The Journal of Neuroscience, October 1995, 15(10): 6957–6962

GABA Antagonists Differentiate between Recombinant $GABA_A$/ Benzodiazepine Receptor Subtypes

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Seventeen rat $GABA_A$ receptor subtypes were transiently expressed in the human embryonic kidney 293 cell line from α1, α2, α3, α5, or α6 variants with any of the three β subunits and γ2S or γ3. We obtained fingerprints in the form of subtype characteristic concentration–response curves of ^{35}S-TBPS binding to GABA and the $GABA_A$ antagonists SR 95531 and bicuculline.

α3β3γ2S/3 and α5β3γ2S/3 containing receptors effectively recognized ^{35}S-TBPS but not when β3 was replaced by the β1 or β2 subunit. This indicates a specific interaction of α and β variants to form high-affinity ^{35}S-TBPS binding sites.

At low levels GABA allosterically increased ^{35}S-TBPS binding to all receptors with the concentration and magnitude depending on the subunit combination. Exchange of the β variant did not alter the concentration–response curves for α1 and α6 containing receptors but did so for α2 containing receptors. α2β1γ3 receptors displayed strong GABA-induced stimulation of ^{35}S-TBPS binding, whereas binding to α2β3γ3 receptors was marginally increased.

SR 95531 and bicuculline decreased ^{35}S-TBPS binding to all γ3 containing receptors. In addition, bicuculline was effective on α1βxγ2 receptors. SR 95531 was threefold more potent than bicuculline in reversing GABA-induced modulation of ^{35}S-TBPS binding in most receptor types, but was 30-fold more potent on α2β1γ3 and α6β1γ2S receptors.

We conclude that the recognition site for GABA analogs on $GABA_A$ receptors is jointly affected by all three subunit classes present in a receptor.

[Key words: GABA, SR 95531, bicuculline, ^{35}S-TBPS, allosteric coupling, subunit interaction]

$GABA_A$ receptors are pentameric proteins, their subunits being encoded by different genes (Seeburg et al., 1990). More than 54 $GABA_A$/benzodiazepine (BZ) receptors may assemble in mammals in subunit combinations of αiβjγk, with i = 1–6, j = 1–3, and k = 1–3, assuming a single stoichiometry for each assembly (Lüddens and Wisden, 1991) and neglecting splice variants (Whiting et al., 1990; Bateson et al., 1991; Kofuji et al., 1991; Harvey et al., 1994; Korpi et al., 1994), the assembly with the δ (Shivers et al., 1989) or ρ subunits (Cutting et al., 1991, 1992). All natural $GABA_A$ receptors appear to recognize the GABA analog muscimol, the GABA antagonists SR 95531 and bicuculline and the cage convulsant ^{35}S-TBPS (Olsen et al., 1990; Korpi and Lüddens, 1993).

^{35}S-TBPS binding is modulated by most, if not all, compounds interacting with $GABA_A$ receptors (for review, see Lüddens et al., 1994). Therefore, ^{35}S-TBPS binding lends itself as a universal tool to analyze $GABA_A$ receptor heterogeneity in combination with compounds recognizing recombinantly expressed receptors (Korpi and Lüddens, 1993; Im et al., 1994; Lüddens et al., 1994; Wafford et al., 1994; Korpi et al., 1995) or native receptors (Gee et al., 1988; Peris et al., 1991; Korpi et al., 1992; Sapp et al., 1992; Liljequist and Tabakoff, 1993). Physiologically relevant concentrations of GABA agonists like GABA itself and muscimol inhibit ^{35}S-TBPS binding and $GABA_A$ antagonists block the GABA-induced decrease of this binding in a concentration-dependent manner (Ticku and Ramanjaneyulu, 1984; Squires and Saederup, 1987; Korpi et al., 1992; Korpi and Lüddens, 1993). Whereas the binding constants for ^{3}H-muscimol and ^{3}H-GABA are similar for a number of native (Olsen et al., 1990; Bureau and Olsen, 1993) and recombinant receptors (Korpi and Lüddens, 1993; Lüddens et al., 1994), ^{35}S-TBPS binding can be used to differentiate $GABA_A$ receptor subtypes due to its sensitivity to allosteric modulation by GABA (Korpi and Lüddens, 1993; Lüddens et al., 1994). ^{35}S-TBPS binding to cerebellar granule cell-specific $GABA_A$ receptors is highly sensitive to GABA and can be blocked by residual endogenous GABA concentrations, but at least 10-fold higher GABA concentrations are needed to inhibit ^{35}S-TBPS binding in other brain areas. The high GABA sensitivity can be mimicked by the coassembly of α6, β2, and γ2 subunits in the heterologous human embryonic kidney 293 (HEK 293) cell system (Korpi and Lüddens, 1993), but lower GABA sensitivity of ^{35}S-TBPS binding can be associated with a number of different $GABA_A$ receptors.

