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A Single Amino Acid in the Second Transmembrane Domain of GABA ρ Receptors Regulates Channel Conductance

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Abstract

GABA_C receptors, expressed predominately in vertebrate retina, are thought to be formed mainly by GABA ρ subunits, each of which exhibits distinct physiological and pharmacological properties. In this study, the receptors formed by perch GABA ρ subunits were expressed in HEK cells, and their single channel conductances were determined using noise analysis techniques. The receptors formed by the perch $\rho 1A$ subunit gate a channel with a conductance of 0.2 pS, whereas the receptors formed by GABA $\rho 2$ subunits exhibit much higher channel conductances, i.e., 3.2 pS and 3.5 pS for perch $\rho 2A$ and $\rho 2B$ receptors, respectively. A comparison of the amino acid sequences of the channel-forming TMII regions of the various subunits suggested that a single amino acid at position 2' was a potential site for the large differential in conductance. We found that switching the serine residue at that site in the GABA $\rho 2$ subunit to the proline residue present in the $\rho 1$ subunit reduced the channel conductance to a level similar to that of the wild type $\rho 1$ receptor. Conversely, mutating proline to serine in the amino acid sequence of the $\rho 1$ receptor significantly increased its unitary conductance. These results indicate that a single amino acid in the TMII region plays an important role in determining the single channel conductance of the GABA_C receptors.

Keywords

GABA_C receptor; ρ subunits; white perch retina; single channel conductance; noise analysis; bipolar cells

Introduction

GABA (γ -aminobutyric acid) is the main inhibitory neurotransmitter in the vertebrate retina and throughout the central nervous system [13,28,33,34]. Three classes of GABA receptor have been identified; i.e., GABA_A, GABA_B, and GABA_C, the distinction based largely on differences in their subunit composition, gating properties, and pharmacological profiles. 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 [1,3,9,12,18,30]. Although capable of coassembling with subunits derived from other members of the Cys-loop superfamily of receptors (e.g., glycine and GABA_A

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receptors) in the *Xenopus* oocyte expression system [21,22,27], native GABA_C receptors are thought to consist primarily of GABA ρ subunits [15,35].

Multiple GABA ρ subunits have been cloned from various species [1,8,24,36], and numerous studies have shown that their GABA sensitivities and response kinetics are often subtype specific [7,11,24]. For example, two members of the group of GABA ρ receptors cloned from perch retina ($\rho 1$ and $\rho 2$) consist of A- and B- subtypes, and the homomeric receptors formed by these subtypes exhibit unique response kinetics and distinct pharmacological properties [20,26]. Although these features have been investigated extensively, there is a paucity of information concerning the single channel properties of the receptors formed by the ρ subunits. In this study, we used noise analysis techniques to examine the channel conductance of the homomeric GABA ρ receptors formed in HEK cells. Our results indicate that the receptors formed by GABA $\rho 2$ subunits exhibit larger single channel conductances than that formed by a GABA $\rho 1$ subunit, and that a single amino acid at the 2' position of the second transmembrane domain region is an important determinant of the channel conductance of GABA_C receptors.

Materials and Methods

Heterologous expression of perch GABA ρ subunits and patch-clamp recording

HEK293 cells (ATCC, Manassas, VA) seeded at a density of 5×10^5 cells/35 mm dish were maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics. The cells were transiently transfected with the desired plasmids using Lipofectamine Plus (Invitrogen, Carlsbad, CA) following the manufacturer's recommended protocol. The perch $\rho 1$ and $\rho 2$ cDNAs were subcloned into a eukaryotic expression plasmid (pcDNA 3.1, Invitrogen), and 1.5 μ g of each plasmid, together with 0.5 μ g pEGFP (Invitrogen) were used per dish for transfection. The transfected cells were identified under an epifluorescent microscope, and patch clamp experiments were performed 24–48 hours after transfection. Prior to recording, the culture medium was replaced with an extracellular solution consisting (in mM) of NaCl (120), KCl (5), NaHCO₃ (25), CaCl₂ (2), MgCl₂ (1), HEPES (10), dextrose (10), pH 7.4. Whole-cell membrane currents were obtained with a patch pipette filled with an intracellular solution containing (in mM): CsCl (130), KCl (4), CaCl₂ (1), MgCl₂ (2), EGTA (11), HEPES (10), MgATP (1), NaGTP (0.2), pH 7.4. Cells were held at -60 mV, and the GABA-elicited responses were recorded with an Axopatch 200B amplifier controlled by pCLAMP software (Axon instrument, Union City, CA); data were analyzed and plotted using Origin software (Microcal, Northampton, MA).

