Tyrosine 62 of the $\gamma$-Aminobutyric Acid Type A Receptor $\beta$2 Subunit Is an Important Determinant of High Affinity Agonist Binding*

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The $\gamma$-aminobutyric acid type A receptor (GABA$_{\text{A}}$R) carries both high ($K_D = 10$–30 nM) and low ($K_D = 0.1$–1.0 $\mu$M) affinity binding sites for agonists. We have used site-directed mutagenesis to identify a specific residue in the rat $\beta$2 subunit that is involved in high affinity agonist binding. Tyrosine residues at positions 62 and 74 were mutated to either phenylalanine or serine and the effects on ligand binding and ion channel activation were investigated after the expression of mutant subunits with wild-type $\alpha$1 and $\gamma$2 subunits in tsA201 cells or in Xenopus oocytes. None of the mutations affected [3H]Ro15–4513 binding or impaired allosteric interactions between the low affinity GABA and benzodiazepine sites. Although mutations at position 74 had little effect on [3H]muscimol binding, the Y62F mutation decreased the affinity of the high affinity [3H]muscimol binding sites by ~6-fold, and the Y62S mutation led to a loss of detectable high affinity binding sites. After expression in oocytes, the EC$_{50}$ values for both muscimol and GABA-induced activation of Y62F and Y62S receptors were increased by 2- and 6-fold compared with the wild-type. We conclude that Tyr-62 of the $\beta$ subunit is an important determinant for high affinity agonist binding to the GABA$_{\text{A}}$R receptor.

The GABA$_{\text{A}}$R is a member of a superfamily of ligand-gated ion channels that includes the nicotinic acetylcholine receptor (nAChR), the glycine receptor, and the serotonin type 3 receptor (1). The GABA$_{\text{A}}$R carries binding sites for a number of therapeutic agents including the benzodiazepines, barbiturates, neurosteroids, some general anesthetics, and possibly also alcohol (1). In brain membranes, there are at least two classes of binding sites for the endogenous neurotransmitter, which differ by more than an order of magnitude in their affinity for GABA or its structural analogues (2–4). This heterogeneity in binding was originally thought to reflect the diversity of GABA$_{\text{A}}$R subtypes in brain tissue. However, the presence of both classes of sites in a stable cell line expressing a specific subtype (5) suggests that both exist in a single receptor molecule. On the basis of biochemical studies, the reasonable correlation between the concentration of agonist required to elicit ion flux and to potentiate the binding of benzodiazepine ligands suggested that the low affinity sites are important for channel gating (see Ref. 6). However, the role(s) of the high affinity binding sites in receptor function remains unclear.

All members of this receptor family are believed to be pentameric complexes formed by homologous subunits assembled to form a central ion channel (7). Recent models (see Ref. 8) predict that ligand binding sites occur at subunit-subunit interfaces. This was first demonstrated in the nAChR in which the $\alpha$-$\gamma$ and $\alpha$-$\delta$ interfaces were implicated in forming non-equivalent binding sites for d-tubocurarine (9). In the GABA$_{\text{A}}$R, low affinity GABA sites (i.e. those that have been implicated in channel activation) are thought to be located at the interfaces between the $\beta$ and $\alpha$ subunits (10–13), whereas the benzodiazepine binding site is predicted to occur at the homologous $\alpha$-$\gamma$ interface (14–19). More detailed analyses of the properties of these sites have led to a “loop model” of ligand binding sites (see Ref. 20) in which amino acid residues from at least three discontinuous regions (denoted “loops” A-C) of one subunit together with residues from at least one region of the adjacent subunit (“loop” D) form the binding pocket (see Fig. 1A).

In the GABA$_{\text{A}}$R, evidence for the location of the high affinity agonist site(s) is derived from a number of experimental approaches. Photoaffinity labeling studies first suggested that the $\beta$ subunit is a major determinant of high affinity binding, because this was the principle site of photoincorporation of [3H]muscimol (21–23), although another report has given some indication that the $\alpha$ subunit can also be labeled (10). Heterologous expression of different GABA$_{\text{A}}$R subunit combinations indicates that coexpression of $\alpha$ and $\beta$ subunits is required for high affinity binding, and that $\alpha_1\beta_3\gamma_2$, $\alpha_1\beta_3\gamma_2\delta$, $\alpha_1\beta_2$, and $\alpha_1\beta_2\gamma_2$ combinations have all been shown to form high affinity binding sites for [3H]muscimol (24, 25). Furthermore, expression of a tandem construct in which the C terminus of $\alpha_6$ was covalently linked to the N terminus of the $\beta_2$ subunit produced high affinity binding sites (26), although the receptors were nonfunctional.

