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Effect of steroids on γ-aminobutyrate-induced currents in cultured rat astrocytes

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Abstract. Cultured astrocytes from rat cortex respond to the inhibitory neurotransmitter γ-aminobutyric acid (GABA) by the activation of Cl^- channels [Bormann J, Kettenmann H (1988) Proc Natl Acad Sci USA 85:9336–9340]. The glial response shares many pharmacological properties with those mediated by neuronal $GABA_A$ receptors, but differs in its sensitivity to inverse benzodiazepine agonists [Backus KH, Kettenmann H, Schachner M (1988) Glia 1:132–140]. To compare glial GABA receptors further with their neuronal counterparts, we analysed the effect of steroids, which have recently been shown to modulate neuronal $GABA_A$-receptor-mediated responses, on GABA-induced currents in astrocytes. The agonist allotetrahydrodeoxycorticosterone (THDOC) at concentrations of 100 nM and 1 μM enhanced GABA-evoked (with 10 μM GABA) currents up to 115% and 162.4% of controls respectively. The antagonist dehydroisoandrosterone 3-sulphate (DHEAS) at concentrations of 1 μM, 10 μM and 100 μM depressed GABA-evoked (10 μM) currents to 72%, 42.8% and 21.4% of controls respectively. The steroids were less effective at higher GABA concentrations. 100 μM DHEAS directly elicited a membrane current, while THDOC (1 μM) did not exert any direct response. This study demonstrates that steroids modulate GABA-evoked currents and thus may interfere with any of the functions of glial GABA receptors that are at present under discussion.

Key words: Steroids – Astrocyte – γ-Aminobutyrate – Receptor – Patch clamp

Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory transmitter in the vertebrate central nervous system acting on two types of receptive sites, $GABA_A$ and $GABA_B$ receptors. $GABA_A$-receptor-mediated currents are modulated by a number of pharmacologically important drugs such as barbiturates and benzodiazepines. Recently Majewska et al. [9] demonstrated that steroids modulate GABA responses in cultured rat hippocampal neurons. Steroids include the steroid hormones as well as intermediate substances, some of them physiologically active. From the main precursor cholesterol, steroids are synthesized through the intermediate formation of pregnenolone and progesterone. Progesterone is the precursor for male and female sex hormones, such as testosterone or estradiol, and for the adrenal corticosteroids. Significant amounts of 3β-steroids including pregnenolone have been found in brain [3]. Steroids can readily pass the cell membrane and trigger changes in mRNA synthesis and the expression of proteins. In addition, these hormones also produce rapid nongenomic actions by interfering with cell membrane receptors, in particular the $GABA_A$ receptor. Steroids can act on GABA receptors both as positive (allotetrahydrodeoxycorticosterone; 5′-pregane-3′,21-diol-20-one: THDOC) and negative modulators of GABA responses (dehydroisoandrosterone 3-sulphate; 5-andrasten-3β-ol-17-one sulphate: DHEAS). From studies on fibroblasts transfected with different combinations of human $GABA_A$ receptor subunits it was suggested that the enhancing action of steroids on GABA receptors is different from those induced by barbiturates or benzodiazepines, indicating a distinct binding site for steroids [14].

It has been demonstrated recently that the expression of $GABA_A$ receptors is not restricted to neurons; astrocytes from mouse brain express GABA receptors with many pharmacological similarities, but also differences from neuronal $GABA_A$ receptors [6]. While in neurons, inverse benzodiazepine agonists such as methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) decrease GABA-activated currents, they exert the opposite effect in astrocytes [1]. It was speculated that the glial receptors are involved in Cl^- homeostasis at GABAergic synapses [2, 8] and in guidance of glial cells during development [7]. In the present study we have further characterized the astrocytic GABA receptor by analysing the ef-

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fect of steroids on GABA-activated currents. We demonstrate that GABA-activated Cl^- currents are modulated by steroids and thus act similarly on glial and neuronal GABA receptors.

Materials and methods

Cell culture. Cultures of astrocytes were prepared from the cerebral hemispheres of newborn rats as previously described [11]. The cells were plated on glass coverslips coated with poly-(L-lysine) and maintained in culture for 2–4 weeks prior to use for electrophysiology.

