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$GABA_A$ receptor currents recorded from Müller glial cells of the baboon (*Papio cynocephalus*) retina

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Abstract

The effect of γ-aminobutyric acid (GABA) application on acutely isolated, non-cultivated Müller glial cells from the baboon retina was studied using the whole-cell voltage-clamp technique. Application of GABA (0.1 mM) generated inward currents at a holding potential of −80 mV as well as an increase in current noise. The GABA-activated current had a reversal potential of 18.6 mV and was therefore supposed to be a Cl^- current (E_{Cl} = 5 mV). The $GABA_A$ receptor agonist muscimol (0.1 mM) elicited an inward current and bicucullin (0.5 mM), a blocker of the $GABA_A$ receptor, diminished the GABA responses in our experiments completely. Baclofen (0.1 mM), a $GABA_B$ agonist, neither had an effect when applied under conditions where the dominant Müller cell K^+ currents were unblocked, nor when the K^+ currents were blocked by application of Ba^{2+} (1 mM). Glycine (0.1 mM) was ineffective as well. From these results we conclude that the baboon retinal Müller cells possess $GABA_A$ receptors. However, these have recently been discovered on skate Müller cells whereas $GABA_A$ receptors could not be found on Müller cells of guinea pig, pig, mouse, rat and rabbit.

Keywords: $GABA_A$ receptor; Müller cells; Glial cells; Primate retina; Voltage-clamp

The retinal Müller cells (MC) are a specialized form of glia since they span the whole retinal thickness. Therefore, they are in close contact with all neuronal retinal cells, whereas the retinal astrocytes are confined to the nerve fiber layer. This morphologic characteristic implies a vast array of functions for the MC during glia–neuron interactions, beyond being simply a mechanical support for the retina. Indeed, we know that the MC play an important part in the clearance of several substances in the retina, e.g. K^+, glutamate and ammonia. The retinal K^+ homeostasis is crucial for the maintenance of normal neuronal membrane potentials and is performed, at least partly, by a special process called 'spatial buffering', which shifts K^+ in the retina along the MC interior [12].

Glutamate, well-known to be neurotoxic in concentrations above physiological levels, is removed from the extracellular space by the high affinity Na^+/glutamate transporter of the MC [1]. Dysfunction of the glutamate transporter under pathological conditions can thus be deleterious for the retina.

A third example of the functional significance of the MC is evident in the detoxification of ammonia, which is performed exclusively by astrocytes in the brain and by the MC (and astrocytes) in the retina. The enzyme glutamine synthetase (GS), a glial marker, catalyzes the reaction of glutamate and ammonia to glutamine [16]. Blocking this reaction leads to generalized seizures in animals, which are attributed to the accumulation of toxic ammonia in the brain. This indicates that the failure of ammonia detoxification in the retina would also be deleterious. Moreover, glutamine synthesized by this reaction, within glial cells, is thought to be released into the extracellular space and provides the main glutamate source of the neurons [14].

From these and other functions the question arises:

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how do the MC adapt their morphological and functional features to the changing requirements of the retina? Neurotransmitter receptors might be a path for the transduction of extracellular signals to the MC interior, as has been shown by receptors whose activation eventually increases the intracellular Ca^{2+} concentration in astrocytes. The subsequent formation of filopodia might demonstrate one possibility of morphological adaptation [5]. However, until now neurotransmitter receptors have not been demonstrated on mammalian MC, with the exception of a dopamine receptor in guinea pig [3].

Therefore, in the present study we demonstrate the presence of γ-aminobutyric acid $(GABA)_A$ receptors on MC of a primate, the baboon.

