ant human T cell epitopes from this protein (*20, 22, 25*). The challenge facing us now is to use the skills of modern molecular immunology to identify means of circumventing immunological nonresponsiveness.

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8. Mature *P. falciparum* gametocytes [3D7 clone (*9*)] were produced in vitro as described (*11*). Gametes were then allowed to exflagellate at room temperature (*11*) and purified by using a Nycodenz gradient [A. N. Vermeulen *et al.*, *J. Exp. Med.* **162**, 1460 (1985)]. Gametes were then further incubated for 5 hours (28°C, 80% humidity) to allow zygote formation. Mice were immunized with a primary intraperitoneal injection of 5×10^6 zygotes emulsified in complete Freund's adjuvant, and two subsequent injections of 5×10^6 zygotes emulsified in incomplete Freund's adjuvant at 21 days and 31 days after the primary injection. Mice were bled before immunization and at day 41, and sera were prepared.
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21. Cells were solubilized and denatured in RIP buffer (100 m*M* tris, *p*H 8.0, 150 m*M* NaCl, 1% NP-40, 1% deoxycholate, 0.1% SDS, 2 m*M* EGTA, 0.1 m*M* phenylmethylsulfonyl fluoride) in a volume of 0.2 ml. A 0.025-ml aliquot was then added to sample buffer [19 m*M* tris, 1.25% SDS, 192 m*M* glycine, 320 m*M* 2-mercaptoethanol, 10% (v/v) glycerol] and boiled at 100°C for 3 min. Samples were separated by SDS–polyacrylamide gel electrophoresis, and the gel was electroblotted overnight in transfer buffer (25 m*M* tris and 192 m*M* glycine in 15% methanol) onto a nitrocellulose membrane (0.2-μm pore size). Nitrocellulose blots were blocked with 0.3% Tween-20 in phosphate-buffered saline (PBS), washed in 0.05% Tween-20/PBS, and incubated for 2 hours with a 1:1000 dilution of peroxidase-conjugated monoclonal antibody to CS protein, 2A10 [F. Zavala *et al.*, *Science* **228**, 1436 (1985)] (Kirkegaard & Perry). Blots were then washed and reacted with 2.8 m*M* 4-chloro-1-napthol and 0.015% hydrogen peroxide in PBS. Reaction was stopped with distilled water.
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Single Subunits of the $GABA_A$ Receptor Form Ion Channels with Properties of the Native Receptor

LESLIE A. C. BLAIR,* EDWIN S. LEVITAN,† JOHN MARSHALL, VINCENT E. DIONNE,* ERIC A. BARNARD‡

The α and β subunits of the γ-aminobutyric acid$_A$ ($GABA_A$) receptor were expressed individually in *Xenopus* oocytes by injection of RNA synthesized from their cloned DNAs. GABA-sensitive chloride channels were detected several days after injection with any one of three different α RNAs (α_1, α_2, and α_3) or with β RNA. The channels induced by each of the α-subunit RNAs were indistinguishable, they had multiple conductance levels (10, 19, 28, and 42 picosiemens), and their activity was potentiated by pentobarbital and inhibited by picrotoxin. The β channels usually expressed poorly but showed similar single channel conductance levels (10, 18, 27, and 40 picosiemens), potentiation by pentobarbital and inhibition by picrotoxin. The finding that both α and β subunits, examined separately, form GABA-sensitive ion channels with permeation properties and regulatory sites characteristic of the native receptor suggests that the amino acid sequences that confer these properties are within the homologous domains shared by the subunits.

GABA, A MAJOR INHIBITORY NEUROTRANSMITTER, binds to specific receptors in the vertebrate central nervous system. Ligand binding to the $GABA_A$ receptor activates chloride channels of multiple conductance states (*1, 2*) that are modulated by barbiturates and the convulsant picrotoxin (*3*). Immunoprecipitation and purification studies indicate that the native $GABA_A$ receptor consists of at least two subunits (α and β) (*4–6*). Photoaffinity labeling with the GABA agonist, [^{3}H]muscimol, suggests that the ligand-binding site is on the β subunit (*7, 8*). Recently, cDNAs encoding one β and three α subtypes (α_1, α_2, and α_3) have been isolated from bovine brain (*9, 10*); their high degree of homology (~35% sequence identity) suggests that α and β subunits arose from gene duplication. We report that the α and β subunits of the $GABA_A$ receptor, expressed separately, are each able to form ligand-sensitive ion channels with normal conductance, ion selectivity, and pharmacological properties.

