$GABA_B$-receptor subtypes assemble into functional heteromeric complexes

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B-type receptors for the neurotransmitter GABA (γ-aminobutyric acid) inhibit neuronal activity through G-protein-coupled second-messenger systems, which regulate the release of neurotransmitters and the activity of ion channels and adenylyl cyclase[1]. Physiological and biochemical studies show that there are differences in drug efficiencies at different $GABA_B$ receptors, so it is expected that $GABA_B$-receptor ($GABA_BR$) subtypes exist[2]. Two $GABA_B$-receptor splice variants have been cloned[3] ($GABA_BR1a$ and $GABA_BR1b$), but native $GABA_B$ receptors and recombinant receptors showed unexplained differences in agonist-binding potencies. Moreover, the activation of presumed effector ion channels in heterologous cells expressing the recombinant receptors proved difficult[3–4]. Here we describe a new $GABA_B$ receptor subtype, $GABA_BR2$, which does not bind available $GABA_B$ antagonists with measurable potency. $GABA_BR1a$, $GABA_BR1b$ and $GABA_BR2$ alone do not activate Kir3-type potassium channels efficiently, but co-expression of these receptors yields a robust coupling to activation of Kir3 channels. We provide evidence for the assembly of heteromeric $GABA_B$ receptors *in vivo* and show that $GABA_BR2$ and $GABA_BR1a/b$ proteins immunoprecipitate and localize together at dendritic spines. The heteromeric receptor complexes exhibit a significant increase in agonist- and partial-agonist-binding potencies as compared with individual receptors and probably represent the predominant native $GABA_B$ receptor. Heteromeric assembly among G-protein-coupled receptors has not, to our knowledge, been described before.

GenBank database searches revealed two expressed sequence tags, T07621 and HSC1HH041, with sequence similarity to the previously cloned $GABA_BR1a/b$ receptors. We used this sequence information to isolate a 5.6-kilobase rat complementary DNA encoding a protein of 941 residues (Fig. 1a). Hydrophobicity analysis showed that this protein had a topological organization typical of a G-protein-coupled receptor (GPCR). The deduced protein sequence is most closely related to that of $GABA_BR1a$ and

