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Correlation of the Apparent Affinities and Efficacies of γ-Aminobutyric Acid$_C$ Receptor Agonists

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ABSTRACT

γ-Aminobutyric acid (GABA), *trans*-4-aminocrotonic acid (TACA), muscimol, imidazole-4-acetic acid (I4AA), *cis*-4-aminocrotonic acid (CACA), and isoguvacine are all GABA$_C$ receptor agonists. These compounds have different apparent sensitivities (EC_{50}) and efficacies (I_{max}) on exogenously expressed human ρ1 homomeric GABA$_C$ receptors. It is not clear if these differences are due to distinct binding affinities and/or distinct gating kinetics. In this study, using a recently developed single oocyte binding technique, we determined the apparent dissociation constants (K_i values) of these compounds from their IC_{50} values for [^{3}H]GABA displacement. The apparent K_i values fell into two distinct groups. The high affinity group was comprised of agonists with longer distances between the nitrogen atom of the amino or imidazole group and the carbon atom of the carboxyl or isoxazole group. The single oocyte binding technique, in conjunction with two-electrode voltage clamp, has allowed a direct correlation of the apparent affinity, efficacy, and potency of agonists on intact functional GABA$_C$ receptors. The correlation and coupling of these parameters are discussed in terms of a simple proposed activation mechanism.

GABA-gated ion channels in the mammalian central nervous system can be classified into GABA$_A$ and GABA$_C$ receptors according to their pharmacological properties (Woodward et al., 1992; Feigenspan et al., 1993; Qian and Dowling, 1993; Macdonald and Olsen, 1994; Johnston, 1996; Lukasiewicz, 1996). GABA$_A$ receptors can be modulated by benzodiazepines, barbiturates, and neurosteroids and can be blocked by bicuculline, whereas GABA$_C$ receptors are insensitive to all of these compounds. These two types of GABA receptors are also distinct in their activation characteristics. For example, GABA$_C$ receptors activate and deactivate more slowly than GABA$_A$ receptors and show minimal desensitization (Polenzani et al., 1991; Feigenspan et al., 1993; Qian and Dowling, 1993, 1994, 1995; Lukasiewicz et al., 1994). These differences between the two types of receptors arise from their different subunit compositions. Recombinant αβγ receptors can reconstitute most of the pharmacological and physiological properties of GABA$_A$ receptors (Pritchett et al., 1989; Sigel et al., 1990; Verdoorn et al., 1990), whereas recombinant homomeric ρ1 GABA receptors have similar properties to native GABA$_C$ receptors (Cutting et al., 1991; Polenzani et al., 1991; Feigenspan et al., 1993; Amin and Weiss, 1994, 1996).

The exogenously expressed ρ1 homomeric GABA$_C$ receptor can be activated by several GABA agonists including *cis*-4-aminocrotonic acid (CACA), *trans*-4-aminocrotonic acid (TACA), muscimol, imidazole-4-acetic acid (I4AA), and isoguvacine (Woodward et al., 1992; Kusama et al., 1993). These agonists differ in their sensitivities (EC_{50}, or concentration of agonist required for half-maximal activation) and efficacies (maximal current). It is unclear whether these differences arise from distinct binding affinities and/or distinct gating kinetics.

In this study, using the intact single oocyte binding technique (Chang and Weiss, 1999) in conjunction with the two-electrode voltage clamp, we have determined the apparent binding affinities (K_i) as well as agonist sensitivities (EC_{50}) and potencies (I_{max}) for TACA, GABA, muscimol, I4AA, CACA, and isoguvacine on ρ1 homomeric GABA$_C$ receptors. The results showed that the apparent affinities of these agonists fell into two groups. The high affinity group was comprised of agonists with longer distances between the nitrogen atom of the amino or imidazole group and the carbon atom of the carboxyl or isoxazole group. This study represents the first direct comparison of agonist affinities, efficacies, and potencies on intact, nondesensitized ligand-activated ion channels.

