A TM2 Residue in the β1 Subunit Determines Spontaneous Opening of Homomorphic and Heteromeric γ-Aminobutyric Acid-gated Ion Channels

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γ-Aminobutyric acid type A (GABA_A) receptors are major inhibitory neurotransmitter-gated ion channels in the central nervous system. GABA_A receptors consist of multiple subunits and exhibit distinct pharmacological and channel properties. Of all GABA_A receptor subunits, the β subunit is thought to be a key component for the functionality of the receptors. Certain types of GABA_A receptors have been found to be constitutively active. However, the molecular basis for spontaneous opening of channels of these receptors is not totally understood. In this study, we showed that channels that contain the β1 but not β3 subunits opened spontaneously when these subunits were expressed homomerically or co-expressed with other types of GABA_A receptor subunits in Xenopus oocytes. Using subunit chimeras and site-directed mutagenesis, we localized a key amino acid residue, a serine at position 265, that is critical in conferring an open state of the β1 subunit-containing GABA_A receptors in the absence of agonist. Moreover, some point mutations of Ser-265 also produced constitutively active channels. The magnitude of spontaneous activity of these receptors was correlated with the molecular volume of the residue at 265 for both homomeric and heteromeric GABA_A receptors, suggesting that the spontaneous activity of the β1 subunit-containing GABA_A receptors may be mediated through a similar molecular mechanism that is dependent on the molecular volume of the residue at 265.

The γ-aminobutyric acid type A (GABA_A) receptors are the major sites of fast synaptic inhibition and the targets of action of a variety of therapeutic agents such as barbiturates, steroids, anesthetics, and benzodiazepines in the brain. These receptors belong to a superfamly of the Cys-loop pentameric ligand-gated ion channels, which includes nicotinic acetylcholine (nACh), serotonin type 3 (5-HT3), and glycine receptors (1). The topology of these receptors comprises a large extracellular N-terminal domain, a large intracellular loop, and four transmembrane (TM) domains (1). The N-terminal extracellular domain contains the specific binding sites for agonists and antagonists (2). The TM2 domain is thought to be a key channel-lining component, which determines channel properties such as conductance, rectification, and desensitization (2).

Molecular cloning has identified a number of receptor subunits including six α, four β, four γ, one δ, one ζ, and one π subunit(s) (3). Among these subunits, the β subunit is thought to be a key component to assemble heterooligomeric functional ion channels, to play a central role in determining the subcellular locations of GABA_A receptors (4), and to bear binding sites for agonists (5, 6) and some clinically important drugs such as general anesthetics (7–9). The β subunits are also found to be capable of forming homomeric functional channels when expressed in Xenopus oocytes or mammalian cells (5, 7, 10, 11). These homomeric GABA_A receptor ion channels have been found to be a valuable approach for localizing molecular determinants of receptor assembly (12, 13) and receptor sensitivity to general anesthetics (14, 15).

Certain types of heteromeric and homomeric GABA_A receptors can form channels that open spontaneously in the absence of agonist (5, 7, 10, 11, 13, 15–18). For homomeric β subunits, the constitutive activity appears to represent a major form of their functionality. Previous studies have reported that the spontaneous channel activity can vary substantially among GABA_A receptor channels that contain different β subunits (11, 17, 19). However, the precise molecular basis for the constitutive activity of GABA_A receptors is not totally understood. Here, we investigated whether the difference in spontaneous activity among different β subunits can be explained by localizing discrete sites on the receptor proteins using subunit chimeras and site-directed mutagenesis. Our data show that a single amino acid residue at position 265 in the second transmembrane domain of the β1 subunit is crucial for conferring increased opening probability of GABA_A receptor ion channels in the absence of agonist. Further molecular analysis found that the magnitude of channel spontaneous opening of GABA_A receptor channels is correlated with the volume of the amino acid residue at 265 of the β1 subunit.