Whole-cell voltage-clamp currents were recorded on 5 days from non-cultured MC, acutely isolated from seven baboons (*Papio cynocephalus*), aged 4–6 years. The animals had a mild experimental infection with *Schistosoma mansoni* of 3 months duration to study the acute phase of schistosomiasis, which is characterized by fever, leukocytosis, cachexia, eosinophilia, diarrhea, hematochezia, elevated inflammatory cytokines and elevated antischistosomal antibodies. They were terminated soon after the acute stage had ended and before much development of chronic schistosomiasis. Six of the animals were sacrificed by exsanguination while under anesthesia maintained by ketamine HCl (10 mg/kg), heparin (45 U/kg) and Na pentobarbital (14 mg/kg), and one by exsanguination while under anesthesia induced with ketamine HCl (10 mg/kg) and maintained with halothane and oxygen. Five animals received atropine sulfate (0.04 mg/kg, intramuscular) before sacrifice. The experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Eyes were enucleated immediately after the animals were euthanized. The MC were isolated according to a procedure that has been described elsewhere [15]. Briefly, retinas were prepared and immersed into Ca^{2+}/Mg^{2+}-free Ringer solution, containing 1 mg/ml papain (Boehringer Mannheim, Germany), for 30 min at 37°C in an atmosphere of 5% CO_2/95% air. The Ca^{2+}/Mg^{2+}-free Ringer contained (in mM): NaCl 110, KCl 3, Na_2HPO_4 1, HEPES 10, glucose 11 and $NaHCO_3$ 25, and was adjusted to pH 7.4 with Tris-base. After incubation the tissue was rinsed four times with Ringer solution (Ca^{2+}/Mg^{2+}-free Ringer plus $CaCl_2$ (2 mM) and $MgCl_2$ (1 mM)), which also served as recording solution; 0.1 mg DNase (Sigma) was added and the tissue triturated several times using a 1 ml-tip Pipetteman until the MC separated from each other. After isolation MC were stored on ice in recording solution and were used for up to 10 h after the animals were sacrificed.

Recordings were performed at 25°C in a chamber mounted on the stage of an inverted microscope containing a volume of 0.2 ml, perfused with recording solution at a flow rate of 6 ml/min. The final pH control was achieved by steadily bubbling with carbogen gas (5% CO_2, 95% O_2). Ba^{2+} (1 mM) was always added before conducting the pharmacological experiments.

The intracellular solution contained (in mM): NaCl 10, KCl 130, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10 and EGTA 10. pH was adjusted to 7.1 with Tris-base. When filled with this solution the patch pipettes had a resistance of 10 MΩ, which was necessary for a high sealing efficacy at the soma of the MC. The compositions of the extra- and intracellular solutions were chosen since in previous experiments on MC they turned out to be well suited for successful whole-cell recording.

The R_s compensation was not used. However, the input resistance of Ba^{2+}-treated MC was higher than 1 GΩ [6]. Therefore, the ratio of series resistance to MC input resistance was very low, causing a 5% maximum voltage error even without series resistance compensation. Recordings were carried out using the patch-clamp amplifier Axopatch 200A (Axon Instruments, Foster City, CA, USA). Currents were on-line (low-pass) filtered at 1 kHz using an eight-pole Bessel filter. All recordings were carried out with a sampling frequency of 5 kHz using the

