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Physiological properties of retinal Müller glial cells from the cynomolgus monkey, *Macaca fascicularis*—a comparison to human Müller cells

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Abstract

Retinae from rabbits and laboratory rodents are often used as 'models' of the human retina, although there are anatomical differences. To test whether monkey eyes provide a better model, a physiological study of Müller glial cells was performed comparing isolated cells and retinal wholemounts from the cynomolgus monkey, *Macaca fascicularis* and from man. The membrane conductance of Müller cells from both species was dominated by inward and outward K^+ currents. Cells displayed glutamate uptake currents and responded to nucleotides by intracellular Ca^{2+} increases. However, there were also species differences, such as a lack of $GABA_A$ receptors and of Ca^{2+}-dependent K^+ currents in monkey cells. Thus, the use of Müller cells from cynomolgus monkeys may be advantageous for investigating a few specific properties; in general, monkey cells are no more similar to human cells than those from standard laboratory animals.

Keywords: Patch-clamp methodology; Ca^{2+} microfluorimetry; K^+ currents; Membrane channels; P2 receptors

1. Introduction

The physiological properties of Müller cells, the dominant macroglia of the retina, have been studied in various mammalian species (Chao et al., 1997). These cells display a species-dependent expression pattern of distinct ion channels and ligand receptors, although their function in glial cells is often not known. Moreover, it has been demonstrated that the functional expression of some ion channels and of nucleotide (P2) receptors may be subject to dramatic changes under pathological conditions (Bringmann & Reichenbach, 2001). These pathophysiological alterations were studied in different diseases of the human eye (Bringmann et al., 2001; Francke et al., 1997) and similar effects were found in rabbits after experimental retinal detachment and proliferative vitreoretinopathy (PVR; Francke et al., 2001, 2002). However, the use of rabbits (as well as of laboratory rodents) as models for human ocular diseases is a matter of ongoing debate. Indeed, there are important

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differences in retinal anatomy such as a strong rod-over-cone dominance in rodent retinae, the lack of blood vessels in most parts of the rabbit retina, and the lack of a fovea in all non-primate retinae. The above-mentioned considerations may lead to the hypothesis that the monkey retina is a desirable model for studies on the physiological and pathophysiological properties of human retinal cells.

Indeed, the retinae of higher primates are strikingly similar in many morphological and functional aspects (for a recent review, see Kremers, 2005). This has also been demonstrated by morphological studies of Müller cells in the macaque retina (Distler & Dreher, 1996). Furthermore, it has been shown that typical changes in filament proteins of Müller cells occur under pathological conditions in monkeys (Guerin, Anderson, & Fisher, 1990; Tanihara et al., 1997), as observed in other species including man (Okada, Matsumura, Ogino, & Honda, 1990). The aim of the present study was to characterize physiological properties of Müller cells from the cynomolgus monkey, *Macaca fascicularis*, using patch-clamp recordings and Ca^{2+} microfluorimetry, and to compare these data with those obtained from human Müller cells. Our patch-clamp experiments extend the existing data about membrane properties of Müller cells from different monkey species (Han, Jacoby, & Wu, 2000; Kusaka & Puro, 1997; Newman, 1987; Reichelt, Hernandez, Damian, Kisaalita, & Jordan, 1996, 1997). In particular, we focussed our interest upon some specific membrane currents which have been well-characterized in human Müller cells but were hardly found in other species. These included a current mediated by an ionotropic nucleotide receptor ($P2X_7$; Pannicke et al., 2000), a current through γ-aminobutyric acid ($GABA_A$) receptors (Biedermann et al., 2004; Reichelt, Pannicke, Biedermann, Francke, & Faude, 1997), and a voltage-activated Na^+ current which was observed in Müller cells from only about two thirds of all mammalian species studied so far, partially at low incidence (Chao et al., 1997).

In conclusion, we wanted to know whether monkey Müller cells might be a better model for human cells than the more widely-used laboratory animals.

2. Materials and methods

Eyes were used from 3 adult (6–8 years) cynomolgus monkeys (*Macaca fascicularis*) that were euthanized in the course of other experiments, and from 8 human organ donors (35–76 years, median: 53 years) without any reported eye disease. All experiments were performed in accordance with the applicable German laws, with the Declaration of Helsinki, and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Human tissue was used after approval by the ethics committee of the Leipzig University Medical School. Eyes were opened, the vitreous was removed and the retina was cut into pieces. Eyes were supplied within 8–24 h after death.

2.1. Electrophysiology

Müller cells were isolated as described previously (Francke et al., 1997; Pannicke et al., 2000). Briefly, retinal pieces were incubated for 30 min in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline containing 0.2–0.5 mg/ml papain (Boehringer, Mannheim, Germany) at 37 °C. After washing with saline containing DNase I (150 U/ml; Sigma, Taufkirchen, Germany; substances were from Sigma unless indicated otherwise), the tissue was triturated with a pipette until single cells were dissociated. Suspensions with isolated cells were stored on ice until use. Electrophysiological recordings were performed in the whole-cell configuration of the patch-clamp technique at 20–24 °C. Cells were suspended in extracellular solution in a recording chamber on the stage of a microscope (Axioskop, Zeiss, Oberkochen, Germany). Extracellular solution contained (mM): NaCl, 110; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 1; Na_2HPO_4, 1; glucose, 11; *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES)-Tris, 10; $NaHCO_3$, 25; and was equilibrated to pH 7.4 by continuous bubbling with 95% O_2/5% CO_2. The $P2X_7$ agonist 2′- and 3′-O-(4-benzoyl-benzoyl)-adenosine triphosphate (BzATP) was applied in a solution where K^+, Ca^{2+}, and Mg^{2+} ions were replaced by Na^+. Additional changes of the solutions are given in the results. The recording chamber was continuously perfused (2 ml/min) after establishing the whole-cell configuration. Recording electrodes were made from borosilicate glass (Science Products, Hofheim, Germany) and had resistances of 4–6 MΩ when filled with a solution containing (mM): KCl, 130; NaCl, 10; $MgCl_2$, 2; $CaCl_2$, 1; ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10; HEPES-Tris, 10; pH 7.1. EGTA was removed from the pipette solution for activation of Ca^{2+}-dependent K^+ currents. When using Ca^{2+}-, Mg^{2+}-, K^+-free extracellular solution, the K^+ ions in the pipette were replaced by Cs^+. For current and voltage recordings, the patch-clamp amplifier Axopatch 200A (Axon Instruments, Foster City, USA) was used. Currents were low-pass filtered at 1 or 2 kHz and digitized at 5 or 10 kHz using a 12-bit A/D converter. Voltage command protocols were generated and data analysis was performed with the software ISO 2 (MFK, Niedernhausen, Germany) and SigmaPlot (SPSS Inc., Chicago, USA). The membrane potential was recorded in the current clamp mode at $I = 0$. The membrane capacitance was calculated from the capacitive artifact evoked by a 10-mV depolarizing step after blocking the K^+ conductance (filtered at 6 kHz, digitization at 30 kHz). In order to evaluate the

