Development of chloride homeostasis in albino and pigmented rat visual cortex neurons

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Albinism has a profound effect on visual development and visual function. Pharmacologically significant alterations of the two most important chloride-transporters – KCC2 (outward transporter) and NKCCl (inward transporter) – functions were found in albino visual cortex neurons, comprising a higher NKCCl and a lower KCC2 action. In this study, we compare the early postnatal development of the reversal potential of γ-aminobutyric acid$_A$R-mediated currents in visual cortex neurons of albino and pigmented rats. At birth we found no differences. At the time of eye opening (second week postnatally) the reversal potential of γ-aminobutyric acid$_A$R-mediated currents is 15 mV more positive and intracellular Cl^- concentration is higher in visual cortex neurons of albinos than of pigmented rats. *NeuroReport* 19:595–598
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Introduction

Oculocutaneous albinism is known to affect various species among vertebrates including mammals. This condition is often caused by a mutation in the Tyrsosianse gene that was recently shown to be responsible for albinism in the albino Wistar rat, according to oculocutaneous albinism type 1 A in humans [1].

All mammals with hypopigmentation of the retinal pigment epithelium have abnormal visual systems. Resulting abnormalities in albinos harm visual processing including cortical binocularity or the direction selective accessory optic system [2–6]. Direction selectivity in turn critically depends on γ-aminobutyric acid (GABA)-ergic mechanisms: although GABA is the main inhibitory transmitter in the adult brain, GABAergic transmission is excitatory during early postnatal development.

The change of GABA and glycine-mediated responses from depolarizing to hyperpolarizing reveals a change in the driving force for Cl^- and a shift in GABA and glycine reversal potential during development [7–10]. It has been shown recently that adult albino Wistar rat's visual cortex neurons have changed GABA$_A$R-mediated currents in comparison with pigmented Long–Evans neurons [11]. This finding, however, has opened several questions. First, it was unclear whether the observed differences are already present at birth or appear during development of the visual system. Second, as this study was performed on different rat strains, it opened up the possibility that strain variations can contribute to the observed differences. To address these questions, we investigated the early postnatal development of chloride homeostasis in visual cortex of pigmented Long–Evans and albino Wistar rats as well as pigmented and albino littermates.

Materials and methods

Experiments were carried out on Long–Evans and Wistar rats of postnatal day (P) 1–40 and second generation of homozygous and heterozygous animals obtained by cross-breeding the rat strains (for detailed method, see [12]). All experimental procedures were strictly in accordance with institutional guidelines and approved by a local ethics committee.

Brain slice preparation

Rats were anesthetized with halothane and decapitated. The brain was taken out of the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF; 123 mM NaCl, 2.5 mM KCl, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, 11 mM D-glucose, 1.8 mM $CaCl_2$, 1.3 mM $MgCl_2$, bubbled with 95% O_2 and 5% CO_2; pH 7.4). Slices of visual cortex (350 μm) were cut on a vibratome (MA752; Campden Instruments, Germany). In the P40 animals the slices were derived from Bregma − 5.8 mm to Bregma − 7.5 mm [13], where the visual cortical areas 18, 17 and 18a extend 7–8 mm from the midline laterally to the temporal cortex. Slices were stored for at least 1 h at room temperature in ACSF and then relocated to a submerged recording chamber.

Gramicidin-perforated patch-clamp recordings

The techniques we used for perforated patch clamp recording have been reported in detail elsewhere [11]. The recording chamber was perfused with oxygenated ACSF at room temperature at a rate of 3 ml/min. Kynurenic acid (2 mM) was added directly to the ACSF to prevent excitatory activity in the neurons tested. Gramicidin perforated patch clamp recordings of pyramidal neurons

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were performed under visual control. All recorded cells were from layer V or pyramidal neurons in cortical plate (P1–3). Borosilicate patch electrodes (5–9 MΩ) were filled with a solution containing 130 mM K-gluconate, 0.5 mM Na-gluconate, 20 mM HEPES, 4 mM $MgCl_2$, 4 mM Na_2ATP, 0.4 mM Na_3GTP, 0.5 mM EGTA (pH 7.2). Gramicidin (30 μg/ml, dissolved in dimethylsulfoxide) was added to the solution as a membrane-perforating agent. The measured membrane potentials were corrected for the junction potential of −10 mV [14]. Inhibitory postsynaptic currents were evoked through a concentric bipolar electrode placed approximately 50–100 μm lateral to the recorded neuron with stimuli (20–100 μA, 50 μs duration, 0.1 Hz) in voltage clamp mode by means of a PC-501A patch clamp amplifier (Warner Institute Corporation, Holliston, Masschusetts, USA) connected via AD/DA-converters (CED 1401+, Cambridge Electronic Design, Cambridge, UK) to a personal computer. Holding potentials were raised from −100 to +30 mV (keeping in mind the junction potential correction of −10 mV) in 10 mV steps in every recording. Recordings underwent low-pass filtering at 3 kHz and were sampled at 10 kHz. For recording and analyzing WinWCP software (John Dempster, University of Strathclyde, Glasgow, UK) was used.

