CHANGES OF GABA$_A$ RECEPTOR ACTIVATION KINETICS IN HIPPOCAMPAL NEURONS CULTURED FOR DIFFERENT PERIODS OF TIME

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Abstract: Cell culture is a convenient model for pharmacokinetic studies, but during the culture period, GABA$_A$ receptors are likely to undergo different modulatory processes. In this study, the current responses to ultrafast GABA applications were recorded from patches excised from neurons cultured for either up to two days (short-term culture) or for more than two weeks (long-term culture). The dose-dependencies of the currentrising phases revealed significant differences between the two groups. In the short-term cultures, the responses to both saturating and non-saturating GABA concentrations were slower than in the case of the long-term cultures. We conclude that the GABA$_A$ receptors in cultured neurons undergo profound kinetic changes involving the modulation of the binding reaction and transitions between bound states.

Key Words: Ultrafast Perfusion, Kinetics, GABA$_A$ Receptor, Hippocampus, Cell Culture, Patch-clamp, Non-equilibrium

INTRODUCTION

GABA$_A$ receptors are heteropentamers that belong to ligand-gated channel superfamily. So far, as many as 20 subunits of this receptor have been cloned [1] indicating a potentially enormous heterogeneity of GABA$_A$ receptor subtypes. It has been demonstrated that different GABA$_A$R subtypes show a large variability of kinetic and pharmacological properties [2-4]. Moreover, several lines of evidence indicate that development [5-7] as well as physiological [8, 9] and
pathological [10, 11] stimuli may result in a subunit switch giving rise to substantial changes in GABAergic signalling in the CNS. Cell cultures are a useful model to study the pharmacokinetic properties of receptors and synaptic currents. However, depending on duration, the receptors may undergo profound changes in their kinetic and pharmacological properties. Recently, the kinetics of GABA_\text{A} receptors was studied in a long-term culture of hippocampal neurons [12]. In particular, the dose-response analysis of the onset of currents elicited by rapid GABA applications provided crucial information both on binding kinetics and transition rates between the bound states of these receptors. In this study, we have analyzed the dose-dependence of the current onset kinetics for GABA-evoked responses in neurons cultured for up to 2 days and for more than two weeks. The dose-dependencies of these currents showed profound modifications, indicating that GABA_\text{A} receptors undergo substantial modulation in culture.

MATERIALS AND METHODS

The primary cell culture was prepared as described in detail in [13]. Briefly, P1-P4 day old Wistar rats were decapitated after being anaesthetised with an intraperitoneal injection of urethane (2g kg\(^{-1}\)). The hippocampi were dissected, sliced, treated with trypsin, mechanically dissociated and centrifuged, plated on Petri dishes and cultured. The experiments were performed on groups of cells cultured for up to 2 days and on neurons cultured for more than 14 days (up to 20 days). Currents were recorded in the outside-out excised-patch configuration of the patch-clamp technique using a EPC-7 amplifier (List Medical, Darmstadt, Germany) at a holding potential (V_\text{h}) of -70 mV. The intrapipette solution contained (in mM) CsCl 137, CaCl\(_2\) 1, MgCl\(_2\) 2, 1,2-bis(2-aminophenoxy)ethane-N,N,N'-tetraacetic acid (BAPTA) 11, ATP 2, HEPES 10 (pH 7.2 with CsOH). The composition of the external solution was (in mM) NaCl 137, KCl 5, CaCl\(_2\) 2, MgCl\(_2\) 1, glucose 20, HEPES 10 (pH 7.4 with NaOH). All the experiments were performed at a room temperature of 22-24°C. The current signals were low-pass filtered at 10 kHz with a Butterworth filter and sampled at 50-100 kHz using a CED micro1401 analog-to-digital converter (Cambridge, U.K.) and stored on hard disk. The acquisition and analysis software were provided by Dr. J. Dempster (Strathclyde University, Glasgow, U.K.). GABA was applied to excised patches using the ultrafast perfusion system based on a piezoelectric-driven theta-glass application pipette [14, 15]. The piezoelectric translator was from Physik Instrumente (Waldbraun, Germany) and the theta-glass tubing from Hilgenberg (Malsfeld, Germany). The open tip recordings of the liquid junction potentials revealed that a complete exchange of solution occurred within 40-80 µs. The standard t-Student test was used for data comparison.
RESULTS AND DISCUSSION

Several previous reports demonstrated that at high (saturating) GABA concentrations, the rising phase of currents is very rapid. In this study, the 10-90% rise time of current responses to saturating [GABA] (10 mM) was 215 ± 6 µs (n = 21, long-term culture > 14 days) which is not much longer than the exchange time of the ultrafast perfusion system (40-80 µs T10-90 % exchange). This raises the question whether the measured current onset rate is biased by the speed of the application system. To address this question, the value of the 10-90% exchange time of the ultrafast perfusion was determined using standard measurements of currents due to junctional (Henderson’s) potential (Fig. 1A, B). The averaged exchange time was 68 ± 14 µs (n = 25). In order to assess whether such an exchange time affects the onset of currents evoked by saturating [GABA], the simulated responses to a theoretical, square-like shape application of saturating [GABA] were compared to simulated responses elicited by [GABA] applied with a finite speed (modeled as [GABA](1-exp(-t/τapplic))). The

