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Kinetic Properties of $\alpha_1\beta_1$ γ-Aminobutyric Acid$_A$ Receptor Channels Expressed in Chinese Hamster Ovary Cells: Regulation by Pentobarbital and Picrotoxin

NADA M. PORTER,[1] TIMOTHY P. ANGELOTTI, ROY E. TWYMAN,[2] and ROBERT L. MACDONALD

Departments of Neurology (N.M.P., R.E.T., R.L.M.), Physiology (R.L.M.), and Pharmacology (T.P.A.), University of Michigan Medical Center, Ann Arbor, Michigan 48104

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SUMMARY

Single-channel recordings from excised outside-out patches were obtained from Chinese hamster ovary cells stably transfected with plasmids containing bovine γ-aminobutyric acid (GABA) type A (GABA$_A$) receptor channel α_1 and β_1 subunit cDNAs. The predominant or main conductance level recorded had a 17-pS chord conductance. There were minor contributions made from 25-pS and 11-pS conductance levels. Average open duration, burst duration, and openings/burst did not change as the GABA concentration was increased from 5 to 25 μM. However, opening frequency increased from 11.0 to 19.5 openings/sec. Pentobarbital increased average channel open duration without increasing opening frequency, whereas picrotoxin slightly reduced average channel open duration and reduced opening frequency. Open duration frequency distributions were fitted best with the sum of two exponential functions, suggesting that the $\alpha_1\beta_1$ GABA$_A$ receptor channel had at least two open states. The time constants and relative proportions of the two components did not vary when GABA concentration was increased from 5 to 25 μM. Closed duration distributions of closures between main conductance level openings were fitted best with multiple exponential functions, suggesting that the $\alpha_1\beta_1$ GABA$_A$ receptor channel had several closed states. Burst duration frequency distributions were fitted best with two exponential functions whose time constants and relative proportions did not change with GABA concentration. A gating kinetic scheme for the $\alpha_1\beta_1$ GABA$_A$ receptor channel was proposed that consisted of a single binding site for GABA and at least two open and five closed states. The kinetic properties of the $\alpha_1\beta_1$ main conductance level differed from those of the spinal cord neuron (native) 27-pS main conductance level and the 19-pS subconductance level. The native main conductance and subconductance levels were characterized by longer openings and at least three open states. Based on the aforementioned observations, it appears that different subunit combinations produce receptor channels with different kinetic properties, but the basic mechanism of regulation by pentobarbital and picrotoxin may be similar for the different receptor channels. Also, it is unlikely that the 19-pS substate of the native GABA$_A$ receptor is produced by an $\alpha_1\beta_1$ dimer.

The mammalian GABA$_A$ receptor, a chloride channel, has structural features similar to those of other ligand-gated ion channels (1). Like the nicotinic acetylcholine receptor (2), it is composed of multiple subunits. At least five different subunit families (α, β, γ, δ, and ρ) have been isolated (1, 3–5). Similar to the neuronal nicotinic cholinergic receptor, multiple cDNAs encoding various isoforms of these subunits have been isolated (6–10). The large number of subunit components suggests that GABA$_A$ receptors may exist *in vivo* in multiple forms.

Studies to date have shown that functional GABA$_A$ receptors are obtained when α and β subunits are expressed in *Xenopus* oocytes (1, 6–8, 11–14) or in various cell lines (15–18). These receptors displayed some of the pharmacological properties typical of GABA$_A$ receptors. Barbiturates enhanced the GABA$_A$ receptor-evoked chloride current and convulsants such as bicuculline or picrotoxin reduced the response. This combination of subunits, however, lacked sensitivity to benzodiazepines. The γ subunit has been shown to be necessary to confer benzodiazepine sensitivity (3). Interestingly, different α subunits modified the benzodiazepine sensitivity and subtype of GABA$_A$ receptor (9, 19).

Homomeric receptors consisting of either α, β, or γ subunits also have been expressed, albeit with lower efficiency, and displayed some of the electrophysiological and pharmacological

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[1] Present address: University of Kentucky, Department of Pharmacology, MS-315 UKMC, Lexington, KY 40536-0084.
[2] Present address: University of Utah, Salt Lake City, UT 84148.

ABBREVIATIONS: GABA$_A$ receptor, γ-aminobutyric acid type A receptor; CHO, Chinese hamster ovary; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

characteristics of GABA$_A$ receptors (4, 15, 20). The maximal response elicited from homomeric receptors was typically much smaller than responses elicited from cells in which at least two subunits were present. Nonetheless, these results suggest that functionally distinct GABA$_A$ receptors can result from different subunit assembly patterns. Support for the notion of distinct subclasses of GABA$_A$ receptors comes from *in vivo* and *in vitro* pharmacological studies and from recent *in situ* hybridization studies examining the localization of mRNAs for different GABA$_A$ receptor subunits in brain. These studies have shown that the subunits and their variants display a heterogeneous distribution in the brain (4, 7, 13, 21, 22). The α_1 subunit appears to be the most widely distributed and abundant subunit in mammalian brain (21). Among other structures, it is found in relatively high levels in the cortex, hippocampus, and cerebellum. Although the β_1 subunit is not as abundant in the brain as the β_2 and β_3 subunits, it is co-localized with the α_1 subunit in some cortical and hippocampal regions (13, 22).

Recently, the α_1 and β_1 subunits have been stably expressed in CHO cells (17). Single-channel patch-clamp recordings from these cells showed that the $\alpha_1\beta_1$ receptor currents were different from GABA$_A$ receptor currents recorded from mouse spinal cord neurons in culture. In spinal cord neurons, the predominant or main conductance level of the GABA$_A$ receptor channel was 27–30 pS (23, 24). However, in CHO cells that stably expressed α_1 and β_1 subunits, the main conductance level of the GABA$_A$ receptor channel was smaller (about 19 pS) (17). This suggested that different combinations of GABA$_A$ receptor channel subunits may produce different conductance levels. Because the α_1 and β_1 subunits are co-localized in certain brain regions, this combination of subunits may represent a form of the GABA$_A$ receptor found in the central nervous system.

There is limited information available on the kinetic properties of channel gating for recombinant receptor channels. Thus, the present study was undertaken to characterize the single-channel kinetic and pharmacological properties of the recombinant $\alpha_1\beta_1$ GABA$_A$ receptor channel. A kinetic model describing the gating properties of the main conductance level of the recombinant $\alpha_1\beta_1$ receptor channel GABA$_A$ was developed based on the kinetic analysis.

