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European Journal of Pharmacology 451 (2002) 103–110

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Comparison of the effects of zaleplon, zolpidem, and triazolam at various $GABA_A$ receptor subtypes

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Received 4 April 2002; received in revised form 24 July 2002; accepted 30 July 2002

Abstract

The pyrazolopyrimidine zaleplon is a hypnotic agent that acts at the benzodiazepine recognition site of $GABA_A$ receptors. Zaleplon, like the hypnotic agent zolpidem but unlike classical benzodiazepines, exhibits preferential affinity for type I benzodiazepine (BZ_1/ω_1) receptors in binding assays. The modulatory action of zaleplon at $GABA_A$ receptors has now been compared with those of zolpidem and the triazolobenzodiazepine triazolam. Zaleplon potentiated GABA-evoked Cl^- currents in *Xenopus* oocytes expressing human $GABA_A$ receptor subunits with a potency that was higher at $\alpha1\beta2\gamma2$ receptors than at α2- or α3-containing receptors. Zolpidem, but not triazolam, also exhibited selectivity for α1-containing receptors. However, the potency of zaleplon at these various receptors was one-third to one-half that of zolpidem. Zaleplon and zolpidem also differed in their actions at receptors containing the α5 or γ3 subunit. Zaleplon, zolpidem, and triazolam exhibited similar patterns of efficacy among the different receptor subtypes. The affinities of zaleplon for [^{3}H]flunitrazepam or *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) binding sites in rat brain membranes were lower than those of zolpidem or triazolam. Furthermore, zaleplon, unlike zolpidem, exhibited virtually no affinity for the peripheral type of benzodiazepine receptor.

Keywords: Zaleplon; Zolpidem; Benzodiazepine; $GABA_A$ receptors; Recombinant; Cl^- current; *Xenopus* oocyte

1. Introduction

Characterization of the oligomeric structure of $GABA_A$ receptors, and the discovery of the presence in mammalian brain of different subpopulations of these receptors comprising various combinations of multiple subunits, have suggested that the sedative-hypnotic, anxiolytic, myorelaxant, and anticonvulsant effects of benzodiazepines might be mediated by different $GABA_A$ receptor subtypes localized in distinct brain regions (Barnard et al., 1998; McKernan and Whiting, 1996). With regard to classical benzodiazepines, $GABA_A$ receptors can generally be classified as either sensitive (those containing α1, α2, α3, or α5 subunits) or insensitive (those containing α4 or α6) (Barnard et al., 1998; Mohler et al., 2002). In addition to α subunits, γ subunits are necessary to form fully functional $GABA_A$ receptors with high-affinity benzodiazepine recognition sites (Pritchett et al., 1989), with the γ2 isoform which confers the highest benzodiazepine sensitivity when combined with an α and β subunit, while receptors containing either γ1 or γ3 exhibit substantial differences with respect to both benzodiazepine affinity and efficacy (Whiting et al., 1995; Barnard et al., 1998).

The generation of transgenic mice harboring point mutations in the α1, α2, or α3 subunit genes revealed that distinct $GABA_A$ receptor subtypes may indeed selectively mediate the various pharmacological effects of benzodiazepines. Thus, whereas the anxiolytic and myorelaxant actions of diazepam appear to be mediated by α2 subunit-containing receptors (Crestani et al., 2001; Low et al., 2000), α1-containing receptors appear responsible for the sedative-hypnotic action and, at least in part, for the anticonvulsant effect of this benzodiazepine (McKernan et al., 2000; Rudolph et al., 1999). Nonbenzodiazepine hypnotic compounds, including the imidazopyridine zolpidem (Arbilla et al., 1985; Benavides et al., 1988; Langtry and Benfield,

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PII: S0014-2999(02)02191-X

1990) and, more recently, the pyrazolopyrimidine zaleplon (*N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide) (Allen et al., 1993; Beer et al., 1997; Day et al., 1992), have also been shown to interact selectively with $GABA_A$ receptors containing the α1 subunit.

