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MOLECULAR PHARMACOLOGY, 48:835–840 (1995).

Subunit Composition Determines Picrotoxin and Bicuculline Sensitivity of *Drosophila* γ-Aminobutyric Acid Receptors

HAI-GUANG ZHANG, HWA-JUNG LEE, THOMAS ROCHELEAU, RICHARD H. FFRENCH-CONSTANT, and MEYER B. JACKSON

Department of Physiology and Center for Neuroscience (H.-G.Z., M.B.J.) and Department of Neuroscience and Entomology and Center for Neuroscience (H.-J.L., T.R., R.H.F.-C.), University of Wisconsin-Madison, Madison, Wisconsin 53706

Received June 2, 1995; Accepted August 1, 1995

SUMMARY

Few γ-aminobutyric acid (GABA) receptor subunits have been cloned from insects. These include *Resistance to dieldrin*, or *Rdl*, and a homologue of the vertebrate $GABA_A$ receptor β subunit. Unlike most vertebrate $GABA_A$ receptor subunits, *Rdl* forms a highly functional homomultimeric receptor. This receptor is picrotoxin (PTX) sensitive but bicuculline (BIC) insensitive and cannot be readily classified within the known $GABA_A$ receptor subtypes. In contrast, functional expression of the β subunit homologue has not been reported. We report that coinfection of cells with recombinant baculoviruses containing *Rdl* plus β subunits induces GABA receptors with distinct pharmacological and kinetic properties. Coinfection produces two separate receptor populations: one highly sensitive to PTX but BIC insensitive (*Rdl* homomultimers) and the other PTX insensitive and BIC sensitive (*Rdl* plus β heteromultimers). Putative *Rdl* plus β channels also show reduced GABA sensitivity, slow desensitization, rapid bursting, and shorter mean open time. These studies not only localize PTX and BIC sensitivity to two distinct GABA receptor subunits but also demonstrate assembly of two highly divergent GABA receptor subunits. Furthermore, the difference in channel conductance and gating between *in vivo* and recombinant channels implies the existence of uncharacterized GABA receptor subunits in *Drosophila*.

Vertebrate $GABA_A$ receptors are complex hetero-oligomers formed from the coassembly of a large number of different receptor subunit subtypes (1, 2). In contrast, only three GABA receptor or GABA receptor-like subunits have been cloned from insects to date: the insecticide resistance gene *Rdl* (3); a homologue of the vertebrate $GABA_A$ receptor β subunit (4); and *GRD* (5). The gene *Rdl* was cloned from a *Drosophila* mutant resistant to the $GABA_A$ receptor antagonists PTX and the cyclodiene insecticides (3). Analysis of amino acid sequence, genomic organization (6), and pharmacology (7–9) reveals that *Rdl* is a member of a novel class of GABA receptor subunits not readily classified within any of the existing $GABA_A$ (or $GABA_C$) receptor subtypes. Interestingly, however, *Rdl* forms highly functional GABA-gated chloride ion channels as a homomultimer in both *Xenopus* oocytes (7) and baculovirus-infected cells (10) and is widely distributed throughout the *Drosophila* central nervous system (11). Furthermore, some GABA receptors in the insect nervous system display a similar pharmacology to that observed in *Rdl* homomultimers (12, 13).

The β subunit was cloned using degenerate PCR primers and, based on its predicted amino sequence, appears to be a homologue of the vertebrate $GABA_A$ receptor β subunit (4). Although coexpression of a β subunit homologue from the pond snail with a vertebrate α $GABA_A$ receptor subunit has been demonstrated in *Xenopus* oocytes (14), functional expression of the *Drosophila* β subunit alone has not been reported. Furthermore, both BIC-insensitive and -sensitive GABA receptors have been identified in the nervous system of other insects (15). It is therefore likely that in certain regions of the nervous system, other receptor subunits contribute to the pharmacology of expressed GABA receptors.

The cloning of these invertebrate GABA receptor-like subunits raised two important questions. First, are there other homologues of vertebrate $GABA_A$ receptors in invertebrates with which the β subunit coassembles, as might be predicted from the apparent coassembly of the snail β homologue with a vertebrate α subunit? Second, if not, can *Rdl* assemble with the β subunit homologue, and is this combination of subunits found in *Drosophila*? To address these questions, we coinfected cells with recombinant baculoviruses containing either *Rdl* or the β subunit, alone or in combination. We then examined GABA responses and the effects of the drugs PTX

This work was supported by National Institutes of Health Grant NS29623 (R.ff.-C.) and Grant NS23512 (M.B.J.).

