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ORIGINAL INVESTIGATION

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Functional characterization of the new human $GABA_A$ receptor mutation β3(R192H)

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Abstract We screened 124 individuals for single nucleotide polymorphisms of the α1, β3 and γ2 genes of the $GABA_A$ receptor in the regions corresponding to the ligand-binding domains on the protein level. In a patient with chronic insomnia, a missense mutation was found in the gene of the β3 subunit. This mutation results in the substitution of the amino acid residue arginine for histidine in position 192 (β3(R192H)). The patient was found to be heterozygous for this mutation. Functional analysis of human α1β3(R192H)γ2S $GABA_A$ receptors using ultra fast perfusion techniques revealed a slower rate of the fast phase of desensitization compared with α1β3γ2S $GABA_A$ receptors. Additionally, current deactivation [a major determinant of inhibitory postsynaptic current (IPSC) duration] was faster in the mutated receptors. This raises the possibility of decreased GABAergic inhibition contributing to insomnia, as some members of the patient's family also suffer from insomnia. The mutation β3(R192H) might, therefore, be linked to this condition. The intron/exon boundaries of the α1 subunit gene were also established and three additional variants were found in the α1 and β3 genes.

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Introduction

$GABA_A$ receptors constitute the major inhibitory neuronal ion channels in the mammalian brain. They belong to the family of ligand-gated ion channels that includes nicotinic acetylcholine, glycine and serotonin type 3 receptors. Biochemical purification was achieved (Sigel et al. 1983) and subsequent cloning of the $GABA_A$ receptors have identified 16 different mammalian subunits: α1–6, β1–3, γ1–3, δ, ε, π and θ (Macdonald and Olsen 1994; Rabow et al. 1995; Davies et al. 1997; Hedblum and Kirkness 1997; Barnard et al. 1998; Bonnert et al. 1999; Sinkkonen et al. 2000). Most $GABA_A$ receptors are thought to be pentameric assemblies of αβγ and αβδ subunits (McKernan and Whiting 1996). The most likely stoichiometry is two α subunits, two β subunits, and one γ or δ subunit (for review see Mehta and Ticku 1999). Upon vesicular release of GABA, post-synaptic $GABA_A$ receptors are activated, resulting in chloride ion influx and hyperpolarization of the cell membrane potential. This inhibitory synaptic current (IPSC) decreases the electrical excitability of the postsynaptic neuron. $GABA_A$ receptors are drug targets for a range of clinically important substances, among them the benzodiazepines and barbiturates (Sieghart 1995; Sigel and Buhr 1997).

Classical benzodiazepines allosterically stimulate α1–3,5β2,3γ2,3 $GABA_A$ receptors in vitro and exert sedative, anxiolytic, muscle relaxant and anticonvulsive effects in vivo. Mutations in exons of human genes coding for $GABA_A$ receptor subunits would, therefore, be expected to have severe consequences if they affect the function of the resulting receptor. Surprisingly, very few such mutations have been described so far. A point mutation in the β1 subunit (H396Q) has been reported to have no overt consequences for the individuals carrying the mutation (Sobell et al. 1996). A point mutation in the α6 subunit (P386S) has been described to be associated with diaze-

pam sensitivity (Iwata et al. 1999, 2000). Both mutations were not investigated for their functional consequences on $GABA_A$ receptors expressed in vitro. Very recently, two different point mutations in the γ2 subunit have been associated with familial epilepsy. Baulac et al. (2001) reported that the mutation K289M in the γ2 subunit segregates in a family with a phenotype closely related to the disease generalized epilepsy with febrile seizures plus. The other point mutation in the γ2 subunit (R43Q) appears to be associated with childhood absence epilepsy and febrile seizures (Wallace et al. 2001).