The affinities for the antagonists SR 95531 and bicuculline differ between native $GABA_A$ receptors (Olsen et al., 1984; McCabe et al., 1988). Therefore, it appeared possible to further subtype low GABA-sensitive ^{35}S-TBPS receptors. A large number of recombinant ternary $GABA_A$ receptors differing in the three subunit components (α, β, or γ), was investigated for the properties of ^{35}S-TBPS binding as affected by GABA, SR 95531 and bicuculline. The identified characteristics of the recombinant receptors allowed us to ascribe pharmacological fingerprints to most subunit combinations which has been impossible when based solely on the benzodiazepine pharmacology of these receptors (Lüddens et al., 1994).

Received Feb. 13, 1995; revised June 9, 1995; accepted June 14, 1995.

This study was funded, in part, by the DFG (SFB 317) and the Academy of Finland. We thank Sabine Grünewald for help in cell culture work and Peter H. Seeburg for his support.

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Table 1. Effect of exchanging β variants in α2βxγ2/3 and α3βxγ2/3 receptors on ^{3}H-Ro15-4513, ^{35}S-TBPS, and ^{3}H-muscimol binding

	γ^2			γ^3		
	β1	β2	β3	β1	β2	β3
α2						
^{3}H-Ro15-4513	113 ± 20	91 ± 4	212 ± 3	51 ± 3	10 ± 5	17 ± 8
^{35}S-TBPS	26 ± 6	41 ± 7	56 ± 7	15 ± 2	35 ± 13	74 ± 28
^{3}H-Muscimol	60 ± 18	49 ± 11	62 ± 6	85 ± 25	70 ± 22	119 ± 46
α3						
^{3}H-Ro15-4513	151 ± 35	83 ± 15	222 ± 136	94 ± 48	17 ± 5	56 ± 12
^{35}S-TBPS	7 ± 4	3 ± 3	27 ± 5	4 ± 2	4 ± 5	34 ± 2
^{3}H-Muscimol	371 ± 80	51 ± 11	191 ± 75	145 ± 52	48 ± 7	320 ± 80

α2βxγ2, α2βxγ3, α3βxγ2, and α3βxγ3 subunit combinations were expressed in HEK 293 cells and membranes were prepared as described. Binding was performed with 6 nM ^{3}H-Ro15-4513, ^{35}S-TBPS, or ^{3}H-muscimol. Results, expressed in fmol/mg protein, are the means ± SEM of three experiments.

Materials and Methods

Materials. All radioligands were purchased from Du Pont-New England Nuclear (Dreieich, Germany). GABA, picrotoxinin, and bicuculline were obtained from Sigma Chemicals (St. Louis, MO) and SR 95531 from Research Biochemicals Inc. (Natick, MA). Ro 15-4513 and Ro 15-1788 were kindly donated by Dr. Hunkeler, Hofmann-LaRoche (Basle, Switzerland).

Transfection and membrane preparation. Expression vectors (Pritchett and Seeburg, 1990) for the α, β, and γ subunits were transfected in triple combination into HEK 293 cells (ATCC CRL 1573) as described previously (Korpi and Lüddens, 1993). For optimal receptor expression, final concentrations (μg of vector DNA per 15 cm tissue culture plate) were: α1, 5; α2 12; α3, 3; α5, 2; β1, 3; β2, 25; β3, 1; γ2S, 0.5; and γ3, 0.2. The γ2S variant is abbreviated γ2 in the remainder of the text. The cells were washed 40 hr after transfection with phosphate-buffered saline, pH 7.4, at 37°C, harvested in ice-cold phosphate-buffered saline and centrifuged at 150 × *g*. The cell pellets were homogenized in an Ultraturrax homogenizer for 15 sec, pelleted at 23,000 × *g* and used immediately or frozen at −80°C and recentrifuged with identical results. The membrane pellets were resuspended in 50 mM Tris/citrate buffer, pH 7.3.

