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Characterization of U-97775 as a $GABA_A$ receptor ligand of dual functionality in cloned rat $GABA_A$ receptor subtypes

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1 U-97775 (*tert*-butyl 7-chloro-4,5-dihydro-5-[(1-(3,4,5-trimethyl)piperazino)carbonyl]-imidazo[1,5-a])quinoxaline-3-carboxylate) is a novel $GABA_A$ receptor ligand of dual functionality and was characterized for its interactions with cloned rat $GABA_A$ receptors expressed in human embryonic kidney cells.

2 The drug produced a bell-shaped dose-response profile in the $\alpha 1\beta 2\gamma 2$ receptor subtype as monitored with GABA-induced Cl^- currents in the whole cell patch-clamp technique. At low concentrations ($<0.5\,\mu M$), U-97775 enhanced the currents with a maximal increase of 120% as normalized to $5\,\mu M$ GABA response (control). An agonist interaction of U-97775 with the benzodiazepine site is suggested, because Ro 15-1788 (an antagonist at the benzodiazepine site) abolished the current increase and [^{3}H]-flunitrazepam binding was inhibited by U-97775 with a K_i of 1.2 nM.

3 The enhancement of GABA currents progressively disappeared as the U-97775 concentration was raised above $1\,\mu M$, and the current amplitude was reduced to 40% below the control at $10\,\mu M$ U-97775. The current inhibition by U-97775 ($10\,\mu M$) was not affected by Ro 15-1788. It appears that U-97775 interacts with a second site on GABA receptors, distinct from the benzodiazepine site, to reverse its agonistic activity on the benzodiazepine site and also to inhibit GABA currents.

4 U-97775 at low concentrations reduced and at high concentrations enhanced [^{35}S]-TBPS binding. Ro 15-1788 selectively blocked the effect of U-97775 at low concentrations. Analysis of the binding data in the presence of Ro 15-1788 yielded a single low affinity site with an estimated K_d of 407 nM.

5 In other $\alpha\beta\gamma$ receptor subtypes, U-97775 at low concentrations enhanced Cl^- currents in the $\alpha 3\beta 2\gamma 2$, but not in the $\alpha 6\beta 2\gamma 2$ subtype. On the other hand, U-97775 at high concentrations reduced Cl^- currents in all the receptor subtypes we examined, including those of two subunits, $\alpha 1\beta 2$, $\beta 2\gamma 2$ and $\alpha 1\gamma 2$ subtypes.

6 Therapeutically, U-97775 could be unique among benzodiazepine ligands because of its ability to limit its own agonistic activity such that, at high doses the appearance of agonistic activity would be delayed until occupancy of its second site wanes. This property should make the total agonistic activity of U-97775 relatively constant over a wide range of drug doses, and may minimize its liability to abuse.

Keywords: U-97775; $GABA_A$ receptor subtypes; GABA-induced Cl^- current; biphasic effect

Introduction

$GABA_A$ receptors are supramolecular receptor-Cl^- channel complexes which are responsible for inhibitory neurotransmission in the brain, and appear to exist in combinations of various subunits (α, β, γ and δ), each of which consists of several isoforms in mammalian brains (Costa & Guidotti, 1979; Barnard *et al.*, 1987; Bormann, 1988; Schofield, 1989; Olsen & Tobin, 1990; Barnard *et al.*, 1993). Allosteric $GABA_A$ receptor ligands of diverse chemical structures have been widely used as therapeutic agents, i.e., numerous hypnotic and anxiolytic agents for the benzodiazepine site, barbiturates and progesterone metabolites (neurosteroids) (Sieghart, 1992). The benzodiazepine site modulators are functionally classified as agonists (positive modulators), inverse agonists (negative modulators), and neutral antagonists (binding, but without functional consequences). Recent studies with cloned rat $GABA_A$ receptors indicate that the allosteric modulation arises from interaction of drugs with distinct modulatory sites on the receptors including a few novel ones (Im *et al.*, 1993). Considering the presence of multiple binding sites for chemicals of diverse structure on $GABA_A$ receptors, it is not surprising to find drugs of dual functionality that interact with more than one modulatory site. In this study, we will describe such a drug, U-97775 (*tert*-butyl 7-chloro-4,5-dihydro-5-[(1-(3,4,5-trimethyl) piperazino) carbonyl]imidazo [1,5-a]) quinoxaline-3-carboxylate) (Figure 1).