subcellular distribution of inward K^+ conductance, a solution containing 50 mM KCl (equimolar replacement of NaCl in the extracellular solution) was pressure-ejected onto different membrane regions of isolated cells; the evoked currents were recorded in the whole-cell configuration at a holding potential of −80 mV by using a patch pipette at the soma of the cells. For the ejection experiments, $NaHCO_3$ in the extracellular solution was replaced by NaCl and CO_2 was omitted. Mean values with standard deviations are given, Student's *t*-test and Mann–Whitney U-test were used for statistical analysis. Differences were considered significant with $P < 0.05$.

2.2. Ca^{2+} microfluorimetry

For Ca^{2+} microfluorimetry, retinal pieces (whole-mounts) were placed, with their vitread surface up, in a perfusion chamber, and loaded for 1 h with Ca^{2+}-sensitive fluorescence dyes (11 μM Fluo-4 AM, 17 μM Fura-Red AM; both from Molecular Probes, Leiden, The Netherlands). Within the tissue, the dyes were preferentially taken up by Müller cells as was shown recently (Uckermann et al., 2004). The same extracellular solution was used as described for patch-clamp recordings. Fluorescence images were recorded every 3.5 s from the endfeet of Müller cells in the nerve fiber/ganglion cell layer, using a confocal laser scanning microscope LSM 510 META (Zeiss, Oberkochen, Germany). The fluorescence dyes were excited at 488 nm; the emission of Fluo-4 was recorded with a band-pass filter between 505 and 550 nm ($F_{\text{Fluo-4}}$), the emission of Fura-Red was recorded with a 650-nm long pass filter ($F_{\text{Fura-Red}}$). To evaluate the Ca^{2+} responses, the fluorescence ratio $F_{\text{Fluo-4}}/F_{\text{Fura-Red}}$ was calculated, and the values were normalized to the mean baseline fluorescence ratio before agonist application. The normalized value is referred to as relative fluorescence and an increase corresponds to an increase in intracellular free $[Ca^{2+}]$.

Moreover, isolated Müller cells were used for Ca^{2+} microfluorimetric recordings. The cell suspension was incubated with Fura-2 AM (1 μM, Molecular Probes). Recordings were performed with Till-vision software using Polychrome IV monochromator and SensiCam camera (Till-Photonics, München, Germany). Fluorescence above 510 nm was excited at 340 nm (F_{340}) and 380 nm (F_{380}), and images were recorded every 3 s. The fluorescence ratio F_{340}/F_{380} was used to describe relative changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).

3. Results

3.1. Electrophysiology

We, first, recorded some basic membrane properties of Müller cells from cynomolgus monkey and human retina (Fig. 1). Both membrane potential and membrane capacitance were found to be very similar in the two species. However, the membrane resistance (evaluated by a 10-mV hyperpolarizing step from −80 mV) was significantly higher (about 4-fold, $P < 0.001$) in human Müller cells. At this voltage step the current is assumed to flow mainly through inwardly rectifying K^+ channels (e.g., Kir4.1; Ishii et al., 1997).

When membrane currents were evoked by depolarizing and hyperpolarizing voltage steps, they displayed similarities between monkey and human cells (Fig. 2A and B). In particular, we found (i) large inward currents due to hyperpolarizing steps from a holding potential of −80 mV (which often inactivated during 250-ms voltage steps at strong hyperpolarization) and (ii) smaller outward currents at weakly depolarizing steps. Taken together, this indicates (iii) an inward rectification of the dominant membrane conductance. The inward currents were found to be smaller in human Müller cells which corresponds to their higher membrane resistance. Moreover, the outward currents at strongly depolarizing potentials differed between the species (see below).

Application of Ba^{2+} (1 mM), a blocker of Kir channels, caused a complete block of inward currents and a strong reduction of outward currents in all cells tested ($n \geqslant 20$ for both species; Fig. 2C and D). The remaining

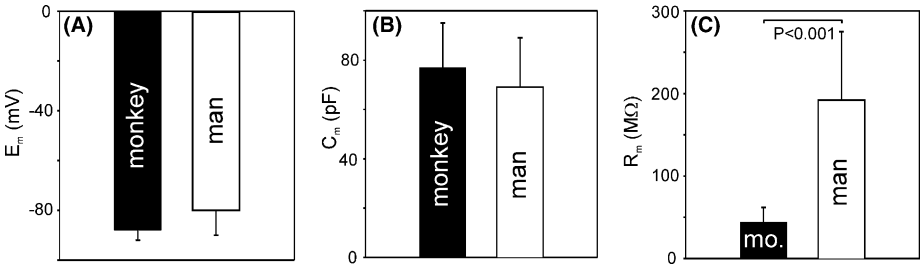

Fig. 1. Basic membrane properties of cynomolgus monkey and human Müller cells. In whole-cell patch-clamp experiments we recorded membrane (zero current) potential, membrane capacitance, and membrane resistance. Whereas the membrane potential E_m (A) and the membrane capacitance C_m (B) of monkey Müller cells (black columns) are very similar to those recorded in human cells (white columns), the membrane resistance R_m was found to be higher in human cells ($P < 0.001$) (C). At least 20 cells were recorded for each column.