Here we report on the finding of several nucleotide substitutions and a 3-bp deletion in the α1 and the β3 subunits. We found these mutations accidentally in a search for possible reasons for an abnormal response to the benzodiazepine-like drug zolpidem. One of the nucleotide substitutions in the β3 subunit, p.R192H, leads to an alteration on the protein level. Functional studies indicated that this missense mutation results in kinetic consequences that would be consistent with a reduced synaptic inhibition. The presence of inherited insomnia in the family of the affected individual suggests a possible link between insomnia and the mutation β3(R192H). If this link can be confirmed, this would represent the third case of a human condition linked to mutations in the $GABA_A$ receptor.

Materials and methods

Characterization of exon/intron borders

Based on the conserved genomic structures of three known GABA receptor genes, $GABA_A$ receptor β1 (*GABRB1*) (Kirkness et al. 1991), β3 (*GABRB3*) and α5 (*GABRA5*) subunits (Glatt et al. 1997), oligonucleotides were designed adjacent to putative exon boundaries of GABA receptor cDNAs. Exon-specific primers were designed from $GABA_A$ receptor α1 subunit (*GABRA1*) and γ2 subunit (*GABRG2*) cDNA sequences. Two primers of opposite direction in respect to the cDNA sequence were used to amplify intron-containing fragments from genomic DNA (200 ng) using the Expand Long Template System (Roche). The size of the PCR products was estimated by agarose-gel electrophoresis. Exon flanking sequences were established by fluorescent automated DNA sequencing of the products using the PCR primers. By sequence comparison of the genomic DNA with $GABA_A$ receptor α1 subunit (*GABRA1*) and γ2 subunit (*GABRG2*) cDNAs, the intron/exon borders were determined and primers to amplify individual exons were designed.

Patients

DNA was obtained from individuals displaying an unusual response to zolpidem (Courtet et al. 1999). More than 115 unrelated controls of the same ethnic background were tested for variants detected in the affected individuals. Informed consent was obtained from all participants of the study.

Mutation detection

Exons of $GABA_A$ receptor genes were individually amplified from genomic DNA by use of primers designed from intron sequences. After an initial denaturation step of 2 min at 94 °C, PCR amplification was performed for 40 cycles of 30 s at 94 °C, 40 s at 54 °C, and 1 min at 72 °C. PCR products were incubated for 2 min at 99 °C to facilitate heteroduplex formation, and they were analyzed by SSCP/HD (single strand conformation polymorphism/heteroduplex), as described elsewhere (Liechti-Gallati et al. 1999).

$GABA_A$ receptor cDNAs

The α1 subunit was provided by P. Seeburg (Schofield et al. 1989). The human β3 and γ2s subunits were cloned from a human Marathon ready cDNA library (Clontech) by 5′ rapid amplification of cDNA ends (5′RACE) using Pfu DNA polymerase (Stratagene). Three independent γ2 clones were cloned and sequenced. All three clones differed from the published cDNA sequence at four positions. Two substitutions are silent on the protein level. Two substitutions result in amino acid exchanges. A c.120T>C transition was found, resulting in p.M81T of the mature subunit. A c.181T>A transversion was found, resulting in p.S142T of the mature subunit. Threonines are also present in both positions of the γ2 subunit from rat. Site-directed mutagenesis of β3R192 to H was done using the QuickChange mutagenesis kit (Stratagene).

Functional tests in *Xenopus laevis* oocytes

Xenopus laevis oocytes were prepared, injected, defoliculated and currents recorded as described (Sigel 1987; Buhr and Sigel 1997). Briefly, oocytes were injected with 50 nl of capped, polyadenylated cRNA dissolved in 5 mM K-HEPES (pH 6.8). This solution contained the transcripts coding for the different subunits at concentrations (calculated from the UV absorption) of 10, 10 and 50 nM for α1, β3, and γ2 subunits, respectively. Electrophysiological experiments were performed by the two-electrode voltage clamp method at a holding potential of –80 mV. Allosteric potentiation via the benzodiazepine site was measured at a GABA concentration (1–3 μM), eliciting 2–10% of the maximal GABA current amplitude by co-application of GABA and diazepam or of GABA and zolpidem. Oocytes were only exposed to a single drug in addition to GABA, to avoid contamination, and the perfusion system was cleaned by washing with dimethyl sulfoxide for the same reason. The GABA EC_{50} was measured at a holding potential of –60 mV. Agonist concentrations between 0.03–3000 μM were applied for 20 s and a washout period of 4–15 min was allowed to ensure full recovery from desensitization. Current responses were fitted to the Hill equation:

$$I=I_{max}/\{1+(EC_{50}/[A])^n\}$$

where I is the peak current at a given concentration of GABA (A), I_{max} is the maximum current, EC_{50} is the concentration of agonist eliciting half maximal current, and n is the Hill coefficient.