EXPERIMENTAL PROCEDURES

Chimeric Receptor—DNA fragments encoding the indicated regions of β1 and β3 subunits were generated by polymerase chain reaction. PCR primers were designed to introduce unique restriction sites into the targeted cDNAs without changing the encoded amino acid sequences. The chimeric β1/β3 cDNAs were constructed by cloning the PCR fragments into appropriate restriction enzyme sites of a pCMV-Script vector (Stratagene). The chimeric C1 and C2 receptors were constructed by introducing an AflII site at position 213 of the β1 subunit. The chimeric C3 and C4 receptors were constructed by introducing a HindIII site at position 314 of the β1 and β3 subunits. The authenticity of the DNA fragments that flank the mutation site was confirmed by double strand DNA sequencing using an ABI Prism 3177 automatic DNA sequencer (Applied Biosystems).

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‡ The abbreviations used are: GABA_A, γ-aminobutyric acid type A; TM, transmembrane; BIC, bicuculline; PTX, picrotoxin; WT, wild type.

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Site-directed Mutagenesis—Point mutations of a cloned rat GABA<sub>A</sub> receptor were introduced using a QuikChange site-directed mutagenesis kit (Stratagene). The authenticity of the DNA fragments that flank the mutation site was confirmed by double strand DNA sequencing using an ABI Prism 377 automatic DNA sequencer (Applied Biosystems).

Preparation of cRNA and Expression of Receptors—Complementary RNAs were synthesized in vitro from linearized template cDNAs with mMACHINE RNA transcription kits (Ambion Inc.). The oocytes of mature Xenopus laevis frogs were isolated as described previously (20). Each oocyte was injected with a total of 20 ng of RNA in 20 nl of diethyl pyrocarbonate-treated water. The injected oocytes were incubated at 19 °C in modified Barth’s solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO<sub>3</sub>, 2.0 mM CaCl<sub>2</sub>, 0.8 mM MgSO<sub>4</sub>, 10 mM HEPES, pH 7.4). Two-electrode Voltage Clamp Recording—After 2–5 days incubation, the oocytes were studied at 20–22 °C in a 90-μl chamber. The oocytes were superfused with modified Barth’s solution at a rate of ~6 ml/min. Agonists and antagonists were diluted in the bathing solution and applied to oocytes for a specified time using solenoid valve-controlled superfusion. Membrane currents were recorded by a two-electrode voltage clamp technique at a holding potential of ~70 mV using a GeneClamp 500 amplifier (Axon Instruments, Inc.). Data were routinely recorded on a chart recorder (Gould 2300S). Average values are expressed as mean ± S.E.

Data Analysis—Statistical analysis of concentration-response curves was performed using the following form of the Hill equation

\[ I = I_{\text{max}} \left( \frac{[A]}{EC_{50} + [A]} \right)^n \]  

(Eq. 1)

where \( I \) is the peak current at a given concentration of agonist \( A \), \( I_{\text{max}} \) is the maximal response, \( EC_{50} \) is the half-maximal concentration, and \( n \) is the slope factor (apparent Hill coefficient). Data were statistically compared by the unpaired \( t \) test or analysis of variance followed by Schef?e’s test as noted. Correlation analysis was carried out using nonparametric regression (Statistica, StatSoft).