ABBREVIATIONS: GABA, γ-aminobutyric acid; *Rdl*, resistance to dieldrin gene; *GRD*, GABA- and glycine-like receptor from *Drosophila*; PTX, picrotoxin; BIC, bicuculline; PCR, polymerase chain reaction; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid;

and BIC (to which *Rdl* homomultimers are sensitive and insensitive, respectively). By prior application of PTX (eliminating *Rdl* homomultimers), we investigated the role of the β subunit in conferring a slow-acting BIC-sensitive response on receptors containing *Rdl* subunits. The conclusion that the two subunits are coassembling is further supported by the changes in single-channel properties observed in putative *Rdl* plus β channels. These results may begin to explain the diversity of BIC and PTX sensitivity observed in different insect GABA receptors (16). Furthermore, they highlight the unprecedented ability of an apparent *Drosophila* $GABA_A$ receptor β subunit homologue to coassemble with a very divergent and novel subtype of GABA receptor subunit, *Rdl*.

Materials and Methods

Cloning of the *Drosophila* GABA receptor subunits. The cloning of the *Rdl* GABA receptor subunit cDNA has been described in detail elsewhere (3). In the present study, we used the *Rdl* cDNA pNB14.1 for mRNA synthesis (7) and the production of recombinant baculoviruses (10). The β subunit was cloned via PCR amplification using primers derived from the published sequence: forward, 5′-ATGACATGTTTTACGCGC-3′; and reverse, 5′-TTCCAGAATATA-AAACAGCC-3′. The resulting PCR product was then cloned into pCR1000 using a PCR product cloning kit (Invitrogen) according to the manufacturer's instructions. The cloned insert was then gel purified, digoxygenin labeled (GeniusII kit, Boehringer Mannheim), and used to screen an embryonic cDNA library constructed in pNB40 (17). Six clones were identified and restriction mapped. One clone of 1730 bp was sequenced in its entirety. The nucleotide sequence of the open reading frame showed only two silent substitutions from the published sequence. The open reading frame was cloned into the baculovirus transfer vector using a *Cla*I site upstream of the first ATG and an *Eco*RI at the 3′ end within the pNB40 polylinker, and recombinant baculoviruses were generated as previously described (10). All baculoviruses were infected at a multiplicity of infection of 15 into cultured *Sf*21 cells grown in TC100 growth medium supplemented with tryptose broth (2.6 mg/ml), amphotericin B (0.6 μg/ml), penicillin G (100 units/ml), streptomycin (207 units/ml), $NaHCO_3$ (0.35 mg/ml), and 10% fetal bovine serum.

Oocyte expression. *Xenopus* oocytes were surgically isolated from ice-anesthetized frogs. After treatment with 2 mg/ml collagenase B (Boehring-Mannheim Co.) for 40 min in calcium-free ND-96 solution and extensive washing with ND-96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.4), the follicle cell layer was removed mechanically with a pair of fine forceps. At least 2 hr later, defolliculated oocytes were then microinjected with ~50 nl of 40 ng/μl mRNA using a Drummond microdispenser. mRNAs were synthesized from the Sp6 promoter of pNB40 and dissolved in RNAase-free water. Their concentrations were estimated by measuring optical density at 260 nm. For coinjection, appropriately diluted aliquots of the different mRNAs were mixed to produce a 1:1 ratio. After injection, the oocytes were maintained at 21° in ND-96 solution with 50 μg/ml gentamicin. Before recording, the vitelline membrane of oocytes was removed mechanically with the use of striping solution (200 mM K-aspartate, 20 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, pH 7.4, with KOH).

Electrophysiology. Electrophysiology was performed at room temperature 2–7 days after virus infection or oocyte injection. Both *Sf*21 cells and oocytes were viewed under a Nikon Diaphot inverted phase contrast microscope and bathed with the same *Drosophila* physiological saline (128 mM NaCl, 2 mM KCl, 4 mM $MgCl_2$, 1.8 mM $CaCl_2$, 35.5 mM sucrose, 5 mM HEPES, pH 7.1). GABA-activated currents were recorded from *Sf*21 cells under whole-cell patch-clamp (18). Patch electrodes were made from borosilicate glass (i.d. 1.10 mm, o.d. 1.70 mm; Garner Glass Co.) coated with Sylgard (Dow Corning, Midland, MI) and filled with a solution consisting of 140 mM KCl, 10 mM HEPES, 10 mM EGTA, pH 7.1. The resistance of patch electrodes was approximately 4 MΩ. Cells were voltage-clamped, and currents were recorded in the whole-cell mode with a List L/M EPC-7 amplifier (List-Electronic, Germany), digitized by a TL-1 DMA interface (Axon Instruments, Foster City, CA), and analyzed on a personal computer with Pclamp software (Axon Instruments).

