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The voltage dependence of GABA_A receptor gating depends on extracellular pH

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Abstract

Recent studies have indicated that changes in extracellular pH and in membrane voltage affect the γ -amino-*n*-butyric acid type A receptor gating mainly by altering desensitization and binding. To test whether the effects of membrane potential and pH are additive, their combined actions were investigated. By analyzing the current responses to rapid γ -amino-*n*-butyric acid applications, we found that the current to voltage relationship was close to linear at acid pH but the increasing pH induced an inward rectification. Desensitization was enhanced at depolarizing potentials, but this strongly depended on pH, being weak at acidic and strong at basic pH values. A similar trend was observed for the onset rate of responses to saturating γ -amino-*n*-butyric acid concentration. These data provide evidence that the voltage sensitivity of GABA_A receptors depends on extracellular pH.

Keywords

gamma-amino-*n*-butyric acid receptor; membrane potential; patch clamp; protons

Introduction

Gamma-amino-*n*-butyric acid type A (GABA_A) receptors have been demonstrated to be sensitive to both extracellular pH [1-6] and the membrane voltage [5,7-12]. Recent studies from this laboratory have that both protons and membrane voltage influenced the receptor gating [6,12]. Interestingly, both factors affected the desensitization and binding rate, although quantitatively these effects were stronger in the case of protons. It thus seems interesting to elucidate how GABA_A receptor functioning is modulated by the combined action of protons and membrane potential. We report that the inward rectification of currents elicited by saturating [GABA] was strongly dependent on pH. Moreover, desensitization kinetics and the onset rate for current responses evoked by saturating [GABA] were found to be less voltage-dependent at acidic pH than those at basic ones. These results indicate that protons might exert a modulatory effect on those sites on the GABA_A receptor macromolecule that are involved in sensing membrane voltage.

Materials and methods

Primary cell culture was prepared as already described [13]. Briefly, P2-P4 old Wistar rats were killed by decapitation, hippocampi were dissected, sliced, treated with trypsin,

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mechanically dissociated and centrifuged twice at 40g, plated in the Petri dishes and cultured. This procedure is in agreement with the Polish Animal Welfare Act and was approved by the Local Ethical Commission. Experiments were performed on cells between 10 and 15 days in culture. Currents were recorded in the outside-out mode of the patch-clamp technique using the EPC-7 amplifier (List Medical, Darmstadt, Germany) at holding potentials varying from -70 to 70 mV. The intrapipette solution contained (in mM) CsCl 137, CaCl₂ 1, MgCl₂ 2, 1,2-bis(2-aminophenoxy)ethane-*N,N,N'*-tetraacetic acid 11, ATP 2 and *N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulfonic acid (HEPES) 10 (pH 7.2 with CsOH). The composition of the standard external solution was (in mM) NaCl 137, KCl 5, CaCl₂ 2, MgCl₂ 1, glucose 20 and HEPES 10 (pH 7.2 with NaOH). HEPES was used to buffer the external solution with pH in the range 6.5-8.0, and at pH 6.0 MES (C₅H₁₃NO₄S) was used (15 mM) and the osmolarity was adjusted with glucose concentration. The current signals were low-pass filtered at 10 kHz using a Butterworth filter, sampled at 50-100 kHz using the analog-to-digital converter CED micro1401 (Cambridge Electronic Design, Cambridge, UK) and stored on the computer hard disk. The acquisition and analysis software was kindly given by Dr J. Dempster (Strathclyde University, Glasgow, UK). GABA was applied to excised patches using an ultrafast perfusion system based on a piezoelectric-driven theta-glass application pipette [14,15]. The piezoelectric translator was from Physik Instrumente (preloaded HVPZT translator 80 μ m; Waldbronn, Germany) and the theta-glass tubing was from Hilgenberg (Malsfeld, Germany). Open-tip recordings of the liquid junction potentials revealed that 10-90% exchange of solution occurred within 40-80 μ s. All experiments were performed at room temperature (22-24°C). Data are expressed as mean $\pm$ SEM. For comparison of data obtained from the same patch, the Student's paired *t*-test was used.

