Characterization of γ-Aminobutyric Acid Receptor GABA$_{B(1e)}$ a GABA$_{B(1)}$ Splice Variant Encoding a Truncated Receptor*

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We have identified a splice variant encoding only the extracellular ligand-binding domain of the γ-aminobutyric acid B (GABA$_B$) receptor subunit GABA$_{B_{1a}}$. This isoform, which we have named GABA$_{B_{1(e)}}$, is detected in both rats and humans. While GABA$_{B_{1(e)}}$ is a minor component of the total pool of GABA$_{B_{1}}$ transcripts detected in the central nervous system, it is the primary isoform found in all peripheral tissues examined. When expressed in a heterologous system, the truncated receptor is both secreted and membrane associated. However, GABA$_{B_{1(e)}}$ lacks the ability to bind the radiolabeled antagonist [3H]CGP 54626A, activate G-protein coupled inwardly rectifying potassium channels, or inhibit forskolin-induced cAMP production when it is expressed alone or together with GABA$_{B_{2}}$. Interestingly, when co-expressed with GABA$_{B_{2}}$, not only does the truncated receptor heterodimerize with GABA$_{B_{2}}$, the association is of sufficient avidity to disrupt the normal GABA$_{B_{1a}}$/GABA$_{B_{2}}$ association. Despite this strong interaction, GABA$_{B_{1(e)}}$ fails to disrupt G-protein coupled inwardly rectifying potassium activation by the full-length heterodimer pair of GABA$_{B_{1a}}$/GABA$_{B_{2}}$.

γ-Aminobutyric acid (GABA) is the primary neurotransmitter responsible for neuronal inhibition. The method by which inhibition is achieved depends upon the type, as well as the anatomical localization, of the GABA receptor. Like other neurotransmitters, inhibition is mediated through both ionotropic (GABA$_A$) and metabotropic (GABA$_B$) receptors. The ionotropic receptor subunits form ion channels that are selectively permeable to chloride. These receptors are responsible for the rapid component of inhibitory postsynaptic potentials. The metabotropic receptors are coupled to heterotrimeric G-proteins which in turn regulate intracellular effector systems. When located at a postsynaptic junction, these receptors manifest long lasting inhibitory postsynaptic potentials by increasing potassium conductance through G-protein coupled inwardly rectifying potassium channels (GIRKs). Those receptors located at a presynaptic junction control neurotransmitter release by inhibiting voltage-gated Ca$^{2+}$ channels.

GABA$_B$ receptors belong to the class C subfamily of G-protein coupled receptors. These receptors, which include the calcium sensing (1), metabotropic glutamate (GRM) (2), vomeronasal (3), and putative taste receptors (4), share low sequence similarity. Like the classical G-protein Cu$^{2+}$ receptors, they are composed of seven-membrane spanning domains, with the intracellular loops being responsible for coupling to heterotrimeric G-proteins. However, they have the distinctive feature of an unusually large extracellular domain (ECD) that is nearly equal in size to the remaining portion of the protein, and bears similarity to the bacterial periplasmic amino acid-binding proteins (5, 6). The ECDs of GABA$_{B_{1a}}$ GRM1, and GRM4 have been successfully expressed as soluble recombinant proteins and possess ligand binding characteristics typical of the full-length receptor (7–9). Furthermore, chimeric constructs between GRM1 and GRM2 have revealed that the ligand binding specificity is dictated by the ECD, with no apparent influence from the membrane spanning domains (10). Thus, the ECD alone appears to be both necessary and sufficient for binding cognate ligand.

The GABA$_B$ receptor is unique among G-protein coupled receptors in that native affinity for agonist ligands, and complete functional activity, rely upon the formation of heterodimers between GABA$_{B_{1a}}$ and GABA$_{B_{2}}$ (11–16). This association occurs, at least in part, through a coiled-coil motif found in the respective carboxyl termini of GABA$_{B_{1a}}$ and GABA$_{B_{2}}$. Although heterodimers have not been detected for other members of the class C G-protein coupled receptor family, they are capable of forming homodimers (17). However, unlike the GABA$_B$ receptor the primary site for this dimerization has been mapped to the amino-terminal ECD.