Materials and Methods

Cell culture. The stably transfected CHO cell line was obtained from Professor Eric Barnard (Medical Research Council, Cambridge, UK) and was maintained under selective pressure in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, 2 mM glutamine, 250 μg/ml xanthine, 5 μg/ml mycophenolic acid, 1× hypoxanthine/aminopterin/thymidine supplement (100 μM hypoxanthine, 400 nM aminopterin, 16 μM thymidine), and 50 units/ml penicillin/streptomycin (17). All media and supplements were obtained from GIBCO. Cells were grown at all times in 5% CO_2/95% air at 37°. In preparation for electrophysiological recordings, cells were split with trypsin/EDTA (0.5%/0.8 mM; Sigma), plated at a density of 500 cells/35-mm tissue culture plates (Corning) in 1 ml of the medium described above, and allowed to recover overnight before induction of GABA$_A$ receptor expression. GABA$_A$ receptor expression was induced in the cell line by exposure to 2 μM dexamethasone (Sigma) for 36–48 hr (a 1/500 dilution of a 1 mM ethanol stock solution into the growth medium).

Solutions and GABA application. All drugs were obtained from Sigma. The medium used to grow and maintain the cultures was exchanged for 2 ml of extracellular salt solution, which consisted of the following (in mM): 142 NaCl, 8.1 KCl, 1 $CaCl_2$, 6 $MgCl_2$, 10 glucose, and 10 Na-HEPES (pH ~7.4). The intrapipette solution contained (in mM) 153 KCl, 1 $MgCl_2$, 10 K-HEPES, and 5 EGTA (pH ~7.4). This combination of extracellular and intrapipette solutions resulted in a chloride equilibrium potential (E_{Cl}) of about 0 mV and a potassium equilibrium potential (E_K) of −75 mV across the patch membrane. All experiments were performed at room temperature (20–23°).

GABA was diluted with extracellular solution from a 1 mM stock solution to a final concentration of 5 or 25 μM on the day of the experiment. Pentobarbital (50 μM) and picrotoxin (1 μM) were prepared on the day of the experiment and diluted with extracellular solution. GABA or GABA and drug were applied to the patch membrane via pressure ejection micropipettes, which were moved adjacent to patches only during the time of GABA or GABA and drug application.

Current recording techniques. Recording techniques were similar to those previously described (24, 25). Excised outside-out patches held at −75 mV were obtained using a model L/M EPC-7 amplifier (LIST Medical Instruments, Darmstadt, Germany) and were simultaneously recorded on a video cassette recording system (SONY SL-2700, modified to 0–20 kHz) via a digital audio processor (SONY PCM-501 ES, 14-bit, 44 kHz) and a chart recorder (Gould Inc., Cleveland, OH). At a later time, the data were played back from the video cassette recorder and digitized (20-kHz sampling rate, 14-bit/0.024-pA resolution, 2-kHz low-pass eight-pole Bessel filter interposed). Current amplitudes and durations were determined by computer using software previously described (24). Openings and closings were detected using the 50% threshold-crossing method and were accepted as valid events if their durations were greater than twice the measured system rise time (rise time = 130 μsec).

Single-channel analysis. Durations of openings, closings, and bursts were placed in either linear or logarithmic bins and plotted in appropriate frequency histograms to minimize bin promotion errors (26). For linear histograms, open durations were binned into 0.5-msec bins with a range of 0.4 to 30 msec and burst durations were binned into 0.5-msec bins with a range of 0.5 to 50 msec. Logarithmic binning used a logarithmic time axis and a square-root ordinate transformation (27). For logarithmic histograms, open durations were binned into 50 bins, with 25 bins/decade resolution and a 400-μsec lower limit. Closed durations were binned using 50 bins and 10 bins/decade resolution, with a 300-μsec lower limit. All event duration histograms were fitted from bins starting from at least twice the system rise time. Curve fitting of histograms was performed as previously described (24, 28). The method of maximum likelihood was used for nonlinear curve fitting of all frequency histograms (29). Error ranges of components were determined by likelihood intervals (m = 2 to approximate 95% confidence levels) (24). The number of significant exponential components was determined by fitting with increasing numbers of exponentials until 1) the χ^2 of the estimated fit and the data were within the 95% confidence interval for accepting the null hypothesis (no difference between the estimated fit and data) and/or 2) the maximum likelihood estimate was no longer improved greatly by the addition of additional exponential components (30–33).

Measured open and closed durations are generally longer than "true" open and closed durations due to undetected closings and openings. Throughout the text the terms "open and closed durations" will refer to measured durations that have not been corrected for unobserved transitions (26). However, mean open durations can be corrected for missed openings by obtaining exponential fits of the open duration frequency distributions and determining mean open durations from the resultant exponential functions. Corrected average open duration was calculated by taking the sum of the relative area of each exponential component in the open duration histogram multiplied by the time constant of the component (corrected average open duration = $a_1\tau_1 + a_2\tau_2$). These "corrected" open durations do not, however, provide correction for missed closures. Similarly, mean closed durations can be corrected for missed closures but not for missed openings. Throughout the text the terms "corrected mean open and closed durations" will

refer to mean open and closed durations that have been corrected for missed open and closed durations, respectively.

Bursts may be defined as openings or groups of openings separated by relatively long closed periods (29). For the purpose of this analysis, a critical closed time, t_c, was chosen such that all openings separated by closures less than t_c belonged within a burst, and bursts were separated by closures greater than t_c. A modification of the equal proportion of misclassification method of Colquhoun and Sakmann (30) was used to select a t_c of 5 msec (24), which resulted in missclassification of <5% of closures. Burst durations can be corrected for missed brief bursts. Corrected average burst duration was calculated by multiplying the sum of the relative area (a) of each exponential component in the burst duration histogram by the time constant (τ) of the component (corrected average burst duration = $a_1\tau_1 + a_2\tau_2$).

Results

$\alpha_1\beta_1$ $GABA_A$ receptor single-channel currents. GABA evoked single chloride currents from 40% (28 of 70 patches) of excised outside-out patches from transfected CHO cells (Fig. 1). No spontaneous currents were observed in patches from transfected CHO cells. At −75 mV at least three current levels were recorded, but one current level (1.3 pA) was predominant. Current levels that were larger (1.9 pA) and smaller (0.8 pA) were recorded infrequently. The single-channel currents reversed at the chloride equilibrium potential (0 mV). Channel chord conductances were 17 pS for the predominant current level and 25 pS and 11 pS for the less frequent current levels. Openings to the main conductance level accounted for 85% of the openings, whereas openings to the larger and smaller conductance levels each accounted for only 7.5% of the openings.