Damgen and Luddens (1999) showed that zaleplon binds to α1-containing receptors with an affinity that is 12, 10, or 27 times that apparent for its interaction with the corresponding receptors containing the α2, α3, or α5 subunits, respectively. A similar, but not identical, receptor selectivity was previously described for zolpidem (Hadingham et al., 1993; Pritchett and Seeburg, 1990). Selectivity for α1-containing $GABA_A$ receptors thus may underlie the predominant sedative-hypnotic actions of these two drugs. This conclusion is further supported by the observation that the sedative effect of zolpidem in vivo was not apparent in transgenic mice harboring an H101R ($His^{101} \rightarrow Arg$) mutation in the α1 subunit of the $GABA_A$ receptor (Crestani et al., 2000).

The intrinsic activity and potency of zaleplon as an allosteric modulator of $GABA_A$ receptor subtypes are relatively uncharacterized. Binding studies in rat brain tissue with *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS), a convulsant ligand that binds to a site closely associated with the $GABA_A$ receptor-coupled Cl^- channel (Squires et al., 1983), suggested that zaleplon possesses a high level of intrinsic activity similar to that of the hypnotic benzodiazepines flurazepam, triazolam, and diazepam (Beer et al., 1997). However, zaleplon was shown to be less effective than was zolpidem in increasing the latency of isoniazid-induced convulsions in rodents (Sanger et al., 1996), a behavioral parameter thought to be predictive of drug intrinsic activity (Perrault et al., 1990), suggesting that the intrinsic activity of zaleplon is less than that of zolpidem. To provide further information with regard to the potency and intrinsic activity of zaleplon at different $GABA_A$ receptor subtypes, we have now evaluated both electrophysiologically and neurochemically the modulatory actions of zaleplon in comparison with those of zolpidem and the triazolobenzodiazepine triazolam.

2. Materials and methods

2.1. Drugs

Triazolam was obtained from Sigma (Milan, Italy), zolpidem was kindly provided by Sanofi-Synthelabo (Bagneux, France), and zaleplon was synthesized as previously described (Follesa et al., 2002).

2.2. Animals

Adult male and female Sprague–Dawley CD rats (Charles River, Como, Italy), with body masses of 200 to 250 g at the beginning of experiments, were maintained under an artificial 12-h-light, 12-h-dark cycle (light on 0800 to 2000 h) and at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for 7 to 10 days before experiments, which were performed between 0800 and 1400 h. Adult *Xenopus laevis* females were obtained from Blades Biological (Cowden, UK), maintained under an artificial 12-h-light, 12-h-dark cycle (light on 0800 to 2000 h), and fed twice a week. Animal care and handling throughout all experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocols were approved by the Animal Ethics Committee of the University of Cagliari.

2.3. Assay of [^{3}H]flunitrazepam binding to rat cerebrocortical membranes

Rats were killed by decapitation, the brain was rapidly removed, and the cerebral cortices were homogenized with a Polytron PT 10 (setting 5, 20 s) in 50 volumes of ice-cold 50 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 20,000 × *g* for 10 min at 4 °C, and the resulting pellet was resuspended in 50 volumes of homogenization buffer and recentrifuged. The second pellet was reconstituted with an equal volume of homogenization buffer and used for the binding assay. [^{3}H]Flunitrazepam binding was determined in a final volume of 1000 μl, comprising 400 μl of membrane suspension (0.4 to 0.5 mg of protein), 400 μl of homogenization buffer, 100 μl of 10 nM (1 nM final concentration) [^{3}H]flunitrazepam (7.4 Ci/mmol; New England Nuclear), and 100 μl of drug solution or solvent. Incubations were performed at 0 °C for 60 min and were terminated by rapid filtration of the binding mixture through glass-fiber filter strips (Whatman GF/B) in a Cell Harvester filtration manifold (model M-24; Brandel). The filters were washed with ice-cold homogenization buffer, after which filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was determined as binding in the presence of 5 μM diazepam and represented ~10% of total binding. Protein concentration was assayed by the method of Lowry et al. (1951) with bovine serum albumin as standard. Drugs were dissolved in dimethyl sulfoxide and serial dilutions were prepared in the same solvent.