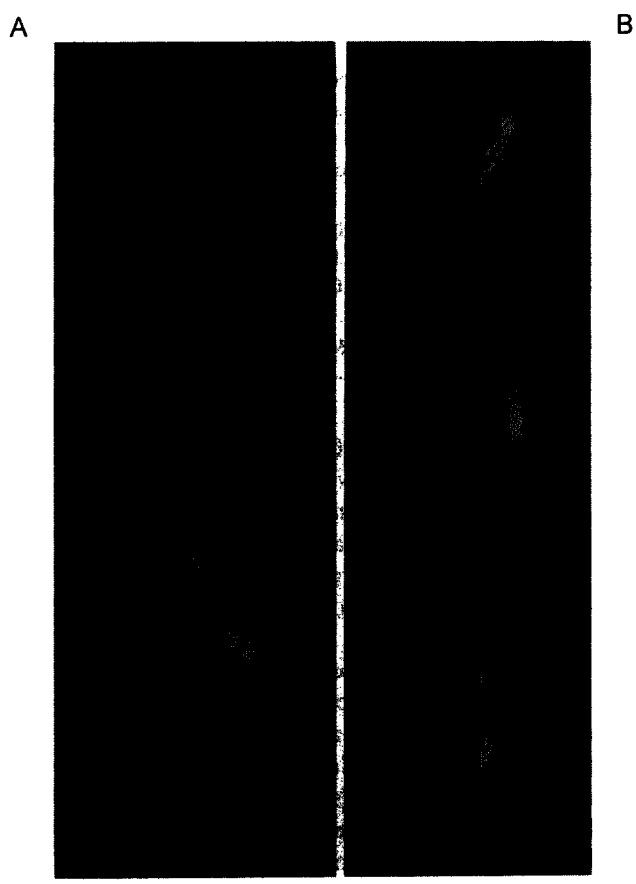

Fig. 1. Photomicrographs of typical isolated baboon MC. (A) A more peripheral MC is displayed characterized by a thicker appearance throughout and their shortness, whereas the more central MC (B) is more slender and longer. We recorded preferentially from MC like that displayed in (B). The recording pipette was always attached to the soma, which is the protrusion marked by an arrow. Scale bar, 10 μm.

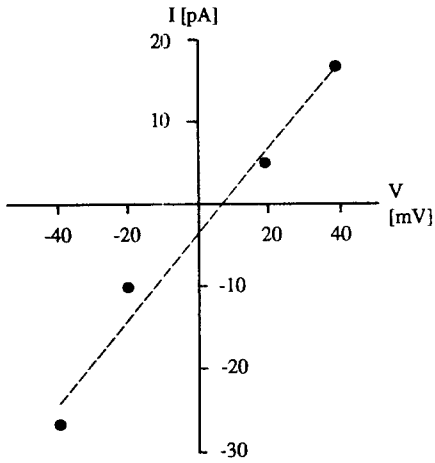

Fig. 2. Typical current/voltage relation of the GABA-evoked MC current. In this MC the zero current potential was at 7 mV. To determine the I/V-curve we applied voltage steps to the cells before, during and after GABA application. Step duration, 100 ms. Membrane voltage, −40, −20, 20 and 40 mV. The currents were measured 90 ms after step onset. Currents from the same voltage steps before and after GABA application were averaged and subtracted from the currents which were elicited by these voltage steps during GABA application. The difference gives the GABA-evoked current. Reversal potentials were determined by linear regression.

pCLAMP software (version 6.0.2; Axon Instruments, Foster City, CA, USA). Data analysis was also done with pCLAMP.

The isolated MC (Fig. 1) of the baboon retina exhibit differences in length, which reflect the differences in the thickness of the retina. We preferentially recorded from the longer cells which were more likely to have a more central retinal location in situ.

GABA (0.1 mM) evoked currents were recorded from 26 MC out of seven animals and the mean current amplitude amounted to 105 pA (SD = 68 pA).

Current noise increased during the presence of GABA on the cell membrane (compare Fig. 3A–C). The zero current potential of the GABA-evoked current was 18.6 mV ($n = 4$, SD = 14.6 mV) (Fig. 2). Cells which were incubated with bicuculline (0.5 mM) were completely insensitive to GABA (0.1 mM) administration, an effect which was reversible ($n = 4$) (Fig. 3A).

Muscimol (0.1 mM) evoked a MC current with a mean amplitude of 91 pA ($n = 4$, SD = 45 pA) (Fig. 3B). The GABA (0.1 mM) elicited current on the same MC had a mean amplitude of 66 pA ($n = 4$, SD = 36 pA).

We tested the effect of glycine (0.1 mM) ($n = 4$) on MC which responded to the application of GABA (0.1 mM). All MC tested were insensitive to glycine (Fig. 2C).

Similarly, the GABA-sensitive MC did not respond to the application of baclofen (0.1 mM), neither in Ba^{2+}-free nor in Ba^{2+}-containing extracellular solution ($n = 4$) (not shown).