GABA (5 μM) evoked complex currents (Fig. 1A) that were often brief and composed of single openings or were prolonged and consisted of a burst of sequential openings and closings. Concentration-dependent increases in channel opening and burst frequencies were recorded as GABA was increased from 5 to 25 μM (Fig. 1A). Average corrected channel open duration did not change when GABA concentration was increased (Table 1). However, opening frequency increased from 10 to 19.5 sec^{-1} (Table 1). As a result of the increase in opening frequency, the percentage of time the channel spent in the open state increased from 1.7 to 3.3%, and the total average current evoked per patch increased from 22 to 44 fA. Consistent with the increase in opening frequency produced by the higher concentration of GABA, the average time the channel spent in the closed state was reduced.

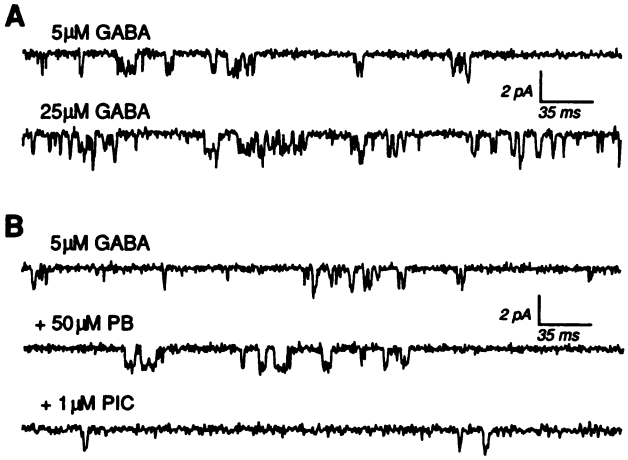


Fig. 1. A, Increasing the GABA concentration from 5 to 25 μM increased the frequency of single-channel currents recorded from excised outside-out patches obtained from CHO cells stably transfected with $GABA_A$ receptor α_1 and β_1 cDNAs. *Tracings*, samples of channel activity selected to demonstrate overall behavior of the evoked responses. B, Pentobarbital (*PB*) (50 μM) increased and picrotoxin (*PIC*) (1 μM) decreased average open channel duration. Data in A and B were from different patches.

Pentobarbital and picrotoxin regulation of $\alpha_1\beta_1$ receptor single-channel currents. Pentobarbital and picrotoxin both altered $GABA_A$ receptor currents recorded from patches obtained from transfected CHO cells (Fig. 1B). Neither pentobarbital nor picrotoxin affected the amplitude of GABA (5 μM)-evoked single-channel currents.

Pentobarbital (50 μM) prolonged GABA receptor single-channel currents, increasing the corrected average channel open duration from 1.2 to 2.4 msec (Table 1). Interestingly, pentobarbital reduced channel opening frequency from 10 to 5.5 sec^{-1} without changing the average percentage of time open or the total average current evoked per patch (Table 1). Consistent with the reduced opening frequency, a 2-fold increase in the average channel closed duration was observed in the presence of pentobarbital.

Picrotoxin (1 μM) decreased the average GABA receptor single-channel currents (Fig. 1B). Picrotoxin reduced average open duration slightly from 1.7 to 1.5 msec but did not reduce corrected average open duration. Picrotoxin reduced channel opening frequency from 10 to 1.7 sec^{-1}, percentage of time open from 1.7 to 0.3%, and the total average current evoked per patch from 22 to 3 fA (Table 1). Consistent with the reduced opening frequency, picrotoxin increased the average channel closed duration.

Stationarity of $\alpha_1\beta_1$ $GABA_A$ receptor currents. Single-channel currents typically vary with time (34). The stationarity of single-channel activity is of particular importance when inferences about the kinetic behavior of the channel are made. Several single-channel parameters were examined over time to determine the stability of the channel kinetic properties (Fig. 2). For patches exposed to either 5 or 25 μM GABA, time intervals were divided into four consecutive 5-sec epochs, beginning 1 sec after onset of GABA application. The average channel open duration, burst duration, opening frequency, and the percentage of time spent in the open state were measured for each 5-sec epoch.

The average channel open and burst durations remained constant over this interval for both concentrations of GABA (Fig. 2). In contrast, channel opening frequency and the percentage of time spent in the open state decreased with time (Fig. 2). The rate of decrease for these two parameters was greater with the higher concentration of GABA. A reduction in opening frequency and the percentage of time in the open state may reflect a decrease in the number of active channels in a patch (34, 35). Channels may become desensitized and, thus, a reduction in channel opening frequency would be observed.

Because bursts usually represent the activity of a single ion channel, the lack of a reduction over time in burst durations suggests that the activity of the channels remaining in the patch was stable. Further evidence for the stationarity of the remaining channels is the stability of the channel open durations over the interval tested. These observations suggest that the data were suitable for kinetic analysis of open and burst durations.

Frequency distributions of open durations. The fre-

TABLE 1

$\alpha_1\beta_1$ GABA$_A$ receptor channel open and closed properties

Open and closed properties of the $\alpha_1\beta_1$ GABA$_A$ receptor channel main conductance level are given. Openings per second, average and corrected average open duration, average current, percentage of analysis time open, average closed duration, and number of openings were derived from detected openings (see Materials and Methods).

	5 μM GABA	25 μM GABA	5 μM GABA + 50 μM phenobarbital	5 μM GABA + 1 μM picrotoxin
Average (corrected) open duration (msec)	1.7 (1.2)	1.7 (1.3)	2.9 (2.4)	1.5 (1.2)
Opening frequency (sec^{-1})	10.0	19.5	5.5	1.7
Average percentage open	1.7	3.3	1.6	0.3
Total average current (fA)	22	44	22	3
Average closed duration (msec)	81.0	42.5	161.0	483.5
Number of applications	25	10	11	5
Number of openings	10,285	9,251	5,868	626

