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SB-205384: a $GABA_A$ receptor modulator with novel mechanism of action that shows subunit selectivity

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1 SB-205384, and its (+) enantiomer (+)-SB-205384 were tested for their modulatory effects on human $GABA_A$ receptor subunit combinations expressed in *Xenopus* oocytes by electrophysiological methods.

2 The slowing of the decay rate induced by SB-205384 on native GABA-activated currents in rat neurones was also seen on $GABA_A$ currents in oocytes expressing human $GABA_A$ subunits. This temporal effect was observed for the $\alpha 3\beta 2\gamma 2$ subunit combination with little effect in subunit combinations containing either $\alpha 1$ or $\alpha 2$.

3 Potentiation of the peak amplitude of the GABA-activated currents by SB-205384 or (+)-SB-205384 was less specific for a particular subunit combination, although the greatest effect at 10 μM drug was seen on the $\alpha 3\beta 2\gamma 2$ subunit combination.

4 In contrast, zolpidem, a benzodiazepine site modulator, did not significantly slow decay rates of $GABA_A$ currents in oocytes expressing the $\alpha 3\beta 2\gamma 2$ subunit combination. Zolpidem, as expected, did selectively potentiate GABA-activated currents on oocytes expressing the $\gamma 2$ subunit compared to those containing the $\gamma 1$.

5 The results show that the novel kinetic modulatory profile of SB-205384 is selective for the $\alpha 3\beta 2\gamma 2$ subunit combination. This suggests that the compound is binding to a novel regulatory site on the subunit complex.

Keywords: γ-Aminobutyric acid; *Xenopus* oocytes; $GABA_A$ subunit; SB-205384

Introduction

γ-Aminobutyric acid (GABA) acting on the $GABA_A$ receptor is the major mediator of inhibitory synaptic transmission in the mammalian central nervous system. The $GABA_A$ receptor complex is composed of subunits arranged as a pentameric structure (Barnard, 1995) which form a chloride ion channel. A number of subunits and isoforms of the mammalian $GABA_A$ receptor have been cloned (6αs, 3βs, 3γs and a δ and ε) giving the possibility of hundreds of different subunit combinations. This potential diversity is unlikely to be fully expressed in native channels and some progress is being made to define which subunit combinations coassemble *in vivo*. $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 2/3\gamma 2$ and $\alpha 3\beta n\gamma 2/3$ combinations are together thought to comprise about 80% of $GABA_A$ receptors in rat brain (for review see McKernan & Whiting, 1996).

The $GABA_A$ receptor is allosterically modulated by a number of compounds of therapeutic interest. The most important class of these is probably the benzodiazepines. Benzodiazepines have been used in the clinic for many years and yet usage results in a number of side effects. In an attempt to reduce these unwanted effects subunit selective compounds have been developed. Studies with expressed subunits have shown that the benzodiazepine binding site appears to be predominantly located on the α subunit (Pritchett *et al*., 1989a), although the γ subunit has also been shown to be required for modulation of channel function (Pritchett *et al*., 1989b). The affinity of the benzodiazepine site ligands for the $GABA_A$ receptor is determined by both the α and γ subunit variants present (Pritchett & Seeburg, 1990; Wisden *et al*., 1991; Puia *et al*., 1991; Wafford *et al*., 1993a,b) with the β subunit having no effect (Hadingham *et al*., 1993a). These data have allowed molecular assignations to be made to the BZ1 and BZ2 sites defined pharmacologically with various benzodiazepine ligands on native preparations. The $\alpha 1\beta 2\gamma 2$ shows BZ1 type pharmacology and $\alpha 3\beta 2/3\gamma 2$ and $\alpha 3\beta n\gamma 2/3$ combinations may represent the BZ2 receptor (McKernan & Whiting, 1996). The subunit selectivity of other modulators of $GABA_A$ receptor function is still unclear, apart from the anticonvulsant, loreclezole, which has been shown to have β subunit selectivity (Wafford *et al*., 1994).

In an effort to develop compounds with novel *in vivo* profiles, we have previously investigated the effects of a non benzodiazepine site ligand, 4-amino-7-hydroxy-2-methyl5,6,-7,8-tetrahydrobenzo[b]thieno[2,3-b]pyridine-3-carboxylic acid, but-2-ynyl ester (SB-205384) on $GABA_A$ receptors in rat cerebellar granule cells (Meadows *et al*., 1997). This compound was found to have a novel mechanism of action. In addition to potentiating the GABA-activated current it prolonged the half-life for decay of current after GABA removal. In this study, we have examined the subunit selectivity of this compound concentrating our studies on the subunit combinations thought to be most abundant in forebrain. We also compared the effects of SB-205384 with those of the benzodiazepine site agonist, zolpidem. We have shown that the effect of SB-205384 on the decay rate of the current was $\alpha 3\beta 2\gamma 2$ selective.