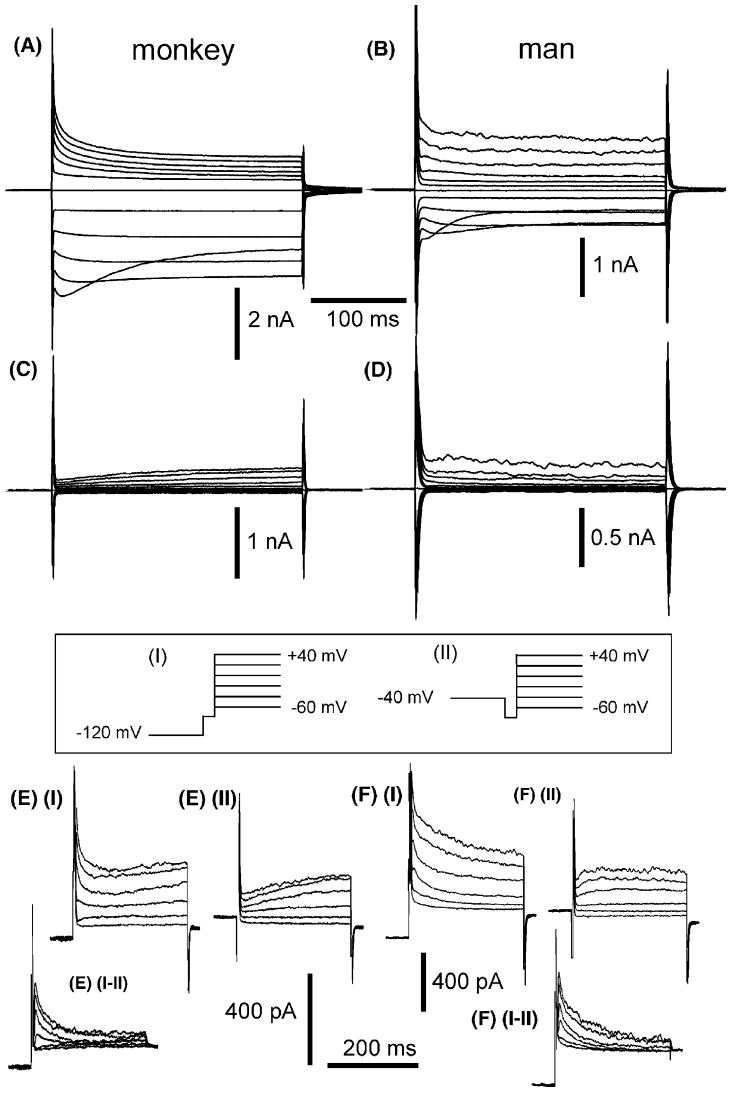

Fig. 2. Membrane K^+ currents in isolated Müller cells. (A–D) Current responses of a monkey (A,C) and a human (B,D) Müller cell to depolarizing and hyperpolarizing voltage steps. Cells were held at −80 mV and 20-mV incremental steps were applied between −180 and +40 mV (control solution, A, B). By application of 1 mM Ba^{2+} the dominating K^+ conductance could be largely blocked (C,D; cells were hyperpolarized only up to −140 mV), only small outward currents could be activated. In the human cell, strong depolarizations evoked a "noisy" current, likely to be a Ca^{2+}-dependent K^+ (BK) current (see J). (E,F) To further characterize the depolarization-evoked outward currents we used special protocols for the isolation of A-type currents. These currents could be maximally activated by a prepulse to −120 mV (inset, I) and inactivated by a prepulse to −40 mV (inset, II). Both prepulses were followed by depolarizing steps (20 mV increment between −60 and +40 mV). In addition to the original traces, the currents resulting from the difference of these protocols (I–II) are shown. Less than 40% of the monkey cells displayed no difference, i.e. no A-type current, whereas the A-type currents found in the remaining cells (E) were similar to those found in most human cells (F). (G–J) Currents through BK channels were not found in monkey (G,H), but were present in human Müller cells (J). Because these currents are sensitive to tetraethylammonium (1 mM; TEA), cells were depolarized from −80 mV to 0, +30, +50, +70, +90, and +110 mV before and after application of TEA and the differences, i.e. the TEA-sensitive currents, are shown. No TEA-sensitive currents were found in a monkey Müller cell (G), whereas outward currents with the typical kinetics could be activated and specifically blocked by TEA in a human cell (J). Moreover, application of the BK channel activator phloretin onto a monkey Müller cell voltage-clamped at +110 mV did not evoke any current (H), therefore the existence of functional BK channels is unlikely. (K,L) Steady-state current–voltage relationships (*I–V*-curves) recorded at the end of 250-ms voltage steps to the respective potentials in protocols as shown in A–D. In both monkey ($n = 18$, K) and human ($n = 16$, L) Müller cells mean values with standard deviations of membrane currents under control conditions (filled circles) and in the presence of 1 mM Ba^{2+} (open circles) are shown.

outward currents were activated upon depolarizing pulses to about −30 mV, indicating that they are likely to flow through voltage-dependent K^+ (Kv) channels. The typical outwardly rectifying component in mammalian Müller cells is a delayed rectifier current (Chao et al., 1997). This was also the dominating outward component in some monkey Müller cells (Fig. 2C). The outward currents of the human Müller cell appeared different in some aspects (Fig. 2D). To investigate this in more detail, the following experiments were performed. Transient, fast inactivating A-type currents were described previously in Müller cells (Bringmann