GABA$_B$ receptors were first detected in the peripheral nervous system (18), however, recent studies have focused on the role of these receptors in the CNS. Interestingly, studies using small molecule GABA$_B$ ligands suggest that the central and peripheral receptors may be functionally and/or structurally distinct (19). For example, N-BAC is a more potent agonist in peripheral tissues than in the CNS (20). This functional distinction may be attributable to differences in the signaling cascade emanating from the receptor, or the particular heterotrimeric G-protein used in each tissue. Alternatively, it is possible that the peripheral receptors are structurally distinct, which in turn influences the ligand binding profile. Here we report the identification of a new GABA$_B$ splice variant that encodes a naturally truncated receptor composed of just the ECD. The tissue distribution, ligand binding profile, and functional attributes of this receptor isoform may contribute to the
aforementioned differences between central and peripheral GABA<sub>B<sub>R<sub> receptors.

**EXPERIMENTAL PROCEDURES**

**Recombinant Receptors**—Complete details of all constructions will be provided upon request. Briefly, full-length GABA<sub>B<sub>(1a), and GABA<sub>B<sub>(2) receptors were amplified by PCR from human brain cDNA (CLONTECH Laboratories, Inc., Palo Alto, CA). The PCR primers were designed to incorporate a Xhol site at the 3' end of the PCR product immediately preceding the translation termination codon. This resulted in the addition of the amino acids Ser, Leu, and Glu at the carboxyl terminus of each recombinant protein. The PCR products were cloned into pcDNA3.1/V5-his (Invitrogen, Carlsbad, CA) and sequenced on both strands with complete identity to the native receptor. The PCR products were cut with Xhol and religated to place the protein in-frame with the COOH-terminal V5-his epitope tag. A FLAG epitope tag (amino acid sequence DYKDDDK) was incorporated at the carboxyl terminus of the GABA<sub>B<sub>(2) clone by replacing the Xhol to Pmel fragment encoding the V5-his tag with a double stranded oligonucleotide containing complementary termini.

The GABA<sub>B<sub>(1a), receptor subunit was amplified from human prostate cDNA by PCR and cloned into pcDNA3.1/V5-his, in-frame with the epitope tag, to generate DSp24. To eliminate the epitope tag, DSp24 was used to generate a PCR product incorporating the native termination codon which was then cloned into pcDNA3.1/V5-his, yielding DSp72. To generate GABA<sub>B<sub>(1e), truncations, DSp24 was subjected to 20 cycles of PCR using sequence specific primers which span the region from amino acids 1–439 and 578–578, and 265–578. The product representing amino acids 1–439 was cloned into pcDNA3.1/V5-his. The remaining products, which incorporated EcoRI and BamHI sites at their termini, were cloned into the corresponding sites of pSecTag2A (Invitrogen).

The truncated mGlur5 clone tGRM5.21 was amplified by PCR from a full-length cDNA clone<sup>2</sup> using primers that incorporate the region encoding amino acids 1–579 of the native receptor. The GIRK-1 and -2 clones were amplified from human heart cDNA using primers that incorporate the entire coding sequence. Each of the above products lack a stop codon and were designed to be cloned into pcDNA3.1/V5-his in-frame with the epitope tag.