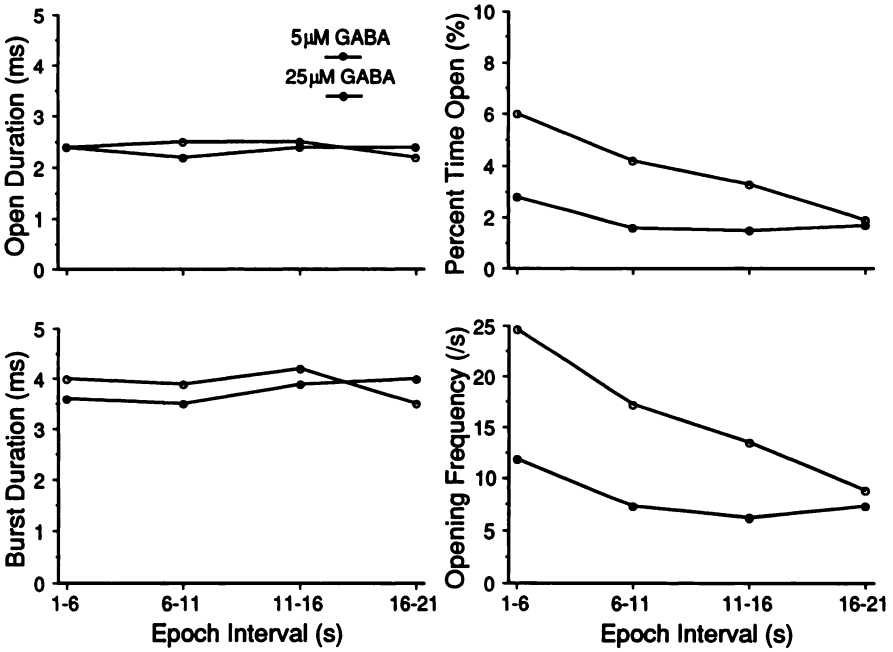


Fig. 2. The properties of the $\alpha_1\beta_1$ GABA$_A$ receptor main conductance level were plotted as a function of GABA application time for two GABA concentrations (5 and 25 μM). Average open and burst durations were stationary, whereas the percentages of time the channel was open (percentage of time open) and the opening frequencies declined with time. Data were analyzed in four consecutive 5-sec epochs from 1 sec after the beginning of agonist application.

quency distributions of open durations produced by the application of GABA (5 and 25 μM) were obtained by pooling open durations from several patches at each concentration. Results from the analysis of individual patches containing large numbers of openings were similar to results from pooled data. For both GABA concentrations, the open duration distributions were similar, consistent with a lack of change in the average open duration observed as the concentration of GABA was increased (Fig. 3A).

The open duration frequency distributions were fitted to sums of exponential functions. For each GABA concentration, a sum of two exponential functions was required to fit best each of the distributions. Exponential components were designated 1 and 2 and corresponded to the functions with the shortest and longest time constants, respectively. The time constants (τ_i) for each component overlapped for both concentrations of GABA and averaged 1.0 and 2.8 msec (Fig. 3B, *top*). The relative areas (a_i) for each component represented the relative frequency of occurrence of the components. For both concentrations of GABA, the relative areas of the open duration distributions represented by components 1 and 2 did not change and averaged 88% and 12%, respectively (Fig. 3B, *bottom*).

When pentobarbital (50 μM) was applied with GABA (5 μM), the open duration frequency distribution was shifted to longer open durations and fitted best by the sum of two exponential functions (Fig. 3A). The shift in the distribution to longer channel open durations was produced primarily by an increase in the relative frequency of occurrence of longer openings (a >3-fold increase in the area of the second component, a_2, from 12% to 39%) (Fig. 3B, *bottom*). In addition, there was a small increase in both time constants and their error ranges did not overlap those found for 5 μM GABA (Fig. 3B, *top*).

When picrotoxin (1 μM) was applied with GABA (5 μM), the open duration frequency distribution was shifted slightly to shorter open durations and was fitted best with only one exponential function (Fig. 3A). The shift in the distribution to shorter open durations was due primarily to an increase in the relative frequency of occurrence of shorter openings (an increase in the area of the first component, a_1, from 88% to 100%) (Fig. 3B, *bottom*). A small decrease in the first exponential function time constant from 1.0 to 0.9 msec was also observed (Fig. 3B, *top*).

Frequency distributions of closed durations. Closed duration frequency distributions were obtained from pooled data. As the concentration of GABA was increased, a shift in the closed duration distribution to shorter closed durations was observed (Fig. 4). Pentobarbital and picrotoxin both shifted the closed duration distributions to longer closed durations (Fig. 4); however, the effect of picrotoxin was much greater.

For each GABA concentration (5 and 25 μM), a sum of five

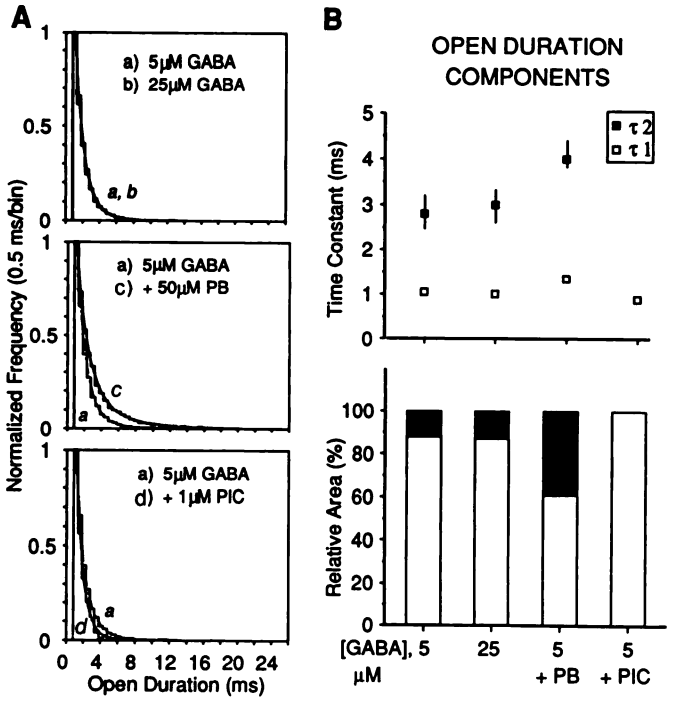


Fig. 3. A, Open duration frequency histograms were obtained for single $\alpha_1\beta_1$ $GABA_A$ receptor channel currents evoked by 5 μM GABA (*a*), 25 μM GABA (*b*), 5 μM GABA with 50 μM pentobarbital (*PB*) (*c*), and 5 μM GABA with 1 μM picrotoxin (*PIC*) (*d*). Open durations were put into linear 0.5-msec bins and displayed for clarity over a range of 1–25 msec. All open duration frequency distributions were fit by the sum of two exponential functions, which were superimposed on the open duration frequency histograms. B, Time constants (*top*) and relative areas (*bottom*) for the two exponential functions for each open duration frequency histogram were unchanged by increasing GABA concentration but were altered by pentobarbital and picrotoxin.