2.4. Assay of [^{35}S]TBPS binding

Rat cerebral cortices were homogenized, as described above (Section 2.3), in 50 volumes of an ice-cold solution containing 50 mM Tris–citrate (pH 7.4) and 100 mM NaCl. The homogenate was centrifuged at 20,000 × *g* for 20 min at 4 °C, and the resulting pellet was then resuspended in an equal volume of Tris–citrate buffer and used immediately for measurement of [^{35}S]TBPS binding. The binding mixture comprised 400 μl of membranes (0.3 to 0.4 mg of

protein), 100 μl of 20 nM (2 nM final concentration) [^{35}S]TBPS (75 Ci/mmol; NEN), 100 μl of 2 M (0.2 M final concentration) NaCl, 5 μl of drug or solvent, and 395 μl of Tris–citrate buffer. Incubations were performed at 25 °C for 90 min and were terminated by rapid filtration of the binding mixture through glass-fiber filter strips as described above (Section 2.3). The filters were washed with two 4-ml portions of ice-cold Tris–citrate buffer, after which filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 100 μM picrotoxin.

2.5. Assay of [^{3}H]PK 11195 and [^{3}H]CB34 binding

After decapitation of rats, the brain was rapidly removed, and the cerebral cortex was stored at −80 °C until assay. The tissue was thawed and then homogenized with a Polytron PT 10 (setting 5, 20 s) in 50 volumes of Dulbecco's phosphate-buffered saline (pH 7.4) (PBS) at 4 °C, and the homogenate was centrifuged at 40,000 × *g* for 30 min at 4 °C. The resulting pellet was resuspended in 50 volumes of PBS and then recentrifuged. The pellet thus obtained was resuspended in 20 volumes of PBS and used for the binding assay. [^{3}H]PK 11195 or [^{3}H]CB34 binding was determined in a final volume of 1000 μl, comprising 100 μl of membrane suspension (0.15 to 0.20 mg of protein), 100 μl of [^{3}H]PK 11195 (85.5 Ci/mmol; New England Nuclear) or [^{3}H]CB34 (127 Ci/mmol; Amersham) at a final assay concentration of 1 nM, 5 μl of drug solution of solvent, and 795 μl of PBS. Incubations were initiated by the addition of membranes, performed at 0 °C for 90 min, and terminated by rapid filtration through glass-fiber strips as described above (Section 2.3). The filters were washed with two 4-ml volumes of ice-cold PBS, and filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 10 mM unlabeled PK 11195.

2.6. Preparation of cDNAs

Complementary DNAs encoding the human α1 to α5, β2, γ2L, and γ3 $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA, USA) (Hadingham et al., 1993) and purified with a Wizard Plus Miniprep kit (Promega, Madison, WI, USA). After resuspension in sterile distilled water, the cDNAs were stored at −20 °C until use.

2.7. Isolation of Xenopus oocytes and microinjection of cDNAs

Stages V and VI oocytes were isolated with the use of fine surgical forceps after manual dissection of the ovary of *Xenopus* females and were exposed to collagenase type IA as described previously (Sanna et al., 1995). Various combinations of cDNAs encoding $GABA_A$ receptor subunits (total of 1.5 ng of DNA in 30 nl) were injected into oocyte nuclei with the use of a 10-μl glass micropipette (tip diameter, 10 to 15 μm). The injected oocytes were then transferred to modified Barth's saline [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM HEPES–NaOH (pH 7.5), 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$] supplemented with 2 mM sodium pyruvate, penicillin (10,000 U/l), streptomycin (10 U/l), gentamicin (50 U/l), and 0.5 mM theophylline, and were maintained at 16 °C until use (usually for up to 5 days, during which time they were transferred to fresh medium each day).