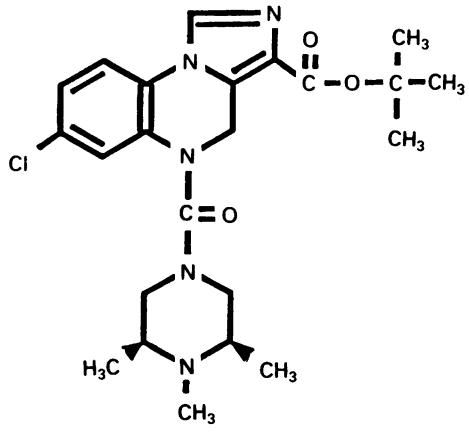

Figure 1 Chemical structure of U-97775.

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Methods

Cloned cells

The stable human embryonic kidney cell lines (HEK-293) expressing the indicated combinations of α1, α3, α6, β2, and γ2 subunits of $GABA_A$ receptors were derived by transfection of plasmids containing cDNA and a plasmid encoding G418 resistance into the A293 cell as described elsewhere (Hamilton *et al.*, 1993). Preparation of baculovirus constructs (AcNPV) carrying rat cDNAs for $GABA_A$ receptor subunits and the growth of SF-9 cells in the presence of the recombinant baculovirus were carried out as described previously (Carter *et al.*, 1992).

Electrophysiology

The whole cell patch clamp technique (Hamill *et al.*, 1981) was used to record the GABA-mediated Cl^- currents in human embryonic kidney cells (A293) expressing various combinations of $GABA_A$ receptor subunits. Briefly, the pipette solution contained (mM): CsCl 140, EGTA 11, $MgCl_2$ 4, ATP 2 and HEPES 10, pH 7.3. Cells were bathed in an external solution containing (mM): NaCl 135, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8 and HEPES 5, pH 7.2. GABA and drugs were dissolved in the external solution and were applied through a U-tube placed within 100 μm of the target cell. The concentration of GABA was varied according to the respective subtype, typically being near EC_{25} values or submaximal levels at which pentobarbitone and 5α-THDOC (3α,21-dihydroxy-5α-pregnan-20-one) produced robust enhancement of Cl^- currents. The current was recorded with an Axopatch 1D amplifier, a CV-4 headstage (Axon Instrument Co.), a Gould Recorder 220, and the holding potential of −60 mV at room temperature (21–24°C).

Binding studies

Binding of radioactive ligands was measured in membranes obtained from Sf-9 cells expressing recombinant receptors, using filtration techniques as described elsewhere (Pregenzer *et al.*, 1993). Briefly, displacement of [^{3}H]-flunitrazepam (α1β2γ2 and α3β2γ2) or [^{3}H]-Ro 15-4513 (ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a]-[1,4] benzodiazepine-3-carboxylate) (α6β2γ2) by test compounds was measured in the medium containing (mM): NaCl 118, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES 20 (pH 7.3), the radioactive ligand (3 nM for flunitrazepam) and 30 μg membrane proteins in a total volume of 500 μl at 4°C for 60 min. The effects of test compounds on [^{35}S]-TBPS ([^{35}S]-t-butylbicyclophosphorothionate) binding were measured in the medium containing 1 M NaCl, and 10 mM Tris/HCl (pH 7.4), [^{35}S]-TBPS (3 nM), and 50 μg membrane proteins in a total volume of 500 μl at 24°C for 120 min. Non-specific binding was estimated and was subtracted to compute specific binding as described earlier (Pregenzer *et al.*, 1993).

Results

Dual effects of U-97775 on GABA-induced Cl^- currents in αβγ subtypes

We tested the effects of U-97775 at various concentrations on GABA-induced Cl^- currents in several subtypes of cloned rat $GABA_A$ receptors expressed in HEK 293 cells, using the whole cell patch clamp technique. Figure 2a shows Cl^- current traces induced by 5 μM GABA alone, with diazepam or U-97775 from 0.2 to 10 μM in the α1β2γ2, α3β2γ2 and α6β2γ2 subtypes of $GABA_A$ receptors. Figure 2b shows composite dose-response profiles obtained after normalization to standard GABA responses. In the α1β2γ2 subtype, U-97775 produced a bell-shaped response. The drug at low concentrations (<0.5 μM) increased GABA(5 μM)-induced Cl^- currents, maximally by 120 ± 22% as normalized to 5 μM GABA response (control), but as the drug concentration was raised, the amplitude of Cl^- currents was gradually decreased, by 39 ± 5% at 10 μM U-97775 from the control. A similar bell-shaped response was observed in the α3β2γ2 subtype. U-97775 enhanced Cl^- currents, maximally by 117 ± 33% at 0.2 μM, but reduced it to the control level at 10 μM. In the α6β2γ2 subtype, where classical benzodiazepines (i.e., diazepam) had no effect, U-97775 at low concentrations (<0.5 μM) produced no appreciable change, but at higher concentrations reduced the current amplitude by 45 ± 7% at 10 μM.