**GABA<sub>B<sub>1 Receptor mRNA Quantitation**—To assess the tissue distribution of each human receptor subunit message, individual mRNAs (CLONTECH) were converted into first strand cDNA by oligo(dT) priming with avian myeloblastosis virus reverse transcriptase. To measure the relative quantity of the 1a versus 1e subunit messages, each sample was subjected to 40 cycles of PCR with the primers DS230 (5'-TCAAGGGTGCTGAGTTCT-3') and DS239 (5'-AACAAGACATCCCGAATTGC-3'). To assess GABA<sub>B<sub>2a, distribution, the above cDNAs were subjected to 30 cycles of PCR with the primers DS73 (5'-CCGCGCCAGGCGCTCC-3') and DS86 (5'-ACATCATCCTGGAGAACG-3'). A fraction of each product was then subjected to 30 additional cycles of PCR with the primers DS72 (5'-CTTACAGGGCACGAGACC-3') and DS87 (5'-TGGGAGGATGCTCTTACC-3'). To assess GABA<sub>B<sub>1a, subunit distribution in rat tissues, single strand cDNAs were subjected to two rounds of PCR amplification (30 cycles each) with the primers DS230 (5'-TCAGGTTGGCCTGTGAGTTC-3') and DS239 (5'-AACAAGACATCCCGAATTGC-3'). The amplified products were cloned into pcDNA3.1/V5-his (Invitrogen) in MES running buffer and transferred to nitrocellulose. Non-specific binding was blocked with 5% non-fat dried milk in Tris-buffered saline (TBS) followed by incubation in TBS, 1% non-fat dried milk containing horseradish peroxidase-conjugated anti-Myc or -V5 antibodies (Invitrogen), or biotin-coupled M2-anti-FLAG (Sigma) in conjunction with avidin-horseradish peroxidase (Pierce, Rockford, IL). Bound antibodies were detected using an enhanced chemiluminescence reagent (ECL, Amersham Pharmacia Biotech).

**Electrophysiology**—50 nl of capped cRNA in H<sub>2</sub>O was injected into defolliculated stage V-VI Xenopus oocytes 2 to 5 days prior to recording. For experiments examining function of GABA<sub>B(1e)</sub> versus GABA<sub>B(1a)</sub> plus GABA<sub>B(2)</sub>, each oocyte was injected with a mixture of 1 ng each GABA<sub>B(1e)</sub> and GIRK-2 and either 2 ng of GABA<sub>B(2)</sub> or 1 ng of GABA<sub>B(1a)</sub>. For experiments examining the effect of excess truncated mGluR5 (tGRM5), each oocyte was injected with 167 pg each GIRK-1 and GIRK-2, 833 pg each GABA<sub>B(2)</sub> and GABA<sub>B(1a)</sub>, and either 6.67 ng of GABA<sub>B(1e)</sub> or 6.67 ng of tGRM5. Current was measured using standard two-electrode voltage clamp with glass microelectrodes containing 3 M KCl (300–800 kOhms tip resistance). The electrodes were amplified using a GenClamp 600 amplifier interfaced to a Digidata 1200, and data were acquired with pClamp6 software (Axon Instruments, Foster City, CA). Oocytes were maintained and voltage clamped to -60 mV in normal extracellular solution consisting of (in mM): NaCl (96), HEPES, hemisodium salt (5), KCl (2), CaCl<sub>2</sub> (1.8), and MgCl<sub>2</sub> (1), pH 7.4. The bath solution was replaced with high potassium extracellular solution consisting of (in mM): NaCl (90), HEPES, hemisodium salt (5), CaCl<sub>2</sub> (1.8), and MgCl<sub>2</sub> (1), pH 7.4. Oocytes were stepped to a range of potentials from -100 to +20 mV for 200 ms, and baclenol-elicted currents were calculated as the difference between currents measured in high potassium containing 100 μM baclenol minus currents measured in high potassium alone.

GABA<sub>B</sub> receptors were transiently co-transfected with cDNA expression constructs encoding the same receptor subunits used in oocytes. Inclusion of an CDS expression construct allowed cells to be identified after decoration with anti-CDS conjugated beads (Dynal Inc., Lake Success, NY). Cells were visualized on an Olympus inverted IX-70 microscope (Tokyo, Japan) and GABA-activated GIRK currents were recorded by standard patch clamp methods using an Axopatch 200B patch clamp amplifier (Axon Instruments). An elevated potassium external recording solution (200 mM) was used to include the GIRK-2 channel, and included NaCl (117.5), KCl (25), CaCl<sub>2</sub> (2.5), MgCl<sub>2</sub> (1.3), glucose (10), and HEPES (10) buffered to pH 7.3. Patch electrodes (3–5 MΩ) were filled with: K-gluconate (120), KCl (20), NaCl (4), MgCl<sub>2</sub> (1), EGTA (1), CaCl<sub>2</sub> (0.1), and HEPES (10) buffered to pH 7.3. Cells were voltage clamped at -80 mV and GABA (100 μM) was applied by bath perfusion (1–2 ml/min). GABA-activated GIRK currents exhibited inward rectification.
and were blocked by 1 mM BaCl$_2$.