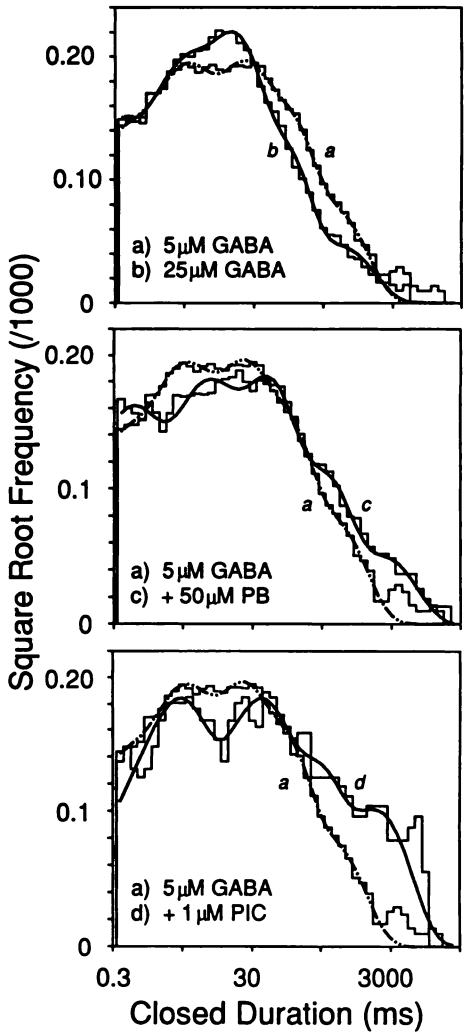


Fig. 4. Closed duration frequency histograms were obtained for single $\alpha_1\beta_1$ $GABA_A$ receptor channel currents evoked by 5 μM GABA (*a*), 25 μM GABA (*b*), 5 μM GABA with 50 μM pentobarbital (*PB*) (*c*), and 5 μM GABA with 1 μM picrotoxin (*PIC*) (*d*). Closed durations were put into logarithmic bins (see Materials and Methods) and displayed for clarity over a range of 0.3 msec to 30 sec. Logarithmic histogram distributions were plotted showing square-root transformed ordinate values (see Materials and Methods). Histograms were fitted best with sums of five exponential functions, and curves were drawn according to the fits (see text).

or six exponential functions was required to fit best each of the closed duration distributions (Fig. 5). As the concentration of GABA was increased, a reduction in the three longer time constants (τ_3, τ_4, and τ_5) was observed. The two shortest time constants (τ_1 and τ_2) did not change significantly with increasing GABA concentration. It should be mentioned that an additional sixth, very long, time constant (2 sec) was detected in the presence of 25 μM GABA. However, it represented only 0.5% of the total closed duration distribution and thus was not considered in further analyses. Small (<15%) changes in the relative areas of the closed duration time constants were observed with an increase in GABA concentration (Fig. 5).

In the presence of GABA and pentobarbital, the closed duration frequency distribution was also fitted best with the sum of five exponential functions. The shift of the closed duration distribution to longer closed durations with pentobarbital was due to an increase in the longer closed time constants (τ_2 through τ_5) (Fig. 5).

In the presence of picrotoxin and GABA, the closed duration frequency distribution was fitted best with only four exponential functions (Fig. 5). No time constant below 1 msec (τ_1; Fig. 5) was detected in the presence of picrotoxin as was found when GABA was applied alone or when GABA was coapplied with pentobarbital. The shift in the closed duration distribution to longer closed durations with picrotoxin was due to an increase in time constants τ_3 through τ_5. The relative areas of the closed duration exponential functions were altered only slightly by pentobarbital and picrotoxin. The significance of these small changes in relative areas is unclear.

Burst properties. Bursts were defined as one or more openings separated by closings greater than a critical time of t_c. A value for t_c was calculated for each closed duration analysis (see Materials and Methods) and determined between the second and the third time constants in the closed duration distributions for each concentration. A t_c of 5.0 msec was used for the burst analyses.

As the concentration of GABA was increased from 5 to 25 μM, average corrected burst duration increased slightly from 3.4 to 3.6 msec, whereas burst frequency increased substantially from 6.1 to 11.5 sec^{-1}. The percentage of analysis time spent in bursts increased from 2.8 to 5.5% (Table 2). The average percentage of intraburst open duration, number of openings per burst, and intraburst closed duration did not change as the GABA concentration was increased. The average interburst closed duration decreased by about 50%.

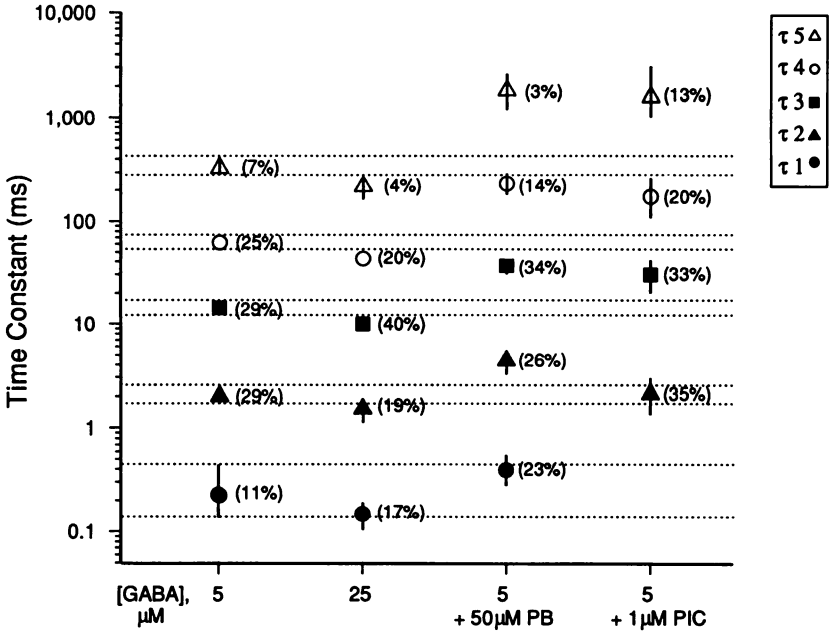


Fig. 5. Closed duration time constants and relative areas for GABA and for GABA with pentobarbital (*PB*) or picrotoxin (*PIC*) for exponential fits to closed duration frequency histograms are presented. Respective areas for each time constant are indicated in parentheses.

TABLE 2

$\alpha_1\beta_1$ GABA$_A$ receptor channel burst properties

Burst properties of the $\alpha_1\beta_1$ GABA$_A$ receptor channel main conductance level are given. The parameters listed were derived from detected bursts and openings and closings within bursts (see Materials and Methods). Bursts were separated by closures of >5 msec. Average interburst closed duration refers to the average closed duration between bursts containing only main conductance openings. The average percentage of intraburst open duration was calculated by taking the percentage of the total open duration in a burst divided by the total duration in a burst (total open duration plus total intraburst closed duration).