Single-channel currents from mRNA-injected oocytes were recorded in outside-out patches. (*Sf*21 cells were not used for single-channel recording because they lifted off the culture dish when patch excision was attempted). Signals were sampled at either 2-msec or 100-μsec intervals and filtered at either 1 or 4 kHz with a low-pass eight-pole Bessel filter (Frequency Devices, Haverhill, MA). All drugs were dissolved in the bathing solution. GABA was applied by pressure (5–10 p.s.i.) from a picrospritzer (General Valve Corp., Fairfield, NJ) using small-tipped (1–2 μm) delivery pipettes positioned near cells or patches. The antagonists PTX (Sigma Biochemicals, St. Louis, MO) and BIC (Research Biochemicals, Natick, MA) were applied by bath perfusion.

Results

GABA responses of infected cells. To examine whether the *Drosophila* subunit can form a functional channel alone or can coassemble with the *Rdl* subunit in a hetero-oligomeric GABA receptor, *Sf*21 cells were infected with the recombinant baculoviruses containing either *Drosophila Rdl* or *Drosophila* β subunits, either alone or in combination. Analysis of cytoplasmic cell RNA produced after baculovirus infection shows that the correct size messages for each of the different GABA receptor subunits were produced after coinfection (Fig. 1). Twenty-four hours after infection, GABA responses were characterized electrophysiologically. No GABA response was detected when the β subunit was expressed alone (24 experiments). In these whole-cell recordings, currents as small as 5 pA can readily be detected, indicating that any expression, if present, must be extremely low. Homomultimeric assembly of this channel therefore seems unlikely. In cells infected with the *Rdl* subunit or coinfected with the *Rdl* plus β subunits, a brief application of 50 μM GABA induced a large inward current when membrane potential was clamped at −70 mV in nearly symmetrical chloride concentration (extracellular Cl^- concentration = 141.6 mM; pipette Cl^-concentration = 140 mM) (Fig 2A).

There was no obvious difference between the GABA responses of *Rdl* and *Rdl* plus β-infected cells when GABA application was brief (200 msec; Fig. 2A). In contrast, sustained application of GABA revealed a striking difference. As expected, we observed rapid desensitization after sustained application of 50 μM GABA in cells expressing *Rdl* (13) (Fig. 2B). However, in *Rdl* plus β-infected cells, the same protocol

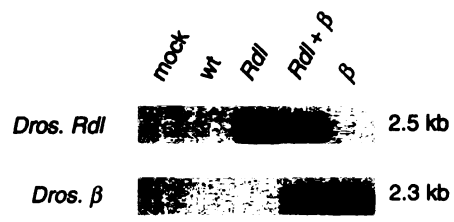

Fig. 1. Infected cells express the correct GABA receptor subunit messages in Northern blot analysis of total cytoplasmic RNA from *Sf*21 cells infected with either *Drosophila Rdl* (*Dros. Rdl*) or the *Drosophila* $GABA_A$ β subunit homologue (*Dros. β*). Note that in all cases, expression of the correct-size message is observed for each subunit on coinfection (3, 4).

revealed a second component of current (Fig. 2B). The first component resembles that of cells expressing *Rdl* alone, and the second component was slow in both activation and desensitization (time constant of activation = 18.6 ± 3.5 sec, mean ± standard error, seven experiments). When the ratio of *Rdl* to β subunit RNA was equal (1:1), the average fraction of the response corresponding to the slow component [$I_{slow}/I_{fast} + I_{slow}$] was 0.46 ± 0.04 (mean ± standard error, 57 experiments). Interestingly, increasing the proportion of β subunit RNA (1:2) did not increase ($p \gg 0.1$) the fraction of the slow component (0.47 + 0.08, mean ± standard error, 10 experiments). Because infection with the β subunit alone could not induce expression of a functional channel, GABA receptors in cells coinfected with *Rdl* plus β must be either *Rdl* homomultimers or *Rdl* plus β heteromultimers. Thus, we conclude that the second slow component of GABA response originates from heteromeric *Rdl* plus β GABA receptor channels.