Based on the above observations and homology considerations, we speculated that a high affinity agonist site in the GABA$_{\text{A}}$R may be located at the $\alpha$-$\beta$ subunit interface, in which the $\beta$ subunit would contribute residues in loop D according to the model described above (see Fig. 1A). Candidate tyrosine residues (at positions 62 and 74) of the $\beta_2$ subunit were identified by amino acid sequence alignment (Fig. 1B) based on previous work that residues in the homologous positions to Tyr-62 in the $\alpha$ and $\gamma$ subunits have been implicated in (low affinity) GABA and benzodiazepine binding, respectively (10, 12, 18). Both tyrosine residues were mutated to phenylalanine.

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1 The abbreviations used are: GABA, $\gamma$-aminobutyric acid; nAChR, nicotinic acetylcholine receptor; BES, $\beta$-N-bis(2-hydroxyethyl)-2-amine-thanesulfonic acid; FNZ, flunitrazepam; WT, wild type; ANOVA, analysis of variance.
Mature Corp. (Madison, WI). The protocol for this system has been described for the GABA _A_ receptor (GABA _A_ R), and A subunit of the serotonin type 3 receptor (5-HT 3 _R_). These results suggest that Tyr-62 of the B 2 subunit is an important determinant for high affinity agonist binding site. The numbering shown is for the mature B 2 subunit. The shaded amino acid residues have been implicated in GABA binding to date.

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis**—The B 2 mutants were generated using the Altered Sites II kit (Promega Corp. (Madison, WI)). The protocol for this system has been described (27). Single point mutants are named according to their position in the mature B 2 subunit (Fig. 1B), which was determined by the calculation of the signal peptide cleavage site according to the algorithm of Nielsen et al. (28). The following oligonucleotides were designed for the mutagenesis procedure and were purified by polyacrylamide gel electrophoresis according to the method previously described (29): Y62F, 5'-TACACCTGAGCATGTTTTTCGCCAACCTTGAGAGATAAGAGA-3'; Y74S, 5'-TACACCTGAGCATGTTTTTCGCCAACCTTGAGAGATAAGAGA-3'; Y74F, 5'-TACACCTGAGCATGTTTTTCGCCAACCTTGAGAGATAAGAGA-3'; ampicillin repair, 5'-CACACGATCTGCTAGAGAATAGC-3'. The incorporation of silent HindIII restriction sites into the mutagenic oligonucleotides facilitated rapid screening of putative mutations and the presence of the mutations was subsequently verified by DNA sequencing. For heterologous expression, all subunit cDNAs were subcloned into the pcDNA3.1 (+) expression vectors (Invitrogen).