Methods

Preparation of human $GABA_A$ receptor cDNAs

Human $\alpha 1$ and $\beta 1$ cDNAs (Schofield *et al*., 1989) were isolated from a human foetal brain λ gt10 cDNA library with subunit-

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specific radiolabelled oligonucleotides. α2 and α3 cDNAs (Hadingham *et al*., 1993a) were isolated from human foetal brain RNA by RT-PCR by use of subunit specific oligonucleotides. β2 cDNA (Hadingham *et al*., 1993b) was obtained by PCR from a human cerebellum λZAPII cDNA library (Stratagene). Human γ1 cDNA was isolated by RT-PCR by use of oligonucleotides based on the rat γ1 cDNA sequence (Ymer *et al*., 1990) and human foetal brain RNA as a template. γ2 cDNA (short form) was obtained by PCR from a human foetal brain λ gt10 library (Clontech) with subunit specific oligonucleotides (Pritchett *et al*., 1989b). The cDNAs were inserted into the polylinker site of the pCIS-2 expression vector (Pritchett *et al*., 1988) by standard recombinant methods. The subunit specific oligonucleotide primers were derived from the 5′ and 3′ untranslated regions of the published sequences. The identities of the cDNA sequences were confirmed by nucleotide sequencing with an Applied Biosystems 373A automated DNA sequencer and dodeoxy chain terminator chemistry. The sequencing primers were designed with the Oligo 4.0 program (National Bioscience, Plymouth, MN).

Oocyte expression

Mature female *Xenopus laevis* were anaesthetized in a 0.4% solution of 3-aminobenzoic acid ethyl ester (MS222, Sigma) until they were unresponsive. A small incision was made and part of the ovary tissue removed into a sterile modified Barth's solution (MBS) containing, in mM: NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 15, $MgSO_4$ 0.82, $Ca(NO_3)_2$ 0.33 and $CaCl_2$ 0.41; pH 7.5 with NaOH. The tissue was disaggregated into small clumps and agitated in calcium-free Barth's solution (direct replacement of calcium with magnesium) containing 1–2% collagenase A (Boehringer Mannheim) for 1–3 h at room temperature. Oocytes were washed in MBS and stage V and VI oocytes removed into MBS plus gentamycin (0.1 mg ml^{-1}: ICN). Injections of different combinations of human $GABA_A$ subunit cDNAs were made directly into the nuclei (1.5 ng cDNA total per oocyte). After injection the oocytes were incubated at 18°C and used for electrophysiological recordings within 1–4 days.

Electrophysiology

Oocytes were placed in a recording chamber and continuously perfused with MBS (14 ml min^{-1}). Solution was applied by use of large bore tubing (internal diameter 1.5 mm) which facilitated rapid solution exchange (half-time 350–1000 ms). Oocytes were held under voltage clamp at −60 mV by use of the two-electrode voltage-clamp technique. Electrodes were low resistance (0.5–3 MΩ) and were filled with 3 M KCl.

Currents were evoked in response to application of GABA or GABA plus modulator applied through the perfusion system until the maximum current amplitude was reached. Thus the effect of SB-205384 appeared to be at equilibrium. Using co-administration in preliminary experiments we checked this by pre-exposure to SB-205384 and found no difference in the decay rates on GABA washout when compared with co-administration. A dose-response curve to GABA was generated on every oocyte, in order to select a concentration of GABA that gave approximately 20% of the maximum response (EC_{20}) for use in experiments investigating the action of the GABA modulators. Dose-response curves were fitted to the logistic equation by use of Origin software. Half-times for decay were measured by calculating the time taken for the current measured at the time of GABA removal to decay to half its amplitude. If the half-time for decay of the GABA response alone was more than 2 s the recording was discarded.

Figure 1 shows an example semi-log plot of the decay of the GABA response upon GABA removal plotted against time. This result was taken from an $\alpha3\beta2\gamma2$ receptor in the absence and presence of (+)-SB-205384. The control response was clearly complex and reflects contributions from the multi-exponential decay of $GABA_A$ currents on a millisecond time scale (Puia *et al*., 1994) and the solution exchange. In the presence of drug the semi-log plot yielded a good fit to a single exponential with $\tau = 13$s. This indicates that a single component with a slow τ dominates the decay in the presence of (+)-SB-205384. The corresponding half-time for decay of the response to GABA alone on this oocyte was 2 s and in the presence of (+)-SB-205384 was 13s. In subsequent experiments half-times were measured rather than applying a more detailed method of analysis, since fitting control GABA responses revealed variable τ values which probably reflect small differences in the perfusion rate between oocytes. In addition the aim of these experiments was to investigate the subunit specificity of modulation produced by SB-205384 rather than carry out a detailed kinetic analysis which would be difficult in an oocyte.