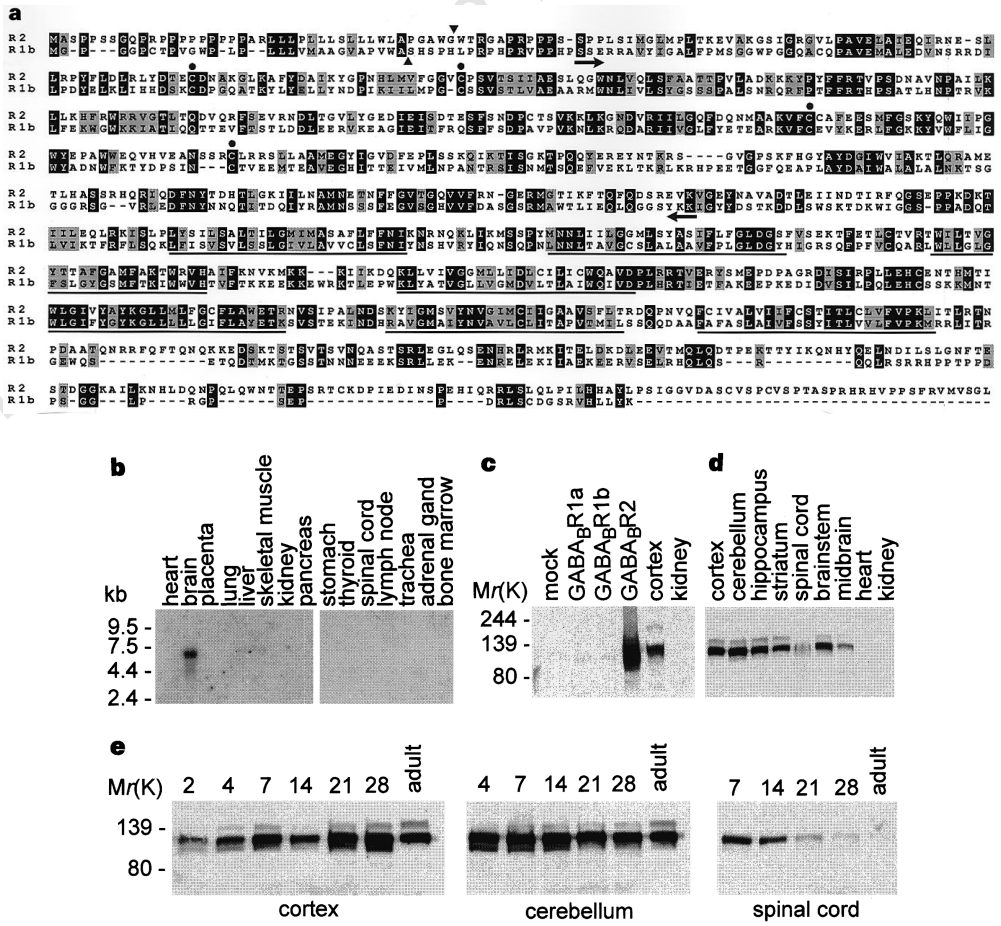

Figure 1 Sequence and expression of $GABA_BR2$ (R2). **a**, Alignment of rat $GABA_BR2$ and $GABA_BR1b$[3] (R1b) amino-acid sequences. Identical amino acids are boxed in black; similar residues are shown in grey; membrane-spanning regions are underlined. Filled circles indicate conserved cysteine residues in the amino-terminal domain. Proposed sites of cleavage of signal peptides[24] are marked with arrowheads. The putative ligand-binding site, which shares homology with the ligand-binding site of bacterial amino-acid-binding proteins[25], is delimited by arrows. **b**, Northern blot analysis of $GABA_BR2$ transcripts. **c**, Antibody AbC22 recognizes native and recombinant $GABA_BR2$ on immunoblots and does not crossreact with $GABA_BR1a/b$. **d**, Immunoblot analysis of $GABA_BR2$ protein in adult rats. **e**, Immunoblot analysis of $GABA_BR2$ in the cortex, cerebellum and spinal cord in rats of postnatal days 2 to 60 (adults).

$GABA_BR1b$ (35% identity). Using northern blot analysis, we detected $GABA_BR2$ transcripts in the brain but not in the other tissues analysed (Fig. 1b). We raised antiserum AbC22, which is specific for the $GABA_BR2$ protein (Fig. 1c), and used it for immunoblot analysis; we detected a protein of relative molecular mass 110,000 (M_r 110K) in transfected cells that is expressed in all major brain areas (Fig. 1c, d). The $GABA_BR2$ protein is abundant in cortex and cerebellum throughout postnatal development whereas its expression in spinal cord gradually decreases (Fig. 1e).

We used membranes from transfected COS-1 cells expressing $GABA_BR2$ for binding assays with the $GABA_B$-receptor radioligands ^{125}I-labelled CGP64213 (0.5 nM), ^{3}H-labelled CGP54626A (50 nM), ^{3}H-labelled APPA (3-aminopropylphosphinic acid) (50 nM) and ^{3}H-labelled GABA (50 nM) and for photoaffinity labelling experiments with ^{125}I-labelled CGP71872 (0.5 nM). None of the ligands bound to $GABA_BR2$ with measurable potency (data not shown). However, recombinant $GABA_BR1a$/$GABA_BR1b$ exhibits a 100- to 150-fold reduced binding potency for agonists as compared with native receptors[3], which precludes a direct measurement of binding to ^{3}H-labelled agonists; therefore, a lack of detectable binding of ^{3}H-labelled GABA to recombinant $GABA_BR2$ protein does not rule out the presence of an agonist-binding site in $GABA_BR2$. When $GABA_BR1a$ or $GABA_BR1b$ is expressed together with $GABA_BR2$, an up to tenfold increase in binding potency is observed, as measured by the inhibition of ^{125}I-labelled CGP64213 binding to the receptor by agonists and partial agonists (Fig. 2a, b). No further increase in binding potency occurred when $GABA_BR2$ cDNA was transfected in more than tenfold excess over $GABA_BR1a$/b cDNAs. In contrast, no significant change in the antagonist-binding pharmacology was detectable (Fig. 2c, d). There is evidence for the existence of dimeric receptors in the $GABA_B$ receptor, metabotropic glutamate receptor and Ca^{2+}-sensing receptor gene family[5,6]. The conspicuous increase in agonist-binding potency observed here on co-expression of $GABA_BR1a$/b and $GABA_BR2$ led us to study the possibility of heteromeric $GABA_B$ receptors.

$GABA_B$ receptors generate late inhibitory postsynaptic potentials by activating Kir3-type K^+ channels[7,8]. Transfected HEK-293 cells that express Kir3 channels together with $GABA_BR1a$/b (ref. 4) or $GABA_BR2$ (Fig. 3a, b) infrequently yield a roughly twofold increase in K^+ current (I_{kir}) after application of 50 μM GABA. Only 1 out of 10 and 9 out of 62 cells expressing a I_{kir} of >200 pA (extracellular K^+ concentration 25 mM) showed I_{kir} upregulation by $GABA_BR1a$/b and $GABA_BR2$, respectively. In $GABA_BR2$-expressing cells, I_{kir} upregulation is not blocked by the $GABA_B$ antagonist CGP54626A (Fig. 3a), consistent with our failure to demonstrate binding of this antagonist to the receptor. This shows that $GABA_BR2$ is a $GABA_B$-receptor subtype with pharmacological properties that are different from those of $GABA_BR1a$/b.