**Equilibrium Binding Assays**—[3H]Ro15–4513 (23.06 Ci/mmol, NEN Life Science Products) binding measurements were carried out using a Hoefer manual filtration apparatus as described (32). In brief, aliquots (200 µl) of cell membranes were incubated with various concentrations (0.01–100 µM) of [3H]Ro15–4513 at 4 °C for 60 min. Nonspecific binding was determined in the presence of 100 µM diazepam (0.07% (w/v) MeSO 4 final). [3H]Muscimol (20.0 Ci/mmol, NEN Life Science Products) binding was performed using a Biologic rapid filtration system (33) and carried out according to the method as described previously (4). Use of this system, in which the filter washing step was reduced to 0.5 s, permits better resolution of the lower affinity (faster dissociating) binding sites (see Ref. 4). Nonspecific binding was determined in the presence of 300 µM muscimol. Data for [3H]muscimol saturation were fit to a two-site saturation model and compared with a one-site hyperbola using GraphPad Software. All K_d values reported were obtained from nonlinear regression analysis using saturation data. Representative Scatchard plots are included for display only (e.g., Figs. 3 and 4). For competition experiments, [3H]muscimol (10–40 nM) was incubated with a 200-µl aliquot of cell membranes and various concentrations of unlabeled GABA (0.1 nM to 1 µM), muscimol (0.1 nM to 100 µM), or bicuculline (0.1–1 µM) for 60–90 min at 4 °C. Nonspecific binding was determined in the presence of 100 µM GABA or muscimol. Experiments to measure the potentiation of [3H]flunitrazepam (FNZ) binding were also conducted using the rapid filtration system. [3H]FNZ (84.5 Ci/mmol, NEN Life Science Products) was incubated with cell membranes and various concentrations of unlabeled GABA (0.01–100 µM) for 90 min at 4 °C.
The effects of the Y62F mutation on the high affinity binding sites were further confirmed by carrying out competition experiments with unlabeled GABA, muscimol, and bicuculline methochloride. These experiments were designed to avoid significant occupancy of low affinity sites ([3H]muscimol < 40 nM), thereby allowing examination of the high affinity sites without complications from the second class of sites. These experiments were not possible with the Y62S mutant, which lacked measurable binding in the high nanomolar range. The Y62F mutation resulted in a 2.8- and 2.6-fold decrease in affinity for muscimol and GABA, respectively (Table II). The $K_D$ value for muscimol-induced displacement of [3H]muscimol from the Y62F receptor is in excellent agreement with the directly measured $K_{D(J)}$ value (Table I), confirming that the mutation significantly reduced the affinity of these binding sites. Two classes of sites for bicuculline methochloride were measured in muscimol displacement experiments using the WT and Y62F receptors, but only one class of sites was seen in the Y74F and Y74S mutants (Table II). This apparent heterogeneity suggests a nonequivalence in bicuculline binding and that Tyr-74 of the β2 subunit may play a role in this. However, this observation has not been further explored.
It has been established that agonist occupancy of their low affinity sites allosterically modulates the binding of benzodiazepine site ligands (38). The ability of GABA to potentiate \(^{3}H\)FNZ binding therefore provides an independent measurement of the presence of these sites and of the integrity of coupling properties. Micromolar concentrations of GABA significantly potentiated 2 nM \(^{3}H\)FNZ binding in all mutants (Table III), and neither the \(E_{\text{max}}\) nor \(E_{\text{C50}}\) value for any recombinant receptor was significantly different from the control values (Table III). Fig. 5 shows the potentiation of \(^{3}H\)FNZ binding by GABA in the wild-type receptor (Fig. 5A) and in the Y62F (Fig. 5B) and Y62S (Fig. 5C) mutants. These data indicate that the \(\beta\) subunit mutations do not compromise low affinity agonist binding, and this provides further evidence for the presence of distinct high and low affinity binding domains in these receptors.

**Two-electrode Voltage Clamp Analysis**—The functional effects of all mutations were investigated using two-electrode voltage clamp analysis of receptors expressed in *Xenopus* oocytes. Concentration-response data for GABA and muscimol are presented in Table IV and Fig. 6. For all receptors, muscimol was, as expected, more potent than GABA in receptor activation (11). The changes observed in potency paralleled the changes seen in binding affinity. Neither of the Tyr-74 mutations affected the concentration dependence of either agonist.

**TABLE I**

The effects of amino acid substitutions in the \(\beta2\) subunit of \(\alpha1\beta2\gamma2\) recombinant GABA\(_{A}\) receptors on high (H) and low (L) affinity \(^{3}H\)muscimol binding

| \(\beta2\) subunit (n) | \(K_{D(H)}\) ± S.E. | \(K_{D(L)}\) ± S.E. |
|------------------------|---------------------|---------------------|
| WT (3)                | 8.9 ± 0.5           | 429.1 ± 40.3        |
| Y62F (3)              | 57.6 ± 11.1**       | 316.9 ± 28.4*       |
| Y62S (3)              | 7.0 ± 5.2           | 360 ± 140           |
| Y74F (3)              | 10.6 ± 5.4          | 1242 ± 741          |

**TABLE III**

However, significant rightward shifts in activation were observed in both Tyr-62 mutants, with the phenylalanine substitution producing a 2-fold shift in \(E_{\text{C50}}\) values for GABA and muscimol and serine giving a more pronounced rightward shift (≈ 6-fold). The Hill slopes (\(n_{H}\)) are not significantly different from the wild-type, with the exception of Y74F, in which the value is significantly decreased for GABA. The significance of this change in \(n_{H}\) is difficult to interpret because the Hill slope is a function of both ligand binding and channel gating (11). However, it is possible that this amino acid substitution shifted the conformation of the receptor such that the observed coop-

Data represent the mean \(K_{D}\) ± S.E. of three to four independent experiments performed in duplicate. Competition experiments were not carried out for Y62S as no specific high affinity binding was observed (Table I and Fig. 4). The number of independent experiments is shown in parentheses. \(K_{D}\) values were analyzed using a one-way ANOVA followed by a Dunnett’s test to determine the levels of significance (*, \(p < 0.05\)). N.D. represents no detectable bicuculline displacement. \(K_{D(H)}\) and \(K_{D(L)}\) represent high and low affinity bicuculline sites.