2.8. Electrophysiological recording

Electrophysiological recording was initiated 18 to 24 h after cDNA injection and was performed as previously described (Sanna et al., 1995). In brief, oocytes were placed in a rectangular recording chamber (volume, 100 μl) and continuously perfused with modified Barth's saline at a flow rate of 2 ml/min and room temperature. Oocytes were impaled at the animal pole with two glass microelectrodes (0.5 to 3 MΩ) filled with filtered 3 M KCl, and were voltage-clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame, CA, USA). The resting membrane potential usually ranged between −30 and −50 mV. GABA was applied to oocytes for 20 s, and intervals of 5 min were allowed between applications of low concentrations of GABA (Sigma, St Louis, MO, USA) alone and of ≥10 min between applications of GABA in the presence of drugs. Drugs were applied for 30 s before their presentation together with GABA. Stock solutions (10 mM) of zaleplon, zolpidem, and triazolam were prepared in dimethyl sulfoxide, divided into small volumes, and maintained at −20 °C until use. Before each experiment, the drugs were diluted to the appropriate concentration (1×10^{-8} to 3×10^{-6} M) with modified Barth's solution. Data from electrophysiological experiments were expressed as a percentage potentiation of the control current response obtained with GABA alone, which was measured before and after each drug application. The control response was determined at the EC_{10} for GABA, defined as the concentration of GABA that induces 10 ± 3% of the maximal GABA response; this concentration was determined experimentally for each cell at the beginning of all experiments. In order to derive the EC_{50} and maximal percent potentiation values for the different drugs, concentration–response curves were fitted (Jandel Sigmaplot 4.01) with the sigmoidal equation:

$$I = I_{max}/(1 + C/EC_{50})^{n_H}$$

were I_{max} is the maximum current observed, C is the drug concentration, EC_{50} is the drug concentration that induces 50% of the maximal response, and n_H is the Hill coefficient.

Oocytes from at least two frog donors were used for each experiment, and the total number of oocytes is given.

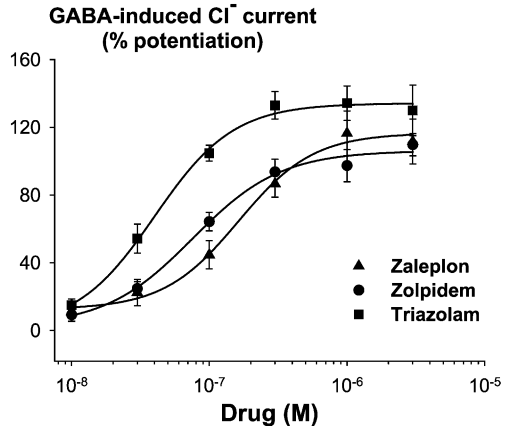

Fig. 1. Modulatory actions of zaleplon, zolpidem, and triazolam on GABA-evoked Cl^- currents measured in *Xenopus* oocytes expressing human $\alpha1\beta2\gamma2$ receptors. Data are expressed as percentage potentiation of the control response to GABA (EC_{10}) and are means ± S.E.M. ($n = 8$ to 15).

2.9. Statistical analysis

Data are presented as means ± S.E.M. and were subjected to one-way analysis of variance (ANOVA), with comparisons between individual means performed with Scheffe's post hoc test.

3. Results

3.1. Differential effects of zaleplon, zolpidem, and triazolam on GABA-evoked Cl^- currents at recombinant $GABA_A$ receptors

We first examined the modulatory effect of zaleplon, in comparison with those of zolpidem and triazolam, on GABA-evoked Cl^- currents in voltage-clamped *Xenopus* oocytes expressing human $\alpha1\beta2\gamma2$ $GABA_A$ receptors. Zaleplon (1×10^{-8} to 3×10^{-6} M), like zolpidem and triazolam over the same concentration range, exhibited marked efficacy in potentiating GABA-evoked Cl^- currents (Fig. 1). The maximal potentiation induced by zaleplon was 113 ± 5%, with an EC_{50} of 169 nM (Table 1). The maximal potentiation induced by zolpidem (100 ± 5%) was similar to that induced by zaleplon, although the potency of zolpidem (EC_{50}, 78 nM) was about twice that of zaleplon. Triazolam also induced a maximal effect (132 ± 6%) similar to those of zaleplon and zolpidem, but its potency (EC_{50}, 52 nM) was much greater than those of the other two drugs.