	5 μM GABA	25 μM GABA	5 μM GABA + 50 μM phenobarbital	5 μM GABA + 1 μM picrotoxin
Average (corrected) burst duration (msec)	3.9 (3.4)	4.1 (3.6)	5.7 (5.4)	2.9 (2.0)
Burst frequency (sec^{-1})	6.1	11.5	3.3	1.2
Average percentage of intraburst open duration	71.2	70.6	83.7	71.4
Percentage of analysis duration in bursts	2.8	5.5	2.1	0.4
Average number of openings/burst	1.6	1.7	1.7	1.4
Average intraburst closed duration (msec)	1.8	1.7	1.4	1.9
Average interburst closed duration (msec)	136	71	266	698
Number of bursts	6290	5449	3528	435

Pentobarbital increased the corrected average burst duration from 3.4 to 5.4 msec but decreased burst frequency from 6.1 to 3.3 sec^{-1} (Table 2). The average percentage of intraburst open duration was increased from 71.2 to 83.7%, but the average percentage of analysis time spent in bursts was reduced from 2.8 to 2.1%. The average number of openings per burst increased slightly from 1.6 to 1.7, and the average intraburst closed duration decreased from 1.8 to 1.4 msec. Consistent with these observations, the mean interburst closed duration increased approximately 2-fold.

In contrast to pentobarbital, picrotoxin reduced the corrected average burst duration from 3.4 to 2.0 msec (Table 2). Burst frequency was reduced from 6.1 to 1.2 sec^{-1}. The average percentage of intraburst open duration was unchanged, but the average percentage of analysis time spent in bursts was reduced from 2.8 to 0.4%. The average number of openings per burst decreased slightly from 1.6 to 1.4. The average intraburst closed duration increased slightly from 1.8 to 1.9 msec. Consistent with the actions of picrotoxin to reduce GABA$_A$ receptor currents, the average interburst closed duration increased approximately 5-fold.

Burst duration frequency distributions were similar for both GABA concentrations and were fitted best with the sum of two exponential functions (Fig. 6). Exponential components 1 and 2 represented bursts with the shortest and longest time constants, respectively (Table 3). The time constants of the exponential functions did not vary with GABA concentration and averaged 0.66 and 5.5 msec for the two components. As GABA concentration increased, the relative proportion of the time constants did not change, averaging 0.41 and 0.59, respectively (Table 3).

In the presence of pentobarbital, the burst duration frequency histogram was shifted to longer burst durations and was fitted best with two exponential functions (Fig. 6). The shift in the distribution to longer burst durations was produced by an increase in both exponential function time constants, from 0.7 to 1.1 and from 5.2 to 7.2 msec, and by an increase in the relative frequency of occurrence of longer bursts (an increase in a_2 from 0.57 to 0.70) (Table 3).

In the presence of picrotoxin, the burst duration frequency histogram was shifted to shorter burst durations and was fitted with two exponential functions (Fig. 6). The shift in the distribution to shorter burst durations was produced by a decrease in the relative frequency of occurrence of longer bursts (a_2 decreased from 0.61 to 0.48) and a reduction in the longer exponential function time constant from 5.2 to 3.6 msec (Table 3).

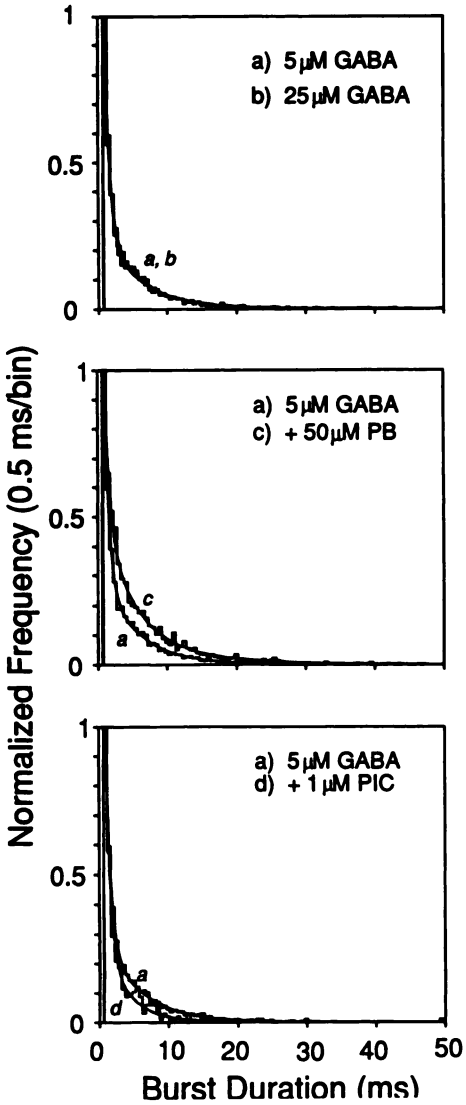


Fig. 6. Burst durations of single-channel $\alpha_1\beta_1$ receptor currents evoked by 5 μM GABA (*a*), 25 μM GABA (*b*), 5 μM GABA with 50 μM pentobarbital (*PB*) (*c*), or 5 μM GABA with 1 μM picrotoxin (*PIC*) (*d*) were placed in frequency histograms. Burst durations were put into 0.5-msec bins and displayed for clarity over a range of 1–50 msec. Histograms were fit best with the sums of two exponential functions, which were superimposed on the histograms.

Discussion

$\alpha_1\beta_1$ $GABA_A$ receptor channel conductance levels. The main conductance level for the stably expressed $\alpha_1\beta_1$ $GABA_A$ receptor channel was about 17 pS, with minimal contribution of 11 and 25 pS levels. In contrast, Levitan *et al.* (11) expressed the bovine $\alpha_1\beta_1$ subunits in *Xenopus* oocytes and reported a main conductance level of 28 pS, similar to the native $GABA_A$ receptor in spinal neurons (24, 36); they also reported the presence of four conductance levels (9, 18, 28, and 44 pS). The basis for this discrepancy is unclear. It is possible that post-translational modification of the receptor may occur either in *Xenopus* oocytes after mRNA injection or in CHO cells after induction with dexamethasone. Alternatively, processing of the receptor might be different in the two cell types. Combinations of differing $\alpha_1\beta_1$ subunit stoichiometries might be expressed in the different cell types to form receptors with different electrophysiological properties. Our results are similar to those of Puia *et al.* (37), who reported that the human $\alpha_1\beta_1$ $GABA_A$ receptor channel had a reduced main conductance level (20 pS). Interestingly, rat $\alpha_1\beta_2$ subunits transiently expressed in human kidney cells had an 11-pS main conductance level (18).