RESULTS

Homomeric β1 but Not β3 Subunits Can Form Channels That Open Spontaneously—In oocytes previously injected with cRNA of rat GABA<sub>A</sub> receptor β1 subunit (Fig. 1A, top), 3000 μM GABA activated a fast inward current. Application of 100 μM bicuculline (BIC), a selective inhibitor of GABA<sub>A</sub> receptors, did not induce any detectable current. However, picrotoxin (PTX), a chloride channel blocker, at a concentration of 100 μM produced a reversible outward current. On the other hand, both BIC and PTX did not induce any response in Xenopus oocytes expressing homomeric β3 subunits (Fig. 1A, bottom). To ensure that we could study the extent of channel opening in the absence and presence of agonist at an equivalent basis, we normalized the magnitude of PTX-sensitive outward current as percentage of maximal response, which is the sum of the amplitude of PTX-sensitive current and that of GABA-activated current (Fig. 1B). The majority of the β1 subunits appeared to be in a spontaneously active state since the maximal amplitude of outward current produced by PTX represented 88% of the normalized maximal response, which is 8-fold higher than the amplitude of inward current activated by 3000 μM GABA. PTX inhibited tonically opened ion channels formed by the β1 subunits in a concentration-dependent manner over a concentration range of 1 μM–300 μM (Fig. 1C). The \( EC_{50} \) value and Hill coefficient of the PTX concentration-response curve for homomeric β1 subunits were 0.3 ± 0.02 μM and 0.6 ± 0.04, whereas PTX in concentrations up to 300 μM did not trigger any detectable current in Xenopus oocytes expressing homomeric β3 subunits (Fig. 1C). The \( EC_{50} \) value of PTX that we found for the β1 subunits is very similar to that of PTX for the β3 subunits reported previously (10).

Certain Types of Rat β1 Subunit-containing GABA<sub>A</sub> Receptors Are Spontaneously Active—The above results suggest that the homomeric β1 but not β3 subunits are constitutively active. Next, we examined whether a similar scenario could occur in heteromeric expression of the α2β1 or β1γ2 subunit combinations. While the average amplitude of maximal GABA-activated current was 27 ± 4 nA (\( n = 17 \)) for the homomeric β1 subunits, the average amplitude of maximal GABA-activated currents was 1370 ± 65 nA (\( n = 12 \)) for α1β1 and 868 ± 45 nA (\( n = 21 \)) for β1γ2 subunit combinations. The differential sen-
Fig. 2. Heteromeric GABA_{A} receptors comprised of the β1 subunits are spontaneously active. A, trace records of inward current activated by 3000 μM GABA and outward current induced by 100 μM PTX in oocytes previously injected with the cRNA of the β1 subunit alone (left) or co-injected with cRNAs of the β1 and γ2 (middle) or the α2 and β1 subunits (right). B, trace records of inward current activated by 3000 μM GABA and outward current induced by 100 μM PTX in oocytes previously injected with the cRNA of the β3 subunit alone or co-injected with cRNAs of the β3 and γ2 or the α2 and β3 subunits. It should be noted that while 3000 μM GABA activated inward currents, application of 100 μM PTX did not induce any detectable current in oocytes expressing different combinations of β3-containing GABA_{A} receptors. The solid bar above each record represents the time of drug application. C, a strong correlation between the magnitudes of normalized spontaneous opening and GABA-activated current in oocytes expressing both homomeric and heteromeric β1 subunit-containing GABA_{A} receptors. Each data point is obtained from at least 5 cells.

A Site for Constitutive Activity of GABA_{A} Receptors

Previously injected with either H_{2}O or cRNAs of the α2β1γ2 subunits on application of 100 μM PTX (data not shown). These observations indicate that certain types of GABA_{A} receptors containing the β1 subunit can form ion channels that are capable of opening independently of GABA. In addition, we found that the magnitude of the GABA-activated current is inversely correlated with that of the PTX-sensitive outward current (Fig. 2C; R = -0.99, a linear regression, p < 0.001), suggesting that the extent of channel opening in response to GABA depends on a preexisting conformational state of these receptor channels.