We also assessed the modulatory actions of zaleplon, zolpidem, and triazolam at $GABA_A$ receptors containing other α subunit isoforms (Table 1). At receptors containing the α2 subunit ($\alpha2\beta2\gamma2$ receptors), zaleplon and zolpidem each potentiated GABA-evoked Cl^- currents with a higher efficacy and markedly lower potency compared with their effects at $\alpha1\beta2\gamma2$ receptors. Again, the maximal effects of the two drugs were similar but the EC_{50} of zaleplon was about three times that of zolpidem. At $\alpha3\beta2\gamma2$ receptors, the maximal potentiation by zaleplon (362 ± 87%) was the highest observed at any of the subunit assemblies examined, but the potency of this drug was further decreased. Zolpidem also exhibited its highest efficacy and a low potency at α3-containing receptors. Triazolam also potentiated the GABA response at both $\alpha2\beta2\gamma2$ and $\alpha3\beta2\gamma2$ receptors with increased efficacy but with a potency not markedly different from that apparent at $\alpha1\beta2\gamma2$ receptors.

The effects of zaleplon, zolpidem, and triazolam were also compared at $GABA_A$ receptors containing the α4 or α5 subunit. Receptors containing either of these subunits were previously shown to be insensitive to zolpidem (Benke et al., 1997; Hadingham et al., 1993; Scholze et al., 1996), whereas α4-containing receptors are insensitive to classical benzodiazepines (Benke et al., 1997; Knoflach et al., 1996; Whittemore et al., 1996). As expected, zaleplon, like zolpidem and triazolam, did not substantially affect GABA-evoked Cl^- currents at $\alpha4\beta2\gamma2$ receptors (Table 1). Unlike zolpidem, however, zaleplon markedly potentiated the

Table 1
Modulatory actions of zaleplon, zolpidem, and triazolam on the function of various recombinant human $GABA_A$ receptor subtypes expressed in *Xenopus* oocytes

	$\alpha1\beta2\gamma2$	$\alpha2\beta2\gamma2$	$\alpha2\beta2\gamma3$	$\alpha3\beta2\gamma2$	$\alpha4\beta2\gamma2$	$\alpha5\beta2\gamma2$
Zaleplon						
EC_{50} (nM)	169 ± 4 *	1360 ± 300 *,**	498 ± 35***	2150 ± 300 *,**	N.M.	2600 ± 700 *,**
Potentiation (%)	113 ± 5	208 ± 21 **	132 ± 4 *	362 ± 87 **	N.M.	109 ± 60
Zolpidem						
EC_{50} (nM)	78± 10	470 ± 110 **	N.M.	750 ± 160 **	N.M.	N.M.
Potentiation (%)	100 ± 5	198 ± 20 **	24 ± 14***	217 ± 34 **	N.M.	15 ± 5 **
Triazolam						
EC_{50} (nM)	52 ± 8	44 ± 5	168 ± 14***	63 ± 7	N.M.	52 ± 5
Potentiation (%)	132 ± 6	255 ± 11 **	61 ± 3***	270 ± 13 **	N.M.	165 ± 8

Data are means ± S.E.M. ($n = 10$ to 16) of EC_{50} values and the maximal percentage potentiation of GABA-evoked Cl^- currents. N.M., not measurable.
* $p < 0.01$ versus zolpidem or triazolam values obtained at the same receptor construct.
** $p < 0.05$ versus values, respective to the same drug, obtained at $\alpha1\beta2\gamma2$ receptors.
*** $p < 0.01$ versus values, respective to the same drug, obtained at $\alpha2\beta2\gamma2$ receptors.

Table 2
Effects of zaleplon, zolpidem, and triazolam on [^{3}H]flunitrazepam binding to rat cerebrocortical membranes

Compound	IC_{50} (nM)
Zaleplon	355 ± 24 *
Zolpidem	111 ± 12
Triazolam	3.4 ± 0.2

Data are means ± S.E.M. of IC_{50} values obtained from three independent experiments, each performed in triplicate.
* $p < 0.01$ versus zolpidem and triazolam.

GABA response at α5β2γ2 receptors, with a maximal potentiation of 109 ± 60% and an EC_{50} value of 2600 nM. In contrast, triazolam modulation of α5β2γ2 receptors was characterized by an efficacy and potency similar to those apparent at α1β2γ2 receptors.