Although 3 out of 40 *Xenopus* oocytes that were injected with both Kir3 and $GABA_BR1a$/b complementary RNAs showed small currents in response to L-baclofen (10 μM) or GABA (100 μM), we did not detect such responses in 28 oocytes expressing $GABA_BR2$ with Kir3 (Fig. 3c). However, when we co-injected cRNAs for $GABA_BR2$, $GABA_BR1a$ and Kir3, application of L-baclofen

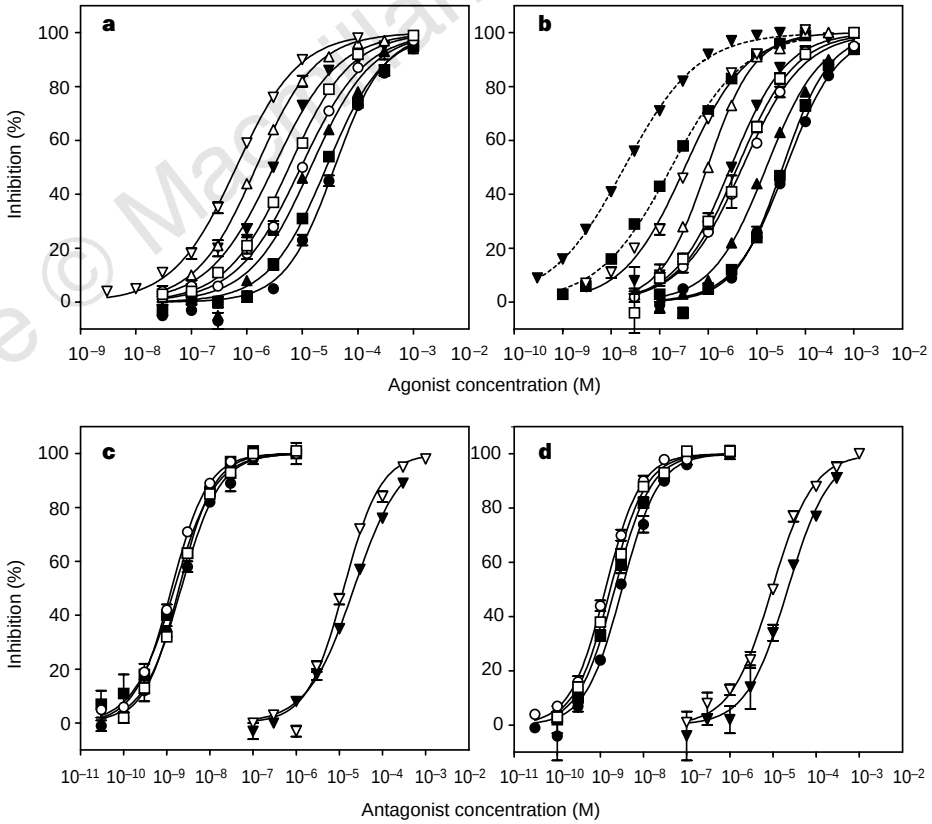

Figure 2 Binding pharmacology of $GABA_BR2$ co-expressed with $GABA_BR1a$ or $GABA_BR1b$. The pharmacology of membranes from COS-1 cells expressing $GABA_BR2$ (20-fold excess) together with $GABA_BR1a$ (**a**, **c**; open symbols) or $GABA_BR1b$ (**b**, **d**; open symbols) was compared with the pharmacology of membranes from cells expressing $GABA_BR1a$ (**a**, **c**; filled symbols) or $GABA_BR1b$ individually (**b**, **d**; filled symbols) and with the pharmacology of brain $GABA_B$ receptors (**b**, dotted lines, parallel assays). **a**, **b**, Inhibition of binding of ^{125}I-labelled CGP64213 to $GABA_B$ receptors by agonists. **c**, **d**, Inhibition of binding of ^{125}I-labelled CGP64213 to $GABA_B$ receptors antagonists. Immunoblotting and photoaffinity labelling confirmed the expression of receptor protein (results not shown). The IC_{50} values for agonists are shifted about 8- to 9-fold ($GABA_BR1b$/$GABA_BR2$) or 2.5- to 5-fold ($GABA_BR1a$/$GABA_BR2$) towards higher potencies in co-transfected cells. The partial agonist CGP47656 (ref. 3) shows a tenfold increase in potency. Antagonist affinities do not differ significantly. Results from typical experiments performed in triplicate are shown. In **a**, **b**, squares indicate that the agonist GABA was used; circles, L-baclofen; inverted triangles, APPA; and triangles, CGP47656. In **c**, **d**, circles indicate the use of the antagonist CGP54626A; squares, CGP64213; and inverted triangles, CGP35348.