Materials

Compounds used were: γ-amino-*n*-butyric acid (GABA; Sigma), zolpidem (Sigma), (±)-4-amino-7-hydroxy-2-methyl-5,6,7,8-tetrahydrobenzo[b]thieno [2,3-b]pyridine-3-carboxylic acid, but-2-ynyl ester (SB-205384; (+)-SB-205384; (−)-SB-205384; Dept of Medicinal Chemistry, SB, Harlow).

SB-205384 is a racemic mixture of (+) and (−) enantiomers. In experiments on native $GABA_A$ channels expressed in rat cerebellar granule cells (Meadows *et al*., 1997), we determined that the two enantiomers were equipotent and equieffective in actions on both current amplitude and decay time. Values for potentiation and $t_{1/2}$ of decay of 0.1 μM $GABA_A$-evoked current were 239±29% and 5.5 s in the presence of 10 μM (+) SB-205384 and 202±38% and 4.5 s in the presence of (−)-SB-205384. Half-time for decay in the absence of modulator was 0.2 s. Most of the experiments presented in this study were performed with (+)-SB-205384, except where stated that the racemate was used.

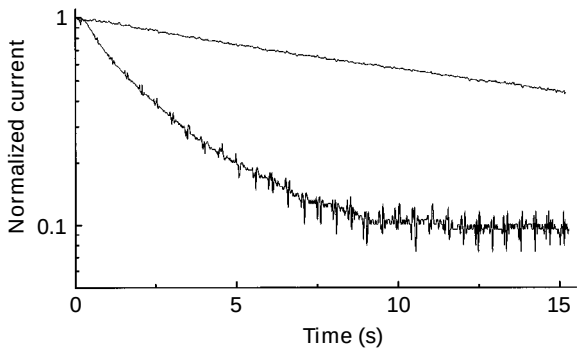

Figure 1 The decay rate of GABA-gated chloride currents in an oocyte expressing $\alpha3\beta2\gamma2$ receptors in the absence and presence of (+) SB-205384 (upper trace). The current amplitudes were normalized to current upon removal of GABA and have been plotted on a semi-log scale against time. Both traces were taken from a single oocyte.

Results

Injection of human $GABA_A$ receptor subunits in combinations each containing one α isoform, one β isoform and one γ subunit isoform results in expression of receptors that show inward currents in response to GABA application. Dose-response curves to GABA indicated that GABA potency varied between the different combinations of receptor subunits expressed over a range of about 100 fold, as shown previously (White *et al*., 1995). Therefore in order to compare modulator effects on more than one subunit combination it was necessary to ensure that the control GABA concentration used for each combination gave a similar percentage of maximum GABA-induced current. This allowed GABA receptor modulators to potentiate the current for any subunit combination to the same degree Table 1 gives values for the concentrations of GABA which produce 20% of the maximum response to GABA (EC_{20}) in the various subunit combinations tested. From this data control GABA concentrations of between 0.3 and 30 μM were assessed to be appropriate for use in experiments investigating the effects of modulatory compounds on GABA-induced responses.

Figure 2 shows typical responses obtained from three of the GABA subunit combinations expressed in oocytes. At 10 μM, (+)-SB-205384 increased the amplitude of the GABA-induced current on all three combinations. Thus, this effect of the compound was not specific, although the degree of potentiation differed between subunit combinations. In oocytes injected with $\alpha3\beta2\gamma2$ subunits, in addition to the effect on the amplitude of the response, (+)-SB-205384 dramatically increased the half-life of decay of the GABA response following washout of GABA (Figure 2c). While control GABA applications showed decay of GABA-induced current with a $t_{1/2}$ of 1–2 s (reflecting washout time of GABA), in the presence of (+)-SB-205384, the decay of current was slowed so that chloride current relaxation took tens of seconds. This effect was specific to the $\alpha3\beta2\gamma2$ combination and was not related to the degree of the potentiation of peak current as for $\alpha1\beta2\gamma2$ (Figure 2a), where the potentiation is greater with only a small effect on timecourse

The subunit selectivity of the timecourse effects was further investigated with eight different subunit combinations, each containing an α subunit isoform, a β subunit isoform and a γ isoform (Figure 3a). The greatest effect on decay time was observed with the $\alpha3\beta2\gamma2$ subunit combination with small but statistically significant effects also seen with $\alpha3\beta2\gamma1$, $\alpha3\beta1\gamma1$ and $\alpha1\beta2\gamma2$ (Dunnett's multiple comparison procedure, significance level 5%). The other four combinations tested showed

Table 1 Effect of GABA on oocytes injected with various $GABA_A$ receptor subunit combinations

Subunit combination	EC_{20} (μM)
$\alpha1\beta2\gamma2$	15.1±5.7
$\alpha2\beta1\gamma1$	5.2±2.4
$\alpha2\beta1\gamma2$	3.4±2.2
$\alpha3\beta1\gamma1$	90.8±7.9
$\alpha3\beta1\gamma2$	42.2±8.9
$\alpha3\beta2\gamma1$	4.2±0.9
$\alpha3\beta2\gamma2$	26.3±8.9

Values shown for EC_{20} are mean±s.e.mean of at least three experiments. Curves were fitted to the logistic equation by use of Origin software.