The presence of the γ3 subunit has been shown to have a marked influence on the receptor affinities of zaleplon, zolpidem, and benzodiazepines (Damgen and Luddens, 1999). In the present study, substitution of the γ2 subunit in α2β2γ2 receptors with the γ3 subunit greatly affected both the potency and intrinsic activity of all three drugs examined (Table 1). This substitution thus resulted in a marked increase in the potency and a decrease in the efficacy of zaleplon. In contrast, both the potencies and efficacies of zolpidem and triazolam were reduced at α2β2γ3 receptors compared with those at α2β2γ2 receptors.

3.2. Affinities of zaleplon, zolpidem, and triazolam for the central type of benzodiazepine receptors: [^{3}H]flunitrazepam binding

The affinity of zaleplon for the central type of benzodiazepine receptors was compared with those of zolpidem and triazolam by measuring the effects of these drugs on [^{3}H]flunitrazepam binding to rat cerebrocortical membranes (Table 2). As expected, zaleplon (10^{-10} to 10^{-4} M) completely inhibited specific [^{3}H]flunitrazepam binding with an IC_{50} that was greater than those of zolpidem and triazolam by factors of about 3 and 100, respectively.

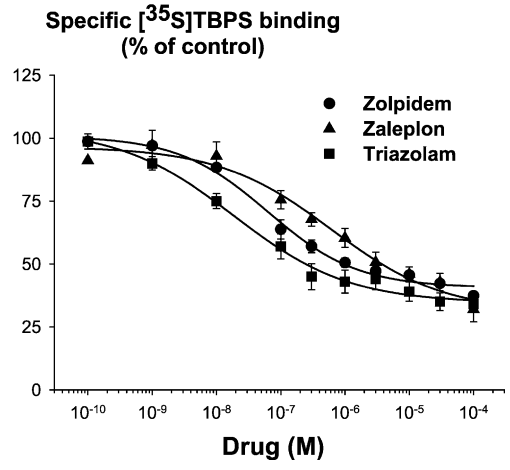

Fig. 2. Inhibition of [^{35}S]TBPS binding to rat cerebrocortical membranes by zaleplon, zolpidem, and triazolam. Data are expressed as a percentage of the specific binding observed in the absence of test drug (control), and are means ± S.E.M. of values from three independent experiments, each performed in triplicate.

Table 3
Effects of zaleplon, zolpidem, and triazolam on [^{35}S]TBPS binding to rat cerebrocortical membranes

Compound	IC_{50} (nM)	Inhibition (%)
Zaleplon	613 ± 87 *	68.1 ± 4.8
Zolpidem	63 ± 11	62.7 ± 0.2
Triazolam	18 ± 1	66.3 ± 3

Data are means ± S.E.M. of IC_{50} and percentage inhibition (at 100 μM) values obtained from three independent experiments, each performed in triplicate.
* $p < 0.01$ versus zolpidem and triazolam.

3.3. Effects of zaleplon, zolpidem, and triazolam on [^{35}S]TBPS binding

In unwashed rat cerebrocortical membranes containing endogenous GABA, zaleplon (10^{-10} to 10^{-4} M) inhibited specific [^{35}S]TBPS binding in a concentration-dependent manner (Fig. 2). Zolpidem and triazolam also inhibited