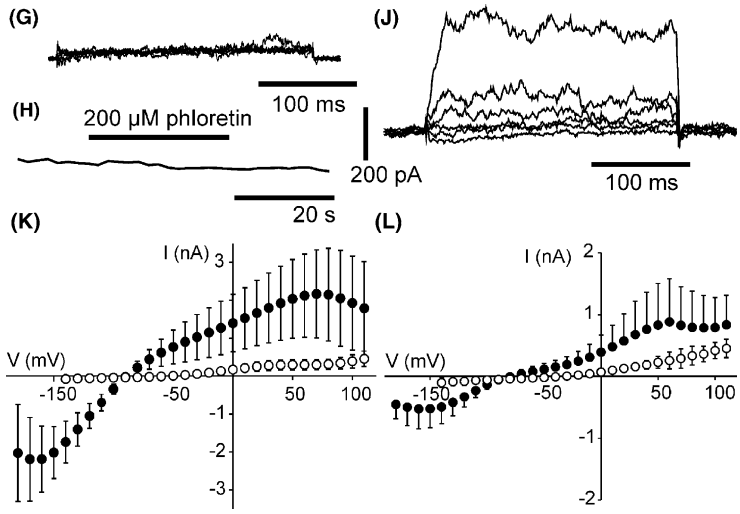

Fig. 2 (*continued*)

et al., 1999). These currents can be activated maximally by depolarizing voltage steps after a hyperpolarizing prepulse, and be steady-state inactivated by a depolarizing prepulse. We tested the expression of A-type currents by the protocol shown in the inset of Fig. 2. This procedure revealed the presence of A-type currents in 8 out of 13 monkey cells (current amplitude at +40 mV: 274 ± 197 pA, current density: 3.1 ± 1.9 pA/pF; Fig. 2E), whereas 14 of 15 human Müller cells expressed this current (amplitude at +40 mV: 345 ± 319 pA, current density: 2.6 ± 0.6 pA/pF, no significant difference between the two species, Fig. 2F). For comparison of the current kinetics the time constant of inactivation (τ) was determined by approximation for the current evoked by the step to +40 mV (using the difference). The τ-values were 27.8 ± 10.4 ms for monkey ($n = 8$) and 35.2 ± 14.0 ms for human cells ($n = 14$), which was not significantly different.

Another K^+ current type in Müller cells is mediated by Ca^{2+}-dependent K^+ channels (BK channels; Bringmann, Faude, & Reichenbach, 1997). These currents can be activated by 200 µM phloretin (Bringmann et al., 2002; Bringmann & Reichenbach, 1997) and blocked by 1 mM tetraethylammonium (TEA; Bringmann et al., 1997). TEA-sensitive currents were recorded in all human Müller cells tested ($n = 7$; Fig. 2J), whereas in monkey Müller cells, no TEA-sensitive currents were found ($n = 5$; Fig. 2G) and no BK currents were activated by phloretin ($n = 4$, Fig. 2H) resulting in smaller outward currents at strongly depolarizing potentials.

The data concerning whole-cell K^+ currents are summarized in the current-to-voltage relationships in Fig. 2K and L. Although the membrane currents were smaller in human Müller cells, their rectification was very similar in both species in the range from −180 to −20 mV in control solution. The ratio between outward currents (evoked by a 60-mV depolarizing step to −20 mV) and inward currents (evoked by a 60-mV hyperpolarizing step to −140 mV) was 0.46 ± 0.15 for monkey cells ($n = 18$) and 0.55 ± 0.31 for human cells ($n = 16$) and, thus, not significantly different. However, when the ratio was calculated for outward currents evoked by a step to +40 mV divided by the inward currents at −140 mV, it was 1.82 ± 1.55 for human and 0.86 ± 0.26 for monkey Müller cells (significantly different, $P < 0.015$). This increase was caused by the additional activation of BK currents in human but not in monkey cells. The difference was still visible under Ba^{2+}, although the BK currents were reduced by this ion.

It is known that the Kir conductance of Müller cells is not evenly distributed over the cell membrane. The concentration of Kir channels in certain regions of the cell may vary between different mammals (Newman, 1987). The subcellular distribution of the Kir conductance was investigated by application of a solution containing 50 mM K^+ onto four different regions of the cell and recording the resulting whole-cell inward currents. Maximum currents were evoked by application of K^+ onto the endfoot of the cell (Fig. 3). Currents were normalized by setting these maxima to 100%. No significant differences were observed when comparing the relative inward current amplitudes between monkey and human Müller cells.

In addition to K^+ channels, Müller cells of a variety of mammalian species express voltage-dependent Na^+ channels (Chao et al., 1997). Detection of fast inactivating Na^+ currents through these channels is possible after blocking the K^+ conductance by Ba^{2+} and using a protocol whereby depolarizing steps are applied after a hyperpolarizing prepulse. Under these conditions, a tiny inward current of not more than 40 pA could be recorded in only 1 of 11 monkey Müller cells (data not shown). In contrast, 6 out of 11 human Müller cells showed such currents with maximum amplitudes

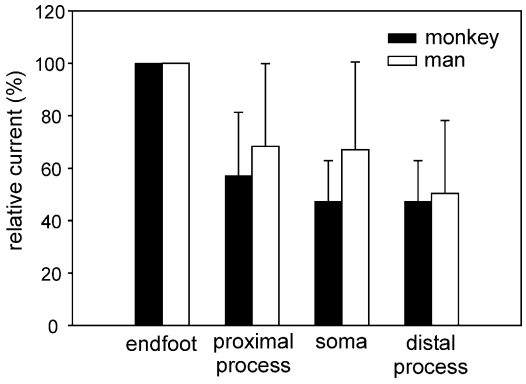

Fig. 3. Subcellular distribution of K^+ conductance in Müller cells. A solution containing 50 mM K^+ was focally applied onto four different regions of Müller cells from monkey (black columns, 13 cells from 2 monkeys) and man (white columns, 10 cells from 3 donors). The resulting whole-cell inward currents (mediated by Kir channels) were recorded. The current amplitude evoked by application onto the endfoot was set to 100%. No significant differences were observed regarding this distribution of conductance between the two species.

ranging from 50 to 400 pA (data not shown but see Francke, Pannicke, Biedermann, Faude, & Reichelt (1996) for a detailed study of the voltage-dependent Na^+ currents in human Müller cells).