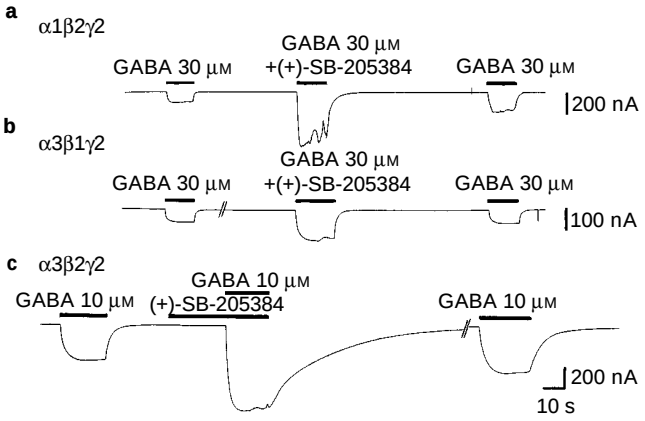

Figure 2 GABA-gated chloride currents and modulation by 10 μM (+)-SB-205384 in three different oocytes injected with cDNA for three different combinations of human $GABA_A$ subunits. Holding potential −60 mV. Horizontal bars indicate the period of GABA or GABA plus (+)-SB-205384 (10 μM) application.

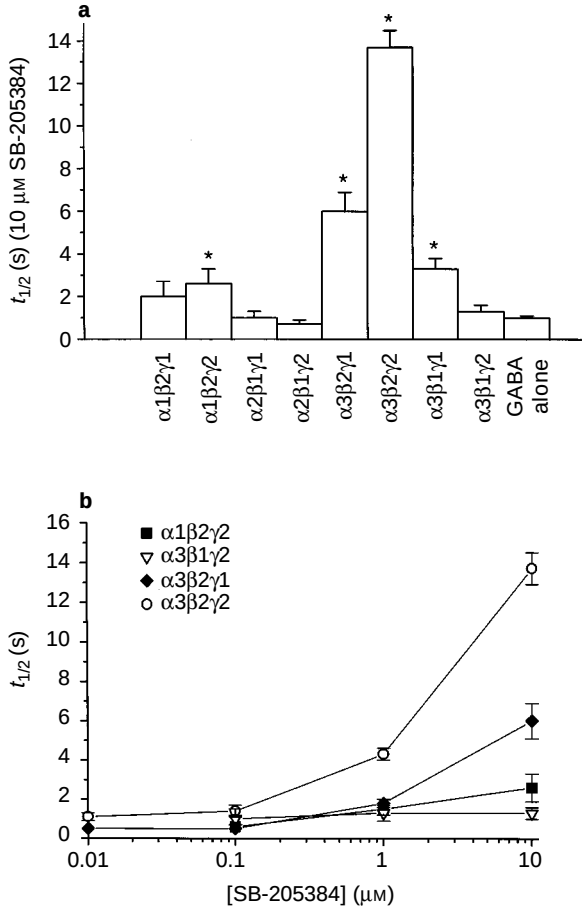

Figure 3 Effect of (+)-SB-205384/SB-205384 on half-time of decay of GABA-induced responses. (a) Effect on oocytes expressing various human $GABA_A$ receptor subtypes. Only $\alpha2\beta1\gamma2$, $\alpha3\beta1\gamma1$ and $\alpha3\beta2\gamma1$ subunit combinations were tested either fully or in part with SB-205384, all other combinations were tested with (+)-SB-205384. Values plotted are the mean of 3–4 oocytes in the presence of the modulator and 28 oocytes expressing the range of GABA subunit combinations for GABA alone; vertical lines show s.e.mean. Asterisks show a significant difference from values for GABA alone (Dunnett's multiple comparison procedure, significance level 5%). (b) Concentration-response data for the effect of (+)-SB-205384 on the half-time of decay of GABA-evoked currents in oocytes expressing various GABA subunit combinations. Values plotted are the mean of 3–4 oocytes. Only the $\alpha3\beta2\gamma1$ subunit combinations was tested in part with SB-205384, all other combinations were tested with (+)-SB-205384.

no significant temporal effect with 10 μM (+)-SB-205384 ($\alpha2\beta1\gamma2$ and $\alpha3\beta1\gamma1$ were both tested with SB-205384). Thus this effect on half-life of decay of the GABA response was specific if not completely selective for $\alpha3\beta2\gamma2$, with significant effects also being observed for (+)-SB-205384 at 1 μM on this combination, while other combinations showed no effect of (+)-SB-205384 at 1 μM. Figure 3b shows dose-response data for four combinations and indicates that potency was reduced by the substitution of $\alpha3$ with $\alpha1$, $\beta2$ with $\beta1$ and $\gamma2$ with $\gamma1$. The study of dose-response relationships at concentrations higher than 10 μM was limited by compound solubility. This prevented a detailed comparison of the absolute efficacy of SB-205384 on each subunit combination.