Cyclic AMP Bioassay—HEK293 cells were seeded into poly-d-lysinetreated 96-well plates at a concentration of 2.5 × 10$^4$ cells per well and cultured overnight. Each well was transfected with 350 ng of plasmid DNA using 1 μl of LipofectAMINE 2000. All conditions were set up in triplicate, and individual DNA components common to different conditions were held constant by supplementing with pBluescript when necessary. Forty-eight hours post-transfection, cells were washed twice with KT buffer and incubated at 37°C in KT buffer + 1 mM of the phosphodiesterase inhibitor isobutylmethylxanathine (Sigma). After 20 min, the isobutylmethylxanathine solution was discarded and replaced with KT buffer containing 1 mM isobutylmethylxanathine, 10 μM forskolin (Sigma) + GABA (either 100 μM or 1 mM). Following 20 min incubation at 37°C, cAMP production was assessed using a Tropix bioassay (Tropix Inc., Bedford, MA) according to the manufacturers recommended procedure. Note that in all assays the treatment solution was discarded and replaced with 200 μl of lysis buffer diluted 1:2 in KT buffer.

RESULTS

GABA$_B$(1e) Is a Novel Splice Variant Derived from the GABA$_B$ Locus—In an attempt to isolate novel GABA$_B$ receptors, GABA$_B$(1a) and GABA$_B$(2) were aligned using the Clustal W sequence alignment tool and two motifs were targeted with degenerate PCR primers. Amplification of adult human brain cDNA generated the expected 1000-bp product composed of both GABA$_B$(1a) and GABA$_B$(2) sequences. In addition, we observed a second amplicon that was 150 bp smaller. Sequence analysis of this product revealed that it was derived from GABA$_B$(1a) but lacked exon 11 (21). The omission of exon 11 results in the introduction of two in-frame stop codons encoded by exon 12, and the consequential production of a truncated protein encoding only the extracellular domain of GABA$_B$(1a).

To assess the tissue distribution of both the GABA$_B$(1e) and GABA$_B$(1a) transcripts, primers were designed that flank exon 11. Because the resulting amplicons differ in size by only 150 bp, the expression of the two transcripts relative to each other could be determined quantitatively. Representative data from the analysis of 26 distinct human tissues are presented in Fig. 1A. To ensure that each cDNA contained approximately equal quantities of amplifiable material, all samples were amplified with glyceraldehyde-3-phosphate dehydrogenase-specific primers (data not shown). In all cases where the cDNAs were derived from the CNS (brain, cerebellum, fetal brain, and spinal cord), the 570-bp amplicon representing the full-length receptor was the predominant species. However, the opposite was true in the peripheral tissues that we tested. In all cases, a 420-bp amplicon representing the truncated receptor was the major species, and depending on the tissue, was often present in vast excess. As shown in Fig. 1B, a similar pattern of distribution was also observed in rat tissues, with the truncated receptor being the predominant isoform in the periphery.

In certain peripheral tissues such as the lung and small intestine (22), application of the agonist baclofen inhibits neurotransmitter release. Because full biological activity of the GABA$_B$ receptor requires dimerization between the 1a and 2 subunits, we used RT-PCR to investigate the expression of GABA$_B$(2) in the same panel of human tissues. One round of PCR (consisting of 30 cycles) detected GABA$_B$(2) mRNA in all the CNS tissues, as well as the salivary gland and thyroid (data not shown). By subjecting these products to a second round of PCR, we were able to detect mRNA for GABA$_B$(2), in a much broader range of peripheral tissues (Fig. 1A). Interestingly, several tissues express message for GABA$_B$(1b), but appear to lack message for GABA$_B$(2), most noticeably the kidney, lung, colon, and small intestine. There were no cases where GABA$_B$(2) was detected in lieu of either GABA$_B$(1a) or GABA$_B$(1e). Unfortunately, because two rounds of amplification were required to detect GABA$_B$(2) mRNA, it is unclear whether the failure to detect a product in these tissues indicates the total absence of GABA$_B$(2) message, or that it is simply expressed at such a low level as to be undetectable in our assay.