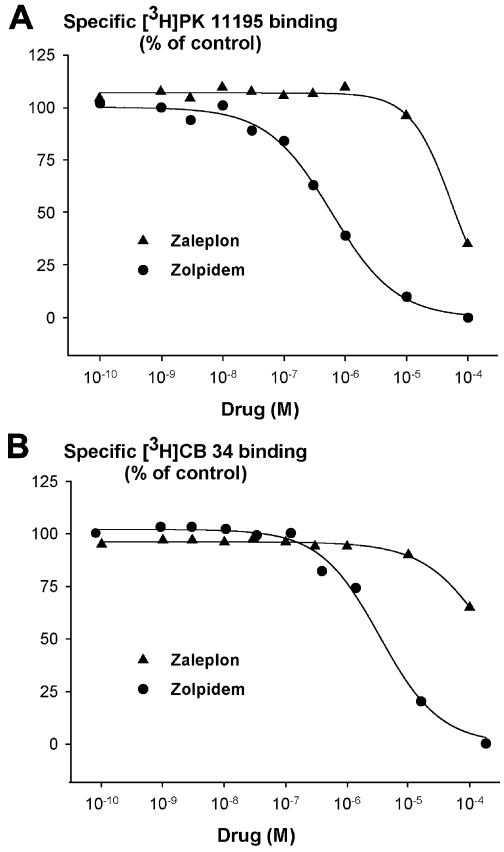

Fig. 3. Effects of zaleplon and zolpidem on the binding of [^{3}H]PK 11195 (A) or [^{3}H]CB34 (B) to rat cerebrocortical membranes. Data are expressed as a percentage of the specific binding observed in the absence of test drug (control), and are means of triplicates from representative experiments.

specific [^{35}S]TBPS binding to similar extents over the same concentration range. Comparison of the IC_{50} values, however, revealed that the affinities of zolpidem and triazolam were about 9 and 34 times, respectively, that of zaleplon (Table 3).

3.4. Binding of zaleplon and zolpidem to the peripheral type of benzodiazepine receptors

Imidazopyridines such as zolpidem bind to the peripheral type of benzodiazepine receptors, albeit with a lower affinity than that with which they interact with the central type of benzodiazepine receptors (Langer et al., 1988). We therefore compared the effects of zaleplon and zolpidem on the binding to rat cerebrocortical membranes of two different radioligands selective for peripheral benzodiazepine receptors, [^{3}H]PK 11195 and [^{3}H]CB34 (Pisu et al., 2001; Serra et al., 1999; Trapani et al., 1999), the latter of which is a new imidazopyridine derivative recently characterized in our laboratory. Zaleplon (10^{-10} to 10^{-4} M) had virtually no affinity for either [^{3}H]PK 11195 or [^{3}H]CB34 binding sites (Fig. 3). In contrast, zolpidem inhibited the binding of [^{3}H]PK 11195 and [^{3}H]CB34 with IC_{50} values of 0.59 and 2.34 μM, respectively.

4. Discussion

Knowledge of the pharmacodynamics of the actions of different benzodiazepine receptor ligands at different $GABA_A$ receptor subtypes is important not only for evaluation of the pharmacological profiles of these drugs but also for rationalization of their therapeutic use. We have now confirmed that the novel hypnotic agent zaleplon exhibits a pharmacological profile that is similar overall to that of the imidazopyridine zolpidem but which differs markedly from that of the triazolobenzodiazepine triazolam. Zaleplon thus acts preferentially at $GABA_A$ receptors containing the α1 subunit. However, zaleplon is distinguishable from zolpidem on the basis of its lower potency with regard to modulation of the function of $GABA_A$ receptors containing α1, α2, or α3 subunits, its ability to potentiate GABA-evoked Cl^- currents at α5-containing receptors, the distinct influence of the γ3 subunit on its action, and its failure to interact substantially with peripheral benzodiazepine receptors.

Our electrophysiological experiments with recombinant human $GABA_A$ receptors expressed in *Xenopus* oocytes revealed that zaleplon, like zolpidem, exhibits a higher modulatory potency at receptors containing the α1 subunit than at those containing either the α2 or α3 subunits. The EC_{50} value for the potentiation of GABA-evoked Cl^- currents by zaleplon thus increased by factors of 8 and 13 at α2β2γ2 and α3β2γ2 receptors, respectively, compared with that apparent at α1β2γ2 receptors. These results are qualitatively similar to those obtained with zolpidem. However, the EC_{50} value (78 nM) for zolpidem at α1β2γ2 receptors was about half that of zaleplon (169 nM), a difference in modulatory potency between the two drugs that is further supported by previous neurochemical data (Beer et al., 1997) as well as by our present results showing that zaleplon competitively inhibits [^{3}H]flunitrazepam binding and allosterically modulates [^{35}S]TBPS binding measured in rat cerebrocortical membranes with affinities markedly lower than those of zolpidem.