(10 μM), APPA (1 μM) and GABA (10 μM) evoked inward currents at −70 mV in 38 of 52 oocytes (Fig. 3c–e). On average, the upregulation of I_{kir} was 32 ± 5%. The half-maximal effective concentration (EC_{50}) value for L-baclofen at $GABA_BR1a/GABA_BR2$ and $GABA_BR1b/GABA_BR2$ receptors expressed in oocytes is 5 μM (Fig. 3e). This EC_{50} is similar to values obtained with $GABA_BR1a$ (11.3 μM)[4] and $GABA_BR1a/GABA_BR2$ (1.5 μM; see Supplementary Information) expressed in HEK-293 cells. It also approximately matches the values obtained for native $GABA_B$ receptors[7,9,10]. CGP54626A blocked I_{kir} upregulation with a half-maximal inhibitory concentration (IC_{50}) of 10 nM (Fig. 3f).

The requirement for two distinct $GABA_B$ receptors for a robust coupling of receptor activation to I_{kir} explains in retrospect why it has been possible to demonstrate modulation of I_{kir} in oocytes injected with cerebellar poly(A)$^+$ RNA[11], but not in oocytes injected with $GABA_BR1a/b$ cRNA[3]. Assembly of heteromeric receptors may be required for effective transport of the receptor to the membrane, as $GABA_BR1a/b$ receptors by themselves fail to reach the cell surface in heterologous cells[12]. The recombinant expression experiments therefore indicate that efficient $GABA_B$-receptor coupling *in vivo* may require assembly of heteromeric receptors. The increased potency of agonist binding observed with heteromeric $GABA_B$ receptors (Fig. 2a, b) could then arise from a more efficient coupling to G proteins[13].

We confirmed the existence of heteromeric $GABA_B$ receptors in immunoprecipitation experiments (Fig. 4). Antibody AbC22, raised against $GABA_BR2$, efficiently co-precipitated the $GABA_BR1a/b$ proteins, tagged with ^{125}I-labelled CGP71872, from cortical membrane preparations (Fig. 4a). Conversely, antibody Ab60696, which recognizes an epitope shared by the $GABA_BR1a/b$ variants, co-precipitated the $GABA_BR2$ receptor (Fig. 4b). Immunoprecipitation experiments with transfected cells expressing receptor combinations showed that $GABA_BR2$ can associate with $GABA_BR1a$ and $GABA_BR1b$ individually (Fig. 4c). We did not find any evidence for the formation of intermolecular disulphide bonds between $GABA_B$ receptors; such bonds do form within dimers of metabotropic glutamate receptor 5 (ref. 6) and the Ca^{2+}-sensing receptor[5].

In situ hybridization shows that there are high levels of $GABA_BR2$ messenger RNA in the cortex and hippocampus (Fig. 5a). Throughout the brain, the patterns of distribution of $GABA_BR1a/b$ and $GABA_BR2$ mRNA largely overlap (Fig. 5a, b). $GABA_BR2$ transcript levels are low as compared with amounts of $GABA_BR1a/b$ transcripts in the basal forebrain (striatum and nucleus accumbens) and the olfactory bulb. In the cerebellum, high levels of both $GABA_BR1b$ (ref. 4) and $GABA_BR2$ transcripts are found in Purkinje cells, demonstrating co-expression in neuronal populations (Fig. 5c–f). Using immunoelectron microscopy, we determined whether $GABA_BR1a/b$ and $GABA_BR2$ co-localize at neuronal membranes in the molecular layer of the cerebellum (Fig. 5g, h). Immunogold labelling of both receptors occurs at extrasynaptic sites of Purkinje cell spines that form type 1 synapses with parallel fibre terminals (Fig. 5g; shown for $GABA_BR2$). Double labelling showed that most