Results

In a recent study from this laboratory, it was reported that in control conditions (pH=7.2), current responses to a saturating GABA concentration (10 mM) showed inward rectification [12]. To check whether extracellular pH exerts an effect on rectification properties, current to voltage relationships (*I-V*) were measured at pH=6.0, 7.2 and 8.0. We found that at acidic pH (6.0) the *I-V* relationship was nearly linear (the ratio of slopes at positive and negative membrane voltages was 0.96 ± 0.04 , $n=3$, $P>0.05$, Fig. 1a). By contrast, the inward rectification clearly increased with increasing pH (at pH=7.2 the slope ratio was 0.71 ± 0.04 , $n=7$, $P<0.05$ and at pH=8.0, 0.59 ± 0.08 , $n=4$, $P<0.05$, Fig. 1c). These results clearly show that the extent of inward rectification increases with pH value. In our recent report, the rectification of *I-V* for currents elicited by saturating [GABA] observed at pH=7.2 was attributed mainly to the voltage dependence of the desensitization rate [12]. It is thus interesting to check whether alteration in extracellular pH affects the voltage sensitivity of the desensitization process. To this end, current responses to long (100 ms) pulses of saturating concentrations of GABA (10 mM at pH=7.2 and 8.0; 30 mM at pH=6.0) were recorded at pH=6.0, 7.2 and 8.0 and at different membrane potentials (Fig. 2a and b). At pH=7.2, the desensitization time constants (τ_{des}) at -70 and $+70$ mV were 2.6 ± 0.1 and 1.9 ± 0.1 ms, respectively ($n=9$, $P<0.05$); at pH=6.0, 5.1 ± 0.3 and 4.3 ± 0.1 ms ($n=3$, $P>0.05$), respectively, and at pH=8.0, 2.3 ± 0.2 and 1.6 ± 0.1 ms ($n=3$, $P<0.05$), respectively. Similarly, as in our previous study [6], we found that acidification of the extracellular medium strongly reduces the rate of desensitization (Fig. 2c). We also found that at acidic pH, however, the voltage dependence of desensitization is markedly reduced (Fig. 2a and c). Interestingly, while the voltage dependence of the desensitization rate was affected by membrane voltage (Fig. 2c), no significant effect on the steady state to peak ratio was found (see examples of traces in Fig. 2a and b). In our previous study, we found that membrane depolarization (at pH=7.2) and increased pH (at $V_m=-70$ mV) resulted in an acceleration of the rising phase of the current response to saturating [GABA] [6,12]. We were interested, however, to know

whether the voltage dependence of the current rise time is dependent on the pH value and vice versa. To address this problem, at each considered pH value, the current rise time was measured at $V_m = -70$ and $+70$ mV. As shown in Fig. 3a, at pH=6.0, there was no significant difference in 10-90% rise time at -70 and $+70$ mV (at -70 mV, 0.47 ± 0.075 ms and at $+70$ mV, 0.38 ± 0.054 ms, $n=4$, $P>0.05$) and the onset rate was considerably slower than in control conditions (pH=7.2, Fig. 3c). This differs qualitatively from what is seen in control conditions (at -70 mV, 0.24 ± 0.015 ms and at $+70$ mV, 0.20 ± 0.015 ms, $n=11$, $P<0.05$). At pH=8.0, both 10-90% rise time and the effect of voltage were similar to those seen in control conditions (0.22 ± 0.013 ms at -70 mV and 0.16 ± 0.023 ms at 70 mV, $n=4$, $P<0.05$, Fig. 3b and c).

Discussion

The major finding of the present study is that the voltage-sensitivity of the GABA_A receptors strongly depends on extracellular pH. As pointed out in our previous reports, the mechanisms by which these factors affect GABA_A receptors are related to desensitization and agonist-binding kinetics [6,12]. This suggests that at least some proton-binding sites on the channel macromolecule are able to interfere with channel structures involved in sensing the membrane voltage. Most interestingly, from the functional point of view, the effects of protons and membrane potential appear not to be additive. Indeed, at acidic pH, the voltage dependence was nearly absent, while the same alteration of membrane potential in control conditions or at pH=8.0 gave rise to profound changes in $I-V$ (Fig. 1), desensitization (Fig. 2) and onset rate (Fig. 3). An enhancement of inward rectification at increasing pH is compatible with an increase in the desensitization rate. Indeed, as pointed out in our recent report [6], a downregulation of amplitude for current responses to saturating [GABA] by increasing pH most likely reflects privileged entry into the desensitized state due to increased desensitization rate. Thus, at saturating [GABA], receptors quickly bind the agonist, reaching fully bound closed state and then, as predicted by Jones and Westbrook's model [16], channels are distributed between open and desensitized states roughly in proportion to the respective rate constants. Taking into account that pH dependence of rectification observed here concerns responses to saturating [GABA] (Fig. 1), it can be hypothesized that the major mechanism is related to the pH sensitivity of the desensitization process. The fact that at acidic pH the desensitization is weak and its voltage dependence is barely detectable whereas at basic pH desensitization is strong and can be further enhanced by depolarization (Fig. 2) is compatible with this hypothesis. These data could thus suggest that entrance into the desensitized conformation requires deprotonation of some residues on the channel macromolecule. An alternative explanation could be that either membrane potential or changes in pH values (or both) might affect the single-channel conductance of GABA_A receptors. This possibility, however, seems unlikely as most investigators did not observe any significant effect of pH [5] or membrane voltage [17-21] on single-channel conductance.