The benzodiazepine site agonist, zolpidem, has been shown to enhance GABA activated currents in cells expressing $\alpha3\beta2\gamma2$ subunits. We tested this compound for comparison under the same conditions as for SB-205384. Figure 4a shows data from the same oocyte in a comparison of the action of the two compounds. As expected zolpidem markedly increased peak currents but had no effect on the half-life of decay of the GABA current for the $\alpha3\beta2\gamma2$ subunit combination up to a concentration of 10 μM (Figure 4a,b). We next checked that zolpidem showed the appropriate $\gamma2$ subunit specificity by substituting $\gamma2$ and $\gamma1$. Figure 5c shows a record from an oocyte injected with $\alpha3\beta2\gamma1$ subunits. Consistent with previous findings, zolpidem was without effect. Pentobarbitone, which shows little subunit selectivity, enhanced current as expected in the same oocyte (Figure 5b), but again with no measurable effect on GABA activated current decay rate, in contrast to SB-205384 which markedly slowed decay (Figure 5a). These results show that the marked decay rate effects were specific to SB-205384 and that the modulatory effects on current amplitude for benzodiazepines and barbiturates were as expected for these subunit combinations.

In the same experiments the effect of (+)-SB-205384 on the amplitude of GABA-activated currents was systematically assessed. Percentage increases in peak amplitude for 10 μM (+)-SB-205384 are plotted in Figure 6 expressed relative to control GABA currents (100%). The greatest effect was seen on the $\alpha3\beta2\gamma2$ subunit combination, although all combinations showed a degree of potentiation. Dose-response curves were constructed to examine the potency of (+)-SB-205384 on different subunit combinations. Comparison of $\beta1$ and $\beta2$ containing combinations (Figure 7a) indicated that (+)-SB-205384 was more potent on $\beta2$ subunit containing combina-

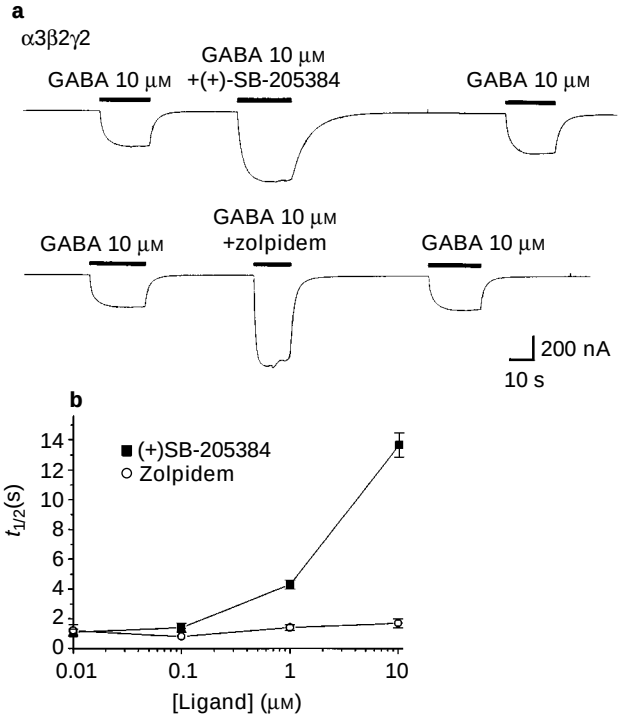

Figure 4 Effects of (+)-SB-205384 (1 μM) and zolpidem (1 μM) on GABA-induced currents in oocytes expressing the $\alpha3\beta2\gamma2$ subunit combination. (a) Sample traces of the effects of (+)-SB-205384 and zolpidem on GABA-induced currents. Holding potential −80 mV. Horizontal bars indicate the period of GABA or GABA plus modulator application. Data were obtained from a single oocyte. (b) Effect of (+)-SB-205384 and zolpidem on half-time of decay of GABA-induced currents in $\alpha3\beta2\gamma2$ subunit expressing oocytes. Values plotted are mean of 4 cells for (+)-SB-205384 and 3 cells for zolpidem; vertical lines show s.e.mean.