Materials and Methods

cDNA and cRNA Preparation. The cDNA of the human ρ1 GABA receptor subunit was cloned into the pALTER-1 vector (Pro-

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ABBREVIATIONS: GABA, γ-aminobutyric acid; CACA, *cis*-4-aminocrotonic acid; I4AA, imidazole-4-acetic acid; TACA, *trans*-4-aminocrotonic acid.

mega, Madison, WI) as previously described (Amin et al., 1994). A silent mutation to remove the *Eco*RI site within the gene was made by site-directed mutagenesis (Promega) using the following mutagenic oligonucleotide: 5′ CTC ATT CAG GAG TTC CAC ACC ACC 3′. The gene was then subcloned into the pGEMHE high expression vector (Liman et al., 1992) between *Eco*RI sites in the T7 orientation. The cDNA was linearized with the *Nhe*I restriction enzyme, and capped cRNA was transcribed by T7 RNA polymerase using standard in vitro transcription procedures. The yield and integrity of the cRNA were examined on a 1% agarose gel.

Oocyte Preparation and cRNA Injection. Female *Xenopus laevis* (Xenopus I, Ann Arbor, MI) were anesthetized by 0.2% MS-222 (3-aminobenzoic acid ethyl ester, methanesulfonate salt). The ovarian lobes were surgically removed from the frog and placed in calcium-free oocyte Ringers-2 (OR2) incubation solution consisting of 92.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, and 5 mM HEPES; 50 U/ml penicillin, and 50 μg/ml streptomycin, pH 7.5. The lobes were cut into small pieces and digested with 0.3% collagenase A (Boehringer Mannheim Biochemicals, Indianapolis, IN) with constant stirring at room temperature for 1.5 to 2 h. The dispersed oocytes were thoroughly rinsed with the above solution plus 1 mM $CaCl_2$. The stage VI oocytes were selected and the follicular layer (if still present) was manually removed with fine forceps. The oocytes were incubated at 18°C.

Micropipettes for cRNA injection were pulled from a borosilicate glass (Drummond Scientific, Broomall, PA) on a Sutter P87 horizontal puller, and the tips were cut with scissors to ~40 μm o.d. The cRNA with dilution in diethyl pyrocarbonate-treated water (for voltage-clamp) or without dilution (for binding) was drawn up into the micropipette and injected into oocytes with a Nanoject microinjection system (Drummond Scientific) at a total injection volume of 20 to 60 nl.

Electrophysiology. One to 2 days after injection, the oocyte expressing ρ1 GABA receptors was voltage-clamped at −70 mV. The agonist-induced currents were in the range of 100 to 1000 nA, except the I4AA-induced current, which had a maximum of 30 to 100 nA. The dose-response relationships were determined by measuring the current induced by a range of agonist concentrations. The EC_{50} and Hill coefficient of the dose-response relationship was determined by fitting the data to the Hill equation in the following form:

$$I = \frac{I_{max}}{1 + (EC_{50}/[A])^n} \quad (1)$$

where I is the current amplitude, I_{max} is the maximum current amplitude for that particular agonist ([A]), EC_{50} is the agonist concentration that induces a 50% maximal response, and n is the Hill coefficient.

Single Oocyte Binding. Two to 3 days after injection, the expression level of the ρ1 GABA receptors in oocytes were examined by two-electrode voltage clamp at −70 mV. Oocytes with a current response (to 10 μM GABA) of more than 3000 nA were selected for the binding assay. Most of the oocytes tested had a maximum current amplitude of 4000 to 6000 nA. Details of the single oocyte binding have been previously described (Chang and Weiss, 1999). Briefly, the oocyte expressing ρ1 GABA receptors was held by gentle suction at the end of a sequencing gel loading pipette tip with a Pasteur bulb on the end. The tip, bulb, and attached oocyte were held by a small clamp attached to a base. In this way, the oocyte could be moved manually between the incubation, rinse, and dissociation solutions as follows. The oocyte was first incubated in 1 μM [^{3}H]GABA (in 100 μl of OR2) for 30 s at room temperature, then rinsed (to remove unbound [^{3}H]GABA) for 6 s in a 150 ml 0°C OR2 bath with constant stirring, and finally placed in 250 μl of OR2 at room temperature for 60 s to let the bound [^{3}H]GABA dissociate. The 250 μl of OR2 containing the dissociated [^{3}H]GABA was thoroughly mixed with 4 ml of scintillation fluid, and the radioactivity of each sample (cpm) was determined in a liquid scintillation counter. For the measurement of the binding affinities of each GABA receptor agonist, the unlabeled GABA receptor agonist was added to the incubation solutions at increasing concentrations. Due to the lack of desensitization of the ρ1 receptor, each oocyte could be examined over the entire range of agonist concentrations without the need for a recovery period between the test concentrations (Chang and Weiss, 1999). The IC_{50} (concentration of agonist that decreases the [^{3}H]GABA binding by 50%) for each GABA receptor agonist was determined by least-squares fit of the following relationship to the data:

$$B = \frac{B_{max}}{1 - ([I]/IC_{50})^n} \quad (2)$$

where specific binding (B) is a function of the inhibitor concentration ([I]). B_{max} is the maximum binding of 1 μM GABA in the absence of unlabeled agonist and n is the slope factor.