Solutions, electrodes and drugs. Substances were applied by bath perfusion. The standard solution contained (mM): NaCl 129, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 5, KCl 5.4, HEPES 5; the pH was adjusted to 7.4 by adding NaOH. Electrodes were fabricated from borosilicate glass tubes using a micropipette puller (Sutter Instrument Co.) and filled with the following solution (mM): KCl 130, $MgCl_2$ 1, $CaCl_2$ 1, ethyleneglycol-bis-(2-aminoethylether)-*N, N, N'*, N'-tetraacetic acid (EGTA) 10, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid (HEPES) 10, titrated with NaOH to pH 7.4 GABA and DHEAS were dissolved in Ringer's solution shortly before use. THDOC was dissolved in dimethylsulphoxide as a stock solution and added to the standard solution at the given concentration. Substances were obtained from Sigma.

Data recording and processing. Cultures were mounted on the stage of an inverted microscope and maintained at room temperature (20 °C–23 °C). Whole-cell currents were recorded using the patch-clamp technique and amplified with an EPC-7 amplifier (List, Darmstadt, FRG [4]). Data were digitized, filtered and analysed on an AT-compatible computer using software developed at the department.

Results

Properties of GABA-activated membrane currents

We tested the effect of the steroids DHEAS and THDOC, on GABA-activated membrane currents in cultured astrocytes from rat cortex. The membrane potential was clamped at −70 mV and GABA was applied by changing the perfusate. As previously described, the amplitude of the GABA-activated current varied among astrocytes and in some cases displayed a run-down [2]. Moreover, large, flat astrocytes were selected, which were predominantly found in this culture system. We compared at least three GABA-activated currents separated by 10-min intervals to select for cells that did not exhibit a significant run-down of GABA responses. Controls (GABA-activated currents without steroids) before and after application of steroids were compared to responses in the presence of the steroid.

Effect of the steroid DHEAS on GABA-activated membrane currents

GABA-activated currents were decreased in the presence of the steroid DHEAS as compared to controls prior or after application of the steroid. In the presence of 1 μM, 10 μM and 100 μM DHEAS, the peak amplitude of GABA-activated currents was decreased to 72% (SD = 8.9%, $n = 5$), 42.8% (SD = 9.7%, $n = 5$) and 21.4% (SD = 8.9%, $n = 5$) of control values, respectively (Fig. 1A). The effect of DHEAS was reversible, in that after a 10-min wash GABA responses reached a peak amplitude similar to controls prior to DHEAS application.

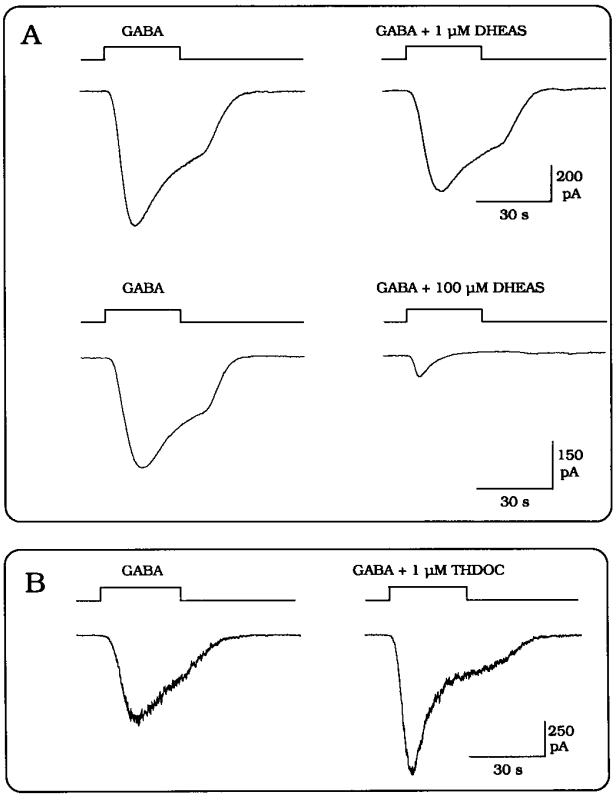


Fig. 1A, B. Effect of dehydroisoandrosterone 3-sulphate (*DHEAS*) and allotetrahydrodeoxycorticosterone (*THDOC*) on γ-aminobutyrate (*GABA*)-activated currents. Inward currents in an astrocyte were activated by bath application of GABA (10 μM, *left traces*) and compared to GABA responses in the presence of the steroid DHEAS (**A**, *right traces*) or THDOC (**B**, *right traces*) at the concentrations indicated. The membrane potential was clamped at −70 mV. The recordings in each line are from different astrocytes