As expected (Hadingham et al., 1993), the potency of zolpidem at α2β2γ2 or α3β2γ2 receptors was reduced by factors of 6 and 10, respectively, compared with that apparent at α1β2γ2 receptors. The receptor selectivity of zaleplon and zolpidem differentiated these drugs from triazolam, whose EC_{50} values for potentiation of GABA-evoked Cl^- currents were similar among the receptors containing α1, α2, or α3.

The intrinsic activity of zaleplon was also influenced by the α subunit isoform coexpressed with the β2 and γ2 subunits. The efficacy of zaleplon at α2- or α3-containing receptors was thus two or three times, respectively, that apparent at receptors containing the α1 subunit. A similar influence of receptor subunit composition was observed for zolpidem and triazolam, although the efficacies of these drugs at α2-containing receptors did not differ substantially from those at α3-containing receptors. A higher efficacy at α2- or α3-containing receptors than at α1-containing receptors has previously been observed for benzodiazepine compounds such as diazepam and flunitrazepam (Puia et al., 1991; Wafford et al., 1993). However, at variance with our results, Puia et al. (1991) showed that zolpidem was equally effective at these three receptor constructs. Comparison of the efficacy of zaleplon with those of zolpidem and triazolam reveals a level of similarity sufficient to suggest that zaleplon be considered a ligand with high intrinsic activity for the BZ_1/ω_1 receptor subtype. Thus, the intrinsic activity at $GABA_A$ receptor subtypes does not distinguish zaleplon from benzodiazepines. This conclusion is also supported by our [^{35}S]TBPS binding data showing that zaleplon, zolpidem, and triazolam negatively modulate this parameter by similar extents.

A further difference between zaleplon and zolpidem was evident at α5β2γ2 receptors. Whereas we confirmed the previous observation that these receptors are insensitive to the modulatory action of zolpidem (Hadingham et al., 1993; Pritchett and Seeburg, 1990), zaleplon potentiated GABA-evoked Cl^- currents at these receptors with a potency similar to that observed at α3β2γ2 receptors and an efficacy similar to that apparent at α1β2γ2 receptors.

Substitution of the γ2 subunit with γ3 has previously been shown to markedly and differentially affect receptor affinity for zaleplon, zolpidem, and benzodiazepines (Damgen and Luddens, 1999; Luddens et al., 1994). Consistent with these neurochemical data, we have now shown that the modulatory action of zolpidem was virtually abolished and that the potency and efficacy of triazolam were reduced at

α2β2γ3 receptors, compared with those apparent at α2β2γ2 receptors, whereas the potency of zaleplon was increased by a factor of 3. Together, the data obtained with receptors containing either α5 or γ3 suggest that the differential effects of these subunits on the actions of zaleplon and zolpidem may contribute to the differences in the behavioral actions of these compounds (Sanger et al., 1996).

The administration of diazepam to transgenic mice harboring a point mutation (H101R) in the $GABA_A$ receptor α1 subunit that results in insensitivity to benzodiazepines was shown to induce the typical anxiolytic, anticonvulsant, myorelaxant, and ataxic effects of this drug, but failed to elicit the sedative and amnesic effects (McKernan et al., 2000; Rudolph et al., 1999). These observations suggest that α1-containing $GABA_A$ receptors mediate the sedative-hypnotic and amnesic effects of benzodiazepines. Drugs with high selectivity for α1-containing receptors, such as zaleplon and zolpidem, may thus be endowed with a greater specificity of action and elicit fewer side effects, compared with classical benzodiazepines.

In contrast to previous evidence of a lack of physical dependence after long-term administration of zolpidem in rodents (Langtry and Benfield, 1990; Perrault et al., 1992; Von Voightlander and Lewis, 1991), recent studies in monkeys have shown that both zaleplon and zolpidem, like benzodiazepines, induce signs of withdrawal after interruption of chronic once-daily administration (Ator et al., 2000; Weerts et al., 1998). These observations suggest that preferential affinity for α1-containing $GABA_A$ receptors may not necessarily be associated with a low liability of dependence.

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