The apparent dissociation constant K_i of an unlabeled competing ligand was determined from the IC_{50} using the following equation (Cheng and Prusoff, 1973):

$$K_i = \frac{IC_{50}}{1 + [L]/K_d} \quad (3)$$

where [L] is the concentration of the radiolabeled ligand and K_d is the affinity of GABA to the receptor [K_d = 0.65 ± 0.22 μM; (Chang and Weiss, 1999)]. Using this competition approach to derive the K_i, we determined a value of 0.58 ± 0.03 μM for the displacement of [^{3}H]GABA by nonradioactive GABA. This agrees well with the value of 0.65 ± 0.22 μM determined from the more direct approach of measuring the amount of binding as a function of [^{3}H]GABA concentration (Chang and Weiss, 1999).

Results

EC_{50} Values and Maximum Currents for Different Agonists on ρ1 Homomeric GABA Receptors. Figure 1A are examples of currents induced by TACA, GABA, muscimol, I4AA, CACA, and isoguvacine in oocytes expressing ρ1 homomeric $GABA_C$ receptors. The normalized average dose-response relationships of these compounds are shown in Fig. 1B. The continuous lines are least-squares fits of eq. 1 to the data points. The resulting EC_{50} values and Hill coefficients are provided in Table 1. Clearly, these compounds activated the receptor with distinct sensitivities of the order: TACA > GABA > muscimol > I4AA ≫ CACA > isoguvacine.

In the measurement of the dose-response relationships, we observed that the maximal currents were different for the agonists (Table 1). Figure 1C is a plot of the dose-response relationship for each agonist normalized to the maximum for GABA, rather than to its own maximum as in Fig. 1B. As evidence indicates that different GABA receptor agonists induce openings to the same conductance level (Mistry and Hablitz, 1990), the data in Fig. 1C suggest that the maximum open probabilities were different.

The Binding Affinities of the Agonists to ρ1 Homomeric GABA Receptors Fall into Two Groups. Using the single oocyte binding technique in a competition assay (Chang and Weiss, 1999), we determined the apparent binding affinities of the different agonists in intact oocytes. Figure 2 shows that nonlabeled GABA agonists could reduce the specific binding of 1 μM [^{3}H]GABA to oocytes expressing ρ1 GABA receptors in a concentration-dependent manner. Note that the dose-inhibition relationships for TACA, GABA, and muscimol were very similar, whereas CACA and isoguvacine required much higher concentrations to displace the [^{3}H]GABA. I4AA displayed the highest apparent affinity and

will be considered separately in a later section. The continuous lines are least-squares fits of eq. 2 to the data yielding IC_{50} values and Hill coefficients (Table 2) for [^{3}H]GABA competition. The apparent binding affinities (K_i) calculated from the IC_{50} values (Cheng and Prusoff, 1973) are presented in Table 2. Note that the binding affinities of these agonists can be divided into two groups: TACA, GABA, muscimol, and I4AA have relatively high apparent binding affinities, whereas CACA and isoguvacine have significantly lower apparent affinities.

The Binding Affinities and EC_{50} Values of the Agonists at ρ1 Homomeric GABA Receptors Are Correlated. The single oocyte binding technique allows us to measure binding under similar conditions as the electrophysiological recording, and in the same set of functional receptors. This has made it possible to directly correlate binding and channel activation. Figure 3 is a plot of the EC_{50} values of these agonists as a function of their apparent dissociation constants (K_i). The continuous line is from a linear regression to all the data points excluding I4AA. The dashed line represents a theoretical exact correspondence between these two parameters. Note that all data points fall below the line. This difference may be due, at least in part, to the requirement that multiple, probably three (Amin and Weiss, 1996), agonist molecules must bind to open the $GABA_C$ receptor.