However, we used a new culture dish for further tests to exclude any influence of long-term changes by the steroid. At concentrations of 100 μM, DHEAS directly activated small outward currents ranging from 20 pA to 44 pA with an average of 33.5 pA (SD = 9.9 pA, $n = 4$; Fig. 2A). We did not investigate this type of current response further. The average effect of DHEAS on GABA-activated currents as a function of DHEAS concentration is summarized in Fig. 3 (lower trace). From this relation, an EC_{50} of 6.6 μM can be deduced. In another set of experiments we compared the effect of DHEAS (10 μM) on currents activated by different GABA concentrations, namely 10 μM, 50 μM and 100 μM. GABA-evoked currents were depressed to 42.8% (10 μM; SD = 9.7%, $n = 5$), 63.4% (50 μM; SD = 3.2%, $n = 5$) and 77.2% (100 μM; SD = 9.1%, $n = 5$) of controls (Fig. 3).

Effect of the steroid THDOC on GABA-activated membrane currents

We tested the effect of THDOC on GABA-activated currents in the presumed physiological range

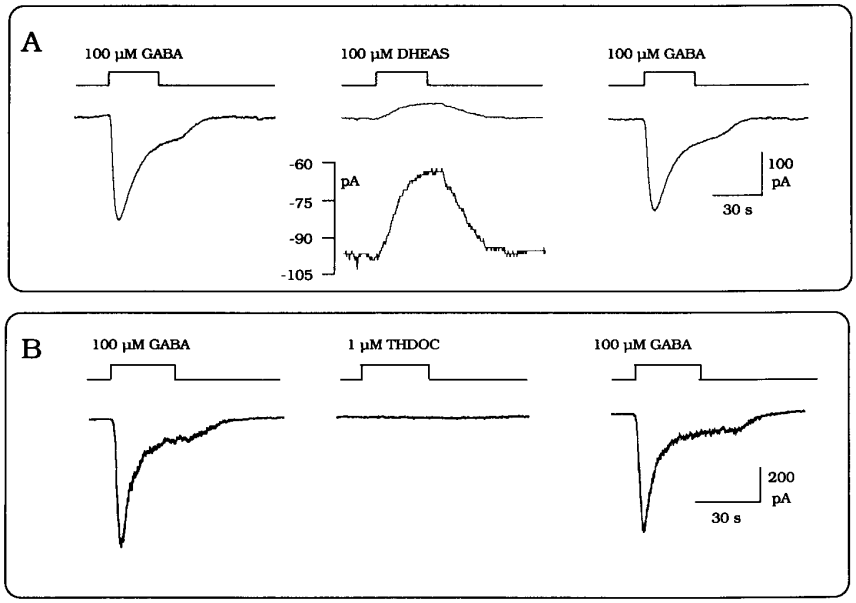


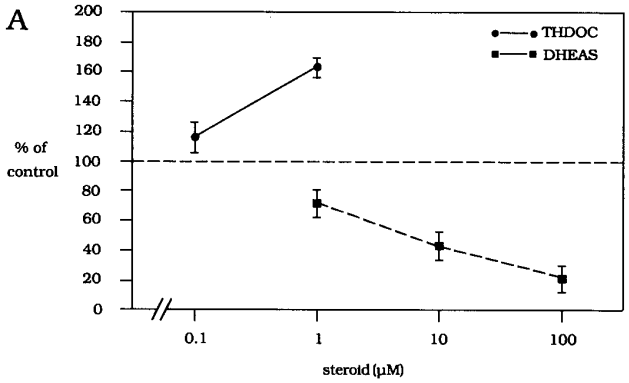
Fig. 2 A, B. Effect of DHEAS and THDOC on resting membrane currents. Currents were recorded as described in the legend to Fig. 1. **A** 100 μM DHEAS was applied (*middle trace*) and GABA-activated currents were compared prior to and after application. The DHEAS-induced current is amplified in the *lower middle trace* with absolute scaling of currents. **B** Similarly the effect of 1 μM THDOC was compared to GABA-activated currents prior to and after application. THDOC did not elicit a detectable membrane current

(100 nM – 1 μM). Figure 1 B shows that in the presence of 100 nM and 1 μM THDOC, the amplitude of GABA-activated (10 μM) currents was increased in comparison to controls. GABA-activated currents were enhanced to 115% (SD = 10.6%, $n = 5$) and 162.4% (SD = 6.5%, $n = 5$) of controls respectively (Fig. 3 upper trace). As observed with the antagonistic steroid DHEAS, the effect of THDOC was reversible. THDOC at a concentration of 1 μM did not elicit any membrane currents directly ($n = 2$; Fig. 2B). However, we did not test higher concentrations because of the limited availability of the drug. As with DHEAS, we compared the effect of 1 μM THDOC on three different GABA concentrations, 10 μM, 50 μM and 100 μM. GABA-evoked currents were enhanced up to 162.4% (10 μM: SD = 6.5%, $n = 5$), 123% (50 μM: SD = 5.8%, $n = 5$) and 111.2% (100 μM; SD = 8.8%, $n = 5$) of controls (Fig. 3).