**TABLE II**

The effects of amino acid substitutions in the \(\beta2\) subunit of \(\alpha1\beta2\gamma2\) recombinant GABA\(_{A}\) receptors on the displacement of \(^{3}H\)muscimol by a number of GABA\(_{A}\)R ligands

| \(\beta2\) subunit | Muscimol \(K_{D}\) (nM) | GABA \(K_{D}\) (nM) | Bicuculline methochloride \(K_{D}\) (nM) |
|-------------------|--------------------------|---------------------|-----------------------------|
| WT (4)            | 16.7 ± 1.2 (4)           | 79.3 ± 4.0 (4)      | 72.9 ± 6.8 (4)              |
| Y62F (4)          | 14.5 ± 1.8 (4)           | 91.8 ± 5.3 (4)      | 68.7 ± 4.0 (4)              |
| Y62S (4)          | 15.7 ± 2.4 (4)           | 94.8 ± 6.5 (4)      | 54.3 ± 4.2 (4)              |
| Y74F (4)          | 17.6 ± 2.5 (4)           | 94.7 ± 5.8 (4)      | 64.2 ± 4.0 (4)              |
| Y74S (4)          | 18.2 ± 2.6 (4)           | 94.7 ± 5.9 (4)      | 68.7 ± 4.1 (4)              |

**TABLE IV**

**Potentiation of \(^{3}H\)FNZ (2 nM) binding by GABA**

| \(\beta2\) subunit (n) | \(E_{\text{max}}\) ± S.E. | Log \(E_{\text{C50}}\) ± S.E. |
|------------------------|---------------------------|-----------------------------|
| WT (5)                 | 175.0 ± 3.9               | -6.48 ± 0.43                |
| Y62F (4)               | 148.8 ± 5.2               | -6.38 ± 0.24                |
| Y62S (5)               | 156.2 ± 7.0               | -6.08 ± 0.31                |
| Y74F (4)               | 139.4 ± 7.7               | -6.41 ± 0.24                |
| Y74S (4)               | 128.8 ± 5.2               | -6.60 ± 0.16                |

**Fig. 4. Equilibrium data for \(^{3}H\)muscimol binding to Y62F (○) and Y62S (□) mutant receptors.** Shown are representative saturation curves (in the 0–150 nM concentration range) and representative Scatchard plots. The binding of \(^{3}H\)muscimol to receptors expressing these mutants was described by a one-site binding isotherm. For the purposes of illustration, a theoretical curve describing two classes of sites (\(K_{D(H)} = 54.5\) nM and \(K_{D(L)} = 400\) nM) of equivalent receptor density is shown by the dashed line for Y62F. Note for the two-site model did not describe the data better than a one-site model (\(K_{D} = 50.8\) nM) within the limits of error of curve fitting by nonlinear regression. The \(K_{D}\) values for muscimol saturation are summarized in Table II.
erativity for GABA activation of the channel is lost, the implication of which may be that these putative high affinity agonist binding sites are coupled, in some fashion, to other GABA binding domains that are essential for gating.

**DISCUSSION**

The elucidation of the mechanisms underlying GABA<sub>A</sub> function is important for the understanding of inhibitory synaptic transmission in the central nervous system. The aim of the present study was to identify residues within a specific domain of the β<sub>2</sub> subunit of the GABA<sub>A</sub> receptor that contribute to high affinity muscimol binding and to define, in part, potential roles of this site in receptor function. Although the subunit stoichiometry of native receptors is unknown, most recent evidence indicates that the recombinant α1β<sub>2</sub>γ<sub>2</sub> GABA<sub>A</sub> receptor contains 2α, 2β, and 1γ subunits (39, 40, 41). One likely arrangement of these subunits within the pentamer has been suggested to be α-β-α-γ-β (40, 42). This arrangement (see Fig. 1A) provides one α-γ interface where the benzodiazepine site is thought to be located and two β-α interfaces, each of which may carry a low affinity agonist site (11, 12). The presence of two low affinity sites would be consistent with a Hill coefficient of ;2 for channel activation (43). This leaves two additional interfaces (α-β and γ-β), which could potentially form high affinity muscimol/GABA sites. According to the popular loop model (see Introduction), loop D contributing to these sites would be