The IC_{50} Values for I4AA Competition of GABA Binding and GABA-Mediated Activation Are Similar. I4AA exhibited the greatest discrepancy between the EC_{50} and K_i values among the agonists we examined (Fig. 3). At the same time, I4AA displayed the lowest efficacy for activation; the maximal I4AA-induced current was ≈2% of the maximal GABA-induced current (Fig. 1C, Table 1). I4AA also exhibited the highest apparent affinity of any of the compounds tested (Fig. 2, Table 2). Therefore, electrophysiologically, if coapplied with GABA, I4AA should act like an antagonist. Figure 4 shows the dose-dependent inhibition of I4AA on the GABA-induced current (1 μM GABA) and [^{3}H]GABA binding. The I4AA-mediated inhibition of the GABA-induced current (1 μM GABA) demonstrated an IC_{50} value of 0.67 ± 0.10 μM (n = 5), which was similar to the IC_{50} value measured in the competitive binding assay: 0.39 ± 0.02 μM (n = 5).

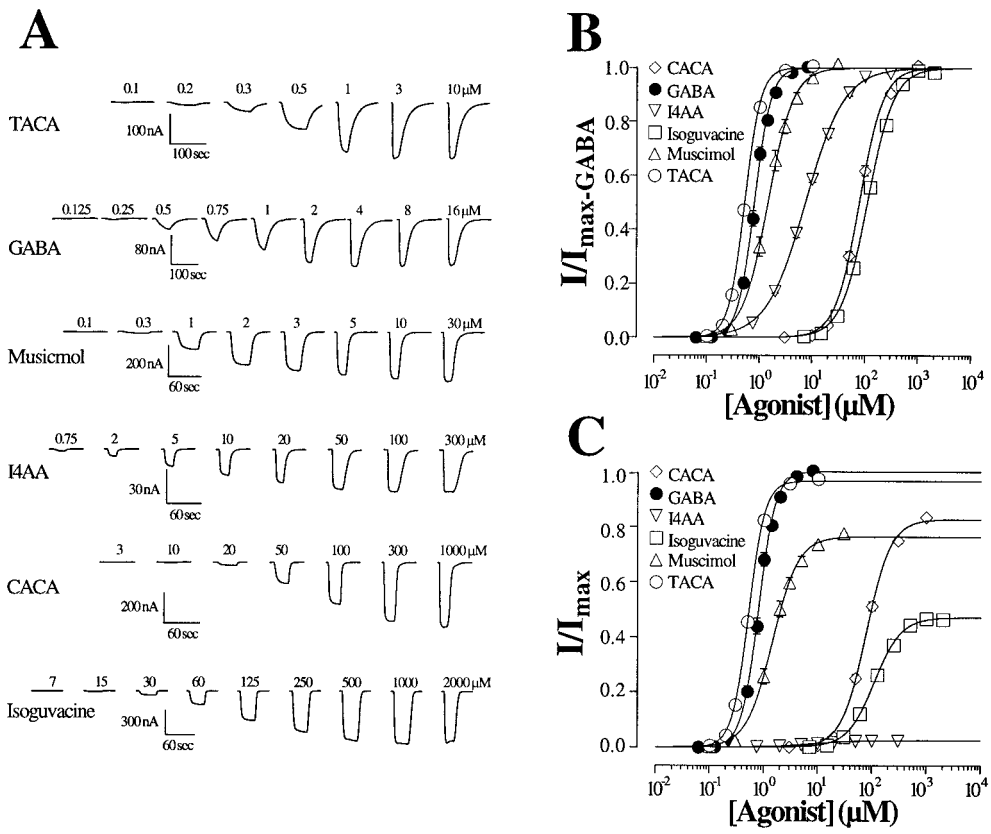


Fig. 1. Dose-response relationships of TACA, GABA, muscimol, I4AA, CACA, and isoguvacine, and efficacies on ρ1 homomeric GABA receptor expressed in *Xenopus* oocytes. A, current traces induced by these compounds. B, average dose-response relationships of different agonists normalized to their own maxima. Continuous lines are least-squares fit of eq. 1 to the data points. The resulting EC_{50} values and Hill coefficients are in Table 1. C, average dose-response relationships of different agonists normalized to the maximum for GABA.

TABLE 1

EC_{50} values, Hill coefficients, and maxima for activation by different agonists

Values are mean ± S.D.