The main conductance level of the native spinal cord $GABA_A$ receptor channel has been shown to be 27 pS, whereas the 19-pS state was the most frequent subconductance level (24, 36). It has been suggested that $\alpha\beta$ $GABA_A$ receptor subunit combinations in central neurons may be responsible for the formation of subconductance levels (17). However, analysis of the native spinal cord 19-pS subconductance level (34) has suggested that that it may not be functionally equivalent to the $\alpha_1\beta_1$ $GABA_A$ receptor channel, because the two channels had different conductance levels and different kinetic properties.

$\alpha_1\beta_1$ $GABA_A$ receptor channel open properties. The $\alpha_1\beta_1$ $GABA_A$ receptor has been expressed in *Xenopus* oocytes and was activated by a half-maximal GABA concentration of 12 μM (6, 14). We applied GABA at concentrations below and above (5 and 25 μM) the half-maximal concentration and found that there was no change in either mean open duration, open time constants, or relative areas of the two exponential functions fitted to the open duration distributions. Because two open state time constants were found, the results suggested that the $\alpha_1\beta_1$ $GABA_A$ receptor channel has at least two kinetic open states. In addition, the concentration independence of open duration time constants suggests that the two open states were gated open after binding of GABA to the receptor. Hill coefficients obtained in studies of transiently expressed $\alpha_1\beta_1$ $GABA_A$ receptors in *Xenopus* oocytes and mammalian cells were ≤1.0, suggesting that only one agonist molecule is required for full receptor activation (1, 6, 14).[3] Taken together, these results suggest that the $\alpha_1\beta_1$ $GABA_A$ receptor may have a single binding site for GABA and that occupation of the binding site results in opening of the receptor channel to two different open states.

$\alpha_1\beta_1$ $GABA_A$ receptor channel closed properties. Maximum likelihood fitting of channel closed duration distributions revealed at least five exponential functions. In the presence of 25 μM GABA, an additional very long time constant of approximately 2 sec was detected. The contribution of this additional long closed state was minor, representing 0.5% of the total closed duration distribution. Because the number of channels in each patch was unknown and because long closed durations may represent the sum of shut intervals for more than one channel, the significance of long closed durations to the gating of single ion channels is uncertain. It is possible, however, that the appearance of this additional very long closed time constant may represent an entry of the receptor into an agonist-induced desensitized state (38).

Similar to the native receptor, concentration-dependent effects of GABA on the closed durations also were observed. When the concentration of GABA was increased from 5 to 25 μM, the three longest time constants were reduced. This reduction in the closed duration time constants was consistent with the increase in channel opening frequency observed when the concentration of GABA was increased. Because these closed states were affected by altering GABA concentration, they likely represent unbound closed states of the receptor.

The two shorter closed duration time constants (τ_1 and τ_2)

[3] T. P. Angelotti and R. L. Macdonald, unpublished observations.

TABLE 3

$\alpha_1\beta_1$ $GABA_A$ receptor channel burst duration exponential components

Time constants (τ_i) (msec) and relative areas (a_i) of burst duration frequency histograms for the main conductance level of the $\alpha_1\beta_1$ $GABA_A$ receptor are given. The fits were performed on burst duration histograms for bursts evoked by GABA alone (5 and 25 μM) and by GABA (5 μM) in the presence of pentobarbital (50 μM) or picrotoxin (1 μM). Histograms were fitted with sums of two exponential functions in all cases (see Fig. 6 for plots). Components 1 and 2 correspond to exponential functions with the short and long time constants, respectively.

	5 μM GABA	25 μM GABA	5 μM GABA + 50 μM phenobarbital	5 μM GABA + 1 μM picrotoxin
τ_1	0.65 (0.58–0.73)	0.67 (0.60–0.75)	1.05 (0.81–1.36)	0.59 (0.45–0.78)
τ_2	5.2 (4.9–5.6)	5.8 (5.31–6.20)	7.2 (6.3–7.9)	3.6 (2.9–4.4)
a_1	0.39 (0.37–0.42)	0.43 (0.40–0.45)	0.30 (0.26–0.34)	0.52 (0.44–0.60)
a_2	0.61 (0.57–0.64)	0.57 (0.54–0.61)	0.70 (0.63–0.77)	0.48 (0.39–0.57)

did not change with concentration. Similarly, concentration-independent brief closed durations were also seen with the native main conductance level of the $GABA_A$ receptor channel recorded from spinal cord neurons in culture (24, 28). It was suggested that the two brief closed durations represented closures of the open state to two "distal" closed states. Taken together, these results suggest the possibility that the two brief time constants found for the $\alpha_1\beta_1$ receptor represent sojourns in two brief closed states that are distal to the two open states. The remaining components may represent closed states before full agonist binding and before channel opening.

$\alpha_1\beta_1$ $GABA_A$ receptor channel burst properties. Increased concentration of GABA produced an increase in burst frequency without an alteration in average burst duration. Burst duration frequency distributions were fitted best by two exponential functions whose time constants and relative areas were unchanged by concentration. The presence of two different burst duration components suggests the presence of two bursting open states. Because the briefest open and burst duration time constants were similar but the longest burst duration time constant was about 2 times the longest open duration time constant, it is likely that the bursts occurred primarily by the opening of the brief open state on average about one time and of the longer open state on average about two times.

Comparison of $\alpha_1\beta_1$ receptors and the native $GABA_A$ receptor main conductance level. The main conductance level of mouse spinal cord neuron $GABA_A$ receptor channel was larger (27 pS) (24) than the main conductance level of the $\alpha_1\beta_1$ $GABA_A$ receptor channel (17 pS). The kinetic properties of the main conductance level of the $\alpha_1\beta_1$ $GABA_A$ receptor channel were also different from those of the native $GABA_A$ receptor channel from spinal cord neurons (24, 28). For the spinal cord $GABA_A$ receptor channel, mean open duration increased with GABA concentration and three exponential functions were required to fit the open duration distributions. It was concluded that a singly liganded receptor opened to a brief open state but that a doubly liganded receptor opened to two additional open states. The results for the $\alpha_1\beta_1$ $GABA_A$ receptor channel were different. Mean open duration did not increase with GABA concentration and only two exponential functions were required to fit the open duration distributions. It was concluded that the $\alpha_1\beta_1$ $GABA_A$ receptor channel likely bound only one GABA molecule and that the bound receptor channel opened to only two different open states. These results suggest that the conductance level and gating properties of the main conductance level of the $\alpha_1\beta_1$ $GABA_A$ receptor channel were different from those of the main conductance level of the native $GABA_A$ receptor channel. The specific subunit composition of $GABA_A$ receptors in spinal cord neurons in cell culture is not clear, but because these $GABA_A$ receptor currents are enhanced by benzodiazepines, it is likely that they contain a γ subunit as well as α and β subunits. Therefore, the difference between the native $GABA_A$ receptor channel and the $\alpha_1\beta_1$ $GABA_A$ receptor channel expressed in CHO cells likely is due to a difference in the specific subunits forming the channels.