Both Homomeric β1 and β3 Subunits Can Form GABA-gated Ion Channels—Whether or not the β1 and β3 homomers can form functional GABA-gated ion channels has been controversial. To address this question, we examined further the function of the β1 and β3 homomers. Oocytes exhibiting inward current in response to 3 mM GABA greater than 25 nA in amplitude were selected for this experiment. Phenobarbital, an allosteric modulator of GABA_{A} receptors, at a concentration of 500 μM directly activated inward current when applied alone (not shown) or increased the amplitude of current activated by 10 μM GABA (Fig. 3A). In addition, GABA directly activated inward currents in a concentration-dependent manner over a concentration range of 0.1–3000 μM (Fig. 3B). The EC_{50} and Hill coefficient values of the GABA concentration-response curves were 7.6 ± 4 μM and 0.8 ± 0.03, respectively, for the β1 subunits and 22 ± 6 μM and 0.8 ± 0.06 for the β3 subunits. The EC_{50} values for the GABA concentration-response curves of the β1 and β3 subunits are significantly different (p < 0.02, unpaired t test, n = 10). Next, we determined the sensitivity of these homomeric receptors to PTX and BIC. In cells expressing β1 homomers, the application of 100 μM BIC slightly reduced the amplitude of current activated by GABA at a concentration of EC_{50}, whereas 3 mM GABA failed to induce inward current in the presence of PTX (Fig. 3C). This suggests that PTX can completely inhibit GABA-activated current in oocytes expressing the β1 homomers and that the outward current induced by PTX is mediated through channels formed by homomeric β1 subunits. In cells expressing β3 homomers, although neither 100 μM BIC nor 100 μM PTX induced detectable outward current, these concentrations of both BIC and PTX significantly inhibited GABA-activated current. The bar graphs in Fig. 3D show the average percentage inhibition of GABA-activated inward current by 100 μM PTX and 100 μM BIC in cells expressing homomeric β1 (solid bars) and β3 subunits (open bars). PTX (100 μM) inhibited currents activated by an EC_{50} concentration of GABA by nearly 100% in oocytes expressing either β1 or β3 homomers (Fig. 3D). On the other hand, 100 μM BIC nearly completely inhibited current activated by an EC_{50} concentration of GABA in cells expressing homomeric β3 subunits but had only a very small inhibitory effect in cells expressing homomeric β1 subunits.

The Constitutive Activity of the β1 Subunits Was Not Affected by the Y205F Mutation—To investigate the molecular mechanisms by which the β1 receptor channels open spontaneously, we first tested whether reduction of agonist binding affinity alters the spontaneous opening of the receptor channels. To do this, we substituted tyrosine at position 205, a previously described agonist-binding site in the extracellular N-terminal domain of the β1 subunit (21), with phenylalanine. Consistent with a previous study (21), the Y205F mutation shifted the GABA concentration-response curve to the right in a parallel manner (Fig. 4A) and increased the EC_{50} value by –10-fold (Fig. 4B; 7.6 ± 4 μM for the wild type (WT) and 82 ± 5 μM for the Y205F receptors, p < 0.001, unpaired t test). However, the sensitivity of the Y205F mutant receptors to PTX-sensitive
current was nearly identical to that of the wild type receptors (Fig. 4, C and D), suggesting that the agonist-binding site is unlikely to be involved in the mechanisms that underlie the constitutive activity of homomeric β1 subunits.

**Chimeric Constructs: TMs (1–3) of the β1 Subunit Are Associated with the Channel Spontaneous Opening—**In view of our observation that homomeric β1 and β3 subunits of GABA<sub>A</sub> receptors exhibit a difference in PTX-induced outward current, we thought that chimeras between the β1 and β3 subunits might be an ideal approach to localize molecular domains that may be involved in the spontaneous opening of GABA<sub>A</sub> receptor channels, and therefore we constructed chimeras between the β1 and β3 subunits. Four chimeric receptors were generated, and the amplitude of PTX-induced outward current was determined. As shown in Fig. 5A, the chimeras that replaced the N terminus of the β1 subunit (C1) and the C terminus of the β1 subunit (C4) with the corresponding segments of the β3 subunit exhibited PTX-induced outward current, suggesting that the extracellular N-terminal domain, the large intracellular loop between transmembrane domains 3 and 4, and the fourth transmembrane domain as well as the extracellular C-terminal domain of the β1 subunit are not essential for spontaneous opening of the channels in the absence of GABA. However, the chimeras that contained a region from TM1 to TM3 of the β3 subunit (C2 and C3) became insensitive to PTX inhibition of channel spontaneous opening (Fig. 5B), suggesting these transmembrane domains of the β1 subunit may be critical for the constitutive activity of the receptors in the absence of agonist. In addition, to determine whether there is a relationship between the amplitude of PTX-induced current in the WT and chimeric receptors and the sensitivity of these receptors to GABA, we first determined the EC<sub>50</sub> values for the GABA concentration-response curves of the WT (Fig. 4), and mutant (open) receptors. C, outward currents induced by application of 100 μM PTX in the absence of GABA. D, bar graph showing average amplitude of the outward current induced by various concentrations of PTX in cells expressing the WT (solid bars) and mutant (open bars) β1 homomers. Each bar is the average from 4–5 cells.