GABA$_B$(1e) Forms Heterodimers with GABA$_B$(2)—Dimerization of the GABA$_B$(1a) and GABA$_B$(2) subunits has been previously demonstrated, with the presumed dimerization domain attributed to a coiled-coil interaction between the cytoplasmic domain of each subunit. However, GABA$_B$(1e) which is the predominant species in the periphery, lacks an intracellular domain and therefore would not be able to dimerize via a coiled-coil interaction. Because other metabotropic receptors dimerize using a motif located within the amino-terminal ECD (17), we sought to determine whether heterodimerization might also occur between GABA$_B$(1e) and GABA$_B$(2) via interactions between the amino termini of their respective receptor subunits. To allow for detection of dimers, VS/polyhistidine epitope tags were incorporated at the carboxyl termini of recombinant proteins representing either full-length GABA$_B$(1a), or the ECD of GABA$_B$(1e) and the structurally related metabotropic glutamate receptor 5 ECD (GRM5). A full-length clone of GABA$_B$(2) was modified to incorporate a FLAG tag at the carboxyl terminus. The resulting fusion proteins, when transiently expressed in HEK293 cells, were all detected in the membrane fraction (Fig. 2 and data not shown). Additionally, each of the ECD constructs could be recovered from the conditioned culture media (Fig. 2 and data not shown) and are presumably secreted. The mobility of the secreted product was reduced relative to the membrane-associated form, suggesting
GABAB(1e) to compete with GABA B(1a) for dimerization with GABAB(2). To accomplish this, we co-transfected fixed quanti-

expression of GABAB(1) subunits.

sequently unrecognized capacity to regulate the overall ex-

of GABA B(1a) and GABA B(1e) in the same reaction. Impor-

tantly, as the concentration of GABA B(1a) was increased, the quantity of bound GABA B(1a) decreased. The magnitude of this

additional post-translational modification. The full-length GABA B(1a) clone was also detected in the membrane fraction, but was not detected in the conditioned media.

Combinations of the above constructs were co-transfected into HEK293 cells and GABA B(2) was immunoprecipitated with the M2 anti-FLAG antibody. To ensure production of the query

randed, parallel samples were captured using nickel-coated beads. As shown in Fig. 3B, the expected heterodimer of GABA B(1a)/GABA B(2) was readily detectable (lane 4). In addition,

we detected heterodimers between GABA B(2) and the ECD of GABA B(1e) (lane 5). In each case, the V5-tagged proteins that were not detected following the anti-FLAG immunoprecipitation were readily detectable following capture with nickel beads (Fig. 3A, lanes 1 and 2), and were not bound by the M2 antibody (Fig. 3C, lanes 1 and 2). Finally, the FLAG-tagged GABA B(2) construct was present in each of the appropriate immunoprecipitated samples (Fig. 3C, lanes 3–5).

The above data, which are representative of several independent experiments, consistently demonstrated a reduction in the amount of full-length receptor protein detected relative to the truncated receptor (see also Figs. 4 and 5). This disparity is particularly noticeable when the full-length 1a and 2 subunits are co-expressed (compare Fig. 3A, lanes 1 and 4). Unlike the truncated receptor which can be secreted, the full-length receptors are integral membrane proteins, and as such, there is a limited capacity for the receptor on the cell surface. In the absence of GABA B(2), GABA B(1a) is retained in the endoplasmic reticulum (23). Only following co-expression of GABA B(1a) is GABA B(1a) efficiently transported to the cell surface (11). This differential localization may account for the reduced protein expression we observe in our assays. Interestingly, co-expression of GABA B(2) also causes a noticeable reduction in the expression of GABA B(1a) (compare Fig. 5B, lanes 2 and 5). Together, these data may indicate that GABA B(2) possesses a heretofore unrecognized capacity to regulate the overall expression of GABA B(1a) subunits.