Isolated retinal bipolar cells

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.

Solitary bipolar cells isolated from the retina of hybrid bass (*Morone chrysops* crossed with *Morone saxatilis*) were used in this study. The cell isolation and recording procedure were the same as described previously [27]. Briefly, animals were anesthetized with Tricaine (0.02%) and sacrificed by double pithing. The retina was isolated from the hemi-sectioned eyeball, and incubated for 40 min in an enzyme solution made up of Leibovitz's L-15 culture medium (GIBCO, Grand Island, NY) containing 2 mg/ml papain (Calbiochem, San Diego, CA) and 1 mg/ml of L-cysteine. Enzyme activity was arrested by washing the tissue 5 times in fresh L-15 medium. After trituration through a sterile pipette, aliquots of the supernatant containing dissociated cells were placed in 35 mm culture dishes containing 3 ml of the L-15 medium,

and stored in an incubator at 16°C. Bipolar cells were readily identified by their characteristic morphology [23], and those with prominent cell bodies were selected for study.

Noise analysis

The procedure for noise analysis of GABA_C receptor-mediated current responses followed a modified protocol described in earlier studies [14,23]. Briefly, a 2 Hz zero-phase digital filter (Matlab, The Math Works, Inc, Natick, MA) was used to separate noise and mean response from recordings of GABA-elicited currents. The responses were divided into several 400 ms segments, from which the mean (I) and variance (σ^2) were calculated. The derived values of I and σ^2 were fitted with a parabolic function to calculate the single channel current (i): $\sigma^2 = iI - (I^2/N) + \sigma_0^2$, where σ_0^2 is baseline variance, and N is the number of GABA_C receptor channels on the cell membrane.

Results

Five subunits have been cloned from perch retina [24], but only three ($\rho 1A$, $\rho 2A$ and $\rho 2B$) formed functional homomeric receptors when expressed in mammalian HEK cells; the other two subunits ($\rho 1B$ and $\rho 3$) failed to form functional receptors on HEK cell membranes. Although this necessitated confining our single channel analysis to the properties of the homomeric perch $\rho 1A$, $\rho 2A$ and $\rho 2B$ receptors, these subunits are fairly representative of the two functional $\rho 1$ and $\rho 2$ subgroups. Fig. 1A illustrates examples of GABA-elicited current responses from HEK cells expressing perch $\rho 1A$, $\rho 2A$ and $\rho 2B$ receptors. As reported previously, the kinetics of the GABA-elicited responses from each homomeric receptor are unique [24].

Noise analysis allowed us to estimate the unitary conductance of the chloride channel gated by each homomeric perch ρ receptor. A 2-Hz zero-phase digital filter was used to separate the noise associated with each GABA response (illustrated above each trace). The variance and means were calculated for each 400 ms segment of the GABA responses, and the resultant data are plotted in Fig. 1B. In each case, the mean current vs variance data could be well-fitted by a parabolic function (thin lines), from which the single channel current was derived. In the examples shown, it is evident that there are large differences in the single channel currents of $\rho 2$ receptors (167 fA and 234 fA for the $\rho 2A$ and $\rho 2B$ subtypes, respectively) compared with that of the $\rho 1A$ receptor (8.6 fA). Single channel conductances were derived by dividing the single channel current by the driving force for the chloride ion (~62 mV), and the averaged results from 5–9 cells are shown in the bar graph of Fig. 2C. The results show the significant differences in the single channel conductances between perch $\rho 1$ receptors ($\rho 1A$) and the $\rho 2$ receptors ($\rho 2A$ and $\rho 2B$), whereas no significant differences were observed between the A- and B- subtypes of the perch $\rho 2$ receptor.