When the concentration of applied GABA was increased to 500 μM, the slow component was not apparent in cells coinfected with *Rdl* plus β subunits (Fig. 2C). However, the pattern of desensitization was changed in cells expressing both subunits. In cells transfected with *Rdl* alone, the desensitizing phase of the response to 500 μM GABA was well fitted by a single exponential function with a time constant of 6.1 ± 1.0 sec (mean ± standard error, seven experiments). In cells infected with both *Rdl* and β subunits, desensitization was not well fitted by a single exponential function. In five of the nine cells examined, a two-exponential function improved the fit, but visual inspection still revealed that discrepancies remained. Apparently, when both subunits are present, it is no longer possible to find a simple function that describes the behavior of the aggregate. To quantify the comparison of desensitization under the two conditions, we therefore fitted the time course of desensitization in cells expressing *Rdl* plus β to a single exponential function, obtaining a time constant of 12.2 ± 2.1 (mean ± standard error, nine experiments). This value is significantly different ($p < 0.013$) from that obtained for cells expressing *Rdl* alone.

The steady state response in 500 μM GABA, after desensitization, was also different between *Rdl* homomultimers and *Rdl* plus β heteromers (Fig. 2C). The ratio of steady state to peak current was 0.11 ± 0.03 (mean ± standard error, seven experiments) for *Rdl* and 0.32 ± 0.02 (nine experiments) for *Rdl* plus β ($p < 0.001$). If we assume that the ratio of 0.32 obtained in *Rdl* plus β-expressing cells is an average of the ratio of steady state to peak current for *Rdl* homomultimeric and *Rdl* plus β heteromeric channels, then because the homomeric ratio was only 0.11 we can conclude that the value for the heteromeric channel is more than 0.32. Because we do not know the fraction of peak current contributed by each of the two types of channels, we cannot obtain a precise ratio of steady state to peak current for the heteromer (the fraction given above for the fast component versus the slow component cannot be used because it was obtained with 50 μM GABA). Nevertheless, a difference clearly exists and indicates that the *Rdl* plus β heteromeric channel exhibits weaker desensitization than the *Rdl* homomultimer.

Pharmacology. The separation of the two components of GABA-activated current in *Rdl* plus β-infected cells was also demonstrated pharmacologically. Our previous work has shown that the predominant GABA receptors of cultured *Drosophila* larvae neurons are PTX sensitive (13) and BIC insensitive (Fig. 4A, five experiments), which is in common with recombinant homomultimeric *Rdl* receptors (7). In contrast, most vertebrate $GABA_A$ receptors are blocked by both these drugs. In the present study, 500 μM PTX completely inhibited the fast component of response to sustained GABA application as well as the response to brief GABA application (Fig. 3). However, the slow component of GABA response was blocked only 44 ± 4.2% (mean ± standard error, seven experiments) by 500 μM PTX (Fig. 3), indicating that the heteromeric *Rdl* plus β channels are less sensitive to PTX. The

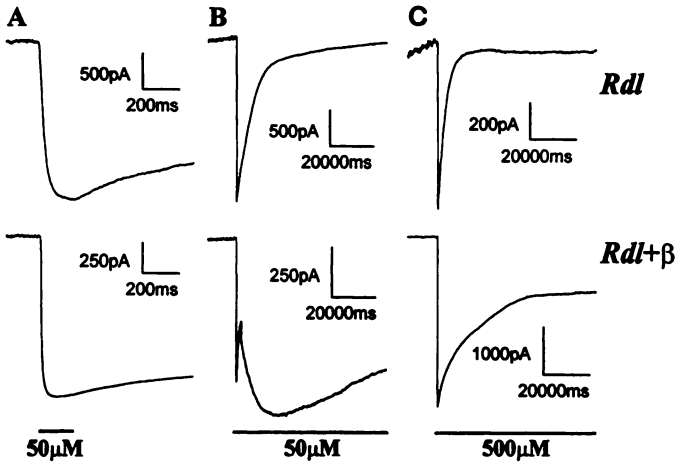

Fig. 2. Expression of *Rdl* and *Rdl* plus β channels in *Sf*21 cells. GABA responses from *Sf*21 cells infected with either *Rdl* alone or *Rdl* plus β subunits after either brief (A) or sustained (B) application of 50 μM GABA (note different time scales) or sustained application of 500 μM GABA (C). A, Brief application of 50 μM GABA to cells expressing either *Rdl* or *Rdl* plus β produce similar responses. B, In contrast to cells infected with *Rdl* alone, sustained application of 50 μM GABA reveals a slower component to the GABA response in cells infected with *Rdl* plus β. C, Sustained application of 500 μM GABA produced responses in cells infected with *Rdl* or *Rdl* plus β that were more similar than 50 μM GABA, but desensitization was slower for the *Rdl* plus β heteromeric channel (see text). *Solid bars*, duration of GABA application. The biphasic response in cells infected with *Rdl* plus β (B) was observed in more than 60 cells coinfected with *Rdl* plus β.