GABA B(1e) Interferes with GABA B(1a)/GABA B(2) Dimerization—Although GABA B(1e) is the primary transcript detected in most peripheral tissues, we also detect the simultaneous expression of the full-length transcript. Because both receptor types will dimerize with GABA B(2), we assessed the ability of GABA B(1e) to compete with GABA B(1a) for dimerization with GABA B(2). To accomplish this, we co-transfected fixed quantities of GABA B(1a)/V5-his and GABA B(2)/FLAG together with a 2-, 4-, or 10-fold molar excess of either GABA B(1a)/V5-his, or the structurally related ECD of the metabotropic glutamate receptor 5 (tGRM5/V5-his). As shown in Fig. 4A, GABA B(2) bound both GABA B(1a) and GABA B(1e) in the same reaction. Importantly, as the concentration of GABA B(1e) was increased, the quantity of bound GABA B(1a) decreased. The magnitude of this

effect was not reproduced when the concentration of tGRM5 was increased, even though the protein was readily produced and bound by nickel beads (Fig. 4B, lanes 3–5). This indicates that not only does GABA B(1e) specifically dimerize with GABA B(2), but it can successfully compete with GABA B(1a) for binding to GABA B(2). As such, this interaction is likely to contribute substantially to the overall avidity of the interaction between the 1a and 2 subunits.

The ECD Dimerization Motif Is Contained within the Amino-terminal Half of GABA B(1a)—To determine the region of GABA B(1a) responsible for dimerization, we developed COOH-terminal truncations that eliminated either exons 4–11 (data not shown) or 7–11 (amino acids 1–439, Fig. 5). The former construct was poorly expressed, perhaps reflecting the loss of one or more regions critical for maintaining protein stability. However, the latter construct readily dimerized with GABA B(2). Within this portion of the protein is a pair of “sushi” repeats (24, 25), the function of which is unknown. These repeats are absent from GABA B(1b), but are retained by GABA B(1e). To determine if dimerization might be mediated by either of the sushi domains, we created a series of progressive NH2-terminal truncations beginning at the region of the protein encoded by exons 1, 2, or 3 (amino acids 166–578, 220–578, and 265–578, respectively). In the absence of a signal peptide, none of these constructs associated with GABA B(2) (data not shown). However, the inclusion of a heterologous signal peptide allowed for the association of all three recombinant proteins with GABA B(2) (Fig. 5, lanes 7–9). Based on the intensity of the

FIG. 2. The GABA B(1e) receptor subunit is both membrane associated and secreted. HEK293 cells were transfected with V5-his-tagged GABA B subunits 1a or 1e, or mock transfected as indicated. The conditioned media (SUP) was captured with Ni-coated beads and analyzed, together with 8 μg of the low speed (LSM) or high speed membrane (HSM) fractions, by Western blotting with an anti-V5 antibody.

FIG. 3. Both GABA B(1a) and 1e receptor subunits heterodimerize with GABA B(2). V5-His (his) or FLAG (flag) epitope tags were incorporated at the carboxyl terminus of each GABA B receptor subunit as indicated. HEK 293 cells were transfiently transfected and lysed 48 h later in RIPA buffer. A, a fraction of each lysate (1/50) was analyzed by Western blotting with an anti-V5 antisera. B and C, the remainder of each lysate was immunoprecipitated with anti-FLAG-coupled beads and 1/2 of the eluted material was analyzed by Western blotting with either V5- or FLAG-specific antibodies.