Discussion

Comparison of steroid effects in neurons, recombinant $GABA_A$ receptors and glial cells

In this study, we report that steroids affect GABA-activated currents in cultured astrocytes from rat cortex. It was reported previously that steroids affect GABA-mediated currents in cultured rat hippocampal neurons [9] and in rat cerebral cortical synaptoneurosomes [12, 13]. In recombinant human $GABA_A$ receptors, which were composed of different subunits, namely α_1, $\alpha_1\beta_1$, and $\alpha_1\beta_1\gamma_2$ combinations, GABA-activated currents were modulated by steroids [14]. Even in homomeric receptors, steroids were effective [14]. From these and other studies on mammalian cells it can be concluded that the different types of $GABA_A$ receptors are all modulated by steroids.



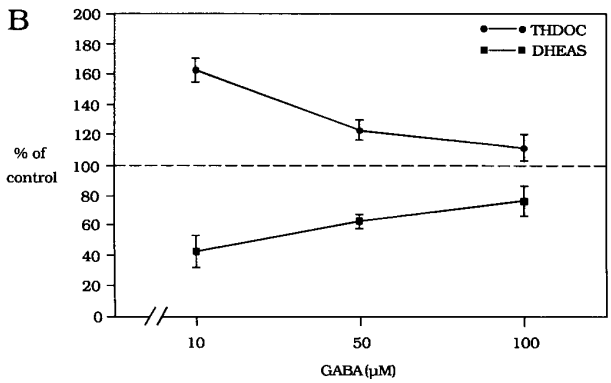


Fig. 3. A Dose/response curve of DHEAS and THDOC. The mean effect on peak currents activated by GABA (10 μM) was plotted as a function of DHEAS (*lower trace*) and THDOC (*upper trace*) concentration. Each data point averages five experiments. The *bars* at each point denote the standard deviation. **B** Dependence of the steroid effect on the GABA concentration. The effect of THDOC (1 μM) and DHEAS (100 μM) on GABA-activated currents was tested. The concentration of GABA was varied from 10 μM to 100 μM and mean effects of the steroids on peak currents were plotted as a function of the GABA concentrations. Each data point averages five experiments. The *bars* at each point denote the standard deviation

It is thus not surprising that steroids also affect GABA responses in cultured astrocytes from rat brain.

Agonistic steroids such as pregnanes act with a threshold concentration of 100 nM and were tested up to concentrations of 10 μM in both neurons [5] and astrocytes and act thus over a similar concentration range. In contrast to our results, the agonist THDOC directly activated currents in cells transfected with cDNA coding for GABA receptor subunits [14]. This difference may reflect the differential actions of steroids on different types of $GABA_A$ receptors; since the subunit composition of the astrocytic receptor is so far unknown, further studies are needed to resolve such a difference on the molecular level. The dose/response curve of the antagonists such as pregnenolone sulphate with an EC_{50} at about 50 μM [10] was similar to our findings using DHEAS with an EC_{50} at about 6.6 μM. In contrast to the experiments with pregnenolone sulphate in neurones [10], DHEAS directly activated an inward current in the astrocytes. The ionic nature of this response was not further analysed in this study. There are thus differences between astrocytes and neurons in the direct action of steroids on membrane currents, while the effect of steroids on GABA-activated currents is similar.

Functional importance of steroid action on astrocytes

The physiological significance of steroid action on the GABA responses in astrocytes is linked to the question of the function of astrocytic $GABA_A$ receptors. All functions attributed to these receptors are subject to modulation by steroids. The steroid concentrations used in this study could match concentrations occurring in the brain under normal or certain behavioural states such as stress or sexual activity [3, 15]. At present, the functional role of glial $GABA_A$ receptors remains speculative. They have been thought to be involved in Cl^- homeostasis in the synaptic cleft [2, 8], in the control of the developmental state of glial cells [7] and in target detection for glial processes contacting GABAergic synapses [6]. All these functions could be modulated by steroids, both positively or negatively depending on an agonistic or antagonistic action of the steroid.

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