Müller cells may express not only voltage-activated but also ligand-gated ion channels. For example, functional $GABA_A$ receptors were described in Müller cells of several vertebrate species (Malchow, Qian, & Ripps, 1989; Reichelt et al., 1996, 1997), including a detailed description of $GABA_A$ receptor-mediated currents in human Müller cells (Biedermann et al., 2004). Whereas we could confirm the existence of $GABA_A$ receptors in man (Fig. 4A), application of GABA onto monkey Müller cells under the same conditions did not evoke similar currents ($n = 8$, Fig. 4A).

The application of neurotransmitters may induce not only channel-mediated currents but also currents via electrogenic transporter systems. Among the transporter proteins described in Müller cells, there is an electrogenic Na^+/glutamate transporter, GLAST-1 (Rauen, Rothstein, & Wässle, 1996). The glutamate transporter-mediated currents have already been characterized in human Müller cells (Reichelt, Pannicke et al., 1997). Here, we obtained similar data from monkey Müller cells ($n = 10$; Fig. 4B). The glutamate-evoked inward currents were dependent on the membrane potential and on the presence of extracellular Na^+. We failed to detect outward currents at positive holding potentials; therefore, these currents are unlikely to be mediated by ionotropic glutamate receptors, and rather represent transporter currents such as those demonstrated in human cells. No differences in the densities of these currents were found between monkey and human cells (Fig. 4C).

Finally, human Müller cells were shown to express functional $P2X_7$ receptors (Pannicke et al., 2000). All

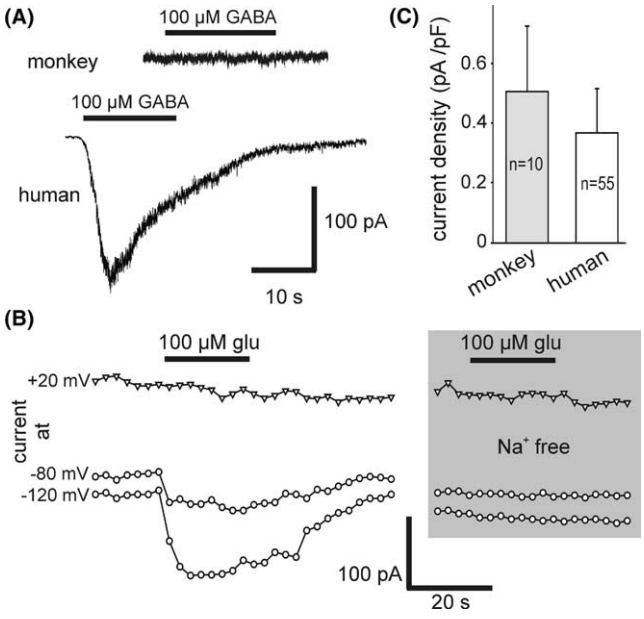

Fig. 4. Effects of neurotransmitters on Müller cells. (A) Application of GABA at a holding potential of −80 mV did not evoke an effect in monkey Müller cells whereas a current (Cl^- outward current) was elicited in a human Müller cell. Representative currents traces are shown. (B) Application of glutamate onto Müller cells evoked inward currents at negative potentials. They are likely to be mediated by the Na^+-dependent glutamate transporter, because no currents were observed at positive potentials and in Na^+-free solution. Representative currents of a single monkey Müller cell are shown. (C) A comparison of the current densities of glutamate transporter-mediated currents revealed no significant difference between monkey and human Müller cells. Number of recorded cells is given within the columns.

human Müller cells responded by inward currents to application of the agonist BzATP (50 µM) in a divalent cation-free solution. For the human cells used in the present study, the current density was 1.36 ± 0.78 pA/pF ($n = 14$), whereas in monkey, BzATP elicited a current with a significantly smaller amplitude (0.28 ± 0.1 pA/pF, $P = 0.01$; Fig. 5) in only 6 out of 12 cells. Application of 100 µM ATP did not change the mem-

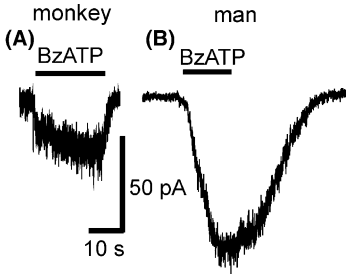

Fig. 5. P2X receptor-mediated currents in Müller cells. (A) In 6 out of 12 monkey Müller cells relatively small inward currents were recorded during application of 50 µM 2′- and 3′-O-(4-benzoyl-benzoyl)-ATP, a $P2X_7$-specific agonist (BzATP). (B) The currents recorded in human Müller cells were significantly larger ($P = 0.01$), and were found in all cells tested. Typical current responses from individual cells at a holding potential of −80 mV are shown.

brane currents of monkey Müller cells at potential steps between −100 and +110 mV ($n = 5$, data not shown). The effects of ATP and related compounds on membrane currents of human Müller cells were described in detail by Bringmann et al. (2002). Therefore, these currents were not further studied here; instead, Ca^{2+} microfluorimetry was performed to investigate the effects induced by P2 agonists.