A

B

C
compared the ability of GABA(B)1a amino acids 166–219 to target the key positions, or the critical domain lies outside of effect on dimerization (data not shown). Thus, either we failed this model, we altered amino acid positions 190 (Pro to Ala), 193 (Glu to Ala), 194 (Met to Arg), 197 (Glu to Ala), 217 (Asp to Ala), and 218 (Ser to Ala). None of these alterations had any this lack of binding was observed regardless of whether GABA(B)1a was expressed in its native form, or with an epitope tag. Although GABA(B)1e is secreted we were also unable to detect binding to His-tagged GABA(B)1e captured from conditioned culture media with nickel beads. Furthermore, we were unable to detect binding to an artificially truncated GABA(B)1a, construct (data not shown) that was previously demonstrated to bind CGP 54626A (7). This discrepancy may be attributable to our use of a different radiolabeled ligand, or a mammalian expression system rather than the insect expression system used in the aforementioned study. Alternatively, the ECD of the human GABA(B)1a subunit, unlike its rat counterpart, may lack the ability to bind CGP 54626A. Regardless,
the apparent inability of human GABA<sub>B(1e)</sub> to bind CGP 54626A does not necessarily indicate an inability to bind all GABA<sub>B</sub> receptor ligands.

**GABA<sub>B(1e)/GABA<sub>B(2)</sub> Heterodimers Do Not Activate GIRK Channels**—A common feature of GABA<sub>B</sub> receptors is their ability to activate GIRKs. While GABA<sub>B(1a)</sub> alone will occasionally activate these channels (27), efficient activation requires co-expression of the GABA<sub>B(1a)/GABA<sub>B(2)</sub> heterodimer (11–15). Since GABA<sub>B(1e)</sub> dimersizes with GABA<sub>B(2)</sub>, we tested the ability of this dimer to activate GIRK channels following heterologous expression in either Xenopus oocytes or mammalian HEK 293 cells. As shown in Fig. 7A, baclofen efficiently activated GIRK channels that were co-expressed with full-length GABA<sub>B(1a)</sub> + GABA<sub>B(2)</sub>. However, no activation was observed in a similar series of experiments using GABA<sub>B(1e)</sub> in place of GABA<sub>B(1a)</sub>. This was true whether data were collected using oocytes or mammalian HEK 293 cells (data not shown) for expression.

Because of the observed competition between the full-length and truncated receptors, and given that GABA<sub>B(1e)/GABA<sub>B(2)</sub> heterodimers do not activate GIRK channels, we postulated that the simultaneous expression of GABA<sub>B(1a)/GABA<sub>B(2)</sub> with an excess of GABA<sub>B(1e)</sub> would interfere with the ability of the full-length receptors to properly activate GIRK channels. As shown in Fig. 7B, a 14-fold molar excess of GABA<sub>B(1e)</sub> failed to significantly reduce the magnitude of GIRK channel activation relative to a parallel transfection with a 14-fold molar excess of tGRM5. To address any potential differences in receptor expression or association between oocytes and mammalian cells, similar experiments were performed in HEK 293 cells (Fig. 7C). However, the data were qualitatively indistinguishable from those obtained from oocytes. Thus, while GABA<sub>B(1e)</sub> competes with GABA<sub>B(1a)</sub> for dimerization to GABA<sub>B(2)</sub>, it does not appear to effect the normal function of the native receptor.

**GABA<sub>B(1e)</sub> Has No Effect on Forskolin-induced cAMP Production**—In addition to regulation of GIRK channel function, both the native and cloned GABA<sub>B</sub> receptors interfere with forskolin-induced adenylyl cyclase activation. This inhibition can be blocked by pertussis toxin and is therefore mediated by heterotrimeric G-proteins coupled to the GABA<sub>B</sub> receptor. While the heterodimeric receptor consistently has been shown to regulate adenylyl cyclase activity, the same cannot be said for the individual subunits. Although the original GABA<sub>B(1a)</sub/> isolate caused a marked reduction in cAMP production (6), other groups have been unable to reproduce this observation (11, 14, 15). Further studies have found that GABA<sub>B(2)</sub> alone can inhibit adenylyl cyclase (14, 16). The basis of these discrepancies is unclear, particularly since most investigators have used the same human cell line (HEK 293) for their studies.