Drugs

The drugs applied were gramicidin D, kynurenic acid (KYN, an ionotropic glutamatergic receptor antagonist), bicuculline (an ionotropic $GABA_A$ receptor antagonist) and picrotoxin citrate (an ionotropic GABA receptor antagonist) (Tocris Cookson, Bristol, UK). Substances were prepared as stock solutions and frozen, then added to the ACSF to reach the desired final concentration.

Statistics

One-way analysis of variance ($P<0.001$), SigmaStat software, was used to test the data for significant disparities. Numerical data are presented as mean ± SD.

Results

Postnatal development of reversal potential of the γ-aminobutyric $acid_A$ receptor-mediated currents (E_{GABA}) in albino visual cortex neurons

Visual cortex neurons $GABA_A$ receptor-mediated currents were measured at different holding potentials and E_{GABA} was determined from these recordings. To investigate the development of inhibitory patterns in albino visual cortex neurons, recordings were taken from four different age groups of albino Wistar rats: P1–5, P7–9, P14–17 and P > 20. Recordings from visual cortex of pigmented Long–Evans rats of the same age groups were taken as a control. Data are presented in Fig. 1. Intracellular chloride levels for the above-mentioned groups were calculated from $GABA_A$R-mediated currents reversal potential according to the Nernst equation (T=25°C) and comprised 29.15 versus 28.09; 20.61 versus 16.34; 21.07 versus 11.47 and 19.73 versus 11.03 mM for albino and pigmented visual cortex neurons, respectively (Fig. 1b). By application of the $GABA_A$R antagonist bicuculline (30 μM) and $GABA_A$R antagonist tetrodotoxin citrate (1 μM) and in Cl-free ASCF all postsynaptic currents disappeared (not shown).

Possible involvement of strain variations to observed changes

To exclude strain variations, we compared the properties of P20–40 visual cortical neurons of the parental and the second generation of homozygous albino and homozygous and heterozygous pigmented littermates. We examined E_{GABA} and resting potentials and calculated intracellular chloride concentration for the above-mentioned groups and found no statistically significant contribution of strain variations to altered $GABA_A$R-mediated currents in albino visual cortex neurons. E_{GABA} in parental and second-generation visual cortex neurons was −65.75 and −67.75 and −50.95 and −49.95 mV for pigmented and albino animals, respectively (Fig. 2a). Resting potentials of these groups were −70.30 and −69.29 and −65.54 and −63.55 mV (Fig. 2b). Calculated values of intracellular chloride presented in Fig. 2c were 11.22 and 10.63 mM in pigmented and 20.23 and 21.03 mM in albino rats.

Discussion

The regulation of intracellular chloride homeostasis plays a crucial role in the maturation of neuronal circuits: although GABA is an inhibitory neurotransmitter in the adult nervous system, it depolarizes cells membranes during development. These actions of GABA rely on different intracellular Cl^- concentrations: depending on this concentration, Cl^- either enters the cell and hyperpolarizes the

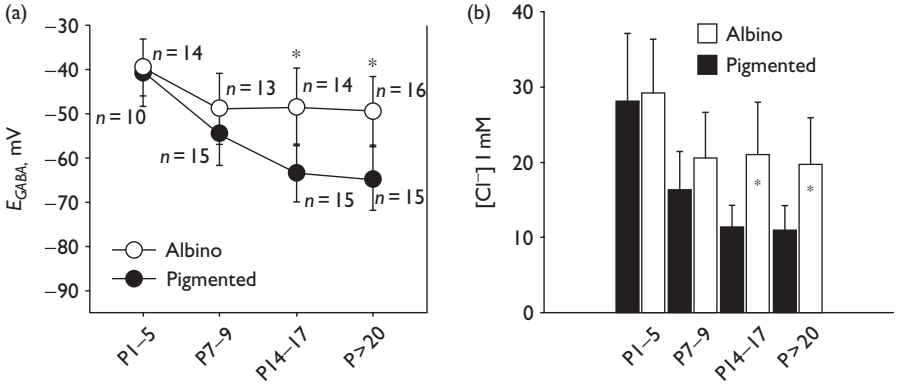

Fig. 1 (a) Postnatal development of γ-aminobutyric acid $(GABA)_A$ receptor-mediated currents reversal potential (E_{GABA}) in visual cortex neurons of albino and pigmented rats. (b) Estimation of intracellular chloride concentration [$(Cl^-)_i$] in visual cortex neurons of albino and pigmented rats, T=25°C. Data are presented as mean ± SD (*$P<0.001$, one-way analysis of variance).