**Point Mutations: A Residue (Ser-265) in the TM2 Confers Spontaneous Activity of the β1 Subunit-containing GABA<sub>A</sub> Receptors—**To identify the site or sites responsible for the spontaneous opening of the homomeric β1 subunits, we aligned the amino acid sequences that flank a segment between TM1 and

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**Fig. 3. Functional properties of the β1 and β3 homomers.** A, records of inward current induced by 10 μM GABA with or without preapplication of 500 μM phenobarbital (PBBT) in cells expressing the β1 and β3 homomers. The phenobarbital was preapplied for 10 s prior to the application of GABA. The solid bar above each record indicates the time of drug application. B, GABA concentration-response curves for homomeric β1 (solid squares) and β3 receptors (solid triangles). The curves shown are the best fit to the equation under “Experimental Procedures.” The error bars not visible are smaller than the size of the symbols. C, trace records of GABA-activated current in the absence and presence of 100 μM bicuculline, a selective GABA<sub>A</sub> receptor antagonist, or 100 μM picrotoxin. D, bar graph showing the average percentage inhibition by BIC and PTX of inward current induced by 10 μM GABA at EC<sub>50</sub> concentrations in cells expressing the β1 or β3 homomers. Each bar is the average from 4–5 cells.

**Fig. 4. Point mutation of an agonist-binding site in the N-terminal domain of the β1 homomer did not alter spontaneous activity of the channels.** A, GABA concentration-response curves for the homomeric wild type (solid squares) and Y205F mutant (solid triangles) β1 homomers. The curves shown are the best fit to the equation under “Experimental Procedures.” The error bars not visible are smaller than the size of symbols. B, the bars represent the average EC<sub>50</sub> values of the GABA concentration-response curves for the WT (solid) and mutant (open) receptors. C, outward currents induced by application of 100 μM PTX in the absence of GABA. D, bar graph showing average amplitude of the outward current induced by various concentrations of PTX in cells expressing the WT (solid bars) and mutant (open bars) β1 homomers. Each bar is the average from 4–5 cells.
TM3 of the β1 and β3 subunits. Within this region, the β1 and β3 subunits differ by only four amino acid residues (Fig. 6A). We then substituted each of these residues of the β1 subunit with the corresponding residue of the β3 subunit. Fig. 6B shows the GABA concentration-response curves for the wild type and mutant β1 receptors. The EC_{50} and Hill coefficient values were, respectively, 30 ± 3 μM and 1.0 ± 0.1 for T255I/L256M receptors (solid circles), 7.7 ± 6 μM and 0.7 ± 0.2 for S265N receptors (solid triangles), and 68 ± 10 μM and 0.8 ± 0.09 for the I283M receptors (solid diamonds) (Fig. 6B). Except for the S265N mutation, which did not alter the sensitivity of the receptor to GABA, the T255I/L256M and I283M mutations significantly decreased the sensitivity of the receptors to GABA by 4- and 9-fold, respectively (p < 0.01, unpaired t test, n = 5). However, of these point mutations, the S265N mutation was the only point mutation that abolished spontaneous activity of the β1 subunits (Fig. 6C), suggesting that the amino acid residue at position 265 in the β1 subunit is critical for the channel spontaneous opening. To determine whether amino acid Ser-265 of the β1 subunit is also important for the spontaneous opening of heteromeric GABA_A receptor channels, we co-expressed β1 (S265N) mutant subunits with γ2 or α2 subunits. The trace records in Fig. 7A show that 100 μM PTX induced an outward current in oocytes expressing the β1γ2 subunits. However, 100 μM PTX did not induce a detectable outward current in oocytes co-expressing β1 (S265N) mutant with γ2 subunits. The S265N mutation of the β1 subunit also blocked the spontaneously opening channels produced by the α2β1 subunits expressed in Xenopus oocytes (Fig. 7B). It should be noted that the N265S mutation of the β3 subunit did not produce spontaneously active channels in cells expressing homomeric β3(N265S) subunits or co-expressing β3/β2(N265S)γ2 subunits (data not shown).