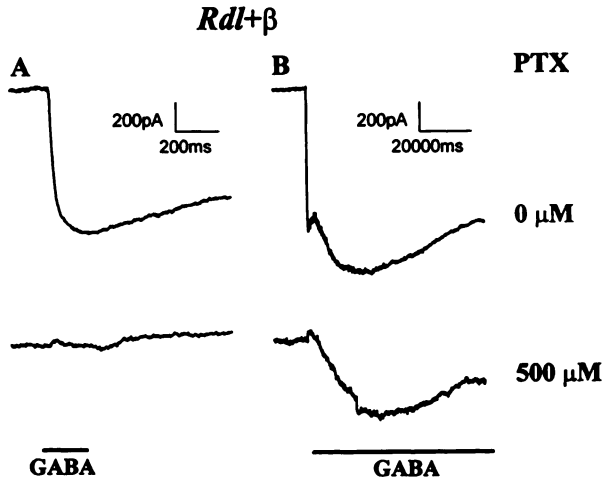

Fig. 3. PTX selectively blocks *Rdl* homomultimers. GABA responses from *Sf*21 cells coinfected with *Rdl* plus β subunits showing responses to brief (A) and sustained (B) application of GABA. Note how PTX completely blocks the rapid response attributable to *Rdl* homomultimers while only partially reducing the slower component attributable to the *Rdl* plus β heteromultimers (seven experiments).

estimated K_i of PTX for the slow component is close to 500 μM compared with the estimated K_i of 1 μM in cells expressing *Rdl* alone.[1]

The action of BIC complemented that of PTX in that BIC spared receptors formed from *Rdl* alone and blocked heteromultimeric *Rdl* plus β receptors. BIC (100 μM) had no effect on the fast component of the GABA response in the *Rdl* plus β subunit-expressing cells (Fig. 4B) but almost completely inhibited the slow component of the GABA response (Fig. 4C, seven experiments). This indicates that heteromeric *Rdl* plus β receptors are BIC sensitive. The two populations of GABA receptors, homomultimeric *Rdl* receptors and heteromultimeric *Rdl* plus β receptors, can thus be readily distinguished by their different pharmacology.

Channel properties. The observation that the *Drosophila* β subunit can coassemble with *Rdl* to form functional GABA channels is further supported by analysis of single-channel properties. We coinjected *Rdl* and *Drosophila* β subunit mRNA into *Xenopus* oocytes and recorded GABA-activated channel currents in outside-out patches. By adjusting the concentration of injected mRNA, expression time, and tip size of patch electrode, we were able to obtain patches containing either hundreds of channels or a few channels. In patches with hundreds of channels, we were able to reproduce the results from infected cells showing responses to 50 μM GABA distinguished on the basis of activation and desensitization kinetics (data not shown), indicating similar receptor assembly in these two different expression systems. In patches with a few *Rdl* channels, brief application of 50 μM GABA activated single-channel currents (Fig. 5A). Patches from *Rdl* plus β-injected oocytes appeared to contain both homomeric *Rdl* channels and heteromeric *Rdl* plus β channels (Fig. 5B). To isolate the heteromeric *Rdl* plus β single-channel currents, we performed experiments in 500 μM PTX and with sustained 50 μM GABA application (Figs. 5 and 6). Again, no GABA-activated channels were detected from oocytes injected with β subunit mRNA alone (18 patches from six oocytes), confirming our result from infected cells that the β subunit alone does not readily form functional GABA receptors.

Single-channel current-voltage plots show reversal potentials near 0 mV, as expected for Cl^- channels in symmetrical Cl^-. The conductance of the homomeric *Rdl* channel was 21.4 ± 0.8 pS for inward current and 12.5 ± 1.1 pS for outward current (mean ± standard error, 21 experiments for inward current and 16 experiments for outward current). Both values differ from the corresponding single-channel conduc-

[1] H.-G. Zhang, R. H. ffrench-constant, M. B. Jackson, unpublished data.