Binding assays. Resuspended cell membranes (50–200 μg protein per tube) were incubated in a final volume of 0.5 ml of 50 mM Tris/citrate buffer, pH 7.3, for ^{3}H-Ro 15-4513 (6 nM) and ^{3}H-muscimol (6 nM), or in 50 mM Tris/citrate buffer, pH 7.3, supplemented with 0.2 M NaCl for ^{35}S-TBPS (2-6 nM). Nonspecific binding was determined by 10 μM Ro 15-1788, 100 μM GABA, and 20 μM picrotoxinin for the three radioligands, respectively. After 1 hr at 4°C (^{3}H-Ro 15-4513 and ^{3}H-muscimol) or 90 min at room temperature (24°C, ^{35}S-TBPS), the assay mixtures were rapidly diluted to 5 ml with ice-cold 10 mM Tris/HCl, pH 7.5, and filtered through glass fiber filters (Schleicher and Schuell, no. 52). Filters were immersed in 4 ml of Packard Ultima Gold scintillation fluid, and the radioactivity determined in a Beckman liquid scintillation counter using external standardization. Nonlinear regression was performed on the INPLOT and PRISM programs (GraphPad Software, San Diego, CA) to calculate the parameters of saturation isotherms and inhibition curves.

Results

β variant selectivity of α2 and α3 containing $GABA_A$ receptors

We expressed ternary $GABA_A$ receptors in HEK 293 cells configured from α2 or α3 subunits with any of the three β subunits and either the γ2 or γ3 variant. These receptors were tested for their ability to bind ^{3}H-Ro 15-4513, an imidazobenzodiazepine recognizing all known BZ receptors (Lüddens et al., 1990; Olsen et al., 1990), ^{35}S-TBPS, the prototypic ligand for the $GABA_A$ receptor channel and ^{3}H-muscimol to identify the GABA-recognition site directly. We observed that all receptors bound high levels of ^{3}H-muscimol and moderate to high amounts of ^{3}H-Ro 15-4513 (Table 1), indicating the presence of GABA and BZ recognition sites. All α2 receptors recognized ^{35}S-TBPS, but only those α3 receptors that contained the β3 subunit bound this ligand to a substantial amount. We therefore characterized the regulation of ^{35}S-TBPS binding to α1βxγ2/3, α2βxγ2/3, α3β3γ2/3, α5β3γ2/3, and α6βxγ2 receptors by GABA, SR 95531, and bicuculline to establish unique properties of these receptor subtypes.

Regulation of ^{35}S-TBPS binding by GABA

GABA modulated the ^{35}S-TBPS binding to all recombinantly expressed $GABA_A$ receptor combinations tested (Fig. 1). For most receptors the neurotransmitter led to a sharp rise in ^{35}S-TBPS binding at concentrations varying from 100 nM (α1β2γ3) and 1 μM (α1βxγ3 and α2β1γ3) to 10 μM (α3β3γ2 and α3β3γ3). The stimulation of the binding by GABA was most prominent on α2β1γ3 receptors (Fig. 1*B*), but very modest in α2β3γ3 receptors (Fig. 1*B*). Exchanging the β variants in α1βxγ2 or α6βxγ2 receptors did not alter the GABA concentration dependency of the binding (Fig. 1*A,D*), but GABA stimulation was pronounced in α1β2γ3 receptors and reduced in α1β3γ3 receptors. ^{35}S-TBPS binding to the three α6βxγ2 receptors was completely blocked by 1 μM GABA. For all receptors besides α3β3γ3 receptors 10 μM GABA significantly reduced ^{35}S-TBPS binding as compared to the respective binding maximum, but binding to α3 or α5 subunit containing receptors was not fully abolished by up to 1 mM GABA. Thus, they represent a recognizable subset of $GABA_A$ receptors.

Since ^{35}S-TBPS binding was greatly stimulated by GABA in some receptor subtypes, we tested whether α3 and α5 containing receptors devoid of evident basal binding, that is, α3β1/2γ2/3 and α5β1/2γ2/3 receptors, would bind the cage convulsant when 10 μM GABA was added. We could not detect any ^{35}S-TBPS binding to α5β1/2γ2/3 receptors in the presence of GABA (data not shown). However, the small amount of picrotoxinin-sensitive ^{35}S-TBPS binding to α3β1γ2 receptors (Table 1) increased threefold with 10 μM GABA, comparable to the relative magnitude of the GABA effect on α3β3γ2 receptors.