Agonist	EC_{50}	Hill Coefficient	Number	$I_{max}/I_{max\text{-}GABA}$	Number
	μM				
TACA	0.53 ± 0.03	2.91 ± 0.21	5	0.97 ± 0.023	5
GABA	0.81 ± 0.07	2.83 ± 0.13	4	1.0	5
Muscimol	1.48 ± 0.26	1.86 ± 0.09	4	0.76 ± 0.09	5
I4AA	7.57 ± 1.08	1.20 ± 0.05	6	0.024 ± 0.0062	5
CACA	79.07 ± 7.93	1.94 ± 0.07	5	0.83 ± 0.028	5
Isoguvacine	111.65 ± 4.31	1.76 ± 0.07	5	0.47 ± 0.061	5

Discussion

Activation Mechanism. The activation mechanism of ligand-gated ion channels includes both binding and gating steps. Investigation of the coupling between binding and gating requires both radioligand binding and electrophysiological recording techniques. We have developed a single intact oocyte binding technique that allows us to study binding and activation properties in functional receptors under identical experimental conditions. In addition, homomeric ρ1 GABA receptors do not desensitize, further simplifying the analysis of the coupling between binding and channel activation. Using the single oocyte binding technique to investigate the ρ1 GABA receptor, we have already provided fundamental insights into the activation mechanism of this receptor by GABA (Chang and Weiss, 1999). Here, we have extended the application of this technique to measuring the apparent binding affinity for other GABA agonists on the recombinant ρ1 $GABA_C$ receptors in an attempt to directly correlate binding and activation of different agonists in functional, intact receptors.

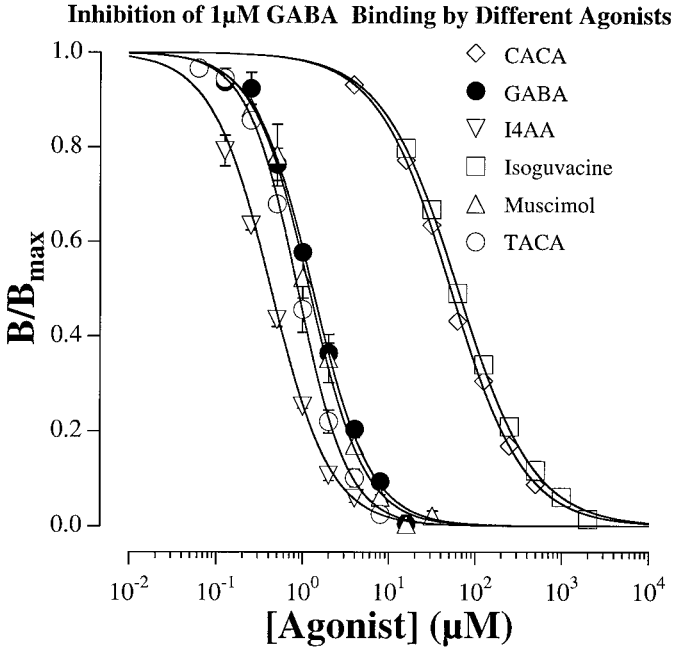


Fig. 2. Dose-dependent inhibition of the [^{3}H]GABA (1 μM) binding to ρ1 GABA receptors by different GABA receptor agonists. Continuous lines are best fit of eq. 2 to the data points. Note that TACA, GABA, muscimol, and I4AA have similar IC_{50} values, whereas CACA and isoguvacine need significantly higher concentrations to inhibit [^{3}H]GABA binding.

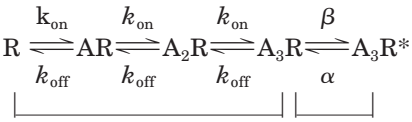
TABLE 2

IC_{50} values and Hill coefficients of the dose inhibition of 1 μM [^{3}H]GABA binding by different nonlabeled ligands and their K_i values

Values are mean ± S.D.

Agonist	IC_{50}	Hill Coefficient	K_i	Number
	μM		μM	
TACA	0.93 ± 0.14	1.56 ± 0.12	0.36 ± 0.06	3
GABA	1.48 ± 0.09	1.44 ± 0.16	0.58 ± 0.03	3
Muscimol	1.17 ± 0.33	1.35 ± 0.15	0.46 ± 0.13	3
I4AA	0.39 ± 0.02	1.20 ± 0.07	0.15 ± 0.01	5
CACA	55.71 ± 2.61	1.06 ± 0.03	21.94 ± 1.03	5
Isoguvacine	75.41 ± 7.08	1.06 ± 0.08	29.71 ± 2.79	5

We consider our data using the following three-bind-to-open concerted kinetic scheme (Amin and Weiss, 1996):