Selective block of the agonistic action of U-97775 by Ro 15-1788

We examined whether Ro 15-1788, a classical antagonist for the benzodiazepine site, influences U-97775 actions in the

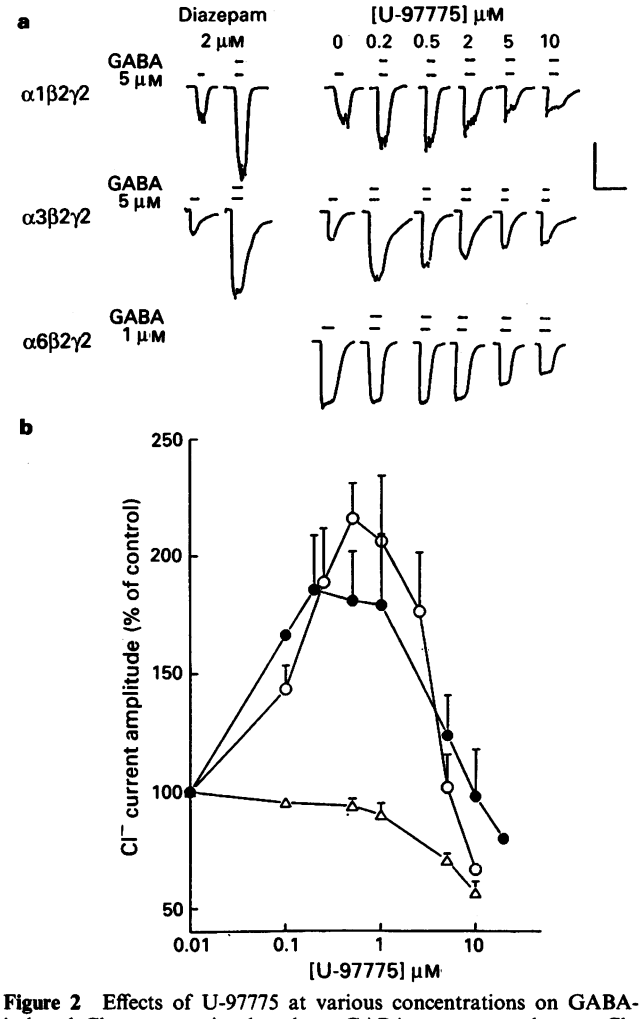

Figure 2 Effects of U-97775 at various concentrations on GABA-induced Cl^- currents in cloned rat $GABA_A$ receptor subtypes. Cl^- currents were measured by the whole-cell patch clamp technique in HEK293 cells expressing the α1β2γ2, α3β2γ2 or α6β2γ2 subtype. The holding potential was −60 mV under a symmetrical Cl^- gradient. GABA at 5 μM in the α1β2γ2 and α3β2γ2 subtypes or at 1 μM in the α6β2γ2 subtype was applied for 10 s with or without U-97775 at the indicated concentration. The GABA concentrations were about equivalent to the EC_{25} value in the respective subtype. Diazepam (2 μM) was used to monitor the ability of benzodiazepine site ligands to enhance Cl^- currents. In the α6β2γ2 subtype, diazepam had no effect on GABA-induced Cl^- currents. (a) Typical traces for GABA-induced Cl^- currents. (b) Plots showing changes in Cl^- current amplitude as a function of U-97775 concentrations: (○) α1β2γ2; (●) $\alpha_3\beta_2\gamma_2$ and (△) α6β2γ2. The currents were normalized to that observed with GABA alone. The data represent the mean ± s.e. from three experiments at least. The vertical calibration bar represents 500 pA and the horizontal bar represents 30 s.

α1β2γ2 subtype. Ro 15-1788 at 10 μM (alone, no effect on GABA-induced Cl^- currents) abolished the current increase by U-97775 at 0.25 μM (Figure 3a), but produced no appreciable effect on inhibition of the Cl^{-1} current by U-97775 at 10 μM (Figure 3b). The extent of inhibition by U-97775 (10 μM) was 35 ± 10% and 39 ± 5% in the presence or absence of Ro 15-1788 (10 μM), respectively. These results indicate that U-97775 interacts with at least two sites on $GABA_A$ receptors, the benzodiazepine site (high affinity, agonistic site) and a second site (low affinity, inverse agonistic site) distinct from the benzodiazepine site.