3.2. Ca^{2+} microfluorimetry

In addition to ionotropic P2X receptors (see above), Müller cells may express metabotropic P2Y receptors which mediate an increase in the $[Ca^{2+}]_i$ following activation. To record these Ca^{2+} responses, we used retinal wholemounts from two monkeys loaded with Ca^{2+}-sensitive dyes. A detailed description of dye uptake by Müller cells and of fluorescence imaging in retinal wholemounts was recently given by Uckermann et al. (2004). Application of ATP (200 μM) evoked transient increases of $[Ca^{2+}]_i$ in virtually all Müller cell endfeet (Fig. 6B and F). Interestingly, the first response was followed by additional $[Ca^{2+}]_i$ transients in a decreasingnumber of endfeet (and with decreasing amplitudes) even after withdrawal of ATP (Fig. 6C–F). To characterize the involved receptors in more detail, pharmacological

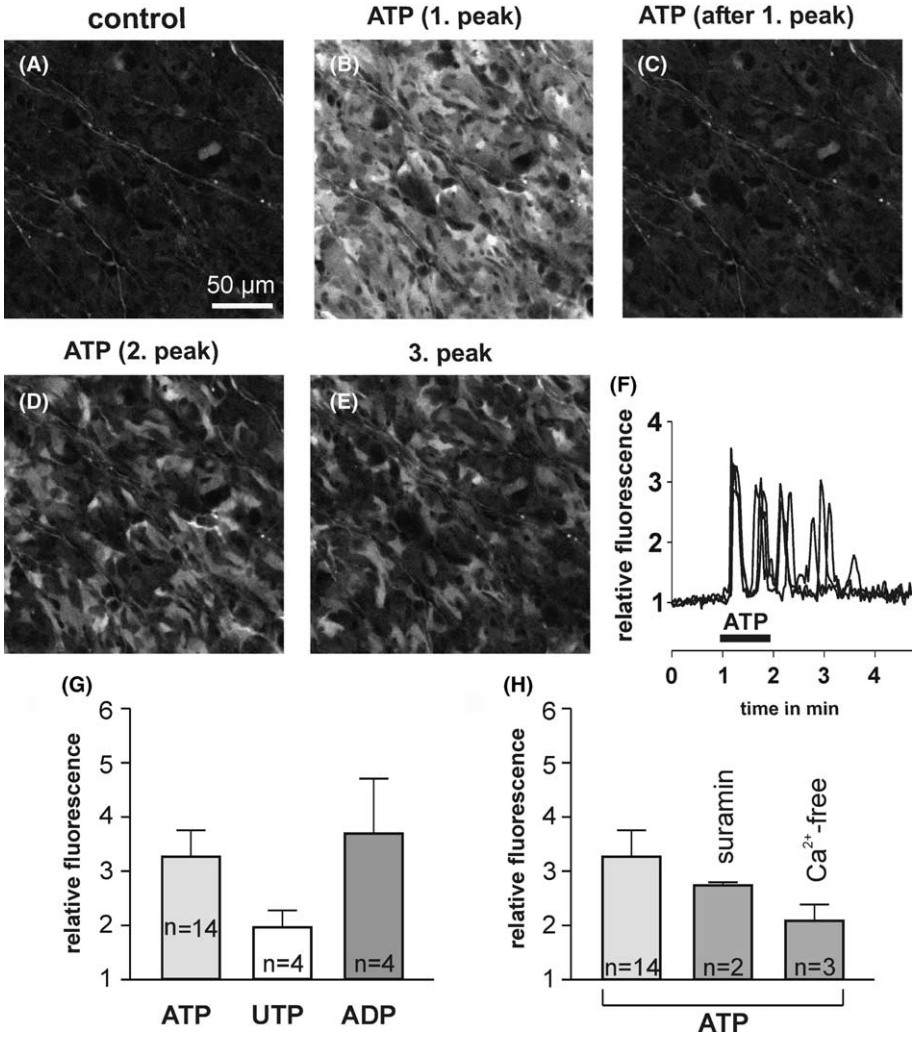

Fig. 6. Effects of extracellular nucleotides on the monkey retina. (A–E) Views onto the vitread surface of a retinal wholemount loaded with Ca^{2+}-sensitive dyes. (A) Fluorescence was low under control conditions. (B) Application of 200 μM ATP evoked transient increases of $[Ca^{2+}]_i$ in virtually all Müller cell endfeet, as indicated by an increased fluorescence. Non-fluorescent areas represent neuronal cells which did not take up a sufficient amount of the dyes. (C) Responses were transient, i.e. although ATP was still present in the bath, the $[Ca^{2+}]_i$ decreased. (D) The first response was followed by additional transient increases in a decreasing number of Müller cell endfeet, even in the absence of ATP (E). (F) The diagram shows the time dependence of responses from three selected Müller cell endfeet. (G,H) Diagrams presenting mean $[Ca^{2+}]_i$ increases from the number of wholemount preparations given within the columns. Responses similar to those evoked by ATP were found after applying the $P2Y_2/P2Y_4$ agonist UTP or the $P2Y_1$ agonist ADP (G). (H) The non-selective P2 antagonist suramin had only a small effect indicating that suramin-resistent receptor subtypes (like $P2Y_4$) might be present. $[Ca^{2+}]_i$ increases were still recordable in Ca^{2+}-free solution, pointing to an intracellular Ca^{2+} release.

experiments were performed (Fig. 6G and H). Application of the $P2Y_2/P2Y_4$ agonist, uridine triphosphate (UTP), or of the $P2Y_1$ agonist, adenosine diphosphate (ADP), caused similar Ca^{2+} responses as those induced by ATP. The P2 antagonist, suramin, which is able to block some but not all P2Y receptors, had only a small effect on the ATP-evoked responses. In Ca^{2+}-free solution, the increases were still recordable, indicating release from intracellular Ca^{2+} stores. However, there was an amplitude decrease in Ca^{2+}-free solution which might be due to a contribution of a Ca^{2+} influx through cation channels in the control solution. No responses were seen when adenosine monophosphate (AMP) or adenosine were used (data not shown). Therefore, the involvement of adenosine (P1) receptors in the recorded effects is unlikely.