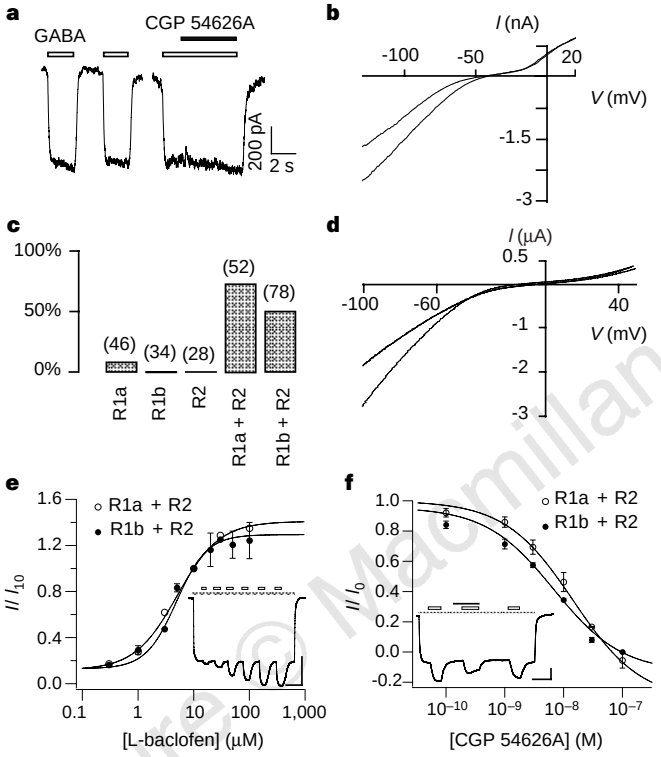

Figure 3 Coupling of $GABA_B$ receptors to Kir3 channels in transfected HEK-293 cells and *Xenopus* oocytes. **a**, **b**, Current recordings from HEK-293 cells co-expressing $GABA_BR2$ (R2) and concatenated pairs of Kir3.1/3.2 channels in an extracellular K^+ concentration of 25 mM. **a**, 50 μM GABA and 1 μM CGP54626A were applied as indicated. Holding potential was 120 mV. **b**, Current–voltage relation (*I*–*V*) in the absence (upper trace) and presence (lower trace) of 50 μM GABA. **c**–**f**, Recordings from *Xenopus* oocytes expressing $GABA_BR1a$(R1a), $GABA_BR1b$ (R1b) or $GABA_BR2$ (R2) or combinations thereof together with Kir3.1/3.2/3.4 channels. **c**, Percentage of oocytes that exhibit an upregulation of I_{kir} of >2% after superfusion of GABA (100 μM) or L-baclofen (10 μM). The numbers of cells tested is shown in parentheses. **d**, *I*–*V* in the absence (upper trace) and presence (lower trace) of L-baclofen (10 μM). **e**, Concentration–response curve (CRC) for L-baclofen. Agonist-induced currents are normalized to those evoked by 10 μM L-baclofen (I_{10}). The EC_{50} values for R1a + R2 and R1b + R2 are 5.0 ± 1.5 μM ($n = 4$) and 4.9 ± 0.7 μM ($n = 6$), respectively. Inset shows raw current trace of a CRC for R1a + R2; the time of application of high-potassium Ringer solution (dotted line) and of (open bars from left to right) 0.3, 1, 3, 10, 30 and 100 μM L-baclofen are indicated. Scale bars, vertical, 500 nA and horizontal, 100 s. **f**, CRC for CGP54626A. Values are normalized to currents evoked by 10 μM L-baclofen in the absence of antagonist (I_0). The IC_{50} values for R1a + R2 and R1b + R2 are 15 ± 6 nM ($n = 3$) and 7 ± 0.8 nM ($n = 3$), respectively. The inset shows the blockage of I_{kir} upregulation by 10 nM CGP54626A (black bar), 10 μM L-baclofen (open bars), and high-potassium Ringer solution (dotted line). Scale bars, 200 nA and 100 s. **e**, **f**, The smooth lines represent Hill equations fitted to the data points.

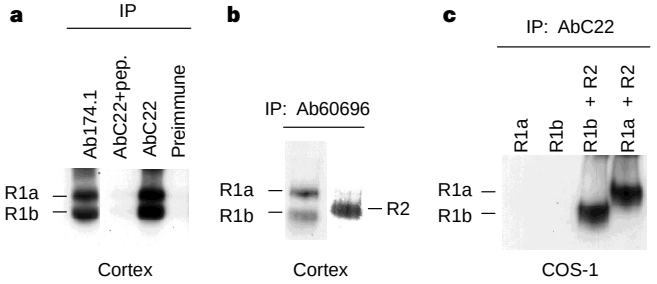

Figure 4 Immunoprecipitation analysis of $GABA_B$ receptors. $GABA_B$-receptor expression in **a**, **b**, cortex and **c**, transfected COS-1 cells. Membranes were labelled with ^{125}I-labelled CGP71872, and proteins were immunoprecipitated (IP) with antibody AbC22, Ab60696 or Ab174.1 and subjected to SDS–PAGE. $GABA_BR1$ and $GABA_BR1b$ proteins labelled with ^{125}I-labelled CGP71872 were detected using phosphorimaging. $GABA_BR2$ was detected on immunoblots using antibody AbC22. **a**, Ab174.1 directed against $GABA_BR1$ precipitates $GABA_BR1a$ and $GABA_BR1b$ labelled with ^{125}I-labelled CGP71872 (lane 1). AbC22 directed against $GABA_BR2$ co-precipitates ^{125}I-labelled $GABA_BR1a$ and $GABA_BR1b$ (lane 3). Immunoprecipitation was blocked by the corresponding antigenic peptide (10 μg, lane 2) and preimmune serum (lane 4). **b**, Ab60696 directed against $GABA_BR1a$ and $GABA_BR1b$ co-immunoprecipitates $GABA_BR2$. **c**, $GABA_BR1a$ and $GABA_BR1b$ are co-immunoprecipitated with AbC22 when expressed in pairwise combination with $GABA_BR2$. AbC22 does not precipitate $GABA_BR1a$ or $GABA_BR1b$ in COS-1 cells that do not express $GABA_BR2$.