RNA was synthesized separately from α and β cDNAs and injected singly into *Xenopus* oocytes. Expression was assayed with two-electrode voltage-clamp and patch-clamp techniques (*11*). We detected GABA-evoked currents in oocytes injected with either α or β RNA (Fig. 1). The responses were dose-dependent, enhanced by 50 μ*M* pentobarbital, and reduced by 2.5 μ*M* picrotoxin. No response was seen to glycine (up to 100 μ*M*). Desensitization increased with elevation of GABA from 1 μ*M* to 1 m*M* (Fig. 1A). Measurements made during

MRC Molecular Neurobiology Unit, University of Cambridge, Hills Road, Cambridge CB2 2QH, United Kingdom.

*Present address: Department of Pharmacology, University of California San Diego, La Jolla, CA 92093.
†Present address: Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.
‡To whom correspondence should be addressed.

voltage ramps revealed that GABA increased a conductance that reversed near the Cl^- equilibrium potential (E_{Cl}) of −24 mV [reversal potential (E_{rev}), −27 ± 4 mV; n = 4 oocytes]. Replacement of 50 or 70% of the external Cl^- by the impermeant anion isethionate caused Nernstian shifts in the reversal potential (E_{rev}, −7 mV and +3 mV, respectively). Uninjected and sham-injected oocytes were insensitive to GABA as well as to picrotoxin and pentobarbital.

Single channel activity in the presence of micromolar concentrations of GABA could first be detected 2 to 3 days after injection of α RNA (Fig. 2A). These channels produced rapid transitions between several current levels (Fig. 2, A and D). For all three α subtypes, the preferred conductance states were 19 ± 1 and 28 ± 1 pS (mean ± SD, n = 79 patches) (Fig. 2E); transitions to 10 ± 1 and 42 ± 2 pS states were also observed but usually were not well resolved as a result of short dwell times. Similarly, β channels had conductance levels of 10 ± 1, 18 ± 1, 27 ± 1, and 40 ± 4 pS (n = 26 patches) (Fig. 3), with preference for the 18 and 27 pS levels. In contrast to α channels, open lifetimes of the β channels were often longer, leading to better resolution of the β conductance levels and suggesting the possibility of additional levels.

Current in these multilevel channels was carried by Cl^- and was blocked by picrotoxin. Replacement of 60% of the Cl^- in the pipette with the impermeant anion methylsulfonate caused a Nernstian shift in the E_{rev} of single channel currents observed in the presence of 10 μM GABA [shift in E_{rev}: 23 ± 2 mV, n = 9 patches (α channels); 23 ± 3 mV, n = 5 patches (β channels)]. Full replacement of Cs^+ with large diameter cations (choline or *N*-methylglucamine) had no effect on the E_{rev} (n = 4 patches). Oocytes that responded well to 10 μM GABA in all patches were incubated for 5 to 15 min in picrotoxin (10 μM) and retested with 10 μM GABA plus 10 μM picrotoxin in the pipette. No activity was seen at hyperpolarized potentials or near 0 mV (n = 6 patches from α RNA–injected oocytes and 7 patches from β RNA–injected oocytes). In several patches at strongly depolarized potentials [membrane potential (E_m) >+100 mV], we saw noisy, ~2 pA openings in the presence of picrotoxin; whether these openings arose from poorly conducting GABA receptor channels was not determined. Chloride channels with 10 to 50 pS conductance states were never observed in uninjected oocytes.

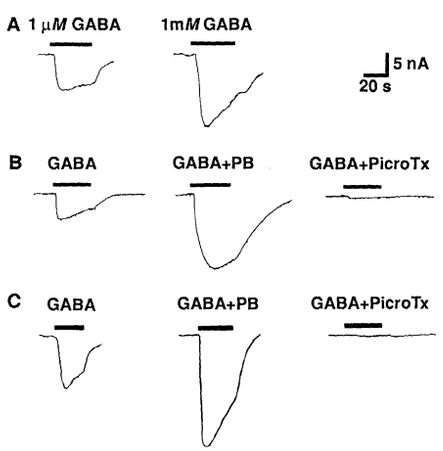

Fig. 1. GABA-evoked currents in *Xenopus* oocytes injected with synthetic α or β RNA. (**A**) Dose dependence and desensitization of currents evoked by 1 μM (left) and 1 mM GABA (right) in an α_2 RNA–injected oocyte. (**B** and **C**) GABA responses were potentiated by pentobarbital (PB) and reduced by picrotoxin (PicroTx) in oocytes injected with either α_2 RNA (B) or with β RNA (C). Left, 300 μM GABA. Middle, 300 μM GABA in the presence of 50 μM pentobarbital. Right, 300 μM GABA in the presence of 2.5 μM picrotoxin. Pentobarbital and picrotoxin were added 2 min prior to GABA applications, which are indicated by bars. E_m, −60 mV (A and B) and −70 mV (C). Traces shown in (A) and (B) are from two different α_2 RNA–injected oocytes.