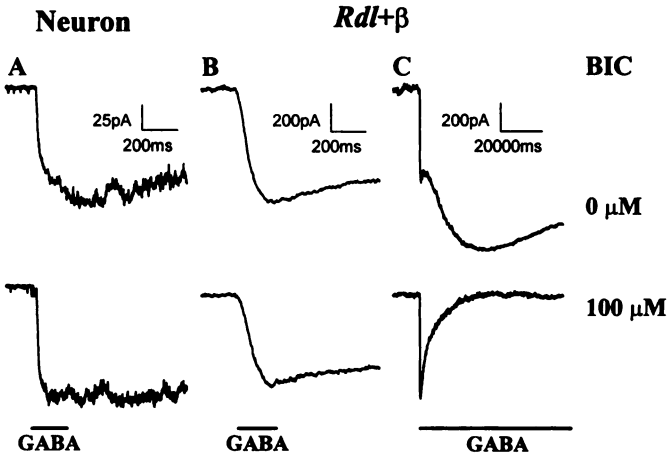

Fig. 4. BIC selectively blocks *Rdl* plus β heteromultimers. GABA responses from (A) cultured *Drosophila* larvae neurons (six experiments) and (B and C) *Sf*21 cells coinfected with *Rdl* plus β subunits (seven experiments) representing a brief (A and B) and sustained (C) application of GABA. Note that neuronal GABA receptors (A) and the response attributable to *Rdl* homomultimers (B) are insensitive to BIC. The slow component attributed to the *Rdl* plus β heteromultimer is blocked by BIC.

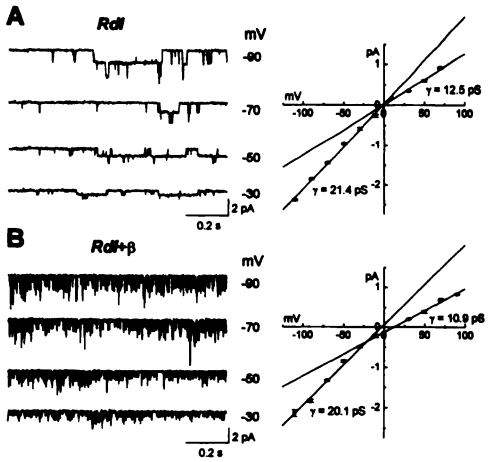

Fig. 5. Single-channel analysis distinguishes *Rdl* homomultimers from *Rdl* plus β heteromultimers. Single-channel currents were recorded in outside-out patches from oocytes injected with either *Rdl* alone (A) or *Rdl* plus β subunit mRNAs (B) in the presence of 500 μM PTX. Note the much more rapid gating of the *Rdl* plus β channels (represented at higher resolution in Fig. 6). Patch holding potentials are indicated to the right of the single-channel traces. Single-channel current was plotted versus voltage for each channel type. For the *Rdl* plus β channels, higher resolution data was used (Fig. 6). Inward and outward currents were fitted separately to lines to give slope conductances. Conductances shown are mean values. See text for standard errors and number of experiments.

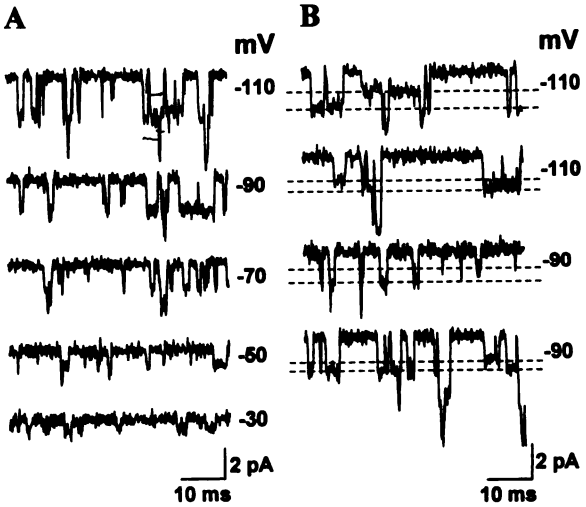

Fig. 6. Higher time resolution of *Rdl* plus β channels reveals subconductance states. Data were acquired with higher time resolution (see Materials and Methods) revealing (A) stable open states with the same amplitude as *Rdl* homomultimers (Fig. 5A). B, Records containing subconductance states. *Broken lines in B* indicate the full open and subconductance state. As in Fig. 5B, recordings from *Rdl* plus β heteromultimers were performed in 500 μM PTX.

tances (28.0 and 19.0 pS for inward and outward currents, respectively) obtained from cultured larval *Drosophila* neurons (13). The heteromeric *Rdl* plus β single-channel current showed conductances similar to those of the *Rdl* homomultimer (20.1 ± 0.9 pS for inward current and 10.9 ± 1.1 pS, mean ± standard error, for outward current in 20 patches). The differences in conductance between *Rdl* and *Rdl* plus β channels were not statistically different ($p = 0.14$ and 0.15 for inward and outward currents, respectively).