of the $GABA_BR2$-positive spines were also labelled for $GABA_BR1a/b$ and vice versa, indicating extensive co-localization (Fig. 5h). Thus our data indicate that $GABA_B$ receptors may form heteromers at select neuronal sites.

The fact that heterologous $GABA_B$ receptors couple to Kir3 (shown here) and to adenylyl cyclase[3], and that $GABA_BR1a/b$-containing receptors inhibit high-voltage-activated Ca^{2+} channels[14], shows that all major effects of native $GABA_B$ receptors could relate to those of the characterized heteromeric receptors. Given the precedent for dimerization among GPCRs[5,6] we expect a heterodimeric configuration for the most abundant native $GABA_B$ receptors, but another stoichiometry is possible. The coupling of individual $GABA_BR1$ (ref. 4) and $GABA_BR2$ (Fig. 3a, b) receptors to Kir3 may be the consequence of the expression of excessive amounts of receptors that force targeting to the cell surface. Nevertheless, the occurrence of homomeric receptors *in vivo* cannot be ruled out. The remaining tenfold discrepancy in apparent agonist-binding potency between heteromeric recombinant and native receptors may be explained by receptor modification (for example, by phosphorylation) or by differences in the relative expression levels of G proteins and receptors[15], and other proteins that assemble with GPCRs may alter the pharmacological properties of $GABA_B$ receptors[16,17]. □

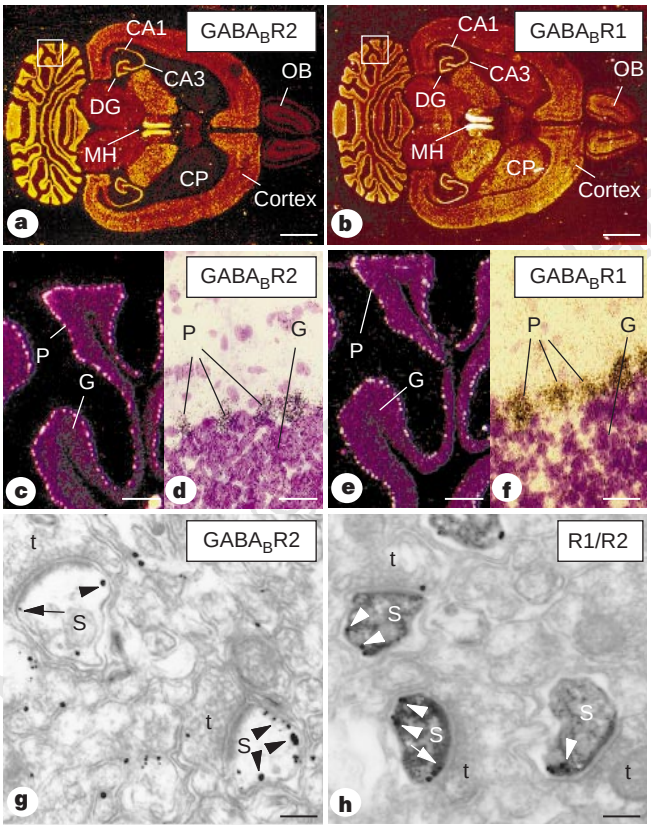

Figure 5 Cellular and subcellular distribution of $GABA_B$-receptor mRNA and protein in rat brain. **a**–**f**, *In situ* hybridization analysis. Autoradiograms of horizontal sections in dark-field (**a**–**c**, **e**) and bright-field (**d**, **f**) illuminations from the dorsal tier of the brain (**a**, **b**) and the lobules of the cerebellar cortex (**c**–**f**). Transcripts of both $GABA_BR1$ and $GABA_BR2$ are abundant in the medial habenula (MH), the pyramidal cells of the CA1–CA3 subfields of the hippocampus, the granular layer of the dentate gyrus (DG) and the cortex. $GABA_BR2$ transcripts are less abundant than $GABA_BR1$ transcripts in the caudate putamen (CP) and the olfactory bulb (OB). $GABA_BR2$ and $GABA_BR1$ transcript expression is higher in Purkinje cells (P) than in the granule layer (G). **g**, **h**, Electron micrographs in the molecular layer showing extensive co-localization of $GABA_BR2$ and $GABA_BR1$ protein in Purkinje cell spines (s) that form type 1 (asymmetrical) synapses with parallel fibre terminals (t): labelling is with immunogold for $GABA_BR2$ (**g**) and $GABA_BR1$ (**h**) and immunoperoxidase for $GABA_BR2$ (**h**). Immunoparticles are present at extrasynaptic sites (arrowheads) of the Purkinje cell plasma membrane. Some particles are found at the edge (arrows) of the postsynaptic density area. Scale bars. **a**, **b**, 1.3 mm; **c**, **e**, 120 μm; **d**, **f**, 10 μm; and **g**, **h**, 0.2 μm.