As shown in Figure 3b, the Cl^- current in the cell treated with U-97775 at 10 μM, upon washing with a drug-free solution, recovered well beyond the level of inhibition. Its amplitude became about double of the control (Figure 3b, washout). The increased current level was observed only with the receptors treated with U-97775 (10 μM) without preincubation with Ro 15-1788 (data not shown), and hardly changed during a 30 min washing period (only about 10% reduction). This could be interpreted to mean that the dissociation rate of U-97775 from the benzodiazepine site was much slower than from its second site, so that the benzodiazepine site remains occupied during washing and is responsible for the current enhancement. The prolonged occupancy of the benzodiazepine site by U-97775 was further supported by the observation that diazepam did not enhance GABA-induced Cl^- currents in the U-97775-treated cells during the washing out period (data not shown). Similar washout effects were observed with the α3β2γ2 subtype upon treatment with U-97775 at high concentrations (10 μM), but not with the α6β2γ2 subtype. This subtype selectivity for U-97775 resembles that for classical benzodiazepines (Luddens *et al.*, 1990).

Another interesting point was noted from the experiments with Ro 15-1788. The level of current inhibition by U-97775 (10 μM) was not appreciably altered in the presence or absence of Ro 15-1788 (see Figure 3b). If the two opposing effects of U-97775 were simply additive, one might expect a much greater degree of inhibition by U-97775 in the presence of Ro 15-1788, which eliminates its agonistic action. The failure to observe such a change here indicates independence of the two opposing effects of U-97775, and further suggests that certain allosteric interactions occur between the first and second sites, which lead to the abolition of its agonistic activity at the benzodiazepine site, in addition to the inhibition of Cl^- currents via the second site. Alternatively, the current inhibition by U-97775 at high concentrations could arise from a direct block of the chloride channel. If so, one would expect not only a voltage-dependence of the inhibition of Cl^- currents by U-97775, but also an ability to abolish the GABA potentiating actions of other allosteric ligands. Contrary to such expectations, the Cl^- current inhibition by U-97775 was not voltage-dependent and its ability to reverse GABA potentiation was restricted to benzodiazepine ligands (Figure 4). For instance, the current reduction by U-97775 at 10 μM was 33 ± 7% at −25 mV and 35 ± 8% at +25 mV (Figure 4a), which were not different from the level of inhibition at −60 mV, 39 ± 5%. Also U-97775 failed to antagonize the potentiating actions of pentobarbitone and 5α-THDOC (a neurosteroid) (Figure 4b and c). In the α1β2γ2 subtype, pentobarbitone at 20 μM and 5α-THDOC at 0.2 μM produced robust enhancements of GABA-induced Cl^- currents, a net increase of 230 and 290%, respectively, as normalized to the control (5 μM GABA response). In the presence of U-97775 at 10 μM, the amplitudes of GABA currents enhanced by pentobarbitone or 5α-THDOC were only partially reduced by the same amount as would be expected if their actions were additive with U-97775. It appears that U-97775 uncouples only the agonistic activity of benzodiazepine site ligands. These results favour the allosteric coupling between the low and the high affinity (benzodiazepine) sites for U-97775 over its direct blocking of Cl^- channels.