Intrinsic modulation of ^{35}S-TBPS binding by SR 95531 and bicuculline

^{35}S-TBPS binding to most of the $GABA_A$ receptor subtypes tested in the nominal absence of GABA was not significantly affected by 30 μM SR 95531 or 100 μM bicuculline (Figs. 2, 3). However, ^{35}S-TBPS binding to α1β2γ3, α2β3γ3, α3β3γ3, and

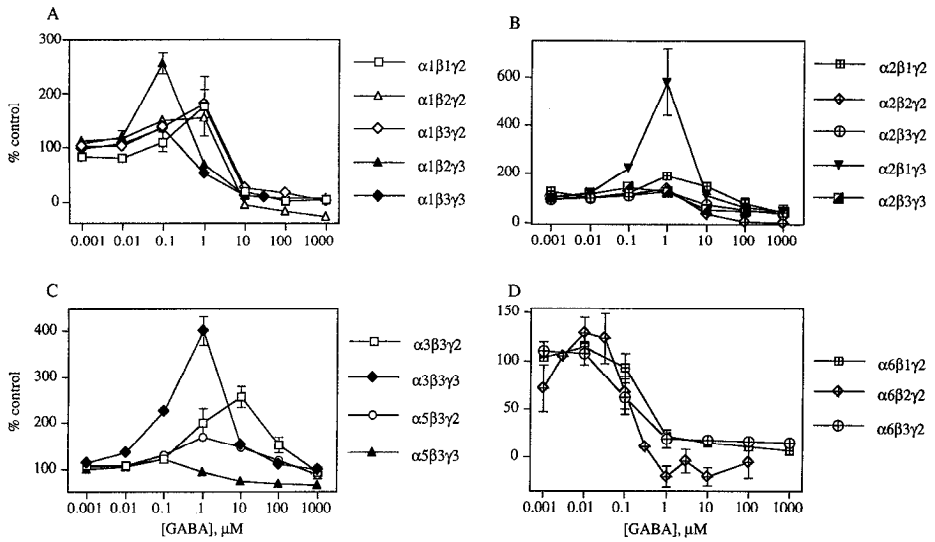

Figure 1. Modulation of ^{35}S-TBPS binding to recombinant receptors by GABA. HEK 293 cells were transfected with the subunit combinations indicated, harvested and the membranes prepared as described in Materials and Methods. The cell membranes were incubated with 2 nM ^{35}S-TBPS for 90 min at room temperature with GABA concentrations ranging from 1 nM to 1 mM. The values are expressed as percentage of control binding in the absence of GABA. Experiments were performed three or four times with duplicate samples and the results expressed as the average ± SEM.

α5β3γ3 receptors was reduced by SR 95531 in a concentration-dependent manner (Figs. 2*A,B*, 3), that is, from the group of γ3 containing receptors only α2β1γ3 receptors were unaffected. Bicuculline differed from SR 95531 in its effects on certain receptor subtypes. It reduced the binding in all α1 containing receptors (Fig. 2*C*), but was less efficacious than SR 95531 in α3β3γ3 receptors (Fig. 3). Thus, the spectrum of receptors that were intrinsically affected by SR 95531 was a subset of those affected by bicuculline.

Antagonism of GABA modulated ^{35}S-TBPS binding by SR 95531 and bicuculline

It is nearly impossible to examine native $GABA_A$ receptors free of contaminating endogenous GABA (Korpi and Lüddens, 1993). We therefore studied ^{35}S-TBPS binding of recombinant $GABA_A$ receptors in the presence of SR 95531 and bicuculline plus GABA concentrations which block ^{35}S-TBPS binding to most receptor types (10 μM GABA in α1, α2, α3, and α5 containing receptors and 1 μM GABA in α6 containing receptors) and obtained concentration–response curves which differed among the receptors.

The shape of the SR 95531 concentration–response curves for α1β1/2γ2 receptors were congruent (Fig. 4*A*), but the curves for α1β3γ2 and α1β2γ3 receptors were right-shifted (Fig. 4*A*) as compared to α1β1/2γ2 receptors.

As little as 300 nM SR 95531 was sufficient (Fig. 4*B*) to convert the inhibitory effect of 10 μM GABA on α2β1γ3 receptors to a stimulatory effect equivalent to 1 μM GABA (Fig. 1*B*), whereas α2β3γ3 receptors required higher antagonist concentrations to recover from GABA inhibition. We found a similar tendency for the three α2βxγ2 receptors (Fig. 4*B*), which points to a higher potency of SR 95531 on β1 containing α2βxγ2/3 receptors than on β2 or β3 containing receptors.