![Fig. 1. An exchange time (T10-90%) of approx. 70-110 µs (τapplic = 33-50 µs) permits the resolution of the onset rate of current response to saturating GABA concentration. (A): Current elicited by a junctional potential induced between the pipette solution (156 mM NaCl) and the solution applied with theta-glass (15 mM NaCl). (B) presents the same current as in A in the expanded time scale. (C) and (D): simulations of current responses evoked either by a square-like pulse of saturating (10 mM) [GABA] (thin lines) or by exponentially rising agonist ([GABA](1-exp(-t/τapplic))– (thick lines). The simulations presented in C and D correspond to τapplic 33 and 50 µs, respectively (T10-90% times approx. 70 and 110 µs). Note that the slower application of the agonist (C, D) has a minor effect on the current rise time (measured as 10-90% increase) and mainly results in a parallel shift of the current response. Insets above the simulated current traces represent the time course of the applied agonist.]
relationship between the T10-90% rise time and the time constant of the exponential rise $\tau_{\text{applic}}$ is $\tau_{\text{T10-90\%}} = \tau_{\text{applic}} \ln(9) \approx 2.197 \cdot \tau_{\text{applic}}$. To model GABA$_A$R responses, Jones and Westbrook’s model [16] was used, with the parameters assessed in our recent study [12]. Figs. 1 C, D show that within the range up to 110 $\mu$s, the T10-90% application time only weakly affects the rise time of currents evoked by saturating [GABA] (10 mM). For the current response to GABA applied with a time constant of $\tau_{\text{applic}} = 50$ $\mu$s (approx. 110 $\mu$s T10-90% exchange time), the simulated T10-90% rise time of the current response was 223.4 $\mu$s, while that of simulated current evoked by instantaneous application of saturating [GABA] was 209 $\mu$s. Thus, the major effect of limited application speed (within the considered range) is a parallel shift in the current onset (Fig. 1 C, D) with a weak effect the onset rate.

The dose-dependence of current responses to different [GABA] within the range of 20 $\mu$M to 10 mM were measured in neurons cultured for up to two days and for cells kept in culture for at least two weeks. The rise time of currents elicited by saturating [GABA] in short-term culture was significantly slower than in neurons cultured for more than two weeks (Fig. 2, 245 ± 17 $\mu$s, n = 9, and 215 ± 22 $\mu$s, n = 21 in short- and long-term culture, respectively). Similarly, for a wide range of non-saturating GABA concentrations, the onset rate of currents recorded from short-lasting cultures was slower than in the case of long-term ones (Fig. 2). The difference in the current onset rates between the two groups of neurons markedly increased when the concentration of the applied agonist was lowered (Fig. 2).

The observed dependence of current onset rates on the time duration of neuronal culture clearly indicates that the GABA$_A$ receptor kinetics undergoes significant changes during the period of culture. While it cannot be expected that the modifications of GABA$_A$ receptor functions in culture closely mimic the physiological processes associated with development, it is of note that the time course of the rising phase kinetics of currents measured in cell culture follows the same trend as those recorded in neurons at different developmental stages. It has been demonstrated that the rising phase of mIPSCs showed a tendency to accelerate during development [6, 17]. Since it is known that the application of the synaptic agonist is very rapid (application is supposed to take tens of microseconds and clearance is described by a predominant fast component of less than a millisecond [18, 19]), it is conceivable that acceleration of current onset in mIPSCs and in our experiments have a similar mechanism. Interestingly, it has been demonstrated that glutamatergic synaptic transmission also accelerates during development [20], indicating that the acceleration of synaptic currents is a common feature of the developing nervous system.
Fig. 2. The rising phases of currents evoked by rapid GABA applications accelerate during the period of culture. (A): Typical examples of current responses to various concentrations of GABA recorded from different neurons in short-term (left panel) and in long-term culture (right panel). Insets above the current traces in the left panel represent the time course of the applied agonist. The current responses in the right panel were elicited by an application protocol identical to that presented in the left panel (B): The dose-dependence of the averaged rise time (T10-90%) for neurons from short-term (filled bars) and long-term cultures (open bars), respectively. Asterisks indicate a significant difference (p < 0.05).

The mechanisms underlying the observed differences in dose-responses of the current onset rates are not clear. It is likely that the observed change in the rising phase kinetics involves a subunit switch of GABA<sub>A</sub> receptors, but this hypothesis would require further studies based on molecular biology techniques. In a recent study from this laboratory, it was shown that the rising phase of current response (both for saturating and non-saturating concentrations) depends on a number of transitions between open, closed and desensitized states [12].
Thus, the description of these mechanisms would require recordings of current responses evoked using several protocols, as well as an extensive quantitative analysis based on model simulations [see, e.g. 12, 16, 19] which is beyond the scope of this report. Nevertheless, the fact that the current responses to saturating GABA concentrations show significant differences indicates that the kinetics of conformational transitions between bound states is changed during the period of culture. Moreover, the observation that the differences between the onset rates increase when lowering GABA concentration clearly indicates that the binding scheme is modified during the period of culture.

In conclusion, we provide evidence that the kinetics of GABA_A receptors is strongly modulated during the period of culture and indicate that the underlying mechanisms involve changes both in binding and transitions between bound conformations.

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