**Table IV**

Concentration-response data for GABA and muscimol activation of wild type and mutant receptors expressed in Xenopus oocytes

Data represent the mean EC<sub>50</sub> ± S.E. of three to five independent experiments performed in duplicate. Values for EC<sub>50</sub> and Hill slope (n<sub>H</sub>) were determined from concentration-response data using Graph Pad Prism Software. The number of independent experiments is shown in parentheses. Hill slope values and log EC<sub>50</sub> values were analyzed using a one-way ANOVA followed by a Dunnett’s test to determine the levels of significance (**, p < 0.01).

| β2 subunit | EC<sub>50</sub> ± S.E. | n<sub>H</sub> ± S.E. | EC<sub>50</sub> (Mutant)/EC<sub>50</sub> (WT) |
|------------|----------------------|----------------|---------------------|
| GABA       |                      |                |                     |
| WT         | 32.8 ± 2.5 (5)       | 1.33 ± 0.11    | 1.00                |
| Y62F       | 65.8 ± 5.5** (3)     | 1.27 ± 0.09    | 2.01                |
| Y62S       | 178.4 ± 16.2** (5)   | 1.26 ± 0.06    | 5.40                |
| Y74F       | 26.4 ± 3.4 (4)       | 0.88 ± 0.07**  | 0.80                |
| Y74S       | 26.9 ± 2.4 (4)       | 1.24 ± 0.09    | 0.82                |
| Muscimol   |                      |                |                     |
| WT         | 7.1 ± 1.0 (3)        | 1.32 ± 0.22    | 1.00                |
| Y62F       | 19.9 ± 3.8** (3)     | 1.21 ± 0.06    | 2.82                |
| Y62S       | 48.9 ± 0.6** (3)     | 1.20 ± 0.40    | 6.94                |
| Y74F       | 4.7 ± 0.3 (3)        | 0.91 ± 0.16    | 0.66                |
| Y74S       | 5.7 ± 1.8 (3)        | 0.92 ± 0.02    | 0.80                |

**Fig. 6.** Concentration dependence of agonist activated Cl<sup>-</sup> conductances for WT (○), Y62F (●), and Y62S (□) receptors expressed in Xenopus oocytes shows responses to GABA (A) and muscimol (B). The data represent the mean ± S.E. of at least three independent experiments performed in duplicate. Data were analyzed using one-way ANOVA followed by the Dunnett’s test to determine levels of significance. The shift in EC<sub>50</sub> for Y62F containing mutants is ~2-fold for GABA and muscimol. The rightward shifts (~6-fold) are more pronounced for Y62S mutants. Data for all mutants are presented in Table IV.
found in the N-terminal domain of the β2 subunit. Previous work has shown that residues within this domain of the γ and α subunits are determinants of benzodiazepine (17) and low affinity GABA (10, 12) binding, respectively. This provided the rationale for targeting homologous residues in the β2 subunit (Tyr-62 and Tyr-74) to investigate their role in high affinity muscimol binding.

The major finding reported here is that Tyr-62 of the β2 subunit is a determinant of high affinity agonist binding. Its substitution by phenylalanine reduced the affinity for both muscimol and GABA (6-fold), whereas its substitution by serine resulted in a dramatic reduction in affinity (>30-fold) such that no high affinity binding was measurable in this mutant. However, receptors containing the Y62S mutation were still functional, albeit with an increased IC50 for channel activation by about 6-fold. Thus high affinity agonist binding does not appear to be obligatory for receptor activation.

In a previous study, Sigel et al. (12) also mutated residue Tyr-62 of the β2 subunit. Although these authors did not investigate receptor binding properties, they found that the Y62L mutation reduced the maximum current elicited by GABA by ~5-fold, leading to the conclusion that the mutation had disrupted receptor assembly. In the present study, we did not observe any reduction of the maximum current as a result of either phenylalanine or serine substitution at this position, suggesting that there were no major effects on receptor synthesis and expression.