Methods

Ligands. $GABA_B$ ligands were synthesized in house. ^{125}I-labelled CGP64213 and ^{125}I-labelled CGP71872 were radiolabelled by ANAWA (Switzerland) to a specific activity of 2,000 Ci $mmol^{-1}$.

Cloning of $GABA_BR2$. Expressed sequence tags were cloned by reverse-transcription with polymerase chain reaction (RT–PCR) using cDNAs synthesized from human cerebellum poly(A)$^+$ mRNA (Clontech). The PCR products were used to screen a rat brain cDNA library as described[3].

***In situ* hybridization and northern blot analysis.** *In situ* hybridization histochemistry using 10-μm cryosections (post-fixed with 4% paraformaldehyde) of rat brain (male Tif RAI f (specific pathogen free), weighing 250 g) was as described[18]. Riboprobes for $GABA_BR2$ were derived from a 724-base-pair *Nhe*I/*Sac*I cDNA fragment subcloned into Bluescript (Stratagene). For $GABA_BR1$ a pan probe that is common to $GABA_BR1a$ and $GABA_BR1b$ was used[3]. Dipped slides were exposed for 15 days. Northern blots using poly(A)$^+$ mRNA (Clontech) were hybridized as described[3].

Cell culture and transfections. COS-1 and HEK-293 cells were obtained from the American Type Culture Collection and transfected with receptor cDNAs as described[3].

Membrane preparations. Membranes from transfected cells were collected 3 days after cell transfection. Cell lysates of transfected cells and synaptic membranes from rat tissues (male Tif RAI f (SPF)) were prepared as described[19,20].

Photoaffinity labelling, antibodies and immunoprecipitation. Photolabelling with ^{125}I-labelled CGP71872 was done as described[3]. Antibodies Ab60696 (Pharmingen) and Ab174.1 are directed against carboxy-terminal epitopes of $GABA_BR1a/b$ (ref. 19). To generate polyclonal antibody AbC22 in New Zealand white rabbits, we expressed a $GABA_BR2$ fragment corresponding to amino acids 806–907 as glutathione-*S*-transferase fusion protein in *Escherichia coli* using plasmid pGEX-2T (Pharmacia). For immunoprecipitation, proteins were solubulized in 50 mM HEPES, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol and 1% Triton-X100 including protease inhibitors (Boehringer) at 4 °C for 1 h and centrifuged for 10 min at 10,000*g*; the supernatant was then centrifuged at 100,000*g* for 1 h. After twofold dilution in buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% Triton-X100, protease inhibitors), protein A–Sepharose pellets were washed twice with the above buffer. Immunoblotting was done as described[19].

Pre-embedding immunoelectron microscopy. Cerebellar sections of Wistar rats were treated for single and double immunolabelling with anti-$GABA_BR2$ (guinea pig) and anti-$GABA_BR1$ (rabbit) antibodies as described[21].

Electrophysiology. Whole-cell patch-clamp[22] and oocyte[23] recordings were done as described. cRNAs were injected into oocytes at a concentration ratio of 4:1:0.2 ($GABA_BR2$:$GABA_BR1a/b$:Kir3.1/3.2/3.4). In high-potassium Ringer solution, 87.5 mM NaCl was replaced by KC1.

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Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

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Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K^+ channels causes epilepsy

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Epilepsy affects more than 0.5% of the world's population and has a large genetic component[1]. It is due to an electrical hyperexcitability in the central nervous system. Potassium channels are important regulators of electrical signalling, and benign familial neonatal convulsions (BFNC), an autosomal dominant epilepsy of infancy, is caused by mutations in the *KCNQ2* or the *KCNQ3* potassium channel genes[2–4]. Here we show that KCNQ2 and KCNQ3 are distributed broadly in brain with expression patterns that largely overlap. Expression in *Xenopus* oocytes indicates the formation of heteromeric KCNQ2/KCNQ3 potassium channels with currents that are at least tenfold larger than those of the respective homomeric channels. KCNQ2/KCNQ3 currents can be increased by intracellular cyclic AMP, an effect that depends on an intact phosphorylation site in the KCNQ2 amino terminus. KCNQ2 and KCNQ3 mutations identified in BFNC pedigrees compromised the function of the respective subunits, but exerted no dominant-negative effect on KCNQ2/KCNQ3 heteromeric channels. We predict that a 25% loss of heteromeric KCNQ2/KCNQ3-channel function is sufficient to cause the electrical hyperexcitability in BFNC. Drugs raising intracellular cAMP may prove beneficial in this form of epilepsy.