Functional expression in HEK293T cells and kinetic analysis

Due to their large size, *Xenopus* oocytes are an unsuitable cell for the determination of fast kinetic phenomena. Therefore, smaller cells had to be used. Human embryonic kidney cells (HEK293T; a gift from P. Connely, COR Therapeutics, San Francisco, Calif.) were maintained in Dulbecco's Modified Eagle's medium, supplemented with 10% fetal bovine serum, at 37 °C in 5% CO_2/95% air. Cells were transfected with 4 μg of each of the cDNAs encoding human α1, β3, and γ2S, $GABA_A$ receptor subunit subtypes, in combination with 1–2 μg of pHOOK (Invitrogen, Carlsbad, Calif.) for immunomagnetic bead separation (Greenfield et al. 1997), using a modified calcium phosphate co-precipitation technique as previously described (Angelotti et al. 1993). This method resulted in reliable co-expression of all cDNAs as indicated by single channel and pharmacological properties (Angelotti et al. 1993). The next day, cells were re-plated and recordings were made at room temperature 18–30 h later.

Cells were bathed in an external solution consisting of 142 mM NaCl, 8 mM KCl, 6 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose (pH 7.4, 325 mOsm). Electrodes were pulled from

thick-walled borosilicate glass (World Precision Instruments, Pittsburgh, Pa.) with a Flaming Brown electrode puller (Sutter Instrument Co., San Rafael, Calif.), fire-polished to resistances of 4–12 MΩ when filled with an internal solution consisting of 153 mM KCl; 1 mM $MgCl_2$; 2 mM MgATP; 10 mM HEPES; 5 mM EGTA (pH 7.3, 300 mOsm). The chloride equilibrium potential was near 0 mV. Membrane patches were excised from positively transfected cells (identified visually by the presence of beads), and voltage-clamped at –40 to –70 mV using an Axon 200A amplifier (Axon Instruments, Foster City, Calif.). Solutions containing GABA were applied (via gravity) to excised outside-out patches using a rapid perfusion system consisting of pulled double-barrel glass flow pipes connected to a Warner Perfusion Fast-Step (Warner Instrument, Hamden, Conn.). This approach allowed rapid switching between control and GABA-containing solutions. Rapid switching is essential to ensure resolution of $GABA_A$ receptor currents that activate rapidly (<1 ms) and desensitize with a fast time constant (<10 ms). To verify the speed and precision of the device, the solution exchange time was determined after each patch recording by stepping a dilute external solution across the open electrode tip to measure a liquid junction current. The 10–90% rise times for solution exchange were consistently ~400 µs or less, faster than the activation rate of the currents. Figure traces were decimated and/or additionally filtered for presentation purposes only.

Currents were low-pass filtered at 2–5 kHz, digitized at 10 kHz, and analyzed using the pCLAMP8 software suite (Axon Instruments, Foster City, Calif.). Multiple (3–15) GABA-elicited responses were acquired for each patch at ~30-s intervals and averaged to form ensemble currents for analysis. Data files were coded such that analysis was performed without knowledge of whether the mutation was present. The desensitization and deactivation time courses were fit using the Levenberg-Marquardt least squares method with one or two component exponential functions of the form $\sum a_n e^{(-t/\tau_n)}$, where n is the best number of exponential components, a is the relative amplitude of the component, t is time, and τ is the time constant. Three component fits were not considered. A second component was accepted only if a significant improvement of the fit occurred, as determined by an F-test performed automatically by Clampfit 8.1 analysis software on the sum of squared residuals. The "fast" component of desensitization was defined as the faster exponential function when the desensitization time course was fitted best by two exponential functions. For comparison of deactivation time courses, a weighted summation of the fast and slow decay components was used. Numerical data were expressed as mean±SEM. Statistical significance, using Student's unpaired t-test (with a Welch's correction for unequal variances when necessary) was taken as $P<0.05$.