Although a responsiveness to nucleotides was also found in Müller cell endfeet in a human retina (Fig. 7), there were some differences to the monkey cells. First, in human cells we never found repetitive responses (which were characteristic of the monkey retina: Fig. 6F); only single transients were recorded when ATP was applied (Fig. 7D). Similar to monkey cells, other P2Y agonists than ATP evoked $[Ca^{2+}]_i$ increases in human Müller cells. In Müller cells from both monkey and man (Fig. 6G, 7E), the responses to UTP displayed smaller amplitudes, whereas the responses to ADP had the same size as the ATP responses. Therefore, the $[Ca^{2+}]_i$ increases seem to be dominantly mediated by an ADP-sensitive subtype (e.g. $P2Y_1$), in cells from both species. In contrast to the monkey cells, human Müller cells also responded to the application of AMP and adenosine (Fig. 7E) suggesting the presence of P1 receptors. Unfortunately, we could not investigate these putative receptors in more detail because of the small amount of fresh human retina available for this study.

Because a variety of P2X receptor subtypes have been described in retinal neurons (Ishii, Kaneda, Li, Rockland, & Hashikawa, 2003), we used isolated Müller cells to determine the direct actions of P2 agonists, thereby avoiding neuron-glia interactions. In isolated monkey cells, we recorded $[Ca^{2+}]_i$ increases after application of 500 μM ATP in normal ($n = 5$) and in Ca^{2+}-free solution ($n = 4$), demonstrating that the responses are not mediated via neuronal activation, and that P2Y receptors are located directly on the Müller cells. However, the oscillating responses found in the retinal wholemounts did not occur in isolated cells. Thus, their appearance might depend on the interaction with neuronal cells. Isolated human cells responded to 500 μM ATP in 98 out of 102 cases, whereas 100 μM UTP evoked $[Ca^{2+}]_i$ increases in 7 out of 8 cells. However, no responses could be recorded when 200 μM adenosine ($n = 6$) was applied.

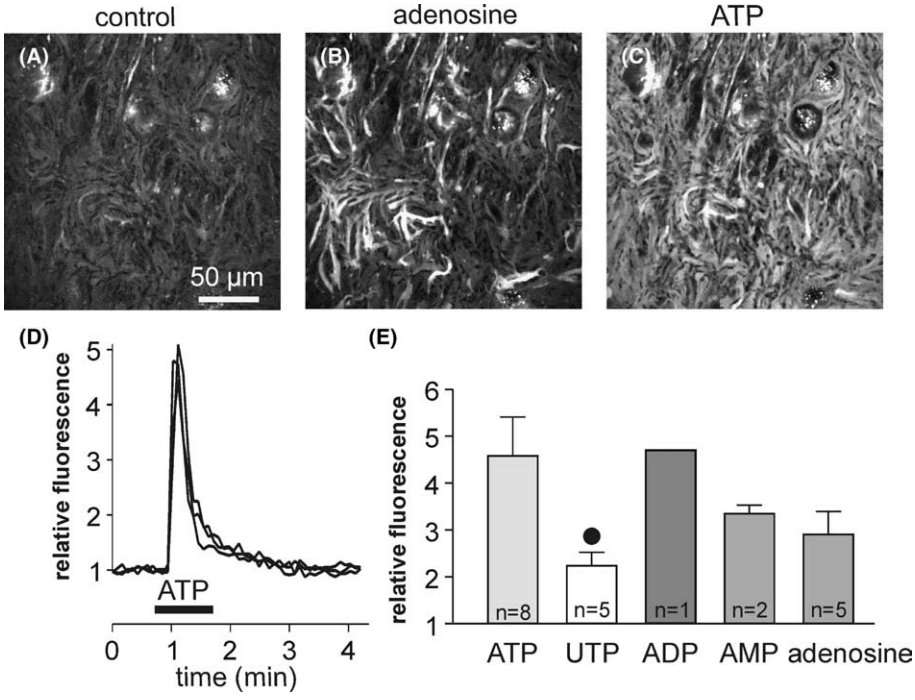

Fig. 7. Effects of nucleotides and nucleosides on human Müller cells. (A–C) Views on retinal preparations as described in Fig. 6. Adenosine and ATP (both 200 μM) evoked $[Ca^{2+}]_i$ increases in Müller cell endfeet. Whereas reactions to ATP were seen in virtually all Müller cells (C), clearly fewer endfeet responded to adenosine (B). (D) Ca^{2+} responses to ATP from three selected Müller cell endfeet. No repetitive responses occurred. (E) Mean $[Ca^{2+}]_i$ increases recorded from the number of wholemounts given inside the columns. The response to UTP is significantly smaller ($P < 0.05$).

4. Discussion

The basic membrane properties between cynomolgus monkey and human Müller cells are very similar (and also similar to those found in cells from other mammals: Chao et al., 1997), the only exception being the relatively large membrane resistance recorded in human Müller cells, which corresponded to the smaller inward currents at hyperpolarizing potentials. This difference does not appear to be due to longer post mortem times in the case of the human retinae because we recorded virtually unchanged low membrane resistances from monkey cells up to two days after the eyes were excised. One possible explanation for this difference may be related to the age of the human donors. Almost half (45%) of the cells used for recording the membrane resistance were from donors older than 60 years and a downregulation of Kir currents has been described under this condition (Bringmann, Kohen, Wolf, Wiedemann, & Reichenbach, 2003).

In Müller cells of both species Ba^{2+} application demonstrated that Kir channels dominate the membrane conductance at potentials close to the resting membrane potential. Müller cells are known to express the Kir4.1 channel subunit which is characterized by a weak inward rectification (Ishii et al., 1997). Therefore, a significant part of Ba^{2+}-sensitive outward currents may flow through these channels, mainly at weakly depolarizing potentials. In addition to the block of Kir currents, 1 mM Ba^{2+} also reduces, but not totally blocks Kv currents which therefore could still be recorded at stronger depolarizations.