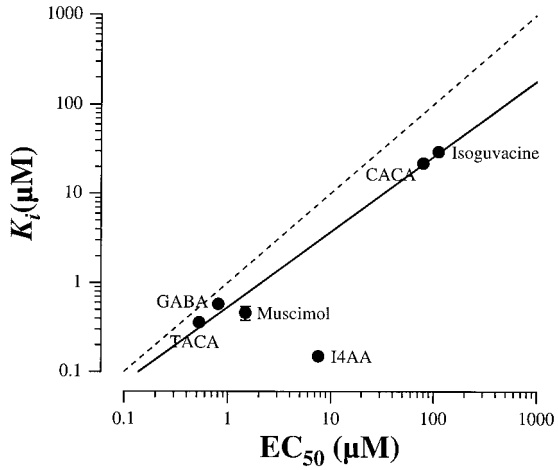


Fig. 3. Relationship between EC_{50} and K_i values. Continuous line is from a linear regression to all the data points excluding I4AA. The dashed line is the prediction assuming an equivalent EC_{50} and K_i. Note that all data points are below the line, indicating that the K_i values are lower than the EC_{50} values.

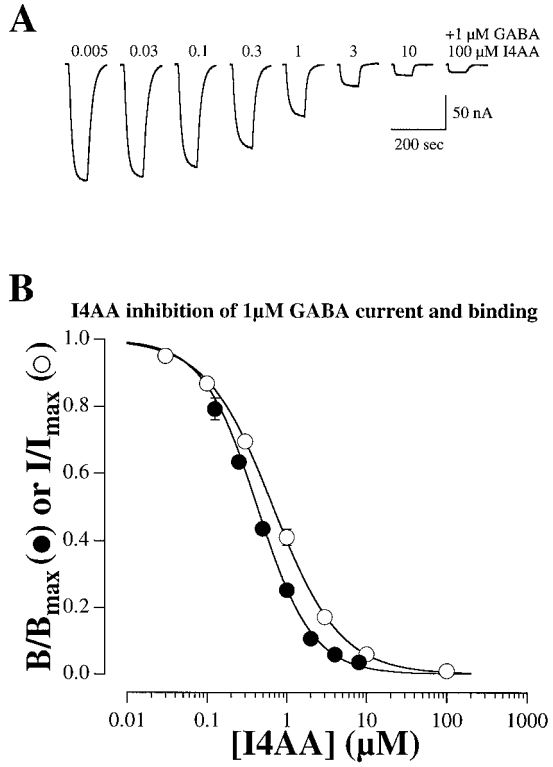


Fig. 4. Comparison of the dose-dependent inhibition of the GABA-activated current and GABA binding by I4AA. A, current traces of I4AA inhibition of GABA-induced current. B, average dose-inhibition relationships of I4AA on GABA-induced current (○) and [^{3}H]GABA binding (●). For the current, the I4AA dose-dependent activation was subtracted from the data points.

In this activation mechanism, pore opening requires three agonist molecules bind to five equal and independent binding sites. In a previous study, using a combination of single oocyte binding and electrophysiology (Chang and Weiss, 1999), we derived the following set of rate constants: $k_{on} = 0.96 \times 10^5\ M^{-1}s^{-1}$, $k_{off} = 0.18\ s^{-1}$, $\beta = 3.6\ s^{-1}$, $\alpha = 0.31\ s^{-1}$.

Using this three-bind-to-open activation mechanism and the determined rates, we investigated whether this proposed activation mechanism could describe our data if we assume an identical affinity of the agonists for the receptor (k_{off}/k_{on}), but variable gating kinetics (β and/or α). The dashed line on the left in Fig. 5 plots the predicted relationship between the EC_{50} and the maximum current using the rate constants provided under *Materials and Methods*, but with a varying opening rate. Thus, decreasing the opening rate increased the EC_{50} and depressed the maximum current. The filled symbols in Fig. 5 plot the experimental EC_{50} values and maxima (normalized to GABA) for the different agonists. TACA, GABA, muscimol, and perhaps I4AA fall along this theoretical line, suggesting that an alteration in the gating kinetics, with a fixed and identical affinity, could account for the observed differences in the EC_{50}. Clearly, the proposed scheme with an agonist affinity identical with that of GABA cannot account for CACA and isoguvacine. The most straightforward interpretation is that these two agonists have a much lower affinity for the receptor. The dashed line on the right is the predicted relationship between EC_{50} and the maximum using the same rate constants as for GABA, but the binding affinity was 75-fold less ($k_{on} = 1.3 \times 10^3\ M^{-1}s^{-1}$). In sum, our results have shown that the six different agonists fall into two affinity classes at ρ1 homomeric $GABA_C$ receptors. Because agonists within a class have a similar apparent affinity, the difference in the EC_{50} values relates to the ability of the agonists to activate the receptor once bound.