Nomenclature

Gene mutation nomenclature used in this article follows the recommendations of den Dunnen and Antonarakis (2001). Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001)

Results

Exon/intron structure of *GABRA1*

Based on the conserved genomic structures of $GABA_A$ receptor β1 (*GABRB1*) (Kirkness et al. 1991), β3 (*GABRB3*) and α5 (*GABRA5*) subunits (Glatt et al. 1997) oligonucleotides were designed adjacent to putative exon boundaries of the α1 subunit (*GABRA1*). Genomic sequences were amplified by PCR and exon intron boundaries were identified by DNA sequencing (Table 1). In addition to the introns between the nine coding exons, there is at least one additional intron in the 5′UTR of *GABRA1*. This additional intron is about 2 kb in size. The primers designed to amplify individual coding exons and the appropriate PCR conditions are listed in Table 2. The exon 6 sequences of *GABRB1*, *GABRB3* and *GABRA1* are homologous to one another, and exon 4 of *GABRG2* is homologous to exon 4 of *GABRA1*.

Table 1 Exon/intron boundary sequences of *GABRA1*

Coding exon	Size (bp)	5′ splice donor	3′ splice acceptor	Intron	Size (kb)
1	99	Arg Ser AGA AG/gtg ggg ...	Ser Tyr ttt cag/C TAT	1	3.5
2	104	Gly Glu GGA G/gta ggt ...	Glu Arg caa cag/AG CGT	2	11.5
3	69	Asp Met GAT ATG/gta agt ...	Glu Tyr acc tag/GAA TAT	3	7.2
4	222	Met Arg ATG AG/gta agg ...	Arg Leu ctt aag/G CTG	4	2.0
5	84	Ser Tyr AGT T/gtg agt ...	Tyr Ala ttt tag/AT GCT	5	6.8
6	145	Thr Gly ACA G/gta agt ...	Gly Glu tct cag/GA GAA	6	8.0
7	154	Phe Gly TTT G/gta agt ...	Gly Val tta cag/GA GTA	7	4.8
8	204	Glu Lys GAA AAG/gta aat ...	Pro Lys cta cag/CCA AAG	8	1.4
9	470[a]				

[a]Minimum size estimate

Table 2 Intron primers for amplification of $GABA_A$ receptor exons

Gene	Coding exon	5′ primer	3′ primer	Product size	PCR conditions	
					Anneal temp (°C)	Mg^{2+} (mM)
GABRA1	4	att atg cac tgt ctg cgt tag a	agt gga agg gaa tga agt aca g	357	54	1.5
GABRA1	6	tga tga aag gta cca act aaa taa	gtg tta ctg aat gat tca ttg ag	291	54	2.0
GABRA1	7	act cat agt aaa cct cag aga tt	ctc ttt gca gtg tca cag ata t	295	56	1.5
GABRB1	6[a]	cac ctt acc act ttt gtg ct	cca atg ttt ctc ttt aga cg	393	56	1.5
GABRB3	6[b]	ttt agc cgt gca ttt ata cg	cat ttt gtc act cca gtc ac	270	56	1.5
GABRG2	4	aag aaa aca gga atg aaa tat acc	ttg ttt tct tcc taa caa tta ctc	350	54	2.5