There is no general consensus as to the number of agonist binding sites on a single GABAAR (44). There is, however, abundant evidence for the presence of high affinity sites in addition to one or more classes of sites having lower affinity (see Refs. 1 and 6). Previous studies have demonstrated that there are approximately twice as many high affinity sites for muscimol as for flunitrazepam (45), suggesting that there are two high affinity sites/receptor. As described above, we predict that these sites are located at the α-β and γ-β interfaces, which by their nature are nonequivalent. Although we have detected no heterogeneity in high affinity [3H]muscimol binding, the bicuculline displacement experiments (Table II) suggest that in the wild type receptor, this antagonist may discriminate between two putative high affinity agonist sites. Although the Y62F mutation caused a significant decrease in affinity for the agonist, bicuculline binding was apparently unaltered. This result is in agreement with the previous observation that the Y62L mutation did not affect the IC50 values for functional antagonism of GABA-mediated chloride conductance by bicuculline (12). Conversely, neither of the Tyr-74 mutations reported here affected agonist binding, but they did alter the characteristics of [3H]muscimol displacement by bicuculline. These observations suggest that although muscimol and bicuculline compete for the same binding sites, different subsets of amino acids may be involved in the recognition of the different ligands. Alternatively, the Tyr-74 mutations may have produced changes in the conformation of the receptor, which indirectly affect the binding of bicuculline. Further complexity arises from the apparent preference of bicuculline for binding to the low affinity agonist sites (46–49). Further studies to explore this novel observation will be required to identify the specific residues with which bicuculline interacts.

The presence of multiple agonist binding sites in the GABA receptor raises the question of their roles in receptor function. Discrepancies between the concentrations of agonists that are required to activate the receptor and agonist affinities that are measured in equilibrium binding assays are generally thought to reflect differences in receptor conformation (i.e. between the activated and desensitized states). In this and many other studies (11–13) it has been found that micromolar concentrations of GABA and muscimol are required to open the ion channel (Fig. 6), suggesting that the sites involved in channel activation are of intrinsically low affinity, indeed lower than can be measured in direct equilibrium binding studies. In recent functional studies, we have found that the concentrations of GABA and muscimol that induce receptor desensitization are in good agreement with the lower affinity binding component measured directly.2 The role of the high affinity sites, however, is less clear.

The Tyr-62 mutations disrupted high affinity agonist binding and also increased the EC50 values for channel activation. It is likely, therefore, that the high affinity binding sites may play a role in the efficiency of channel activation. The EC50 value is a macroscopic constant that depends on several microscopic processes, including ligand binding and channel gating (50). It is, therefore, difficult to discriminate among the various contributing factors on the basis of concentration-response curves alone. This is particularly true when complications arising from multiple classes of agonist sites are introduced. One possibility is that the high affinity sites are allosterically coupled to other domains intimately involved in channel activation and that their occupancy at low concentrations of agonists increases the affinity of the latter sites to enhance the efficiency of synaptic transmission. It has been theorized that two nonequivalent sites, in nAChR, provide an ideal kinetic mechanism to enhance and potentially accelerate receptor activation, which may satisfy physiological requirements for rapid activation and termination of response (51).

In the present study, substitution of the tyrosine residue at position 62 by serine had a more dramatic effect than the phenylalanine substitution. Although we have not made multiple amino acid substitutions at this position, the aromaticity of the residue in this position appears to be particularly important in agonist binding. As has been previously reported for agonist binding to the nicotinic acetylcholine receptor (52) and for benzodiazepine binding to the GABAAR (32), aromatic residues may be involved in a π–π stacking interaction with the ligand.

Detailed analyses of structure-function relationships without knowledge of the crystal structure of the protein should be interpreted with caution (53). As with all site-directed mutagenesis studies, the major limitation of the present study is that we cannot state with any degree of certainty that implicated residues are directly or indirectly involved in ligand binding. However, the mutations do appear to be specific for the high affinity agonist site and this study provides the first evidence for the structural basis of high affinity binding that has been noted for more than 20 years.

In conclusion, we have identified residue Tyr-62 of the β2 subunit as a determinant of high affinity [3H]muscimol binding in the recombinant α1β2γ2 GABAAR. Further, we have shown that the reduction in affinity of high affinity binding site(s) does not have a large effect on receptor activation (54). It has previously been suggested that the nicotinic acetylcholine receptor carries sites of low and high affinity, and although the former are involved in channel activation, the latter may be important in mediating receptor desensitization (55). By analogy to the nAChR, the high affinity site(s) of GABAAR may fulfill the same role. Other investigators have likewise suggested that two molecules of GABA are required for activation, and two independent molecules of neurotransmitter are required for desensitization (56). Experiments to examine the consequences of the above mutations on the desensitization of GABAAR are currently in progress.

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2 J. G. Newell and S. M. J. Dunn, unpublished observations.
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