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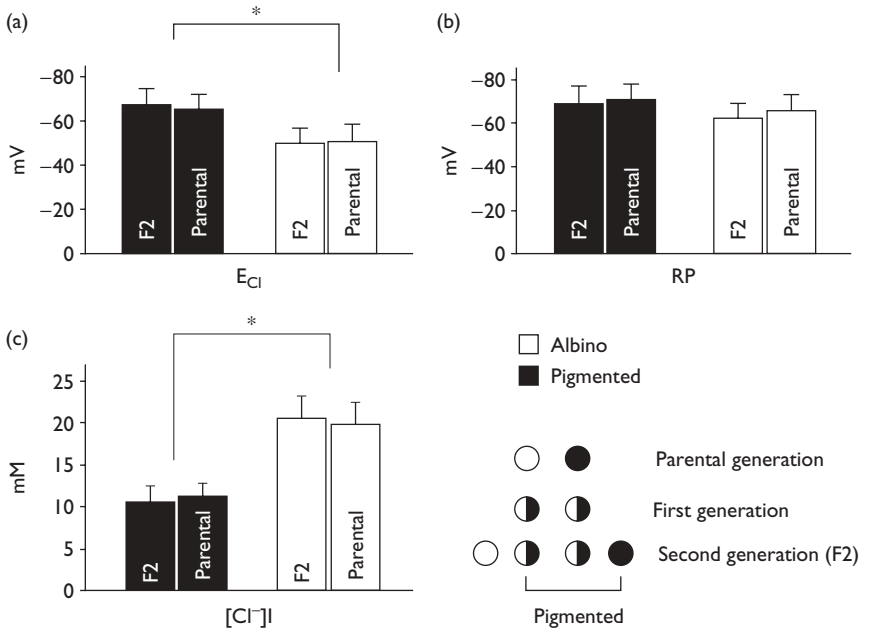

Fig. 2 (a) and (b) Comparison of E_{GABA} and resting potential (RP) in visual cortical neurons between parental and the second generation (F2) of homozygous albino and heterozygous and homozygous pigmented animals, $P > 15$. (c) Corresponding estimation of intracellular chloride concentration [$(Cl^-)_I$], $T = 25°C$. Data are presented as mean ± SD (*$P < 0.001$, one-way analysis of variance).

membrane or leaves the cell and depolarizes it [15]. Owens *et al.* [16], using the perforated patch clamp technique, showed a decrease in neuronal Cl^- concentration during postnatal development in GABAergic neurons from the neocortex. The same pattern was reported for glycinergic neurons from the auditory brain stem [17].

This study describes and compares inhibitory postsynaptic currents of pyramidal neurons in slices of visual cortex from pigmented and albino rats. We monitored reversal potential of $GABA_A$ receptor-mediated currents during postnatal development in both albino and pigmented visual cortex neurons and showed that after the second week of postnatal development albinos start to demonstrate an inhibitory deficit compared with pigmented animals. This finding supports the hypothesis of an aberrant maturation of albino visual circuits rather than that of inborn abnormalities. Interestingly, the calculated intracellular chloride concentration in albino visual cortex neurons exceeded almost twice that in pigmented animals after postnatal day 14 (19.73 versus 11.03 mM, respectively), a fact that confirms the recent findings published by Barmashenko *et al.* [11]. In the study, using furosemide and bumetanide as blocking agents of the two most important chloride-transporters-KCC2 (outward transporter) and NKCC1 (inward transporter), significant alterations of these cotransporter functions were revealed in albino visual cortex neurons, comprising a higher NKCC1 and a lower KCC2 action. Although upregulation of KCC2 and downregulation of NKCC1 are consistent with the significant decrease in intracellular Cl^- observed during postnatal development [10,15,18,19], additional experiments involving direct measurements of cotransporter actions or even the presence of NKCC1, the inward cotransporter, which has not yet been found in unpathological mature mammalian neocortical neurons in a functional state [10,20,21], are needed to resolve this problem.

To exclude the possible involvement of strain variations in the observed changes in the intracellular chloride level in albino visual cortex neurons, we examined visual cortex neurons of albino Wistar and pigmented Long–Evans rats that were crossbreed according to Mendel's law. We did not find statistically significant differences in the reversal potential of $GABA_A$-mediated currents between parental and second generation of homozygous albino and homozygous and heterozygous pigmented animals. Thus, strain variations do not contribute to the pathology of intracellular chloride homeostasis observed in adult albino visual cortex neurons.

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