[a]The 3′ primer is within exon 7
[b]Glatt et al. 1997

Variations in $GABA_A$ receptor genes

Exons of the α1, β1, β3 and γ2 subunits coding for amino acid positions putatively involved in the interaction of GABA or the modulatory drugs acting at the benzodiazepine binding site were screened for single nucleotide polymorphisms. Nine individuals who showed an unusual response to the administration of zolpidem were analyzed. PCR products were analyzed for point mutations by SSCP/HD. Two different conformers of the β3 subunit exon 6 PCR product were found in two different patients (Fig. 1). Sequencing demonstrated a transition g.G>A (Fig. 2) in exon 6 of patient 1 (Courtet et al. 1999), resulting in p.R192H of the mature β3 subunit. In the second patient, a transition g.C>T was found that is silent at the protein level (p.T176T). We also analyzed 115 control samples (230 chromosomes) of uncharacterized healthy individuals. In the controls, p.R192H and p.T176T were not present, indicating that the allele frequencies for both mutations was <0.1%. In two control samples, we detected additional conformers of the PCR products of exon 6 α1 subunit. A transition, g.T>C, was found in intron 5 and a deletion of three bases was found in intron 6, respectively. Since these sequence changes did not appear in other control samples, these non-coding mutations are considered rare variants. The nucleotide variations described are summarized in Table 3.

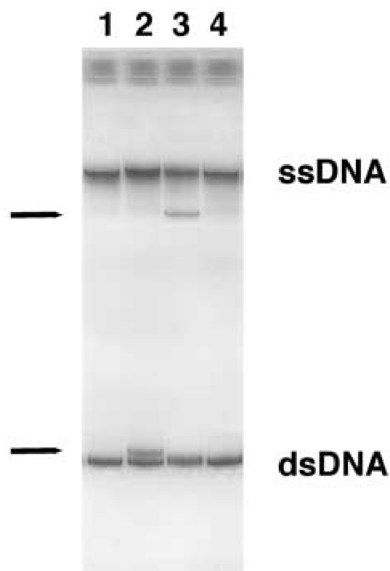


Fig. 1 SSCP/HD analysis of *GABRB3* exon 6. *Lanes 1, 4* wild type; *lane 2* patient 2 displaying an additional band of heteroduplex DNA (*ds*); *lane 3* patient 1 with an unusual conformer of ssDNA (*ss* single strand)

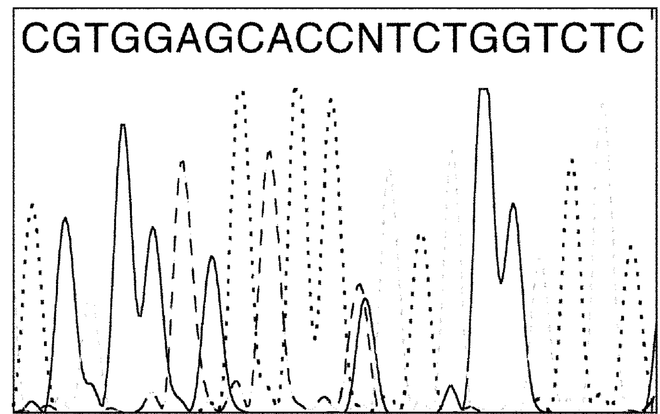


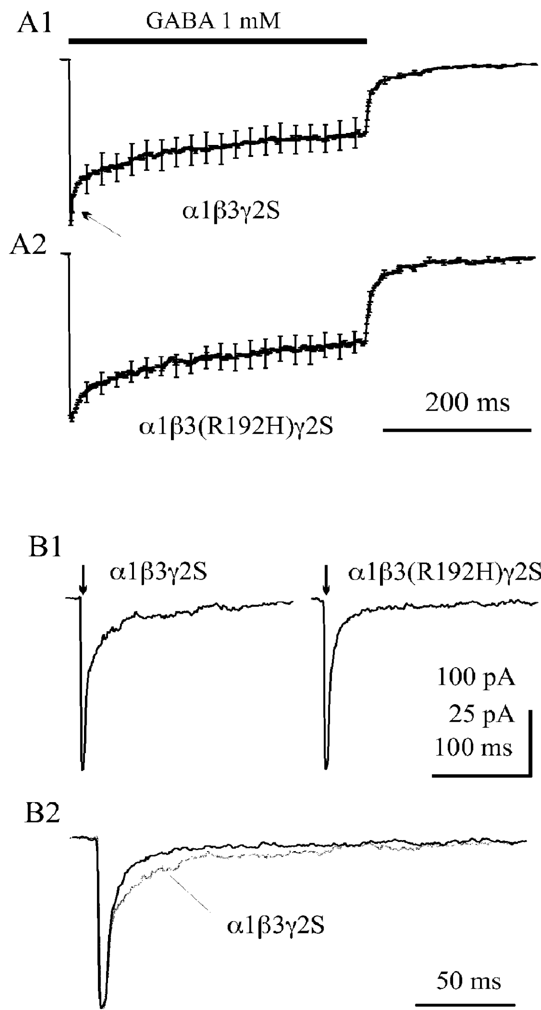
Fig. 2 *GABRB3* missense mutation. The sequence of exon 6 demonstrates that patient 1 is heterozygous for a g.G>A transition