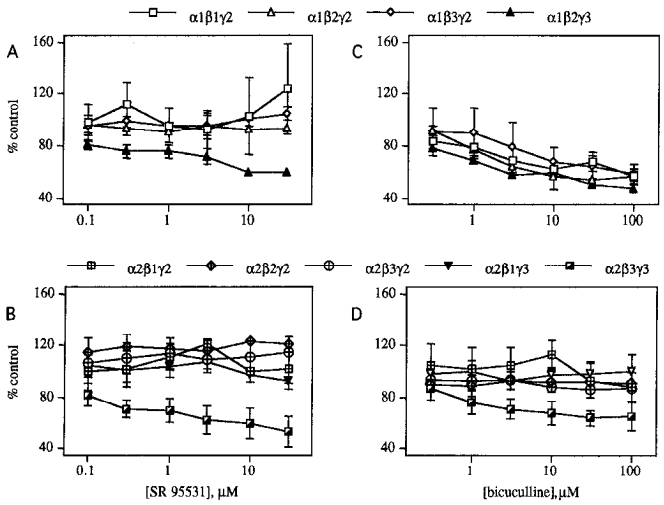

Figure 2. Intrinsic effects of SR 95531 and bicuculline on ^{35}S-TBPS binding of α1 and α2 subunit containing recombinant receptors. HEK 293 cells were transfected with α1 and α2 subunit combinations as indicated, harvested and the membranes prepared as described in Materials and Methods. The cell membranes were incubated with 2 nM or 6 nM (α2 containing receptors) ^{35}S-TBPS for 90 min at room temperature with 0.1 μM to 30 μM SR 95531 (*A, B*) or 0.3 μM to 100 μM bicuculline (*C, D*). The values are expressed as percentage of control binding in the absence of GABA. Experiments were performed three or four times with duplicate samples and the results expressed as average ± SEM.

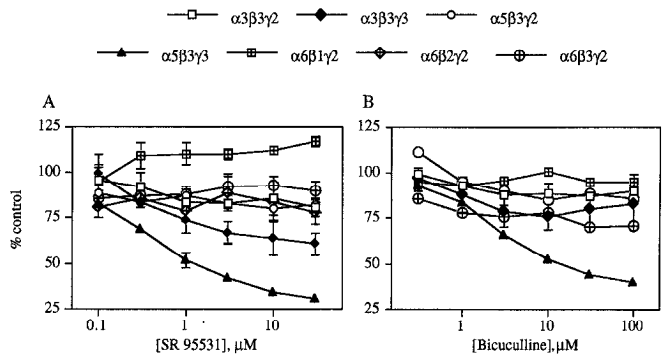

Figure 3. Intrinsic effect of SR 95531 and bicuculline on ^{35}S-TBPS binding of α3, α5 and α6 subunit containing recombinant receptors. HEK 293 cells were transfected with α3, α5, and α6 subunit combinations as indicated and harvested, and the membranes were prepared as described in Materials and Methods. The cell membranes were incubated with 2 nM ^{35}S-TBPS for 90 min at room temperature with 0.1 μM to 30 μM SR 95531 (*left panel*) or 0.3 μM to 100 μM bicuculline (*right panel*). The values are expressed as percentage of control binding in the absence of GABA. Experiments were performed three or four times with duplicate samples and the results expressed as average ± SEM.