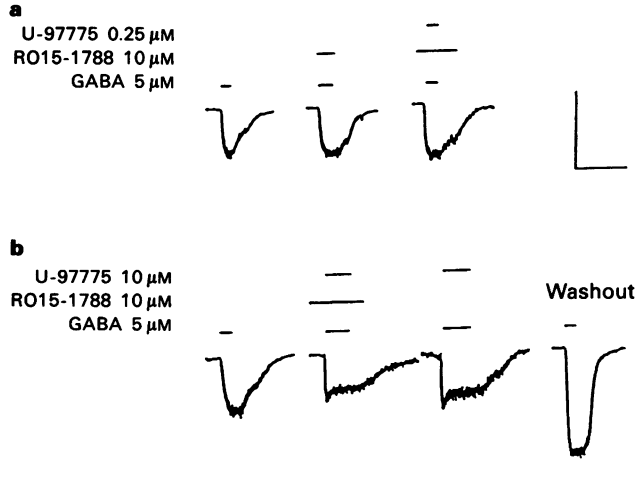

Figure 3 Differential sensitivity of U-97775 action on the Cl^- currents at low and high concentrations to Ro 15-1788. GABA-induced Cl^- currents in the whole cell configuration were measured with the α1β2γ2 subtype. Ro 15-1788 alone had no effect on Cl^- currents, but abolished the enhancement of Cl^- currents by U-97775 at 0.25 μM (a). In order to ensure occupancy of the benzodiazepine site with Ro 15-1788, the receptors were exposed to Ro 15-1788 for 5 min before the coapplication with U-97775. Ro 15-1788, on the other hand, had no effect on the current inhibition by U-97775 at 10 μM (35 ± 10%, the mean ± s.e. from three experiments) (b). Between drug applications, the patch was washed until the original GABA response was restored. It should be noted that the GABA response was restored to the initial value following application of the mixture of Ro 15-1788 and U-97775, but increased more than two fold following application of U-97775 at 10 μM due to the slow dissociation rate of the drug from the benzodiazepine site. The vertical calibration bar represents 500 pA and the horizonatal bar represents 30 s.

Monophasic inhibition of GABA-induced Cl^- currents by U-97775 in the α1β2, α1γ2 and β2γ2 subtypes

Receptor subtypes made of only two types of receptor subunits have often been useful to study the modes of interactions for novel ligands (Im *et al.*, 1993). Here we examined the effects of U-97775 on GABA-induced Cl^- currents on the α1β2, β2γ2 and α1γ2 subtypes (Figure 5). In all these subtypes, U-97775 at low concentrations (<0.5 μM) did not appreciably enhance GABA-mediated Cl^- currents, but at high concentrations (>0.5 μM) reversibly inhibited the currents in a concentration-dependent manner (Figure 5a). The U-97775 potency in blocking Cl^- currents was the highest in the β2γ2 subtype, followed by the α1γ2, α1β1 and α1β2γ2 subtypes (in the presence of Ro 15-1788); the IC_{50} values were 1.5 ± 0.3, 3.7 ± 0.2, 3.9 ± 0.3 and 5 ± 1 μM for the respective subtypes in the same order as above (Figure 5b). The variation in the IC_{50} values, albeit marginal, suggests the influence of quaternary interactions among subunits on the second site for U-97775.

Effect of U-97775 on benzodiazepine and TBPS binding

Interaction of U-97775 with the benzodiazepine site was further supported by equilibrium binding studies with the cloned α1β2γ2 receptor expressed in SF-9 insect cells which were infected with recombinant baculovirus carrying the $GABA_A$ receptor cDNAs. U-97775 displaced [^{3}H]-flunitrazepam with a K_i value of 1.2 ± 0.1 and 0.3 ± 0.04 nM for the α1β2γ2 and α3β2γ2 subtypes, respectively, but had no effect on [^{3}H]-Ro 15-4513 binding in the α6β2γ2 subtype (K_i> 10,000 nM), where classical benzodiazepines (i.e., diazepam) also failed to interact (Luddens *et al.*, 1990).

TBPS is another high affinity ligand specific for $GABA_A$ receptors and sensitive to allosteric modulators (Squires *et al.*, 1983; Gee *et al.*, 1986). Agonists and inverse agonists for $GABA_A$ receptors, for instance, have been shown to decrease

and increase TBPS binding, respectively, in the presence of 2 μM GABA (Im & Blakeman, 1991). In this study we tested the effect of U-97775 on TBPS binding in the membranes of SF-9 cells expressing the α1β2γ2 subtype of $GABA_A$ receptors (Figure 6). In the presence of GABA, U-97775 at low concentrations (<0.2 μM) reduced TBPS binding by 25 ± 7%, but at higher concentrations (>0.5 μM) reversed the early inhibition and increased TBPS binding by about 39% above the control. Ro 15-1788 (5 μM) abolished the inhibition of TBPS binding by U-97775 at low concentrations, but had no effect on the TBPS binding enhancement by the drug at high concentrations. Again, the maximal enhancement of TBPS binding by U-97775 at high concentrations (10 μM) was not appreciably changed by Ro 15-1788 (10 μM). In the absence of Ro 15-1788, U-97775 (10 μM) increased TBPS binding by 39 ± 6% above the control, a net shift of about 64%, including the 25% inhibition observed at low concentrations (0.02 μM). However, the maximal enhancement by U-97775 was still 35 ± 10% in the presence of Ro 15-1788, which abolished the inhibition of TBPS binding by U-97775 at low