To examine the ability of GABA<sub>B(1a)</sub> and GABA<sub>B(1e)</sub> to inhibit forskolin-induced adenylyl cyclase activity, we expressed the respective cDNAs in HEK 293 cells either alone, or co-transfected with a 2-fold molar excess of GABA<sub>B(2)</sub>. To address any potential reduction in the affinity of GABA for the 1e subunit, we tested both 1 mM and 100 μM concentrations of GABA in those cells co-expressing both the GABA<sub>B(1a)</sub> and GABA<sub>B(2)</sub> subunits. As shown in Fig. 8, none of the GABA<sub>B</sub> subunits, when expressed alone, had any significant effect on cAMP produc-
tion. However, the coexpression of GABA$_B$ subunits 1a and 2 caused a 44% reduction in cAMP (1888 fmol with forskolin alone, 1057 fmol in the presence of 100 μM GABA). In the case of cells co-expressing the 1e and 2 subunits, there is a minor reduction in the amount of cAMP produced in the presence of 100 μM GABA, but this effect does not reproduce in similar experiments, nor is there any effect using 1 mA GABA.

**DISCUSSION**

We have identified a new GABA$_B$ splice variant encoding a truncated receptor composed of only the ligand binding ECD. The GABA$_B_{1(1e)}$ transcript is widely distributed in human and rat tissues, but its abundance relative to the full-length receptor differs dramatically when comparing the CNS and peripheral tissues. Thus, in the CNS, GABA$_B_{1(1e)}$ is a minor contributor to the overall level of GABA$_B$ transcripts. However, in peripheral tissues we find that GABA$_B_{1(1e)}$ is typically the primary isoform expressed. Although we have yet to directly demonstrate the existence of protein in vivo, it is likely that the protein is produced since GABA$_B_{1(a)}$ and GABA$_B_{1(1e)}$ transcripts only differ with respect to the possession of exon 11 sequences. Furthermore, we readily detect both GABA$_B_{1(1a)}$ and GABA$_B_{1(1e)}$ proteins in heterologous expression systems.

We have compared the function of the truncated receptor to its full-length counterpart with respect to binding the antagonist CGP 54626A, GIRQ activation, and inhibition of adenylyl cyclase activity. By each of these assays we were readily able to measure the expected functional activity of the full-length receptor. However, in none of these assays were we able to detect any biological response from the truncated receptor, regardless of whether it was expressed alone or in combination with GABA$_B_{2(c)}$. Given that the GABA$_B_{1(1e)}$ isoform has been conserved in rats and humans, it seems unlikely that it represents a splicing artifact. However, because the truncated receptor appears to lack the traditional biological activities ascribed to the full-length receptor, its biological function in vivo presumably fulfills a hitherto unknown regulatory function.

If we assume that, as found in the present study, GABA$_B_{1(1e)}$ lacks the ability to bind ligand, then its function in the peripheral may be to regulate the availability of functional GABA$_B_{1(a)}/$GABA$_B_{2(c)}$ heterodimers by competing for GABA$_B_{2(c)}$ dimerization. This type of regulation could explain the observation that certain small molecule ligands exhibit differential affinity for different GABA$_B$ subunits, and that the GABA$_B_{1(a)}$ subunit is primarily retained within the endoplasmic reticulum when expressed alone in a heterologous system, and that the GABA$_B_{2(c)}$ subunit is required for it to be efficiently secreted from the cell surface. Because the GABA$_B_{1(1e)}$ is very efficiently secreted from the cell, it is clear that the residues responsible for endoplasmic reticulum retention do not reside with the ECD.

The above observations regarding dimerization are very intriguing when considered in light of the fact that overexpression of GABA$_B_{1(1e)}$ in the presence of the normal GABA$_B_{1(a)}/$GABA$_B_{2(c)}$ heterodimer potently interferes with dimerization, yet has no obvious effect on GIRQ activation. This implies that there is a pool of the receptor heterodimer which is either unaffected by the truncated receptor, or which does not intersect the cellular transport pathway used by GABA$_B_{1(1e)}$. Curiously, Benke et al. (28) observed a subset of native GABA$_B$ receptors with an apparent molecular mass greater than would be expected for the heterodimer alone. It will be interesting to determine not only the composition of this subset, but what function, if any, it has in the normal biology of the native GABA$_B$ receptor.

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