Since GABA ρ subunits are predominately expressed on retinal bipolar cells, we also estimated the single channel conductances of the GABA_C receptors present on hybrid bass retinal bipolar cells. An example of the GABA-elicited response is shown in Fig. 2A, and the corresponding digitally-isolated noise is shown above the trace. A similar protocol was used to calculate the variance and means for each segment of the response, and the data were fit by a parabolic function (Fig. 2B). The derived single channel conductance averaged from 7 bipolar cells is shown in Fig. 2C. The averaged value (1.36 pS) is similar to that found in our previous study [23], as well as the single channel conductance reported for GABA_C receptor mediated responses on goldfish bipolar cells [19]. Note that the single channel conductance estimated from retinal bipolar cells (1.36 pS) lies between the value derived from perch $\rho 1$ receptors (0.2 pS) and that from perch $\rho 2$ receptors (3.2 and 3.5 pS for perch $\rho 2A$ and $\rho 2B$ receptors, respectively). These data are consistent with the view that native GABA_C receptors are formed by heteromers of GABA ρ subunits [21].

To identify the molecular mechanism for the different single channel conductances of receptors formed by $\rho 1$ and $\rho 2$ receptors, we compared the amino acid sequences for the channel-forming TMII domain of the GABA ρ subunits. As shown in Fig. 3, position 2' of that domain is the only site to exhibit a consistent difference in amino acid content between $\rho 1$ and $\rho 2$ subunits. It is noteworthy that this dichotomy persisted for all $\rho 1$ and $\rho 2$ subunits, from fish to mammals. To establish whether the amino acid at this position is a major factor in determining the single channel conductance of the GABA ρ receptor, we used site-directed mutagenesis in which the residue at the 2' site in the perch $\rho 1$ subunit was exchanged for the corresponding amino acid in the $\rho 2$ subunit, and vice versa. The estimated single channel conductances resulting from these substitutions are shown in Fig. 4. When the proline in the perch- $\rho 1A$ subunit was switched to a serine, there was an approximately 5 fold increase in single channel conductance. Conversely, when the serine residue in the perch- $\rho 2A$ receptor was replaced by a proline, the single channel conductance was reduced about 14 fold, to a value similar to that observed for the perch- $\rho 1A$ receptor. These results provide clear evidence that the amino acid in the 2' position in the second transmembrane domain of GABA ρ subunit plays an important role in determining the conductance of the ionic channel in the GABA_C receptor.

Discussion

We used noise-analysis to estimate the single channel conductance of the homomeric receptors formed by perch $\rho 1$ and $\rho 2$ subunits. Our results indicate that the conductance of the channels formed by $\rho 2$ subunits are more than an order of magnitude greater than the conductance of the $\rho 1$ receptor channel (Fig. 2). On the other hand, the estimated channel conductance of the GABA_C receptors on retinal bipolar cells lies between the values obtained for the two homomeric receptors. These results are consistent with the notion that native GABA_C receptors on retinal neurons are formed as heteromeric ρ subunits [21].

Although noise analysis is a powerful technique, and widely used for estimating single channel properties, it tends to underestimate the value of the single channel conductance. As shown in Fig. 2, the single channel conductance of the $\rho 1$ receptor we obtained from noise analysis was approximately 0.2 pS, which is somewhat lower than the 0.65 pS value reported for homomeric $\rho 1$ receptors measured directly by single channel patch recordings [6,31]. On the other hand, noise-analysis methods are technically easier to implement, and are especially useful in cases where small conductance channels, such as those of GABA_C receptors, make single channel recordings challenging. More importantly, we should stress that this study is focused mainly on a comparison of the single channel properties of the $\rho 1$ and $\rho 2$ receptors; any bias inherent in the noise-analysis approach can be expected to affect the measurements for both receptor types.