Point Mutations: The Molecular Volume of the Residue at Position 265 of the β1 Subunit Is Correlated with the Spontaneous Activity of GABA_A Receptors—The observations above suggest that residue 265 in the β1 subunit is critical for the spontaneous opening state of these receptor channels in the absence of agonist. To gain molecular insight into the structural-functional role of the residue at position 265, we replaced Ser-265 with multiple amino acid residues and examined the spontaneous activity of mutant β1γ2 subunits. Among seven mutant receptors, the β1(S265W)γ2 and β1(S265G)γ2 mutant receptors were constitutively active. Next, we used correlation analysis to compare the magnitude of the PTX-induced outward current with the hydropathicity (22), polarity (23), hydrophilicity (24), and molecular volume (25) of the amino acid residues replaced at position 265. In general, there is no significant difference among these variables (Fig. 8, A, B, C, and D). However, because these receptors clearly fall into two distinct groups based on whether they open or not in the absence of agonist, we classified the receptors into two groups, those that exhibit spontaneous activity, group 1, and those that do not, group 2. For the group 1 receptors, the strongest correlation was found between the side chain molecular volume of the residues at position 265 and the extent of spontaneous channel opening (Fig. 8D; R = 0.99, p < 0.0001, nonparametric analysis). A similar scenario was observed for other spontaneously opening β1-containing heteromeric GABA_A receptors (Fig. 8, E and F).

**DISCUSSION**

In this study we have demonstrated that homomeric and heteromeric expression of rat GABA_A receptors that contain the β1 subunit were constitutively active, whereas GABA_A receptors that contain the β3 subunit were inactive in the absence of GABA. These observations are consistent with previous studies showing that homomeric GABA_A receptor channels formed by β1 but not β3 subunits are constitutively active (7, 10, 15). In addition, we found that the magnitude of spontaneous opening of homomeric β1 receptor channels was predominant as the channel spontaneous activity accounted for 88% of total extent of the opening probability. In line with previous studies (5, 26), we also observed spontaneously opening β1-containing heteromeric GABA_A receptor channels. It is
the amino acid sequences of a segment between TM1 and TM3 of the oocytes expressing either homomeric study, we found that GABA activated an inward current in subunits were insensitive to GABA (5, 11, 19). In the present was due to insensitivity of the homomeric /H9252 finding that a TM2 residue is critical for channel spontaneous opening was inversely correlated with magnitude of GABA-activated current in cells expressing homomeric β3 subunits. It has been well documented that homomeric β subunits can form functional channels that either can open spontaneously or can be directly activated by some general anesthetics (5, 7, 11, 19, 27). However, whether or not homomeric β subunits can form functional GABA-gated ion channels is still controversial and appears to depend on different species. For instance, while human and bovine β receptors were found to form channels that can be gated by GABA (27–30), rat and mouse β and β subunits were insensitive to GABA (5, 11, 19). In the present study, we found that GABA activated an inward current in oocytes expressing either homomeric β or β subunits. The amplitude of inward current was increased by barbiturate and inhibited by a selective GABA receptor antagonist, bicuculine, suggesting that the inward current activated by GABA was mediated through homomeric β1 and β3 subunits. Although the precise reason behind the different results from our laboratory and other laboratories is not totally understood, there are a number of possibilities that could contribute, at least in part, to this discrepancy. First, it could be due to different levels of homomeric expression of the β1 subunits. Second, it may depend on the level of posttranslational modulation of the subunits, which could vary pronouncedly among different batches of Xenopus oocytes. Consistent with this hypothesis, we found that the major differences in the amino acid sequences between human and rat β subunits occur within the large intracellular loop between TM3 and TM4 domains. Another possibility to reconcile this discrepancy could be due to different levels of spontaneous activity of the β1 subunits expressed in oocytes under different experimental conditions. We and others have found that the magnitude of spontaneous activity appeared to be so predominant that it nearly overshadowed the magnitude of GABA-activated current in cells expressing homomeric β subunits (27–30). Overall, the magnitude of PTX-sensitive current was 5–9-fold larger than that of GABA-activated current. We also observed that the magnitude of spontaneous opening was inversely correlated with magnitude of GABA-activated current in oocytes expressing different combinations of homomeric and heteromeric GABA receptors that contain the β1 subunit. This indicates that the tendency of homomeric β subunits to open spontaneously increases with a decrease in the sensitivity of these receptors to GABA. It is therefore plausible to predict that when homomeric β receptor channels open spontaneously at the maximal probability, these receptors might no longer respond to activation by GABA.

