higher density of peak Na\textsuperscript{+} currents ($r = 0.604, P < 0.001$). As a mean, the more membrane features were altered the stronger the upregulation of P2X\textsubscript{7} receptor-mediated currents by human Müller cells. This may implicate a causal relationship between purinoceptor activation and the strength of gliosis.

**I\textsubscript{BK} and ATP-Induced Müller Cell Proliferation**

A specific upregulation of P2X\textsubscript{7} receptor currents in cells from patients with PVR may indicate that this type of purinergic receptors is involved in processes that are activated when gliotic Müller cells become proliferative. A role of P2X\textsubscript{7} receptors in induction and/or maintenance of proliferation was previously described for lymphocytes.\textsuperscript{30,31} Moreover, many tumor cell lines are characterized by high P2X\textsubscript{7} receptor expression levels.\textsuperscript{52} We found that both ATP and BzATP stimulated the DNA synthesis rate of cultured human Müller cells (Fig. 6A). Coapplication of iberiotoxin fully reversed the effects of the purinergic agonists. When we excluded an unspecific effect of iberiotoxin on Müller cells, the data indicated that the activation of I\textsubscript{BK} may be a step that is necessary for the purinergic induction of Müller cell proliferation. The mechanism of involvement of I\textsubscript{BK} in the regulation of the proliferation is unclear. One mechanism may be the regulation of the strength of P2X\textsubscript{7} entry into Müller cells by BK channels,\textsuperscript{55} as it was shown for the agonist-induced Ca\textsuperscript{2+} entry in other cell types.\textsuperscript{54} Because elevated intracellular Ca\textsuperscript{2+} concentration is necessary for both gliosis and maintenance of proliferative activity, the increased expression of P2X\textsubscript{7} receptors and the subsequent stronger activation of I\textsubscript{BK} may promote the induction of both processes in cells from PVR retinas. However, further experiments are necessary to investigate the relationships between purinergic receptor activation, BK current stimulation and induction and/or maintenance of Müller cell proliferation.

**References**


3. Bringmann A, Faude F, Reichenbach A. Mammalian retinal glial cell (Müller) cells express large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels that are modulated by Mg\textsuperscript{2+} and pH, and activated by protein kinase A. *Glia*. 1997;19:311–323.