All members of the Cys-loop family of ligand-gated ion channels share a common feature, namely, that the cytoplasmic region of the TMII domain forms the narrowest region of the channel. Residues between the -3' and 2' positions contribute to the receptor pore [10] and play major roles in channel gating, ion permeability and selectivity [2]. In addition to contributing to the channel pore, mutational analysis of the TMII domain of ρ receptors has shown that the region governs receptor desensitization [17], ion selectivity [32], and other pharmacological properties [17]. In the present study, we observed that alignment of the amino acid sequences in the TMII domain of $\rho 1$ and $\rho 2$ subunits from various species revealed a strikingly consistent difference in a single residue at the 2' position, where proline was present in $\rho 1$ receptors, and serine occupied the site in $\rho 2$ receptors (Fig. 3). By interchanging the amino acids of perch $\rho 1$ and $\rho 2$ subunits at this site we confirmed that the residue at this position is a major determinant of channel conductance (Fig. 4). This is in keeping with earlier studies showing that the amino acid at position 2' of GABA ρ subunits influences response kinetics [25], receptor pharmacology [4,29], and ion selectivity [5]. The importance of the amino acid at the

2' position has also been reported in a recent study on the GABA_A receptor, in which mutating the serine residue in the γ 2 subunit significantly altered the single channel conductance of the receptor [16]. Thus, the results of the present study are consistent with the view that residues at position 2' influence the conformational geometry of the channel and significantly affect receptor gating. It is interesting to note that the amino acid at the 2' position exhibits large variations among members of the Cys-loop family of ligand-gated channels, perhaps to serve a diversity of functions that characterize the different receptors.

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References

1. Albrecht BE, Breitenbach U, Stuhmer T, Harvey RJ, Darlison MG. In situ hybridization and reverse transcription-polymerase chain reaction studies on the expression of the GABA_C receptor ρ 1- and ρ 2-subunit genes in avian and rat brain. *European Journal of Neuroscience* 1997;9:2414–2422. [PubMed: 9464935]
2. Barry PH, Lynch JW. Ligand-gated channels. *IEEE transactions on nanobioscience* 2005;4:70–80. [PubMed: 15816173]
3. Boue-Grabot E, Roudbaraki M, Bascles L, Tramu G, Bloch B, Garret M. Expression of GABA receptor rho subunits in rat brain. *J Neurochem* 1998;70:899–907. [PubMed: 9489708]
4. Carland JE, Moore AM, Hanrahan JR, Mewett KN, Duke RK, Johnston GA, Chebib M. Mutations of the 2' proline in the M2 domain of the human GABA_C ρ 1 subunit alter agonist responses. *Neuropharmacology* 2004;46:770–781. [PubMed: 15033337]
5. Carland JE, Moorhouse AJ, Barry PH, Johnston GA, Chebib M. Charged residues at the 2' position of human GABA_C ρ 1 receptors invert ion selectivity and influence open state probability. *J Biol Chem*. 2004
6. Chang Y, Weiss DS. Channel opening locks agonist onto the GABA_C receptor. *Nat Neurosci* 1999;2:219–225. [PubMed: 10195213]
7. Chebib M, Mewett KN, Johnston GA. GABA_C receptor antagonists differentiate between human ρ 1 and ρ 2 receptors expressed in *Xenopus* oocytes. *Eur J Pharmacol* 1998;357:227–234. [PubMed: 9797041]
8. Cutting GR, Lu L, O'Hara BF, Kasch LM, Montrose-Rafizadeh C, Donovan DM, Shimada S, Antonarakis SE, Guggino WB, Uhl GR, Kazazian HH Jr. Cloning of the γ -aminobutyric acid (GABA) ρ 1 cDNA: a GABA receptor subunit highly expressed in the retina. *Proc Natl Acad Sci U S A* 1991;88:2673–2677. [PubMed: 1849271]
9. Enz R, Cutting GR. GABA_C receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. *Eur J Neurosci* 1999;11:41–50. [PubMed: 9987010]
10. Filippova N, Wotring VE, Weiss DS. Evidence that the TM1-TM2 loop contributes to the rho1 GABA receptor pore. *J Biol Chem* 2004;279:20906–20914. [PubMed: 15007065]
11. Greka A, Lipton SA, Zhang D. Expression of GABA_C receptor ρ 1 and ρ 2 subunits during development of the mouse retina. *Eur J Neurosci* 2000;12:3575–3582. [PubMed: 11029627]
12. Johnston GAR. GABA_C receptors: relatively simple transmitter-gated ion channels? *Trends Pharmacol Sci* 1996;17:319–323. [PubMed: 8885697]
13. Kalloniatis M, Tomisich G. Amino acid neurochemistry of the vertebrate retina. *Prog Retin Eye Res* 1999;18:811–866. [PubMed: 10530752]
14. Knapp AG, Schmidt KF, Dowling JE. Dopamine modulates the kinetics of ion channels gated by excitatory amino acids in retinal horizontal cells. *Proc Natl Acad Sci U S A* 1990;87:767–771. [PubMed: 1689053]
15. Lukasiewicz PD. GABA_C receptors in the vertebrate retina. *Mol Neurobiol* 1996;12:181–194. [PubMed: 8884747]