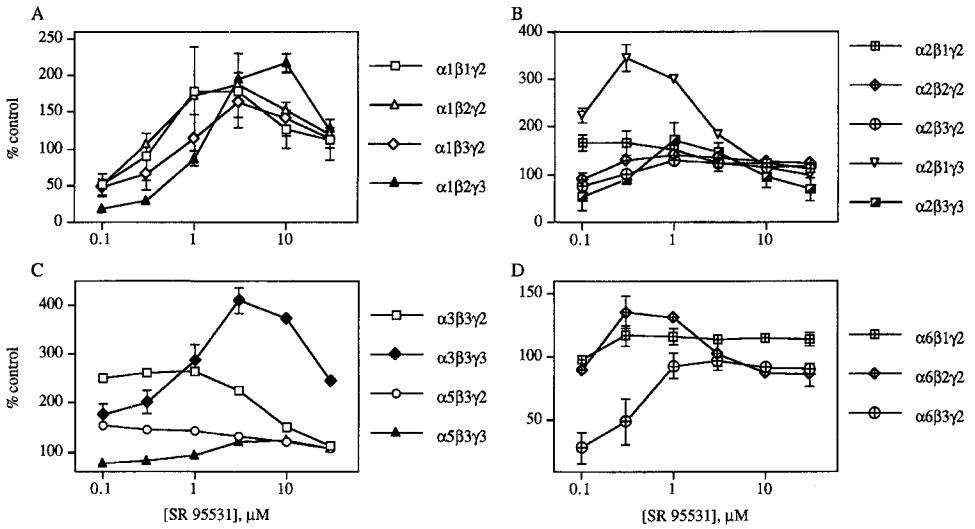

Figure 4. Modulation of ^{35}S-TBPS binding to recombinant receptors by SR 95531 in the presence of GABA. HEK 293 cells were transfected with the subunit combinations indicated and harvested, and the membranes were prepared as described in Materials and Methods. Cell membranes were incubated with 2 nM or 6 nM (α2 containing receptors) ^{35}S-TBPS for 90 min at room temperature with 1 μM GABA (α6 containing receptors) or 10 μM GABA (all other receptors) plus SR 95531 concentrations ranging from 0.1 μM to 30 μM. The values are expressed as percentage of control binding in the absence of GABA. Experiments were performed three or four times with duplicate samples and the results expressed as the average ± SEM.

In α3 and α5 containing receptors, SR 95531 was more potent in γ2 than in γ3 containing subtypes in reversing the GABA effect (Fig. 4*C*). However, GABA sensitivity was greater in γ3 than in γ2 containing receptors (Fig. 1*C*), which could indicate that the affinity of SR 95531 is similar for the four receptor subtypes.

Already 100 nM SR 95531 reversed the inhibitory effect of 1 μM GABA on α6β1γ2 and α6β2γ2 receptors (Fig. 4*D*). Tenfold higher antagonist concentrations were needed to achieve a similar result for α6β3γ2 receptors.

In the presence of GABA threefold higher concentrations of bicuculline were needed for most recombinant $GABA_A$ receptors to obtain concentration–response curves congruent to those of SR 95531 (Fig. 5*A–D*), which indicates a constant ratio of potency for SR 95531 and bicuculline. The two most pronounced exceptions were α2β1γ3 receptors (Figs. 4*D*, 5*D*) and α6β1γ2 receptors (Fig. 4*D*, 5*D*), where SR 95531 was much more potent than bicuculline.

Discussion

This study presents a novel pharmacological classification of a number of $GABA_A$ receptor subtypes. The data outline the importance of particular subunits on receptor domains and their functional allosteric interactions. Furthermore, they can be applied to simulate pharmacological brain regional heterogeneity and predict subunit combinations of native $GABA_A$ receptors.

The formation of the ionophore binding site recognized by ^{35}S-TBPS in α3γ2/3 and α5γ2/3 containing receptors depended on the presence of the β3 variant. Receptors containing the γ3 variant were generally more sensitive to GABA than γ2 containing receptors but the former receptors were more strongly modulated by the $GABA_A$ antagonists SR 95531 and bicuculline in the absence of GABA. The present results, thus, indicate roles for the β as well as the γ subunits in the transduction of the ligand binding process from the GABA recognition site to the Cl^--ionophore, and agree with the idea of a ternary receptor complex with intimate interactions between the subunits. The pharmacological fingerprint of α2β1γ3 receptors deviated from that of other recombinant receptors as its ^{35}S-TBPS binding was strongly enhanced by GABA, and SR 95531 antagonized the GABA action 30-fold more potently than bicuculline. This makes the α2β1γ3 subunit combination an excellent candidate to explain some of the rat brain regional heterogeneity that can be seen in the actions of the antagonists on ^{35}S-TBPS binding. The clear features of α2β1γ3 receptors should make it possible