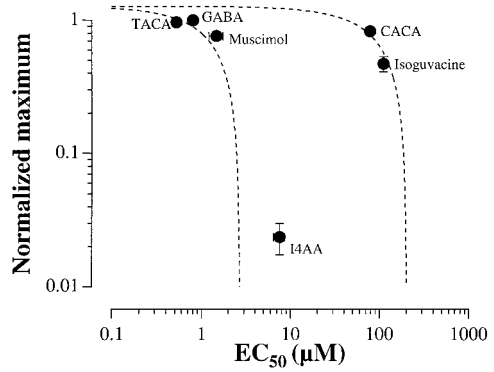


Fig. 5. Relationship between the GABA-activated current (normalized to the maximum for GABA) and the experimentally observed EC_{50} values. The dashed lines represent the predicted relationship between the maximum current and the EC_{50} using the rate constants and three-bind-to-open kinetic model provided under *Materials and Methods*. The dashed lines were produced by holding all rate constants fixed and varying only the opening rate (β). The shift along the abscissa for the two dashed lines results from the different forward binding rates. The predicted EC_{50} and maximum current for each set of parameters was determined by using a Q-matrix algorithm to generate a dose-response relationship, which was then fitted with eq. 1 (Colquhoun and Hawkes, 1981; Chang and Weiss, 1999). The dashed lines were generated by holding all rates fixed except for β (opening rate), which was varied to produce the relationship between maximum activatable current and EC_{50}. The maximum for GABA is plotted as one, which is not the efficacy because the maximum open probability for GABA is ≈0.92 (Chang and Weiss, 1999).

Although we have determined the K_i values for *these* agonists, we have used the term "apparent K_i values" throughout to distinguish this parameter from the theoretical "true" dissociation constant, which is the ratio of the off rate to the on rate for agonist. This is because the measurement of the binding affinity is influenced by the gating, or opening and closing, of the pore (Colquhoun, 1998; Chang and Weiss, 1999). The extent to which the gating will influence the binding will vary for the different agonists as these agonists certainly differ in their ability to gate the receptor. This differential efficacy of the agonists would also affect the observed K_i values as determined by the Cheng-Prusoff correction (eq. 3) and therefore the specific K_i values must be interpreted with caution. In a previous study, based on a simple activation mechanism, we were able to determine the extent to which the gating influenced the binding measurements (and vice versa) using GABA as the agonist. Although one would like to repeat this type of analysis for the other agonists, obtaining these compounds in a radiolabeled form is cost-prohibitive (Chang and Weiss, 1999).

Structure/Activity Relationship for the Agonists. A conformational analysis to explain the structure/activity relationships of these GABA agonists for ρ1 receptors has been published (Kusama et al., 1993; Chebib and Johnston, 2000). Using the nomenclature of Kusama et al. (1993), the high affinity agonists GABA, TACA, muscimol, and I4AA are in extended planar conformations when bound to ρ1 receptors (Fig. 6A). The lower affinity agonist CACA is in a folded planar conformation and isoguvacine is in a nearly planar conformation (Fig. 6B). For the un-ionized forms of the agonists shown in Fig. 6, the distances from the nitrogen atom in the amino or imidazole group (I4AA) to the carbon atom in the carboxyl or isoxazole group (muscimol) vary between 4.29 and 5.02 Å. For the high affinity agonists, the C—N distance varies from 4.63 to 5.02 Å. For the low affinity agonists, the C—N distance varies from 4.29 to 4.51 Å. Thus, the high affinity agonists have a larger C—N distance than the low affinity agonists.

In Fig. 6, C and D, TACA is superimposed on muscimol and I4AA, respectively. Part of the imidazole ring of I4AA, which has high affinity but low efficacy, occupies a unique region of space that is not occupied by the agonists TACA and muscimol. One possibility is that this region of space is accessible when I4AA binds to the unopened conformation of the receptor, but inaccessible when the receptor is in the open conformation (i.e., the channel cannot open unless parts of the receptor are allowed to move into the space occupied by part of the imidazole ring of I4AA). This explanation also accounts for the antagonist actions of I4AA.