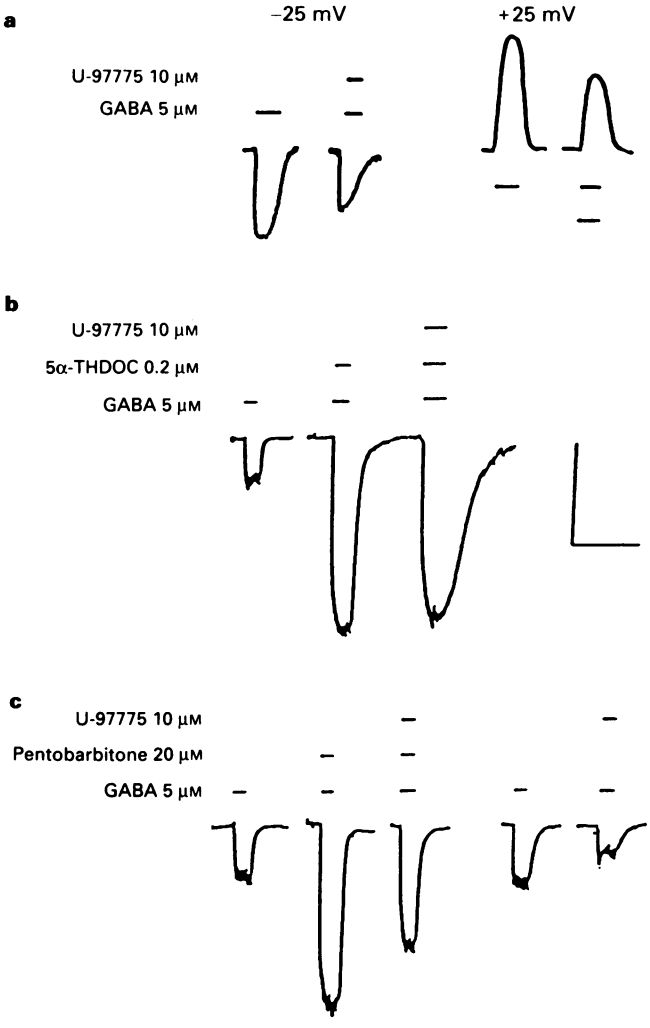

Figure 4 Voltage-independence of the Cl^- current inhibition by U-97775 at high concentrations and the absence of an interaction of U-97775 with binding sites for 5α-THDOC and barbiturates. GABA (5 μM)-induced Cl^- currents in the whole cell patch were measured with the α1β2γ2 subtype. U-97775 (10 μM) decreased Cl^- currents by 33 ± 7 and 35 ± 8% (the mean ± s.e. from six experiments) at the holding potential of −25 and +25 mV, respectively (a). The inhibition level was not different from that observed at −60 mV (39 ± 5%). Also we examined the effect of 0.2 μM 5α-THDOC or the mixture of 5α-THDOC and U-97775 on GABA(5 μM)-induced Cl^- currents (b); similarly the effect of 20 μM pentobarbitone alone or in combination with U-97775 was examined (c). Enhancement of GABA-induced Cl^- currents by 5α-THDOC or pentobarbitone was reduced in the presence of U-97775 (10 μM) by the same amount as the inhibition of GABA currents by U-97775 alone (about 30% as normalized to the 5 μM GABA response). This additiveness of the drug effects, which was consistently observed in three experiments, indicates no coupling between the binding sites for U-97775 and those for 5α-THDOC or pentobarbitone. The vertical calibration bar represents 500 pA and the horizontal bar represents 30 s.