In contrast to the similar conductance behavior, the *Rdl* plus β heteromeric channel exhibited totally different gating kinetics (Fig. 5). The homomultimeric *Rdl* channel had a mean open time of 62 ± 8.0 msec (mean ± standard error from six patches). Whereas, the heteromeric *Rdl* plus β channels display a bursting activity with shorter open times (1.00 ± 0.15 msec, mean ± standard error, from six patches; $p < 0.0001$). It should be noted that the homomeric channel formed from the *Rdl* subunit has a mean open time that is shorter than the mean open time of GABA gated channels in *Drosophila* larvae neurons, where a value of 118 msec was obtained (13). This together with the difference in single-channel conductance described above suggests that the native *Drosophila* GABA receptor is not an *Rdl* homomultimer. The details of the single-channel gating behavior were better viewed in data collected with a digitization frequency of 10 kHz and filtered with a high frequency cutoff of 4 kHz (Fig. 6). This reveals that the heteromeric *Rdl* plus β channels have at least two subconductance states (Fig. 6B), which are not evident in GABA-gated channel currents from *Drosophila* larvae neurons (13) or homomultimeric *Rdl* channels (Fig. 5A). The approximate conductances of these subconductance states were 11 and 14 pS.

Discussion

It is well established that a number of different vertebrate $GABA_A$ receptor subtypes are required for assembly of functional receptors and that single vertebrate $GABA_A$ receptor polypeptides (except the ρ subunit, also previously classified as a $GABA_c$ subunit (19)) express inefficiently in heterologous expression systems. *Drosophila Rdl*, however, functions as a highly efficient homomultimeric GABA receptor in heterologous systems. Because *Rdl* differs in sequence identity, alternative splicing, genomic organization (6, 20), and pharmacology (7, 8), it is not readily classified within any of the existing $GABA_A$ (or $GABA_C$) receptor subtypes. *Rdl* thus represents a novel class of GABA receptor subunits. In contrast, the *Drosophila* β subunit is clearly a homologue of vertebrate $GABA_A$ β receptor subunits. This means that the two *Drosophila* GABA receptor subunits studied here are only distantly related; *Rdl* and the β subunit share only 26% amino acid identity (4), and *Rdl* is in fact more closely related to glycine than to GABA receptors at the sequence level (6). Therefore, the present study demonstrates a surprising coassembly of two widely divergent ligand-gated channel subunits.

Coexpression of these two subunits was investigated at two different levels relating to either pharmacology or biophysical analysis of receptor activation and channel gating. Using the two standard $GABA_A$ receptor blockers PTX and BIC, we resolved two responses. *Rdl* homomultimers are PTX sensitive and BIC insensitive. In contrast, *Rdl* plus β heteromers are BIC sensitive and much less sensitive to PTX. Furthermore, previous recordings made in this laboratory from cultured *Drosophila* larvae neurons revealed receptors with *Rdl*-type pharmacology, i.e., receptors sensitive to PTX and insensitive to BIC. This strongly suggests that the *Drosophila* β subunit does not contribute to the formation of the predominant GABA receptor found in larvae neurons. However, studies in other insects have revealed both BIC-sensitive and -insensitive GABA responses in the insect nervous system (16). Therefore, the β subunit may be responsible for conferring BIC sensitivity on *Rdl*-containing invertebrate GABA receptors in an analogous fashion to the vertebrate γ $GABA_A$ receptor subunit conferring sensitivity to benzodiazepines and insensitivity to zinc on vertebrate $GABA_A$ receptors (21).