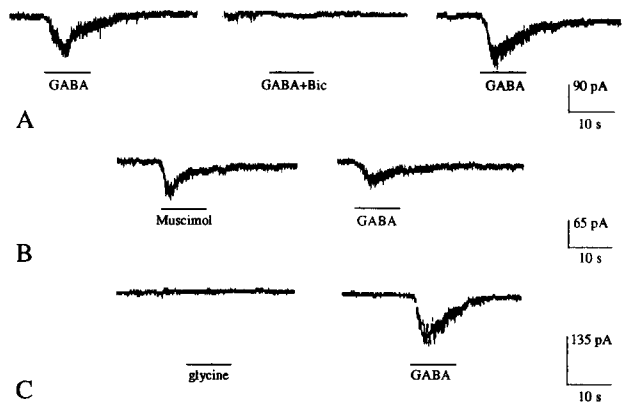

Fig. 3. Baboon MC currents under different conditions. (A) GABA (0.1 mM) evoked currents can be blocked by application of bicuculline (0.5 mM) which was prewashed for 30 s. The bicuculline effect is reversible (right trace). (B) Muscimol (0.1 mM) elicits a current on GABA-sensitive cells, whereas in (C) glycine (0.1 mM) is ineffective on the baboon MC. Note the increase of current noise during application of GABA, which completely disappeared under bicuculline. The membrane potential was −80 mV during all recordings.

Pharmacology as well as the reversal potential of the GABA-evoked currents in baboon MC unequivocally point to the presence of $GABA_A$ receptors on these cells.

Bicuculline is a typical $GABA_A$ receptor blocker, whereas muscimol is a typical agonist [13]. Since baclofen is a $GABA_B$ receptor agonist, its ineffectiveness excludes the possibility that the recorded currents were $GABA_B$ receptor-mediated. This was confirmed when, during baclofen application, the MC K^+ currents were unblocked and blocked, respectively, which would have permitted us to observe both the suppression of K^+ currents and the activation of Ca^{2+} currents. Both effects are described as being generated by activation of $GABA_B$ receptors [4].

The $GABA_A$ character of the currents is further strengthened by the relative vicinity of the measured GABA current reversal potential and the Cl^- equilibrium potential of 18.6 and 5 mV, respectively. This is a strong hint that Cl^- carries the GABA-evoked currents.

Additionally, the observed current noise increase during GABA application is a characteristic of opening ion channels, in contrast to membrane transporters. Thus, the current noise increase supports the pharmacological characterization of the GABA currents as receptor-mediated. The question arises from our experiments whether the baboon MC possess a high affinity Na^+-dependent GABA transporter, as described for the guinea pig MC [2]. We were not able to observe currents caused by such a transporter during our recordings. However, from results on MC of other species, it is known that the transporter current cannot be evoked under our recording conditions, i.e. with nearly symmetrical intra- and extracellular Cl^- (Biedermann, personal communication). This could be explained by the Cl^- dependency of the high affinity Na^+/GABA transporter [8]. Thus, our results regarding the

GABA transporter of MC are not contradictory to a previous report about GABA uptake into baboon MC using [^{3}H]GABA autoradiography [10].

On the contrary, however, it is interesting to compare the $GABA_A$ response of baboon MC with the GABA response of MC from other species. Surprisingly, Biedermann (personal communication) was not able to find $GABA_A$ currents in several mammals, i.e. guinea pig, pig, mouse, rat and rabbit. It is rather improbable that the existence of $GABA_A$ receptors on our baboon MC was due to schistosomiasis the animals were suffering from, since we recently found $GABA_A$ receptors on human MC (unpublished results). Thus, $GABA_A$ receptors on MC are seemingly a primate characteristic instead of a mammalian one. The evolutionary and functional significance for the differences between primates and other mammals cannot yet be explained. In addition, the finding of $GABA_A$ receptors on baboon as well as skate MC [9], an organism which is relatively low on the evolutionary scale, is confusing.

The molecular composition of the primate $GABA_A$ receptors remains unknown, since immunocytochemical staining of $GABA_A$ receptor subunits in the macaque retina did not identify MC [7].

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