We cloned the complete complementary DNA of the human KCNQ3 K^+ channel by homology to KCNQ1 (also known as KVLQT1 (ref. 5)) and determined its genomic structure. We identified two intronic CA nucleotide repeats which represent microsatellite markers linked previously to the long arm (q) of chromosome 8 at band 24, one of the known loci[6,7] for BFNC. Indeed, a partial KCNQ3 cDNA was cloned recently and a KCNQ3 mutation was identified in a BFNC family[3]. The KCNQ3 protein (Fig. 1) is 41% identical to KCNQ2, the other K^+ channel mutated in BFNC, and 31% identical to KCNQ1, a K^+ channel subunit mutated in the long QT syndrome[5] (LQTS). It displays the typical structure of a K^+ channel with six transmembrane domains and a pore-forming P-loop.

KCNQ3 shows similarities to KCNQ2 (ref. 2) in being highly specific for brain (Fig. 2a). Northern blot analysis and *in situ* hybridization revealed that both genes are co-expressed in most brain regions, but there are some regional quantitative differences (Fig. 2b). Potassium channels are tetramers of identical or homologous α-subunits[8–10], raising the possibility that KCNQ2 and KCNQ3 form heteromeric channels. After co-expressing KCNQ2 and KCNQ3 that had been N-terminally tagged with haemagglutinin (HA) or Flag epitopes in *Xenopus* oocytes, we were able to co-

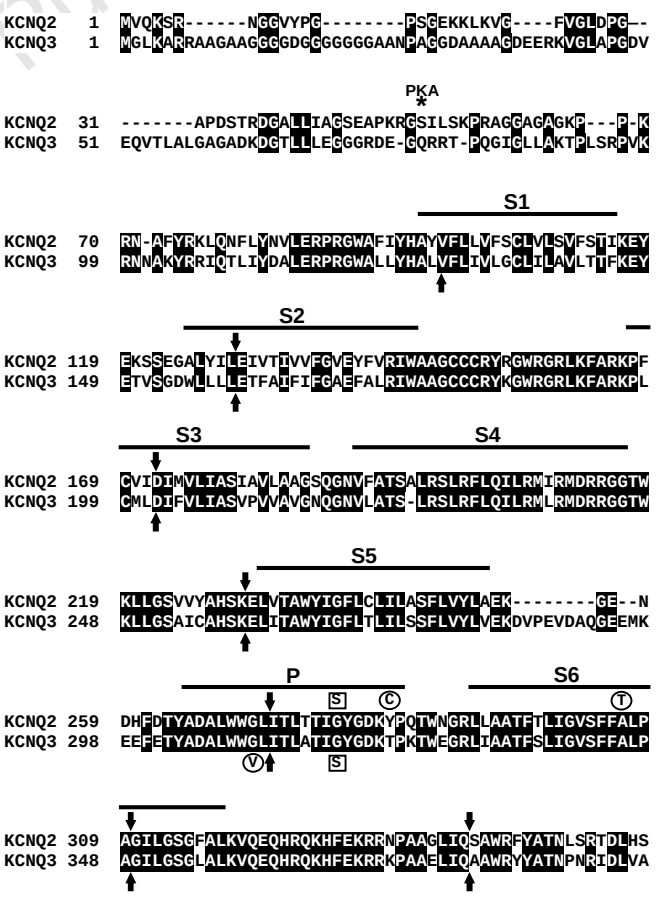

Figure 1 Partial alignment of KCNQ2 and KCNQ3 potassium channel subunits showing the newly identified KCNQ3 N terminus, the cAMP-dependent phosphorylation site (*) in KCNQ2, and the point mutations introduced here. Identical residues are marked by black background. Predicted transmembrane spans S1–S6 and the P-loop (P) are indicated. Positions of introns in both genes are shown by arrows, but the KCNQ2 exon-intron structure is incomplete[2]. The Y284C and A306T (KCNQ2) and G310V (KCNQ3) mutations (circles) were found[3,4] in BFNC pedigrees, whereas the G279S (KCNQ2) and G318S (KCNQ3) mutations (squares) were modelled on mutations in KCNQ1 found[14] in dominant LQTS patients.