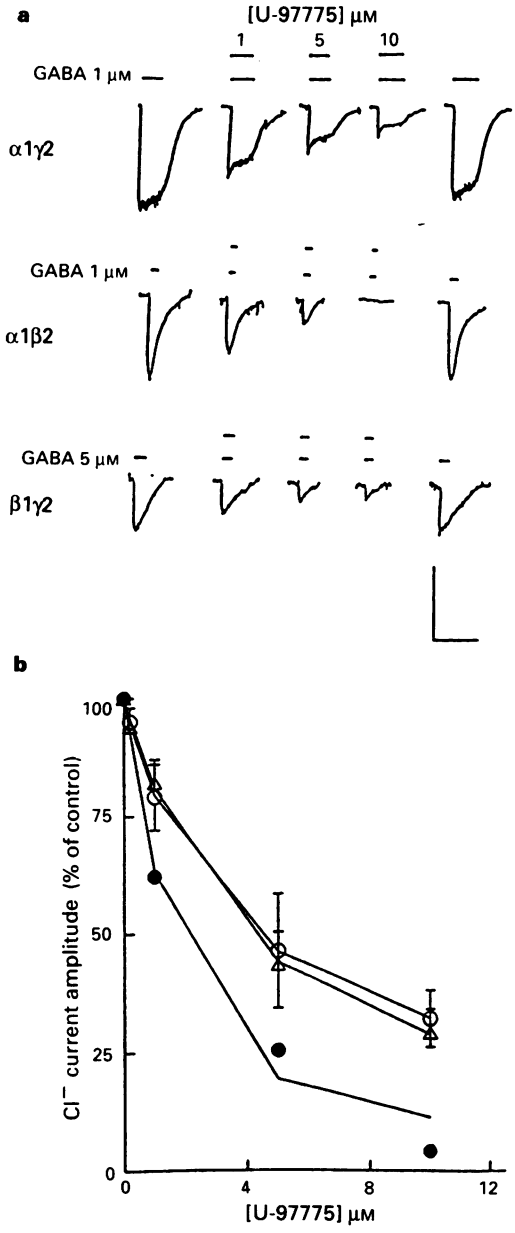

Figure 5 Concentration-dependent inhibition of GABA-induced Cl^- currents by U-97775 in the α1β2, β2γ2 and α1γ2 subtypes. Cl^- currents were induced with 1 μM GABA in the α1β2 subtype and with 5 μM in the β2γ2 and α1γ2 subtypes in the presence or absence of U-97775 at the indicated concentrations (a). The GABA concentrations represent submaximal levels at which pentobarbitone and 5α-THDOC produced robust enhancement of Cl^- currents. In these subtypes, the GABA response was immediately restored to the initial level following washout of U-97775 (10 μM), suggesting the absence of high affinity sites for the drug such as occur in the α1β2γ2 subtype. Changes in Cl^- current amplitude by U-97775 at various concentrations were normalized to the GABA response and plotted as a function of the drug concentration (b): (○) α1β2; (●) β2γ2; (△) α1γ2. The solid lines represent the data fitted with a logistic equation (see text). The currents were normalized to that observed with GABA alone. The data represent the mean from two or more experiments (with s.e.). The vertical calibration bar represents 500 pA and the horizontal bar represents 30 s.

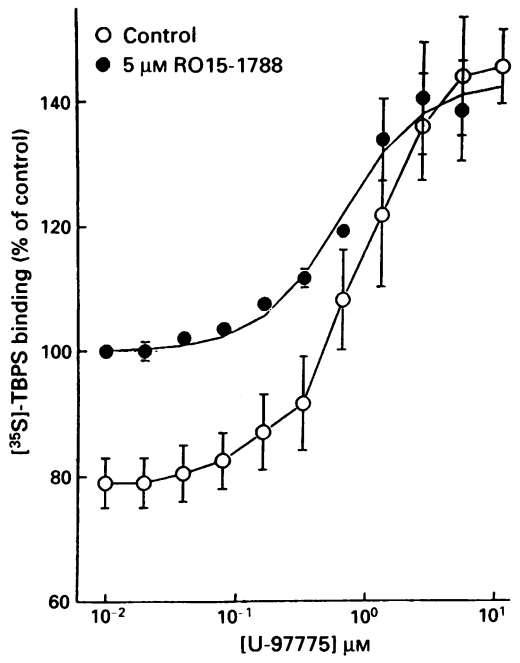

Figure 6 Effect of U-97775 on [^{35}S]-TBPS binding to the α1β2γ2 subtype of $GABA_A$ receptors. [^{35}S]-TBPS binding (3 nM) at equilibrium was measured in the membranes from Sf-9 cells expressing the α1β2γ2 subtype in the presence of 2 μM GABA, U-97775 at the indicated concentrations with (●) or without (O) 5 μM Ro 15-1788. U-97775-induced changes in TBPS binding were normalized to the level without the drug. The data representing changes in TBPS binding as a function of the U-97775 concentration in the presence of Ro 15-1788 were fitted with a logistic equation (see text). The data represent the mean ± s.e. from three experiments.

concentrations. This further supports the independence of the two opposing effects of U-97775, as noted above with electrophysiological data. The data in the presence of Ro 15-1788 was analyzed with a logistic equation, $E = E_{max} * [U\text{-}97775]/(K_{0.5} + [U\text{-}97775])$, which provided the half maximal concentration of U-97775 ($K_{0.5}$) of 407 ± 37 nM, and E_{max} of 39 ± 6%. Overall, the effects of U-97775 on TBPS binding are consistent with the functional characteristics observed in the electrophysiological studies described above, including its concentration-dependent dual action, sensitivity to Ro 15-1788, and independence of the two opposing effects.