16. Luu T, Cromer B, Gage PW, Tierney ML. A role for the 2' residue in the second transmembrane helix of the GABA A receptor gamma2S subunit in channel conductance and gating. *The Journal of membrane biology* 2005;205:17–28. [PubMed: 16245039]
17. Martinez-Torres A, Miledi R. A single amino acid change within the ion-channel domain of the γ -aminobutyric acid $\rho 1$ receptor accelerates desensitization and increases taurine agonism. *Arch Med Res* 2004;35:194–198. [PubMed: 15163459]
18. Milligan CJ, Buckley NJ, Garret M, Deuchars J, Deuchars SA. Evidence for inhibition mediated by coassembly of GABA_A and GABA_C receptor subunits in native central neurons. *J Neurosci* 2004;24:9241–9250.
19. Palmer MJ. Functional segregation of synaptic GABAA and GABAC receptors in goldfish bipolar cell terminals. *J Physiol* 2006;577:45–53. [PubMed: 17008372]
20. Pan Y, Khalili P, Ripps H, Qian H. Pharmacology of GABA_C receptors: responses to agonists and antagonists distinguish A- and B-subtypes of homomeric rho receptors expressed in *Xenopus* oocytes. *Neurosci Lett* 2005;376:60–65. [PubMed: 15694275]
21. Pan Y, Qian H. Interactions between ρ and γ_2 subunits of the GABA receptor. *J Neurochem* 2005;94:482–490. [PubMed: 15998298]
22. Pan ZH, Zhang D, Zhang X, Lipton SA. Evidence for coassembly of mutant GABA_C $\rho 1$ with GABA_A γ_2S , glycine $\alpha 1$ and glycine $\alpha 2$ receptor subunits *in vitro*. *Eur J Neurosci* 2000;12:3137–3145. [PubMed: 10998097]
23. Qian H, Dowling JE. GABA_A and GABA_C receptors on hybrid bass retinal bipolar cells. *J Neurophysiol* 1995;74:1920–1928. [PubMed: 8592185]
24. Qian H, Dowling JE, Ripps H. Molecular and pharmacological properties of GABA- ρ subunits from white perch retina. *J Neurobiol* 1998;37:305–320. [PubMed: 9805275]
25. Qian H, Dowling JE, Ripps H. A single amino acid in the second transmembrane domain of GABA ρ subunits is a determinant of the response kinetics of GABA_C receptors. *J Neurobiol* 1999;40:67–76. [PubMed: 10398072]
26. Qian, H.; Ripps, H. The GABA_C receptors of retinal neurons. In: Kolb, H.; Ripps, H.; Wu, S., editors. *Progress in Brain Research*. 131. Elsevier Science B.V; 2001. p. 295-308.
27. Qian H, Ripps H. Response kinetics and pharmacological properties of heteromeric receptors formed by coassembly of GABA ρ - and γ_2 -subunits. *Proc R Soc Lond B Biol Sci* 1999;266:2419–2425.
28. Sivilotti L, Nistri A. GABA receptor mechanisms in the central nervous system. *Prog Neurobiol* 1991;36:35–92. [PubMed: 1847747]
29. Wang TL, Hackam AS, Guggino WB, Cutting GR. A single amino acid in γ -aminobutyric acid $\rho 1$ receptors affects competitive and noncompetitive components of picrotoxin inhibition. *Proc Natl Acad Sci U S A* 1995;92:11751–11755. [PubMed: 8524842]
30. Wegelius K, Pasternack M, Hiltunen JO, Rivera C, Kaila K, Saarma M, Reeben M. Distribution of GABA receptor rho subunit transcripts in the rat brain. *Eur J Neurosci* 1998;10:350–357. [PubMed: 9753143]
31. Wotring VE, Chang Y, Weiss DS. Permeability and single channel conductance of human homomeric $\rho 1$ GABA_C receptors. *J Physiol (Lond)* 1999;521(Pt 2):327–336. [PubMed: 10581305]
32. Wotring VE, Miller TS, Weiss DS. Mutations at the GABA receptor selectivity filter: a possible role for effective charges. *J Physiol* 2003;548:527–540. [PubMed: 12626678]
33. Wu SM. Functional organization of GABAergic circuitry in ectotherm retinas. *Prog Brain Res* 1992;90:93–106. [PubMed: 1631312]
34. Yazulla S. GABAergic mechanisms in the retina. *Progress in Retinal Research* 1986;5:1–52.
35. Zhang D, Pan ZH, Awobuluyi M, Lipton SA. Structure and function of GABA_C receptors: a comparison of native versus recombinant receptors. *Trends Pharmacol Sci* 2001;22:121–132. [PubMed: 11239575]
36. Zhang D, Pan ZH, Zhang X, Brideau AD, Lipton SA. Cloning of a γ -aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxinin channel block. *Proc Natl Acad Sci U S A* 1995;92:11756–11760. [PubMed: 8524843]