Table 3 Variants in human $GABA_A$ receptor subunit genes. Nucleotide position is based on the cDNA sequence (GenBank accession numbers NM_000814 and NM_000806 for β3 and α1, respectively)

Gene	Location	Change	Consequence
GABRB3	Exon 6	g.703G>A	p.R192H
GABRB3	Exon 6	g.656C>T	p.T176T
GABRA1	Intron 5	g.875–40T>C	–
GABRA1	Intron 6	1018+46_48delAAT	*Ssp*I del

Functional consequences of the β3(R192H) mutation

The mutation p.R192H was introduced in a clone of the human β3 subunit. Wild-type α1 and γ2S subunits and the mutant β3 subunit were co-expressed in *Xenopus* oocytes.



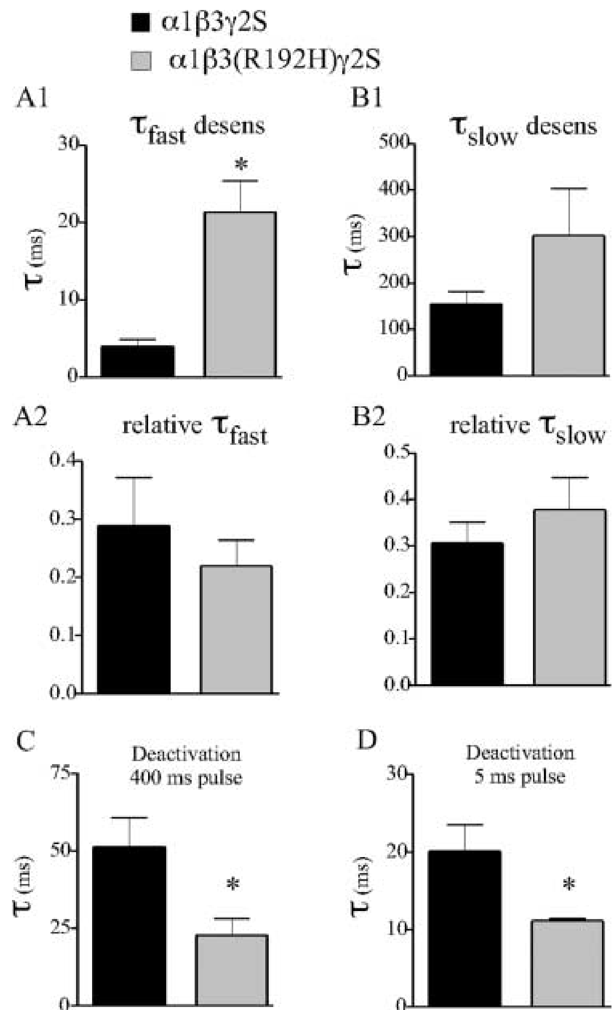


Fig. 3A, B Concentration jump experiments on α1β3γ2S and α1β3(R192H) γ2S $GABA_A$ receptors. Membrane patches were excised from transfected HEK293T cells. GABA was applied to patches by rapidly switching between solutions flowing from two parallel flow pipes, one of which contained GABA (1 mM). **A1, A2** Traces are the mean+SEM current responses to 400 ms pulses of GABA (1 mM). The *vertical axis* is current with arbitrary units, as individual patch records were normalized to peak current amplitude prior to averaging. *Horizontal axis* is time. *Solid bar* indicates duration of GABA pulse. Currents activated rapidly (sharp downward deflection) upon GABA application. Desensitization was observed, as indicated by the fading of current amplitude after reaching peak. Note the fast phase of desensitization (arrow in **A1**). Upon removal of GABA, currents relaxed toward baseline levels, a process known as deactivation. **B1** Representative current response to a brief (<5 ms; *arrow*) application of GABA (1 mM) to α1 β3 γ2 S (*left trace*) and α1 β3 (R192H) γ2S (*right trace*) $GABA_A$ receptors. The larger vertical scale value applies to left trace. **B2** The same traces from **B1** are overlaid and time-expanded to show the faster deactivation rate of α1β3 (R192H) γ2S $GABA_A$ receptors (*dark trace*) compared to α1 β3 γ2 S $GABA_A$ receptors (*gray trace*, labeled)

Fig. 4A–D Summary of macroscopic kinetic properties. In each panel, the *solid and gray bars* indicate α1β3γ2S and α1β3 (R192H) γ2S $GABA_A$ receptors, respectively. **A1, B1** The τ (time constant) for the fast and slow phases of apparent desensitization are shown. The mutation resulted in significant slowing of the fast phase; *asterisk* $P<0.004$. **A2, B2** The relative contribution of the fast and show phases of desensitization, respectively, were not significantly different. Weighted deactivation rate was significantly faster for the mutated receptors following a 400-ms (**C**) and 5-ms (**D**) pulse of GABA (1 mM); *asterisk* $P<0.05$. Data are the mean±SEM from six patches for each construct