From the biophysical perspective, the homomultimeric *Rdl* channel has a mean open time of 62 msec. In contrast, single-channel recordings from the *Rdl* plus β heteromultimer show the hybrid channel opening for 1 msec. Analysis of channel activation also reveals striking differences. With 50 μM GABA, the *Rdl* homomultimer activates in <100 msec and desensitizes with a time constant of ~1 sec. In this regard, the *Rdl* homomultimer resembles the neuronal *Drosophila* GABA receptor (13). However, addition of the β subunit leads to the formation of a receptor with very different properties. The *Rdl* plus β heteromultimer requires 16 sec for activation and desensitizes with a time constant on the order of 1 minute after application of 50 μM GABA, with kinetics of both processes becoming markedly faster with increasing GABA concentration. Receptors with such different properties are likely to serve very different functions, depending on the synaptic and extrasynaptic concentrations of GABA. For example, rapidly gated Cl^- channels should participate in rapid circuit activity, whereas Cl^- channels with slow gating may function in development or in modulation of circuit behavior. Interestingly, GABA-activated Cl^- currents with kinetics comparable to the slow kinetics of activation of the *Rdl* plus β heteromeric channel by 50 μM GABA have recently been described in neonatal rat hippocampus (22).

The pharmacological differences between the two receptors raise the important question of how different subunits of a heteromeric receptor influence drug sensitivity. In relation to BIC sensitivity, the *Rdl* polypeptide clearly lacks an effective BIC binding site; thus, concentrations as high as 100 μM had no effect. The receptor gains a BIC binding site through coassembly with the β subunit. However, PTX sensitivity is reduced by coassembly of the *Rdl* polypeptide with the β subunit. In a similar fashion, the addition of a glycine receptor β subunit removes the sensitivity to PTX of a homomeric glycine receptor formed from an α subunit (23). There are several possible explanations for this. (a) The β subunit could physically obstruct PTX binding. Because this region is located in M2, near the center of the channel (7, 13, 23), it should be close to other subunits and thus could be sensitive to steric interference. (b) The PTX binding site may be formed from the interface of two *Rdl* subunits and that interface may fail to form when the β subunit is present. (c) We previously suggested that reductions in PTX sensitivity can also be explained in terms of allosteric interactions (13) in which a drug-preferred desensitized state (24) is destabilized. From the allosteric perspective, the reduced desensitization of the *Rdl* plus β heteromultimer therefore also offers

an explanation for the reduced sensitivity to PTX. PTX (500 μM) blocks the response attributable to the *Rdl* plus β heteromultimer by 44%, whereas an equivalent level of block of the *Rdl* homomultimer is achieved by only 1 μM (to give a 500-fold difference in sensitivity). The differences in steady state desensitization of 0.11 versus >0.32 at 500 μM GABA is not sufficient to account for a 500-fold reduction in sensitivity to PTX. Thus, the allosteric effect on binding is not likely to play an important role in reducing PTX sensitivity.

Although we report the coassembly of two cloned invertebrate GABA receptor subunits in both baculovirus-infected cells and *Xenopus* oocytes, whether these two subunits coassemble *in vivo* remains unclear. The *Rdl*-containing GABA-activated Cl^- channel in cultured *Drosophila* larvae neurons has a conductance of 28 pS for inward current and 19 pS for outward current (13). The conductance of the *Rdl* homomultimer expressed in *Xenopus* oocytes is 21 pS for inward current and 12 pS for outward current. The mean open time and steady state to peak current ratio of the homomultimer are also different than those of the native *Drosophila* GABA receptor (13). It is unlikely that these differences are due to the different *Rdl* alternative splice forms (20), as the conductance for inward current of homomultimers expressed from a different splice form (cDNA NB4.1, containing alternative exons 3b and 6d) was 19.8 ± 0.1 pS (mean ± standard error, four experiments) and indistinguishable ($p = 0.2$) from those formed by cDNA NB14.1 (containing alternative exons 3a and 6c). To the extent that one can expect such channel properties to be preserved in heterologous expression systems, these differences suggest that the *Rdl*-containing receptors are not homomultimers *in vivo*. Furthermore, the difference in conductance between these two channels indicates that the β subunit cannot account for the difference because the conductance of the *Rdl* plus β heteromeric channel is virtually identical. Because post-translational modifications and other expression system-specific functions are not known to affect single-channel conductance, these differences imply that the *Rdl* polypeptide coassembles *in vivo* with another uncharacterized invertebrate GABA receptor subunit.

Acknowledgments

We thank C. Czajkowski, L. Trussell, and N. Anthony for helpful comments on the manuscript and G. Robertson for provision of oocytes and help with oocyte injection.

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Send reprint requests to: Dr. Meyer B. Jackson, Department of Physiology, University of Wisconsin-Madison, Madison, WI 53706-1532.