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HIGH pH ACCELERATES GABA DEACTIVATION ON PERCH-ρ1B RECEPTORS

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Abstract—The ionotropic $GABA_C$ receptor, formed by GABA ρ subunits, is known to be modulated by a variety of endogenous compounds, as well as by changes in pH. In this study, we explore the proton sensitivity of the GABA ρ subunits cloned from the perch retina, and report a novel action of high pH on the homomeric receptor formed by one of the GABA ρ subunits, the perch-ρ_{1B} subunit. Raising extracellular pH to 9.5 significantly accelerated GABA deactivation responses elicited from oocytes expressing the perch-ρ_{1B} subunit, and reduced its sensitivity to GABA. The change in the kinetics of the GABA-offset response occurred without altering the maximum response amplitude, and the reduced GABA sensitivity was independent of membrane potential. Although acidification of the extracellular solution also accelerated GABA deactivation for all other GABA ρ receptors examined in this study, the effects of high pH were unique to the homomeric receptor formed by the perch-ρ_{1B} subunit. In addition, we found that, unlike the effects on the response to the naturally occurring full agonist GABA, the responses elicited by partial agonists (imidazole-4-acetic acid (I4AA) and β-alanine) in the presence of the high pH solution showed a significant reduction in the maximum response amplitude. When considered in terms of a model describing the activation of $GABA_C$ receptors, in which pH can potentially affect either the binding affinity or the rate of channel closure, the results were consistent with the view that external alkalization reduces the gating efficiency of the receptor. To identify the proton sensitive domain(s) of the perch-ρ_{1B} receptor, chimeras were constructed by domain swapping with other perch-ρ subunits. Analysis of the pH sensitivities of the various chimeric receptors revealed that the alkaline-sensitive residues are located in the N-terminal region of the perch-ρ_{1B} subunit.

Key words: protons, response kinetics, channel gating, *Xenopus* oocyte, GABA ρ subunits.

GABA, the main inhibitory neurotransmitter in the CNS, activates three classes of GABA receptor: the $GABA_A$, $GABA_B$, and $GABA_C$ receptors. The classification is based largely on differences in the subunit composition, gating properties, and pharmacological profiles of the various receptor types (Barnard et al., 1998; Bormann, 2000; Johnston et al., 2003; Zhang et al., 2001). The newest member of this family, the $GABA_C$ receptor (referred to also as $GABA_{A0r}$), is expressed predominantly on retinal neurons, although recent studies indicate a wide distribution in many parts of the CNS (Enz and Cutting, 1999; Qian and Ripps, 2001; Rozzo et al., 2002; Wegelius et al., 1998; Zhang et al., 2001). The $GABA_C$ receptor is thought to consist primarily of GABA ρ subunits (Lukasiewicz, 1996; Zhang et al., 2001), but there is evidence indicating that GABA ρ subunits are able to co-assemble with subunits of the $GABA_A$ receptor to form a receptor with unique kinetic and pharmacological properties (Milligan et al., 2004; Pan and Qian, 2005; Qian and Ripps, 1999).

A variety of substances, including endogenous cations such as protons (H^+) and zinc (Zn^{2+}), has been shown to affect the responses of both $GABA_A$ and $GABA_C$ receptors (Krishek et al., 1998; Qian and Ripps, 2001; Rivera et al., 2000). In the case of the $GABA_A$ receptors, proton modulation of receptor function involves a complex mechanism, and the effects are dependent upon the subunit composition of the receptor. Thus, lowering pH enhances GABA-activated responses on receptors formed by $\alpha_1\beta_1$ or $\alpha_1\beta_1\delta$ subunits, whereas $\alpha_1\beta_1\gamma_{2S}$ receptors are not affected by changes in external pH (Krishek et al., 1996). In addition, multiple residues are involved in mediating the proton sensitivity of $GABA_A$ receptors. A histidine residue at position 267 of the β subunit has been shown to be critical in modulating GABA-activated currents by acidic pH (Wilkins et al., 2002), whereas residues in both the GABA binding pocket and in the TM2–TM3 linker have been suggested as the molecular basis for proton modulation of receptor activity (Huang et al., 2004; Wilkins et al., 2005). The possibility that protons modify ligand-binding or channel gating has also been proposed (Feng and Macdonald, 2004; Huang et al., 2004; Krishek and Smart, 2001; Wilkins et al., 2005), and it is possible that these mechanisms play a role in proton modulation of $GABA_C$ receptors.

The receptors formed by mammalian GABA ρ subunits are also sensitive to proton modulation (Rivera et al., 2000; Wegelius et al., 1996). Interestingly, the homomeric receptors formed by human and rat GABA ρ_1 subunit exhibit

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Abbreviations: CHES, 2-(cyclohexylamino)ethanesulfonic acid; Hepes, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); I4AA, imidazole-4-acetic acid.

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different sensitivities to extracellular pH despite the high degree of homology in their amino acid sequences (Rivera et al., 2000). In the present study, we examined the pH sensitivity of the homomeric receptors formed by four of the GABA ρ subunits cloned from perch retina (Qian et al., 1998), and compared their GABA-elicited responses to those observed on the homomeric human ρ_1 receptor. Although protons affected the kinetics of the GABA-elicited responses on all of the ρ subunits, high pH accelerated GABA deactivation only at receptors formed by the perch-ρ_{1B} subunit. Using partial agonists to probe the mechanism of this novel effect provided evidence that high pH alters the gating efficiency of the ρ receptor. To identify the proton sensitive domain(s) of the perch-ρ_{1B} receptor, chimeras were constructed by domain swapping with other perch-ρ subunits, and analysis of their pH sensitivities indicated that the alkaline-sensitive residues are located in the N-terminal region of the perch-ρ_{1B} subunit.