Although the presented data clearly indicate the lack of independence between modulatory effects of protons and membrane voltage, that neuronal GABA_A receptors are greatly diversified and their modulation by these factors may differ from one receptor subtype to the other need to be taken into consideration [4]. In particular, there is growing evidence that GABA_A receptors mediating phasic and tonic inhibition show different subcellular distribution and clear differences in their kinetics as well as susceptibility to modulators [22]. In the present study, we have investigated currents measured from somatic excised patches in which the extrasynaptic receptors (implicated in tonic inhibition [22]) are likely to predominate.

The physiological significance of the link between pH and voltage sensitivity of GABA_A receptors reported here is not clear. It can be speculated that an inward rectification and related enhancement in desensitization might provide a negative feedback for GABAergic inhibition at physiological (or basic) pH values. Acidic pH, as pointed out in the present report, appears to decrease such feedback, making the GABAergic drive potentially more efficient at strongly depolarized potentials. It can be speculated that this could be of advantage in some pathological conditions such as ischemia or hypoxia that are known to be associated with acidification of extracellular media [23]. Although the role of interdependence between pH and voltage sensitivities is not clear, the observations presented above may provide a hint regarding the structural determinants of voltage sensitivity, desensitization and protein protonation sites.

Conclusion

The present report provides evidence that the voltage sensitivity of GABA_A receptors strongly depends on extracellular pH. The effects of these two factors are not additive and suggest an interaction of proton-binding sites with channel structures involved in voltage sensitivity.

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Fig. 1.

Increase in extracellular pH enhances inward rectification of current to voltage (I - V) relationships for responses elicited by saturating γ -amino- n -butyric acid concentration. A typical example of an I - V relationship at pH=6.0 (a), pH=7.2 (b) and pH=8.0 (c). Dashed and solid lines are added to better visualize the difference in slopes at negative and positive membrane potentials, respectively. (d) Average values of slopes relative to the slope obtained at negative voltages at a different pH. Asterisk above the bar indicates a significant difference with respect to the value obtained at -70 mV at a given pH.

Fig. 2.

Extracellular pH affects the voltage dependence of desensitization time constant. (a, b) Typical normalized current responses (at -70 mV - thin line and at $+70$ mV - thick line) elicited by a long (>30 ms) pulse of saturating γ -amino-*n*-butyric acid concentration at pH=6.0 (a) and at pH=8.0 (b). At pH=6.0 (a), 30 mM was used to ensure saturation. For clarity, the two responses have been separated slightly. Note that depolarization induces acceleration of the desensitization onset rate at basic pH. Insets above the current traces indicate the time course of the applied agonist. (c) Averaged values of the desensitization time constant (τ_{des}) at pH indicated above the bars at negative (black bar) and positive (white bar) voltages. Asterisks above the bars indicate a significant difference with respect to the value obtained at -70 mV at adequate pH.

Fig. 3.

Membrane depolarization accelerates the rising phase of currents elicited by saturating γ -amino-*n*-butyric acid (10 mM) concentration at basic pH. A typical example of normalized current traces evoked by rapid application of saturating γ -amino-*n*-butyric acid concentration at negative (thin line) and positive (thick line) potentials at pH=6.0 (a) and pH=8.0 (b). (a) For clarity, the two responses have been slightly separated. Inset above the current trace indicates the time course of the applied agonist. (c) Average values of the 10-90% rise times measured at -70 mV (black bar) and 70 mV (white bar) at pH indicated above bars. Asterisk above the bar indicates a significant difference with respect to the value obtained at -70 mV at adequate pH.