The activity of both α and β channels increased as the concentration of GABA was increased in the micromolar range; at higher concentrations both types of channels were desensitized. Channel activity, np (n = number of channels in a patch, p = open probability), was estimated from the time channels spent open at $E_m \cong -80$ mV. For α channels at steady state, np increased as a function of GABA concentration: 1 μM GABA, np = 0.02 ± 0.02; 2 μM GABA, np = 0.09 ± 0.03; 10 μM GABA, np = 0.29 ± 0.07 (mean ± SEM, n = 7 to 11 patches at each concentration). At desensitizing concentrations (100 or 500 μM GABA), np = 0.01 ± 0.004 (n = 10 patches). Similar to observations on acetylcholine receptors (*12*), desensitized channel activity consisted of occasional bursts separated by silent intervals up to 10 s without openings (Fig. 2B). Increasing the GABA concentration did not appear to alter substrate preference. For β channels at steady state in 1 μM GABA, np = 0.08 ± 0.06; 2 μM GABA, np = 0.13 ± 0.07; 10 μM GABA, np = 0.19 ± 0.11 (n = 4 to 5 patches at each concentration). These values are not significantly different from those obtained with α channels ($P > 0.4$). For β channels exposed to 100 μM GABA, $np < 0.01$ (n = 3 patches), with bursts of activity separated by long periods of no openings. In most cases, no activity was observed in the absence of GABA [n = 6 patches from α RNA–injected and 5 patches from β RNA–injected oocytes, where all patches contained either α or β channels (*11*)]. In two patches with α channels, spontaneous openings were observed; these occurred at low frequency as brief, unclustered events (Fig. 2C).

Channel activity was strongly voltage-dependent. This was evident in all patches regardless of the number of channels but

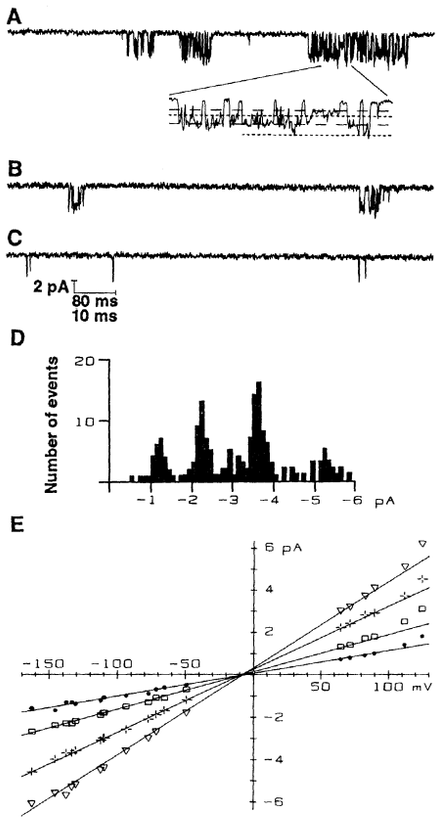

Fig. 2. GABA-sensitive channels in an oocyte injected with α_2 RNA. Each of the panels in this figure is derived from patches on the same oocyte; resting potential, −21 mV. (**A**) An unusually long burst of openings in the presence of 2 μM GABA. Membrane potential, −133 mV. A short section is shown on an expanded time scale (bar, 10 ms) and the four most common current levels are indicated by dashed lines. Transitions between levels were extremely rapid, and it was seldom possible to resolve all levels in short sections of data. This patch appeared to contain only one channel; multiple openings were never observed and direct transitions between the closed and highest conducting states were common. (**B**) In the presence of 500 μM GABA, channel activity was infrequent. The rare openings occurred as bursts. Membrane potential, −133 mV. (**C**) In two patches, brief infrequent openings of GABA receptor–like channels occurred in the absence of GABA. Membrane potential, −125 mV. (**D**) Amplitude histogram of single channel currents for the patch shown in (A). Openings of ≥1 ms were compiled, resolving four peaks at −1.3, −2.3, −3.7, and ~−5.3 pA. (**E**) Current-voltage relation for the same patch shown in (A). At hyperpolarized membrane potentials the slope conductances were 11 pS (●), 18 pS (□), 29 pS (+), and 41 pS (▽). At strongly depolarized potentials, current amplitudes deviated from these conductance values, indicating open channel rectification. Similar curves were obtained for all test concentrations of GABA.