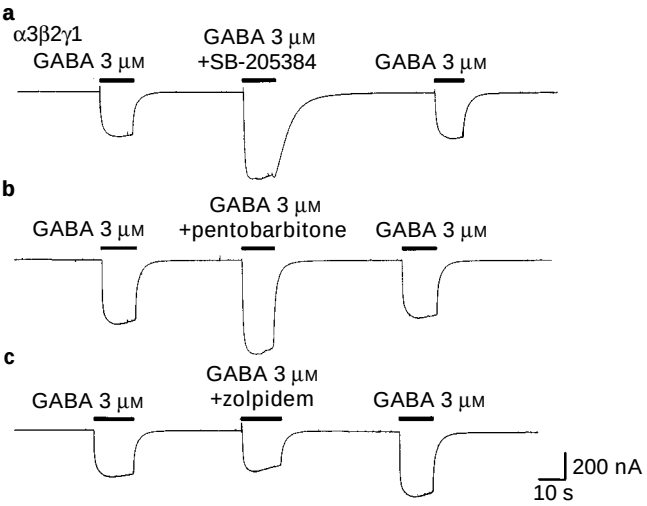

Figure 5 Effects of SB-205384 (10 μM), pentobarbitone (10 μM) and zolpidem (10 μM) on GABA-induced inward currents in oocytes expressing the $\alpha3\beta2\gamma1$ subunit combination. Holding potential −80 mV. Horizontal bars indicate the period of GABA or GABA plus modulator application. All data were obtained from one oocyte.

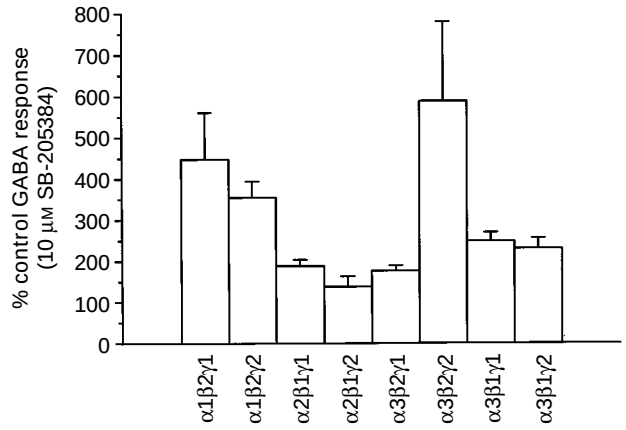

Figure 6 Effect of (+)-SB-205384/SB-205384 on peak current amplitude of GABA-induced responses recorded from oocytes expressing various human $GABA_A$ receptor subtypes. Values of peak amplitude are expressed as % control GABA response i.e. >100% indicates potentiation. Only $\alpha2\beta1\gamma2$, $\alpha3\beta1\gamma1$ and $\alpha3\beta2\gamma1$ subunit combinations were tested either fully or in part with SB-205384, all other combinations were tested with (+)-SB-205384. Plotted values are the mean ± s.e.mean of 3–5 oocytes.

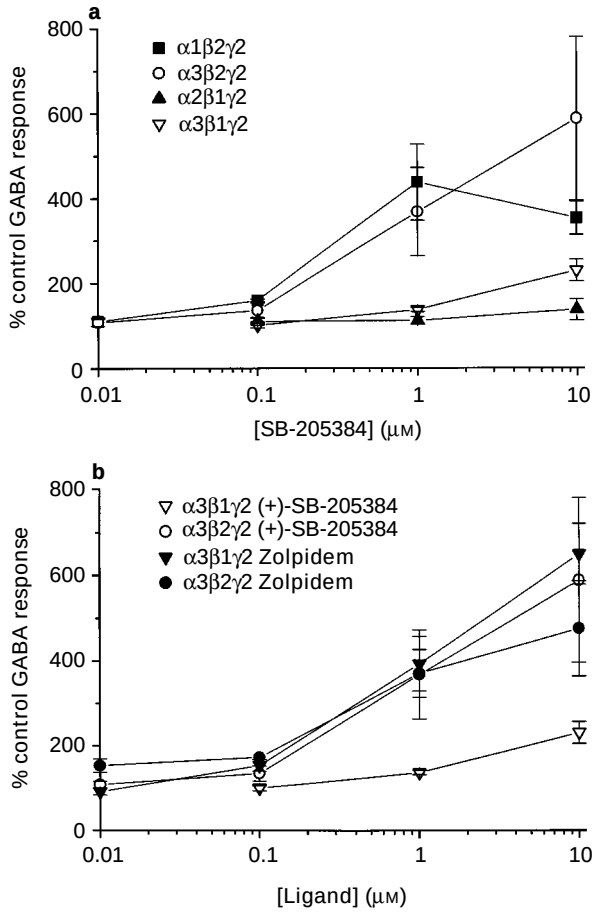

Figure 7 Effect of β subunit of modulation of peak GABA-induced current. (a) Effect of modulation by (+)-SB-205384/SB-205384. Only the $\alpha 2\beta 1\gamma 2$ subunit combination was tested with SB-205384, all other combinations were tested with (+)-SB-205384. Values plotted are mean of 3–8 oocytes; vertical lines show s.e.mean. (b) Effect of modulation by zolpidem. Values plotted are mean of 3–4 cells.