EXPERIMENTAL PROCEDURES

Oocyte expression and recording

Methods for oocyte preparation and recording followed procedures described previously (Qian et al., 1999). Briefly, stages V–VI oocytes were isolated and defolliculated after removal from gravid female *Xenopus* (Xenopus One, Dexter, MI, USA), and kept at 16 °C in a Ringer's solution containing (in mM): NaCl (100), KCl (2), $CaCl_2$ (2), $MgCl_2$ (1), Hepes (5), glucose (10), at pH 7.4. Plasmids containing perch and human ρ subunits were linearized, and capped mRNAs were synthesized with SP6 RNA polymerase using the mMessage mMachine (Ambion Inc., Austin, TX, USA) according to the manufacturer's instructions. Each oocyte was injected with 50 nl mRNA (0.5 mg/ml), and after 2–5 days of expression, GABA-activated currents were monitored with the cell held at −70 mV using a two-microelectrode voltage clamp amplifier (GeneClamp 500, Axon Instruments, Inc., Foster City, CA, USA). With the cell mounted in a small chamber (vol ~20 μl), GABA was applied by a computer-controlled custom-designed perfusion system that rapidly exchanged solutions in approximately 0.3 s. When the current–voltage relation was determined with a ramp protocol, the voltage range (−120 to +60 mV) was scanned at a rate of 40 mV/s. To test the effects of extracellular pH on GABA elicited responses, Ringer's solutions were prepared in buffers appropriate to the desired pH range (Ripps et al., 2004): Hepes (pH 7.5 and 8.5), 2-(cyclohexylamino)ethanesulfonic acid (CHES) (pH 9.5), and 1,4-piperazinediethanesulfonic acid (PIPES) (pH 6.5). Experiments carried out in Ringer's solution with Hepes buffer titrated to pH 9.5 gave similar results to those obtained with CHES buffer.

All experimental procedures conformed to the statement on animal care of the Association for Research in Vision and Ophthalmology, and adhered to the guidelines for the Care and Use of Laboratory Animals formulated by the Animal Care Committee of the University of Illinois College of Medicine. Every effort was made to minimize the number of animals used and their suffering.

Chimeric ρ receptors

Construction of chimeric receptors formed by white perch GABA ρ subunits followed published protocols. To generate $\rho_{1A/1B}$ and $\rho_{1B/1A}$ chimeras, a *Pst*I site at the beginning of the first transmembrane domain was used in combination with either *EcoR I* or *Xho*I to cut out N- and C-terminal regions of the subunits. The chimeras were obtained by ligation of a 5′ fragment from one subunit with the 3′ fragment of the other. It should be noted that $\rho_{2A/1B}$ and $\rho_{1B/2A}$ chimeras are the same as those referred to as C-1 and C-2 in Qian et al. (1999).

Data analysis

Data from each experiment were collected from at least four oocytes, and the current amplitudes and response kinetics were derived from the recordings using pCLAMP programs (Axon Instrument). Dose-response curves for drug–receptor interactions were fitted using Origin software (Microcal, Northampton, MA, USA) to a Hill equation of the form:

$$\frac{I}{I_{max}} = \frac{[C]^n}{[C]^n + [EC_{50}]^n}$$

where I is the current response to a drug concentration [C], I_{max} is the maximum current elicited, n is the Hill coefficient, and EC_{50} is the concentration at which a half-maximal response is obtained. Data were presented as mean±S.E.M.

RESULTS

pH and GABA deactivation

As shown previously (Qian et al., 1998), the homomeric perch-ρ_{1B} receptor is characterized by slow response kinetics, in particular with respect to the time course of deactivation when GABA application is terminated, i.e. the current decay at drug offset. Fig. 1A shows examples of the membrane currents elicited by 2 μM GABA applied to oocytes expressing the perch-ρ_{1B} subunit in the presence of various extracellular proton concentrations. The values for the time constant (τ) of the decay phase associated with each response show that the kinetics of deactivation were accelerated when pH_o was either raised or lowered from its value (pH 7.5) in normal frog Ringer's. In addition to the kinetic changes, alkalinization of the extracellular media produced a consistent reduction in the amplitude of the GABA response. When tested with a higher concentration (10 μM) of GABA, similar effects on the kinetics of the GABA-elicited responses were seen (data not shown). However, at this concentration, the amplitude of the response was maximal and exhibited little change whether elicited in normal (pH 7.5) or in alkaline (pH 9.5) media (Fig. 1B). A quantitative analysis of the kinetics of GABA-evoked responses in various pH environments is presented in Fig. 1C, where the bar graphs show the averaged values (n=5) for the time constant of GABA deactivation compared with the value obtained in normal Ringer's solution (pH 7.5). The data indicate that acidification to pH 6.5 produced a significant reduction of ~34% in the rate of decay, whereas GABA deactivation was only moderately accelerated when the medium was switched to pH 8.5; the 24% decrease due to the slightly alkaline medium was not statistically significant. However, a Ringer's solution titrated to pH 9.5 produced about a fivefold reduction (or ~80%) in the GABA deactivation time constant compared with that recorded at pH 7.5.

Whereas altered response kinetics in acidic pH have been reported for $GABA_A$ and $GABA_C$ receptors, the acceleration of the GABA-offset response on the perch-ρ_{1B} receptor has not been reported previously. The present study focused on the mechanism of this novel effect. In