Figure 1.

GABA (10 M)-elicited responses from homomeric perch GABA ρ receptors expressed in HEK cells. (A) Current responses and associated noise isolated by a high-pass digital filter shown above each trace. (B) The relationship between the mean and variance of the current responses for each GABA-elicited response. The data were fitted by a parabolic function (see text for details).

Figure 2.

(A) An example of the GABA_C receptor-mediated current response and associated noise obtained from white perch retinal bipolar cells. (B) Relation between the mean and variance of the responses shown in (A). Data were fit with a parabolic function. (C) Bar graph illustrates the averaged single channel conductance derived from homomeric perch $\rho 1A$ ($n = 9$), $\rho 2A$ ($n = 7$), and $\rho 2B$ ($n = 5$) receptors expressed in HEK cells, and from the GABA_C receptors on retinal bipolar cells ($n = 7$). Error bars represent the SEM.

Position	-4'	0'	2'	5'	10'	15'
Human- ρ 1	A	V	P	A	R	V
Rat- ρ 1	---	---	P	---	---	---
Mouse- ρ 1	---	---	P	---	---	---
Perch- ρ 1A	---	---	P	---	---	---
Perch- ρ 1B	---	---	P	---	---	---
Human- ρ 2	---	---	S	---	---	T
Rat- ρ 2	---	---	S	---	M	---
Mouse- ρ 2	---	---	S	---	M	---
Perch- ρ 2A	---	---	S	---	---	---
Perch- ρ 2B	---	---	S	---	---	---

Figure 3.

A comparison of the amino acid sequences of the second transmembrane domains of perch and mammalian GABA ρ 1 and ρ 2 subunits. There is a consistent difference between ρ 1 and ρ 2 subunits at position 2', where a proline is in the ρ 1 subunits and serine is in the ρ 2 subunits.

Figure 4.

Effects of exchanging a single amino acid in the 2' position of the TMII domain on the single channel conductances of the GABA_C receptor subunits. (A) Bar graphs show the averaged single channel conductances derived from noise analysis of the GABA-elicited responses of homomeric perch ρ receptors. (B) Schematic diagrams of the corresponding wild type and mutant GABA ρ subunits