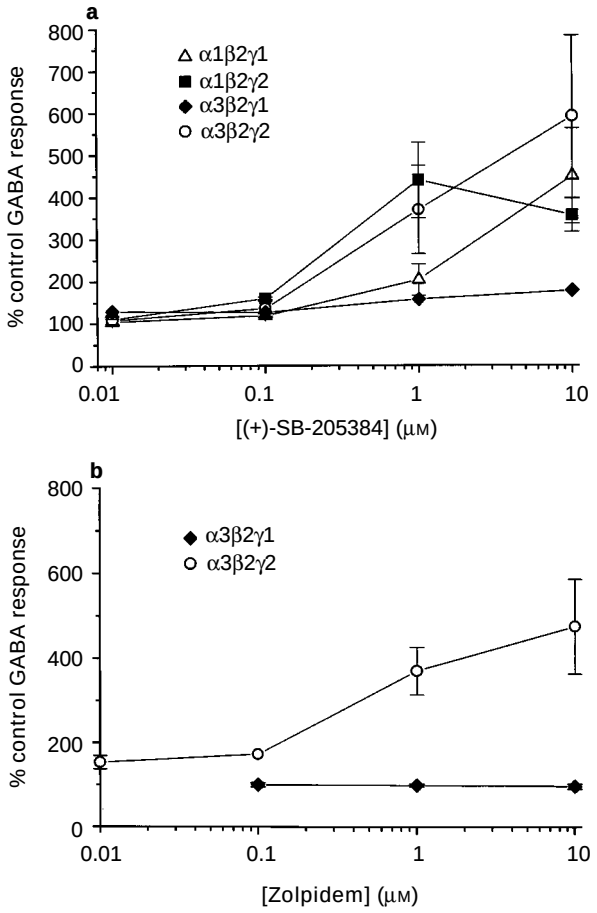

Figure 8 Effect of γ-subunit on modulation of peak GABA-induced current amplitude. (a) Effect of modulation by (+)-SB-205384. Values plotted are mean of 3–8 oocytes. (b) Effect of modulation by zolpidem. Values plotted are mean of 3–4 cells. Vertical lines show s.e.mean.

tions. At 1 μM, activity was only seen if $\beta 2$ was present with approximately 4 fold increases in current. For comparison the effect of zolpidem on the $\alpha 3\beta 1/2\gamma 2$ subunit combinations are shown in Figure 7b. In contrast to (+)-SB-205384, zolpidem potentiated both $\beta 1$ and $\beta 2$ containing combinations with similar potency.

Figure 8a shows data examining the contribution of $\gamma 1$ and $\gamma 2$ subunits to SB-205384 sensitivity. More potent activity of (+)-SB-205384 was seen with $\gamma 2$-containing combinations than $\gamma 1$ (compare data at 1 μM, Figure 8a) whether $\alpha 3$ or $\alpha 1$ subunit was present. However, this pattern was less clear at 10 μM, indicating selectivity for $\gamma 2$ was not absolute. Zolpidem was also shown to be selective for $\gamma 2$ over $\gamma 1$, as expected (Figure 8b, also compare Figures 4a and 5).

Discussion

SB-205384 is one of a group of benzothiophene compounds that act as allosteric modulators of $GABA_A$ receptor function, but which do not bind to the benzodiazepine binding site (Benham *et al*., 1994). The mechanism of action also differs from that of benzodiazepines or any of the other known $GABA_A$ channel modulators. SB-205384 slowed the decay rate of the GABA response in addition to the more usual $GABA_A$ receptor modulator effect on current amplitude (Meadows *et al*., 1997). In addition no direct agonist effect of SB-205384 was observed. This study shows that the same modulatory effect is seen on human $GABA_A$ channel currents expressed in *Xenopus* oocytes. We have partially characterized the subunit specificity of these effects and revealed a unique profile of activity on subunit combinations making up the majority of functional channels in the forebrain (McKernan & Whiting, 1996).

The effect of SB-205384 on the decay rate of the GABA response showed selectivity for the $\alpha 3\beta 2\gamma 2$ subunit combination, with much smaller effects observed on $\alpha 1$ or $\alpha 2$ containing combinations. Thus in addition to having a unique modulatory action, the subunit specificity is also novel when compared with the potentiating actions of other modulators, being specific for one of the combinations that makes up the BZ2 pharmacological grouping. $\alpha 3$ subunit selectivity has not previously been shown for any of the other allosteric modulators of the $GABA_A$ receptor complex. Benzodiazepine site ligands show varying degrees of α selectivity; flunitrazepam has similar affinities at $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ containing receptors when expressed along with $\beta 1$ and $\gamma 2$, whereas zolpidem has a higher affinity at $\alpha 1$ containing receptors, than at $\alpha 2$ or $\alpha 3$ and virtually no effect on $\alpha 5$-containing receptors (Puia *et al*., 1991; Hadingham *et al*., 1993b).