Discussion

In this study we have shown that U-97775 is unique among $GABA_A$ receptor ligands in having two interaction sites of opposing functionality on the receptors. The drug produced a bell shaped dose-response profile as measured with GABA-induced whole cell currents in α1β2γ2 and α3β2γ2 $GABA_A$ receptors. The enhancement of the currents by U-97775 was attributed to the drug interaction with the benzodiazepine site as an agonist, because of its disappearance in the presence of Ro 15-1788 and its absence in the α6β2γ2 subtype where diazepam also failed to interact (Luddens *et al.*, 1990). Furthermore, U-97775 inhibited [^{3}H]-flunitrazepam binding with nanomolar affinity only in α1β2γ2 and α3β2γ2 subtypes, but not [^{3}H]-Ro 15-4513 binding in the α6β2γ2 subtype.

The second site (low affinity site) which was responsible for the descending limb of the bell shaped dose-response profile for U-97775, on the other hand, appeared to be distinct from the benzodiazepine site, judging from its insensitivity to Ro 15-1788 and its presence in all the subtypes tested, including α6β2γ2, α1β2, α1γ2 and β2γ2 subtypes. The interesting point, however, was that Ro 15-1788 neither abolished, nor enhanced the current inhibition by U-97775 (10 μM), although it eliminated the agonistic activity of U-97775 in the α1β2γ2 subtype. Thus, the disappearance of agonistic activity for U-97775 could not be explained by an algebraic sum of its positive and negative effects resulting from occupancy of its high and low affinity sites, respectively. Furthermore, the inhibition of Cl^- currents by U-97775 at high concentrations seems not to arise from a direct block of Cl^- channels, judging from the voltage-independence and the failure of U-97775 to antagonize the GABA potentiating action of pentobarbitone and 5α-THDOC (Figure 4). Also U-97775 blocked TBPS binding, which has been shown to be sensitive to modulation of Cl^- channels by allosteric ligands (Squires *et al.*, 1983; Gee *et al.*, 1986). Apparently, occupancy of the second site by U-97775 allosterically influences not only the GABA site (resulting in inhibition of GABA-induced Cl^- currents), but also the benzodiazepine site (leading to the abolition of its own agonistic action at this site). These two types of negative allosteric actions of U-97775 could conceivably arise from two distinct low affinity sites on the receptor, but that is highly unlikely since only one low affinity site with a K_d of 407 nM was detected from analysis of TBPS binding data at equilibrium in the presence of Ro 15-1788. At present, we cannot identify the location of the low affinity site, but it may not be localized at a selective subunit or subunit interface, because the drug effectively blocked GABA-induced Cl^- currents with an IC_{50} value ranging from 1 to 3 μM in α1β2, α1γ2 and β2γ2 subtypes. The low affinity site is likely to consist of common regions among the three subunits. This subunit nonselectivity has been observed with several $GABA_A$ receptor ligands, such as picrotoxin (also TBPS), barbiturates and neurosteroids (Im *et al.*, 1993), and may stem from 30 to 40% indentity in the amino acid sequence among $GABA_A$ receptor subunits (Olsen & Tobin, 1990).

The therapeutic potential of U-97775 deserves some consideration. At doses leading to occupancy of only the high affinity site, U-97775 would be expected to behave as a benzodiazepine site agonist (anxiolytic or hypnotic agent). However, at higher doses the drug should behave like a benzodiazepine site antagonist (or a weak inverse agonist at extremely high concentrations) because of its occupancy of the low affinity site. A delayed agonistic activity would then appear as drug occupancy of the second site wanes. These characteristics of U-97775 should result in an agonistic activity that is relatively constant over a wide range of the drug concentrations, unlike typical benzodiazepines. On the basis of the K_d for the low affinity site (407 nM as estimated from TBPS binding in the α1β2γ2 subtype), one would expect considerable occupancy of the low affinity site at the plasma concentration of 0.5 mg l^{-1} which is reachable under various clinical situations. Thus, the dual action of U-97775 on $GABA_A$ receptors may minimize its liability to abuse of the type associated with most of the benzodiazepines currently available on the market.

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