The pK_a of the imidazole group of I4AA is 7.46 (Bowery and Jones, 1976). Hence, at pH 7.4 the imidazole ring of I4AA is ~50% protonated, possibly only ~50% of I4AA is binding to the ρ1 receptor, and the affinity of I4AA may be underestimated by about 2-fold. Additionally, the delocalization of the positive charge over the imidazolium ring could affect the affinity and efficacy of I4AA.

Isoguvacine is a conformationally constrained molecule. The double bond in the six-membered ring of isoguvacine and its conjugation with the carboxylic acid group greatly flattens the ring. Although isoguvacine does not mimic the conformation of CACA in the folded conformation, C—N distances for these molecules are similar and the structures are easily

superimposed as shown in Fig. 6E. We hypothesize that it is the short C—N distance found in these molecules that is responsible for the lower binding affinity of these compounds. As discussed previously (Kusama et al., 1993), steric hindrance caused by parts of the heterocyclic ring of isoguvacine may also contribute to the low affinity of isoguvacine.

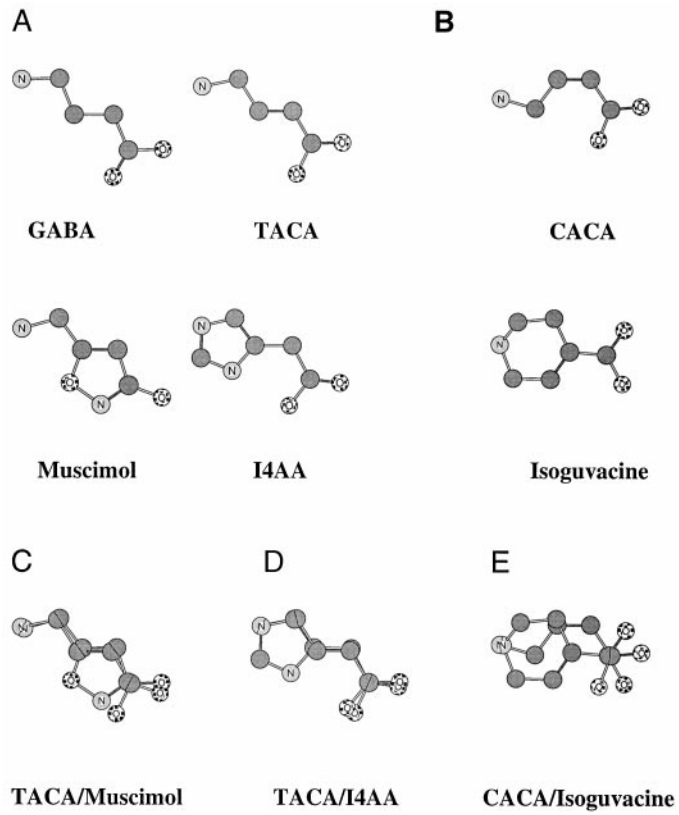


Fig. 6. Three-dimensional structures of GABA agonists. The conformations of the structures shown were generated using the CS Chem 3D Pro molecular modeling software from CambridgeSoft Corp., Cambridge, MA. For clarity, hydrogen atoms are not shown. A, extended conformations of GABA, TACA, muscimol, and I4AA. The distance between the amino group nitrogen and the carboxyl group carbon in GABA (C—N distance) is 5.02 Å. The distances between the corresponding atoms in TACA, muscimol, and I4AA are 4.90, 4.63, and 4.83 Å, respectively. B, folded conformation of CACA and isoguvacine conformation. The C—N distances are 4.51 and 4.29 Å, respectively. C, superimposition of muscimol on TACA. The intermolecular distances between the superimposed nitrogen and carbon atoms of TACA (starting at N and ending with the carboxyl C) and the corresponding atoms in muscimol are 0.14, 0.11, 0.22, 0.25, and 0.13 Å, respectively. D, a superimposition of I4AA on TACA. The intermolecular distances, as defined in panel C, for the TACA/I4AA superimposition are 0.03, 0.09, 0.21, 0.18, and 0.03 Å, respectively. Part of the imazole ring of I4AA occupies a region of space that is unoccupied by TACA and muscimol. E, a superimposition of CACA and isoguvacine. The intermolecular distances, as defined in panel C, for the TACA/I4AA superimposition are 0.11, 1.69, 0.49, 1.35, and 0.11 Å, respectively.

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