Receptors were analyzed using the two electrode voltage clamp method. No difference between wild-type α1β3γ2 $GABA_A$ receptors and mutant α1 β3(R192H) γ2 receptor was observed regarding maximally expressed current amplitude, GABA sensitivity for channel opening, apparent desensitization, and sensitivity to stimulation by diazepam or zolpidem (not shown).

To determine whether the mutation affected rapid kinetics that might influence synaptic transmission, we used the concentration jump technique applied to outside-out patches excised from acutely transfected HEK293T fibroblasts (see methods). In contrast to the oocyte expression system, this technique allows resolution of $GABA_A$ receptor function on a time-scale that is relevant for synaptic events and is sensitive to even subtle changes in rapid kinetic properties. Four-hundred-millisecond pulses of GABA (1 mM) were used to investigate the impact of the mutation on receptor activation, desensitization and deactivation. This saturating concentration of GABA is thought to

be present in the synaptic cleft during neurotransmission. Averaged current responses evoked by this protocol were shown in Fig. 3A1 and A2 for α1β3γ2S and α1β3 (R192H) γ2S receptors, respectively. No differences were observed in peak current amplitudes (wild type: 160±40 pA, *n*=6; mutant: 227±102 pA, *n*=6; data not shown). The 10–90% current rise time (activation) was unchanged by the mutation (wild type: 0.88±0.17 ms; mutant: 0.68±0.15 ms; data not shown). Although the currents desensitized to a similar extent and with a biphasic time course in both cases, the time constant of the fast component of desensitization was significantly slower for the mutated receptors (Fig. 4A1) (wild type: 4.0±1.0 ms; mutant: 21.3±4.0 ms; $P<0.004$). The time constant of the slow component of desensitization was not significantly altered (Fig. 4B2) (wild type: 165.0±29.7 ms; mutant: 303.0±101.0 ms). The relative contribution of the fast and slow phases of desensitization were unchanged by the mutation (Fig. 4A2, B2) (wild type: 0.29±0.08 and 0.31±0.45, respectively; mutant: 0.22±0.04 and 0.38±0.07, respectively). Finally, the β3(R192H) mutation accelerated deactivation after the 400 ms pulse of GABA (Fig. 4c) (wild type: 51.3±9.5 ms; mutant: 22.8±5.4 ms; $P<0.03$).