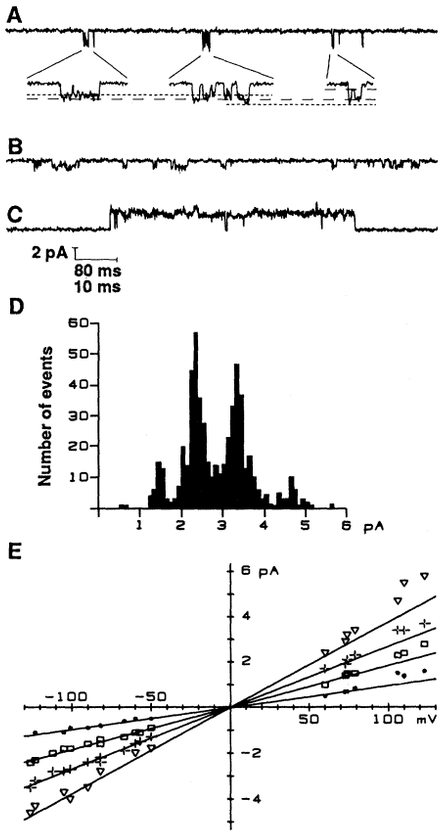

Fig. 3. Single channel activity from an oocyte injected with β RNA. These data, obtained in the presence of 2 μ*M* GABA, show the voltage-dependent gating and conductance properties of β channels. The panels are from a patch that contained one channel, as indicated by the absence of multiple openings even at potentials where the channel was almost continuously open. (**A**) At hyperpolarized potentials openings were brief, rare events. Because only a single channel was present, the activity coefficient *np* equals the open probability of the channel, here 0.016. $E_m = -105$ mV. Several openings are shown on an expanded time scale (bar, 10 ms); the best resolved current levels are indicated by the four dashed lines. (**B** and **C**) Burst duration increased dramatically with depolarization. At a membrane potential of −50 mV (B), bursts were approximately three times as long as at −105 mV; $p = 0.10$. At strongly depolarized potentials (C), bursts of several seconds were common; $E_m = +105$ mV; $p = 0.84$. (**D**) Amplitude histogram of single channel currents at +105 mV. The four peaks, at 1.5, 2.4, 3.3, and ~4.7 pA, correspond to the four conductance levels shown in (E). (**E**) Current-voltage relation for the same patch. Slope conductances were 10 pS (●), 18 pS (□), 27 pS (+), and 38 pS (▽). At strongly depolarized potentials, single channel currents showed outward rectification. The E_{rev} and E_m were both 0 mV, which implies that intracellular and extracellular Cl^- concentrations were approximately equal. The lack of resting potential was due to damage incurred when removing the vitellin membrane.

was shown most clearly by two patches (one from an α_2 oocyte and one from a β oocyte) that had only one channel each; for both, GABA concentration was 2 μ*M*. In these two patches *p* increased from 0.01 at $E_m \cong -130$ mV to 0.99 at $E_m = +125$ mV (Fig. 3, A to C). The increased activity was largely due to increased burst duration. At $E_m = -105$ mV, bursts were rarely longer than 20 ms, whereas at +105 mV, burst durations ranged from 150 ms to more than 10 s. Because of the effect of prolonged depolarization on channel activity (*11*) only the first 5 to 10 s of records were used at those potentials. At mildly hyperpolarized potentials (Fig. 3B) the opening frequency rose, suggesting that ligand binding or gating properties associated with opening the receptor are also voltage-dependent.

Outward rectification of single channel currents was observed at depolarized potentials when current amplitudes for each conductance state fell above the lines fitted at hyperpolarized potentials (Figs. 2E and 3E). Alternatively, rectification could also have been produced by a voltage-dependent shift to conductance states not detected during hyperpolarization.

In summary, individual α or β subunits of the $GABA_A$ receptor can form GABA-sensitive anion channels with normal conductance levels and gating properties (*1, 2, 13*), as well as some pharmacological binding sites (*3*). This is an unexpected result for a neurotransmitter receptor composed of different subunits; conventional ideas based on the study of the acetylcholine receptor suggest that the assembled complex of subunits is needed to produce the normal complement of functions. At present, it is not known how the individual subunits interact in the native receptor. The available biochemical and immunological evidence, as well as the lower expression of the single subunits in oocytes (*9, 10, 14*), suggests that $GABA_A$ receptors exist in vivo as heterooligomers. Electrophysiological studies of coexpressed α and β subunits even suggest that additional subunits may be required for complete reconstitution of function (*10, 15*). The finding that individual subunits can reproduce many properties of the native receptor supports the idea that the subunits arose from gene duplication and suggests that the sites that confer each of these properties lie within the most homologous regions, that is, in the putative transmembrane segments or in short stretches of the extracellular NH_2-terminal domain.

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