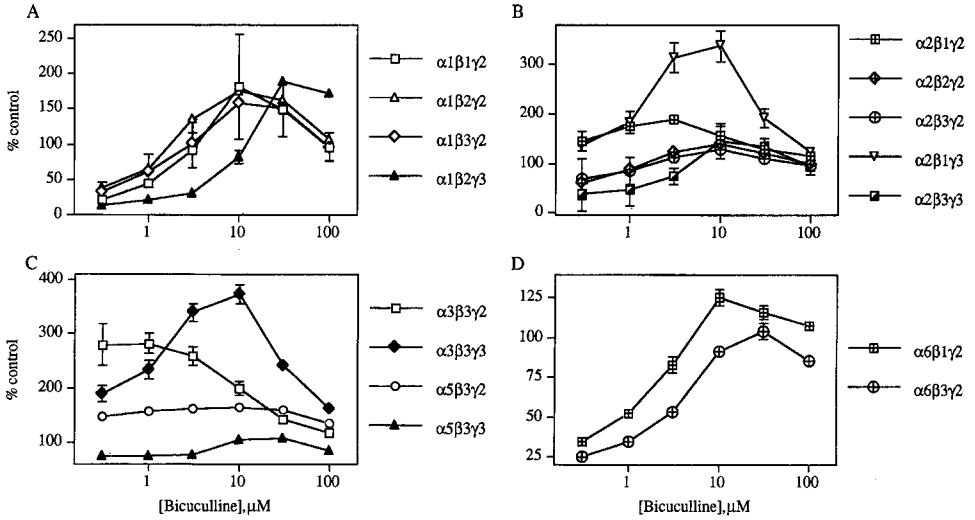

Figure 5. Modulation of ^{35}S-TBPS binding to recombinant receptors by bicuculline in the presence of GABA. HEK 293 cells were transfected with the subunit combinations indicated and harvested, and the membranes were prepared as described under Materials and Methods. Cell membranes were incubated with 2 nM or 6 nM (α2 containing receptors) ^{35}S-TBPS for 90 min at room temperature with 1 μM GABA (α6 containing receptors) or 10 μM GABA (all other receptors) plus bicuculline concentrations ranging from 0.3 μM to 100 μM. The values are expressed as percentage of control binding in the absence of GABA. Experiments were performed three or four times with duplicate samples and the results expressed as the average ± SEM.

to identify brain regions enriched in this subunit combination using autoradiography on rat brain sections.

Role of β variants in forming ^{35}S-TBPS binding site

We previously reported on the β variant selectivity of α5 containing ^{35}S-TBPS receptors (Lüddens et al., 1994). Neither α1 nor α6 containing receptors are β subunit selective (Lüddens et al., 1994; Korpi et al., 1995), and now we show that the α2 variant could also form ^{35}S-TBPS recognizing receptors with all three β subunits (Table 1). The α3 variant resembles the α5 variant since α3β1/2γ2/3 and α5β1/2γ2/3 receptors bound little ^{35}S-TBPS when compared to the corresponding β3 containing receptors. However, in contrast to α5β1/2γ2/3 receptors ^{35}S-TBPS binding to the corresponding α3 receptors was significantly increased by 10 mM GABA which could indicate a lower affinity of ^{35}S-TBPS to α3β1/2γ2/3 receptors than to α3β3γ2/3 receptors rather than differences in the number of binding sites. Though it is still open whether our findings are specific for HEK 293 cells (compare Im et al., 1994) or for $GABA_A$ receptor subunits from rodents (compare Wafford et al., 1994), our data indicate that the ability of $GABA_A$ receptors to bind ^{35}S-TBPS with high affinity requires the proper interaction of two of the three different subunits participating in the formation of the pentamer and hence is prone to subtle alterations in any of the variants.

Role of subunits in the allosteric interaction with the ^{35}S-TBPS binding site

Modulation of ^{35}S-TBPS binding by GABA or its analogs appears to be a function of all participating subunit classes. Most γ3 containing receptors were more sensitive to GABA modulation of ^{35}S-TBPS binding than γ2 containing receptors. The transition from γ2 to γ3 caused a left shift of otherwise fairly congruent concentration–response curves in the α1βxγ2/3, α3β3γ2/3 and α5β3γ2/3 receptor pairs (Fig. 1*A,C*), but affected only the magnitude of the GABA effect in α2 receptors (Fig. 1*B*).

α1β2/3γ3 receptors were more sensitive to GABA than the corresponding γ2 containing receptors, but the affinity of these receptor subtypes for GABA is identical (Korpi and Lüddens, 1993; Lüddens et al., 1994). Hence, the observed difference between the receptors reflects variations in allosteric coupling between GABA and ^{35}S-TBPS binding site. The same mechanism of action may hold true for the higher sensitivity of γ3 receptors in the α3β3γ2/3 and α5β3γ2/3 receptor pairs.

Only limited data are available on GABA concentration–response of recombinant $GABA_A$ receptors, but the known EC_{50} values determined in *Xenopus laevis* oocytes, α1β2γ2, 20 μM (Korpi et al., 1995); α3β1/2γ2, 300 μM (Sigel et al., 1990); α5β1/2γ2, 15 μM (Sigel et al., 1990); and α6β2γ2, 2 μM (Korpi et al., 1995) correlate well with the deflection point of the concentration–response curves of ^{35}S-TBPS binding for GABA, that is, they are 10–30-fold higher than the GABA concentration needed to achieve maximal ^{35}S-TBPS binding. If this pattern holds true, an EC_{50} value of approximately 1 μM can be estimated for α1βxγ3 receptors, that is, 10-fold lower than for α1βxγ2 receptors. The EC_{50} values for α3β3γ3 and α5β3γ3 receptors would then be about 30 and 1 μM, respectively. These predictions, except for α1βxγ3 receptors, which was not evaluated, have been recently confirmed (Ebert et al., 1994).