A further similarity between both species was that the cells may express fast-inactivating A-type K^+ channels and voltage-gated Na^+ channels. A major difference was the occurrence of BK channel-mediated currents in human but not in cynomolgus monkey Müller cells. On the one hand, the lack of these currents in monkey Müller cells is no unique feature of this species but is shared with rat (Felmy, Pannicke, Richt, Reichenbach, & Guenther, 2001) and murine Müller cells (Pannicke, Bringmann, & Reichenbach, 2002). On the other hand, BK channel-mediated currents are reliably recordable from rabbit (Francke et al., 2002) and porcine Müller cells (Bringmann et al., 1997).

The uneven distribution of K^+ conductance (mainly of weakly rectifying Kir4.1 channels) is assumed to be a prerequisite for effective K^+ buffering in the retina by the process of K^+ siphoning (Newman, Frambach, & Odette, 1984). Excess K^+ due to neuronal activity is believed to flow from the plexiform layers via the endfeet of Müller cells into the vitreous body (Newman et al., 1984), and into retinal blood vessels which are ensheathed by glial cell processes (Newman, 1987). Thus, the endfoot membrane and the membrane at the soma and distal process, where the cell may have contact with intraretinal blood vessels, should be highly permeable for K^+. Until now, the only study about the distribution of K^+ conductance in primate Müller cells was done using the owl monkey, *Aotus trivirgatus* (Newman, 1987). Here, we present data on Müller cells from cynomolgus monkey and man, demonstrating that in both species the K^+ conductance is highest in endfeet (normalized to 100%), whereas the current-responses recorded during ejection onto the soma and the distal process ranged between 47% and 67%. The main difference compared to data from the owl monkey is the localization of the maximum K^+ conductance close to the soma in owl monkey Müller cells. However, the endfoot conductance in owl monkey was still larger than the conductance of most other cell regions, pointing to the possibility that a significant fraction of K^+ is siphoned from the endfoot into the vitreous body and into blood vessels at the retinal surface (Newman, 1987). Although certain species differences between the primates seem to exist, this model may also apply to the retinae studied here. In contrast, there is a dramatic difference to the distribution of K^+ conductance in Müller cells from rodent retinae, which displayed maximal current-responses of more than 200% at the distal process in rat (Pannicke et al., 2004) and mouse (Connors & Kofuji, 2002).

Currents mediated by $GABA_A$ receptors were recorded from Müller cells of man here and in previous studies (Biedermann et al., 2004; Reichelt, Pannicke et al., 1997) and from baboon, *Papio cynocephalus* (Reichelt et al., 1996) but not from cynomolgus monkey, *Macaca fascicularis*. This latter observation disproves the hypothesis of Reichelt et al. (1996) who supposed the existence of $GABA_A$ receptors to be a characteristic of primate (in contrast to other mammalian) Müller cells. Without knowledge about the function of this receptor on Müller cells, it is difficult to speculate about the reason for these species differences. This applies also to the expression of ionotropic P2X receptors. $P2X_7$-mediated currents and immunoreactivity for the $P2X_7$ receptor were demonstrated in isolated human Müller cells (Pannicke et al., 2000), whereas Ishii et al. (2003) found only retinal neurons to be immunolabeled for this receptor in rhesus monkeys. Very small currents, evoked by the agonist BzATP, were recorded in some monkey Müller cells (Fig. 5A); their small amplitudes and low incidence prevented a detailed study of these currents. Macaque Müller cells might display these receptors at such a low expression level that they could not be demonstrated immunohistochemically by Ishii et al. (2003). Due to the pharmacological profile (Bianchi et al., 1999) the existence of another P2X subtype than $P2X_7$ is unlikely.

Transporters in Müller cells are known to play an important role in removing neurotransmitters, such as glutamate, from the extracellular space (Newman & Reichenbach, 1996). Here we present data on the physiology of this transport in monkey Müller cells and show

its similarity to those obtained in cells from other mammals (Rauen, Taylor, Kuhlbrodt, & Wiessner, 1998; Sarantis & Attwell, 1990) including man (Reichelt, Pannicke et al., 1997).

There are a growing number of studies suggesting and/or demonstrating important functional roles of nucleotides—and P2 receptors—in the retina (Bringmann et al., 2001; Francke et al., 2002; Newman, 2001, 2003), among them also data obtained from monkey retina (Cowlen et al., 2003; Ishii et al., 2003). Some of these reports present evidence for the existence of P2 receptors on Müller cells, but also for clear differences in receptor expression between species. Here we demonstrate that Müller cells in the retina of a non-human primate respond to application of different P2 agonists with an increase of $[Ca^{2+}]_i$ similar to human (Bringmann et al., 2002), rat (Li, Holtzclaw, & Russell, 2001), and rabbit Müller cells (Uckermann, Grosche, Reichenbach, & Bringmann, 2002). Unfortunately, a more detailed characterization of these receptors is hampered by the lack of highly specific pharmacological tools.

In the context of purinergic receptors, we observed the occurrence of adenosine-evoked $[Ca^{2+}]_i$ responses in Müller cells from human but not from monkey retina. The failure of adenosine to increase the $[Ca^{2+}]_i$ in isolated human cells is in agreement with data from patch-clamp experiments (Bringmann et al., 2002). This raises the question how the $[Ca^{2+}]_i$ increases recorded in human retinal wholemounts (Fig. 7E) can be explained. It cannot be excluded that the dissociation procedure destroyed putative P1 receptors, and/or prevented their activation, in isolated cells. The other possibility is that the responses in human retina were indirectly induced by an activation of neuronal (or astrocytic) receptors. Adenosine receptors and their mRNA have already been described in the retina (Braas, Zarbin, & Snyder, 1987; Kvanta, Seregard, Sejersen, Kull, & Fredholm, 1997). An activation of neuronal receptors may thus cause a secondary glial response.

In conclusion, we cannot support the idea that Müller cells of the cynomolgus monkey constitute the 'ideal model' for investigating the pathophysiological roles of Müller cells in human ocular diseases. As in many cases, different experimental animal species must be chosen depending on the question(s) to be answered.

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