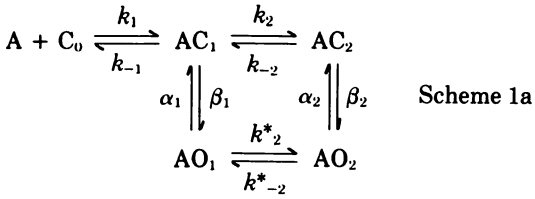
Comparison of $\alpha_1\beta_1$ receptors and the native $GABA_A$ receptor subconductance level. The properties of the $\alpha_1\beta_1$ $GABA_A$ receptor channel were also different from those of the spinal cord subconductance level $GABA_A$ receptor channel (34). The main conductance level of $\alpha_1\beta_1$ $GABA_A$ receptor channel (17 pS) was somewhat smaller than that of the spinal cord $GABA_A$ receptor channel (19–20 pS) subconductance level. The kinetic properties of the $\alpha_1\beta_1$ receptor channel were also different from those of the subconductance level of the spinal cord $GABA_A$ receptor channel. The $\alpha_1\beta_1$ receptor channel had briefer average open durations than the spinal cord $GABA_A$ receptor channel. The $\alpha_1\beta_1$ receptor appeared to have a minimum of two different open states, whereas the native receptor subconductance level had at least three different open states (34). Therefore, the conductance levels and gating kinetics of the two receptors were different, suggesting that the subunit composition of the two receptors is different.

To date, no studies have examined the localization of $GABA_A$ receptor subunits in cultured fetal mouse spinal cord by *in situ* hybridization. Localization of receptor subunits has been determined for adult rat cervical and lumbar spinal cord (39, 40). The most abundant α and β isoforms were α_2, α_3, α_5, and β_3. These results suggest that the $\alpha_1\beta_1$ subunit combination may not represent a type of $GABA_A$ receptor found in high abundance in the spinal cord.

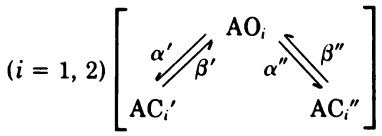
Preliminary kinetic model for the $\alpha_1\beta_1$ $GABA_A$ receptor channel. Based on the observations reported above and by analogy with the main conductance level of the $GABA_A$ receptor channel from mouse spinal cord neurons, a preliminary scheme for gating of the $\alpha_1\beta_1$ $GABA_A$ receptor may be proposed. Because the mean open duration of the receptor did not change with increasing concentration and Hill numbers of $\alpha_1\beta_1$ $GABA_A$ receptors expressed in *Xenopus* oocytes and mammalian cells were about 1, it was assumed that only one agonist molecule was required for activation of the receptor. Because open duration distributions were fitted with two exponential functions, it was assumed that two open states exist after GABA binding. The burst duration was fitted best by two exponential functions. It was assumed that the two open states opened repetitively to burst with an average number of openings/burst of

about one and two openings/burst (scheme 1a). Furthermore, by analogy with the main conductance level of the $GABA_A$ receptor channel from mouse spinal cord neurons, it was assumed that the presence of two concentration-independent short closed durations represents intraburst closures to two distal closed states (scheme 1b). Therefore, bursts are formed when an open state occurs followed by repeated closures into a distal closed state. The burst terminates after entry into an extraburst closed state (scheme 1a).

In this scheme, O_1 and O_2 represent the brief and long open states and C_i' and C_i'' represent the two brief concentration-independent closed states. There is no direct evidence that



Scheme 1a



Scheme 1b

these two brief closed states are distal to the openings. However, the kinetic properties of these two brief closed states were similar to those reported for the main conductance level of the $GABA_A$ receptor of spinal cord neurons (28). In spinal cord neurons analysis of intraburst kinetics suggested that these two states were located distal to the open states. We, therefore, tentatively suggest a similar location for the $\alpha_1\beta_1$ receptor, although intraburst kinetics were not studied in detail for the $\alpha_1\beta_1$ receptor. C_0 represents the unbound receptor and C_1 and C_2 represent extraburst, singly bound, closed states.

$\alpha_1\beta_1$ $GABA_A$ receptor regulation by pentobarbital and picrotoxin. The kinetic model in scheme 1 can be used to suggest the sites of action of pentobarbital and picrotoxin on the $\alpha_1\beta_1$ $GABA_A$ receptor channel. Neither pentobarbital nor picrotoxin had a large effect on the open time constants but both had large effects on the relative areas of the two open components. Pentobarbital produced a 3-fold increase in the relative area of the longest component and slightly increased the time constants. Channel openings in picrotoxin were shifted completely to shorter duration openings (first component openings). A small reduction in the time constant of the first component also was observed. Neither pentobarbital nor picrotoxin had clearly demonstrable effects on the short closed durations. Pentobarbital and picrotoxin also had opposing effects on burst durations. Pentobarbital increased the relative proportion of longer bursts and slightly increased both burst duration time constants. In contrast, picrotoxin shifted the burst durations toward briefer durations and slightly reduced both time constants.

Based on these findings, the major actions of pentobarbital and picrotoxin appeared to be on the relative magnitude of the transition rates (β_1, β_2) into brief and long open states or on the transition rates between the extraburst closed states (k_2, k_{-2}). The two drugs appeared to regulate the relative rate of entry into either the brief unstable open state (O_1) and the long stable open state (O_2). Pentobarbital, which prolonged and enhanced GABA responses, shifted the relative frequency of opening to the longer openings, whereas picrotoxin had the opposite action. These results were qualitatively similar to those previously reported for the main conductance level of the $GABA_A$ receptor from mouse spinal cord neurons (41, 42). In spinal cord neurons it was noted that pentobarbital and picrotoxin had reciprocal actions on the native $GABA_A$ receptor, resulting in opposing actions on open duration histograms and burst duration histograms. It was hypothesized that pentobarbital had its major action on those rate constants regulating the entry into the open states. The present study confirms that the same qualitative results can be inferred for the $\alpha_1\beta_1$ $GABA_A$ receptor.

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Send reprint requests to: Robert L. Macdonald, M.D., Ph.D., University of Michigan, Neuroscience Laboratory Building, 1103 East Huron, Ann Arbor, MI 48104–1687.