Most receptor subtypes are insensitive to the intrinsic effects of SR 95531 and bicuculline, but with the exception of α2β1γ3 receptors all γ3 containing $GABA_A$ receptors are affected by one or both of the GABA antagonists in the absence of GABA. Bicuculline seems to have the same potency as SR 95531 but a broader intrinsic specificity than SR 95531, leaving only α3β3γ3 receptors as more sensitive to SR 95531 than bicuculline. In the absence of GABA the antagonists could act on the GABA recognition site in a manner analogous to the inverse agonist action at BZ recognition site, but the possibility remains that the action(s) proceeds through a novel, independent recognition site(s).

We evaluated the effects of SR 95531 and bicuculline on ^{35}S-TBPS binding in the presence of 1 μM GABA (α6βxγ2) or 10 μM GABA (all other receptors) to compare the potency of the two antagonists in reversing the GABA induced effect on ^{35}S-TBPS binding. For most receptors threefold higher concentrations of bicuculline than SR 95531 were needed to obtain congruent SR 95531 and bicuculline concentration–response curves in the presence of GABA (compare Figs. 4, 5). A ratio of three for the potency of SR 95531 and bicuculline has been reported earlier for ^{35}S-TBPS binding to whole rat brain membranes (Squires and Saederup, 1987). However, all β3 variant receptors exhibited a lower sensitivity to SR 95531 than the corresponding β1- or β2-containing receptors. In contrast, α2β1γ3 and α6β1γ2 receptors were striking examples of highly SR 95531 sensitive receptor subtypes. For these receptor types a 30-fold lower concentration of SR 95531 than of bicuculline was needed to reach similar levels of GABA-modulated ^{35}S-TBPS binding.

The concentration–response curves for bicuculline (plus constant GABA) were generally similar when the β variants were exchanged in a αxβxγx combination, arguing for similar bicuculline affinities of receptor combinations differing in the β component. Indeed, the affinity of GABA and bicuculline seems to vary within a narrow range (Lüddens et al., 1990, 1994; Bureau and Olsen, 1993). Together with our data on SR 95531 concentration–response, this leads to the conclusion that the affinity of β1 containing receptors for SR 95531 is 10-fold higher than of the corresponding β2 or β3 containing receptors.

The γ3 subunit was associated with high GABA sensitivity (see above), which was corroborated by the concentration–response curves of SR 95531 and bicuculline, that is, generally higher concentrations of GABA antagonists were needed to counteract the GABA modulation of ^{35}S-TBPS binding in αxβxγ3 receptors than in αxβxγ2 receptors. Since most γ3 containing receptors displayed a more pronounced inhibition of ^{35}S-TBPS binding by the antagonists in the absence of GABA, the results clearly dissociate the intrinsic actions of the antagonists from their GABA antagonism.

GABA antagonist profiles as an aid to identify native $GABA_A$ receptor subtypes

α6β1γ2 receptor and α2β1γ3 receptors are the only recombinant receptors with a potency ratio of SR 95531 to bicuculline around 30, but in a study employing furosemide as a tool to differentiate $GABA_A$ receptors we could exclude the α6β1γ2 combination as a naturally occurring $GABA_A$ receptor subtype (Korpi et al., 1995). Therefore, native $GABA_A$ receptors, which are drastically more sensitive to SR 95531 than to bicuculline should be composed of α2, β1, and γ3 subunits. We are currently investigating whether the properties of recombinant α2β1γ3 receptors are present in rat brain regions with high α2 mRNA expression. These receptors should constitute a minor fraction of all brain $GABA_A$ receptors, but could be identified

by comparison of pharmacological fingerprints obtained by ^{35}S-TBPS autoradiography of rat brain sections and by ligand binding assays of recombinant $GABA_A$ receptors. Our data indicate that SR 95531 differentiates this receptor from other $GABA_A$ receptor subtypes based on its potent GABA antagonism and not on its strong intrinsic activity on ^{35}S-TBPS binding.

GABA analogs regulate ^{35}S-TBPS binding of a wide range of recombinant $GABA_A$ receptors. Besides being used to determine the subunit composition of native $GABA_A$ receptors, our data yielded new insights into the pharmacology of these receptors which provide starting points to analyze the contribution of single subunits to the GABA recognition site and to develop novel $GABA_A$ receptor subtype selective compounds not recognizing the BZ binding site.

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