We observed that the magnitude of channel spontaneous opening was not affected by the Tyr-205 mutation, a distinct agonist-binding site of the β1 subunits. This observation raises the possibility that molecular mechanisms by which the GABA receptor channels open in the absence and presence of ligands may be different. This hypothesis is consistent with our finding that a TM2 residue is critical for channel spontaneous opening of the β1 subunits and is also in line with a previous study showing that the Y205F mutation did not alter spontaneous activity induced by substitution of a highly conserved
The amino acid residues at position 265 and the constitutive activity of GABAA receptors to ethanol, general anesthetics, and anticonvulsant agents may be mediated through a molecular mechanism that involves the residue Ser-265 of the β1 subunit (35, 36). This particular residue has been found to be critical for channel spontaneous opening, previous studies also showed that such a spontaneous activity of the receptor channels could depend on receptor assembly and stoichiometry (12, 13). It is also unclear which combinations of GABAA receptor subunits may form channels that can open spontaneously in vivo. Although there is evidence suggesting that other types of GABAA receptor subunits also could be involved in channel spontaneous activity of GABAA receptors (5, 43), the results from this and previous studies suggest that such spontaneous activity of the wild type GABAA receptors may, at least in part, rely on the presence of distinct β subunits (5, 7, 10, 11, 27). There is also evidence showing that spontaneous opening of GABAA receptor channels can be detected in spinal cord neurons (44) and in pituitary cells (45, 46). However, the physiological significance of spontaneous activity of GABAA receptors remains unclear, given the fact that spontaneously opening GABA-gated ion channels are somehow difficult to identify in vivo because of background GABA release.

In summary, we have identified a particular amino acid residue, Ser-265, in the TM2 of the β1 subunit as a critical site that confers spontaneous opening of GABAA receptor channels. The magnitude of spontaneous activity of these receptor channels is correlated with the molecular volume of the residue at position 265. We have proposed that this particular residue in the β1 subunit may serve as a key structural element, which confers an open state of GABAA receptor channels in the absence of agonist by lowering the energy barrier that is required for channel opening. These observations should help to enhance our understanding of molecular mechanisms by which GABAA receptor channels can open spontaneously. The study reported here also provides some molecular details for the structural/functional role of the residue at position 265 in determining the preexisting conformational state of GABAA receptor channels. Finally, our analysis together with others of the residue at position 265 of GABA-A receptor subunits should raise the possible argument against a proposed hypothetical “anesthetic binding pocket” that involves the residue Ser-265.

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