$\alpha 1\beta 2\gamma 2$ receptors deactivate more slowly than $\alpha 1\beta 2$ receptors indicating the $\gamma 2$ subunit induces slower deactivation (Tia *et al*., 1996). $\alpha 3\beta 2\gamma 2$ receptors deactivate three times slower (0.68 ± 0.1 s) than their $\alpha 1$ counterparts (0.22 ± 0.1 s) (Gingrich *et al*., 1995), indicating that the α subunit variant is

also an important determinant of deactivation. The most prominent modulatory effect produced by SB-205384 on decay rate was on the $\alpha3\beta2\gamma2$ subunit combination which appears to naturally have slower deactivation kinetics. It is possible therefore that in the presence of SB-205384 there is an enhancement of the conformational change that is stabilized by this subunit combination and which causes the slower deactivation.

Potentiation of the GABA response by SB-205384 or (+)-SB-205384 does not appear to show any α subunit-selectivity but instead shows selectivity for the $\beta2$ subunit over $\beta1$, a similar selectivity as has previously been shown for loreclezole (Wafford *et al*., 1994), indicating that the effect on potentiation of the GABA response could be occurring through the loreclezole binding site. No modulation of the decay rate of GABA responses in the presence of loreclezole has been observed and potentiation was not α subunit-selective. This suggests that if SB-205384 bound to the loreclezole binding site on the β subunit, it must also have a binding site on the α subunit through which the modulatory effect on decay rate occurs. The β-carbolines have been shown to bind to two sites, the loreclezole site as well as the benzodiazepine site (Stevenson *et al*., 1995). However, it has previously been shown that SB-205384 does not bind to the benzodiazepine binding site (Benham *et al*., 1994). No β subunit selectivity has yet been shown for benzodiazepine site modulators, neurosteroids or barbiturates (Hadingham *et al*., 1993b). In addition, SB-205384 and (+)-SB-205384 showed little selectivity for the $\gamma2$ subunit over $\gamma1$. This is in contrast to the much greater $\gamma2$ selectivity which has previously been observed for zolpidem, other benzodiazepine site ligands and to some extent for loreclezole. This combination of selectivity between β subunits and minor selectivity between γ subunits is unique to this class of compounds.

SB-205384 has previously been shown to prolong the half-life of decay of GABA-induced currents in rat cerebellar granule cells in culture (Meadows *et al*., 1997). *In situ* studies have shown that rat cerebellar granule cells contain mRNAs for $\alpha1$, $\alpha4$, $\alpha6$, $\beta1$, $\beta2$, $\beta3$, $\gamma2$, $\gamma3$ and δ subunits (Wisden *et al*., 1992).

Immunofluorescence staining with antibodies for the $\alpha1$, $\alpha3$, $\alpha6$, $\beta2$, $\beta3$, $\gamma2$ and δ subunits has also revealed the presence of $\alpha1$, $\alpha6$, $\beta2,3$, $\gamma2$ and δ subunits, but not the $\alpha3$ subunit in rat cerebellar granule cells (Gao & Fritschy, 1995). In transfected oocytes the temporal effect appeared to be selective for $\alpha3$ subunit-containing combinations. This would indicate that the effect observed on native currents in granule cells may be due either to the smaller increases observed for $\alpha1$ subunit-containing receptors or that the compound also shows selectivity for $\alpha4$ or $\alpha6$ containing receptor combinations that we have not tested. The temporal effects on the heterogeneous granule cell current were much smaller in magnitude than those found here for $\alpha3\beta2\gamma2$ combinations. Species effects might also explain these data.

The $\alpha3$ subunit is expressed throughout the forebrain and is thought to be a component of approximately 17% of $GABA_A$ receptors (Wisden *et al*., 1992; McKernan & Whiting, 1996). Receptors containing the $\alpha3$ subunit appear to be predominantly located in layer VI of the cortex (Wisden *et al*., 1992; Dunn *et al*., 1996). The expression of the $\alpha3$ subunit has also been specifically localized to monoaminergic and cholinergic neurones. In contrast receptors expressing the $\alpha1$ subunit or the $\alpha1/\alpha3$ subunit combination were mostly localized on GABAergic neurones (Fritschy *et al*., 1992; Gao *et al*., 1993; 1995). The subunit-selective effect of SB-205384 along with the specific localization of particular subunit-containing $GABA_A$ receptors may produce a novel therapeutic profile for compounds acting at this binding site.

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