Since it is thought that the fast phase of desensitization is critical for shaping the response to brief exposure to GABA, such as might occur at synapses, we used 5-ms pulses of GABA (1 mM) to confirm that the mutation would accelerate deactivation, the major determinant of IPSC duration. Brief pulses of saturating GABA are thought to be a reasonable model of the brief synaptic transient of GABA at synapses. Typical currents evoked by brief GABA pulses were shown in Fig. 3B1. To illustrate the accelerated deactivation time course, the traces were overlaid in Fig. 3B2. The synaptic GABA transient is thought to be very brief, and the IPSC time-course has been closely reproduced by brief (1–10 ms) pulses of 1 mM GABA (Jones and Westbrook 1995; Haas and Macdonald 1999). As expected, the rate of deactivation for mutated channels was significantly faster than observed in patches containing wild-type receptors (Figs. 3B2 and 4D) (wild type: 20.1±3.4 ms, *n*=6; mutant: 11.1±0.3 ms, *n*=4; $P<0.05$).

Discussion

We describe here the identification and characterization of variants in the α1 and β3 genes of the $GABA_A$ receptor. Two point mutations were found in the β3 subunit. One is silent at the protein level, whereas the other results in an amino acid residue change from arginine to histidine in position 192. The mutation in the β3 subunit that involves an amino acid substitution has an influence on the functional properties of expressed receptors and might be related to some forms of familial insomnia.

We screened 115 healthy individuals by SSCP for the presence of these point mutations. Both point mutations were absent in these 230 chromosomes. Interestingly, the mutations were detected in patients showing an abnormal response to zolpidem (Courtet et al. 1999, and unpublished observation by P. Courtet, V. Pignay and J.P. Boulenger). The zolpidem sensitivity of $GABA_A$ receptors depends on the presence of the α1 and γ2 subunits. The presence of point-mutated β3 subunits might, therefore, be a coincidence.

Kinetic analysis indicated a specific effect of the β3 (R192H) mutation on the fast phase of current desensitization. The fast phase of $GABA_A$ receptor desensitization has been suggested to prolong the duration of IPSCs by trapping GABA on the receptor, allowing additional channel openings to occur before GABA eventually unbinds (Jones and Westbrook 1995, 1996; Bianchi and Macdonald 2001a). Consistent with this theory, the deactivation of mutated channels was faster than that of wild-type channels. Specifically, the slow phase of deactivation, thought to depend heavily on desensitization, was attenuated (Fig. 4B2). However, other kinetic parameters could accelerate deactivation, such as decreases in either binding affinity or gating efficacy. The lack of difference in GABA EC_{50} argues against the former. Moreover, slower apparent desensitization rate (such as that observed here) may be caused by increased gating efficacy (Bianchi and Macdonald 2001b), but this predicts a prolonged deactivation, the opposite of what we observed for the mutation. Therefore, this change may represent partial attenuation of specific "fast" desensitized states and the resulting increase in deactivation rate. The impact of faster deactivation on synaptic transmission would be a decrease in the duration of IPSCs mediated by receptors containing the mutated β subunit. The resulting decrease in carried charge would decrease the neuronal inhibition achieved by a given synaptic event. For individuals heterozygous for this mutation, it is possible that functional $GABA_A$ receptors might contain zero (wild type), one ('heterozygous') or two ('homozygous') β3 (R192H) subunits. Assuming unaltered expression and assembly of the mutant subunit, the predicted IPSC duration would be intermediate between our in vitro results for the wild-type receptors and those 'homozygous' for the β3 (R192H) mutation. Whether this shortening of IPSC duration contributes to the clinical phenotype remains unknown.

In the family of the affected individual there is a history of sleep problems. Unfortunately, the family members refuse to collaborate. It is very tempting to hypothesize the existence of a link between the missense mutation in β3 (R192H) and the insomnia. It will only be possible to confirm this hypothesis if this mutation is found in other families affected by insomnia and the link will be established there. It is interesting to note that β3-subunit-containing $GABA_A$ receptors have been implicated in sleep processes independently, by the observation that β3-less mice lose the hypnotic response to oleamide (Laposky et al. 2001).

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