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Biochemical Evaluations of the Effects of Loreclezole and Propofol on the $GABA_A$ Receptor in Rat Brain

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ABSTRACT. The effects of loreclezole on the function of the γ-aminobutyric acid type A ($GABA_A$) receptor complex in rat cerebral cortical membrane preparations were compared with those of propofol and diazepam. Loreclezole and propofol modulated [^{3}H]muscimol binding and t-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) binding to washed and unwashed membranes with potencies and efficacies greater than those of diazepam. Loreclezole and propofol enhanced [^{3}H]flunitrazepam binding to washed membranes with efficacies lower than those of GABA and muscimol. Both loreclezole and propofol showed biphasic effects on [^{35}S]TBPS binding to washed membranes: at low concentrations (5 to 10 μM), both drugs, with different efficacies, enhanced [^{35}S]TBPS binding whereas, at higher concentrations (30 to 100 μM), they inhibited this biochemical parameter. In contrast, diazepam enhanced [^{35}S]TBPS binding to washed membranes at all concentrations tested. The combination of loreclezole with GABA, at a concentration (0.3 μM) that only slightly increased [^{35}S]TBPS binding to washed membranes, reversed the increase in binding elicited by loreclezole (5 to 10 μM) and significantly potentiated the inhibitory effect exerted by higher concentrations (30 to 100 μM) of this drug. Similar effects were observed with the combination of GABA and propofol. However, GABA had no effect on the enhancement of [^{35}S]TBPS binding induced by diazepam. The ability of GABA to reverse and potentiate the effects of loreclezole and propofol on [^{35}S]TBPS binding to washed membranes was shared by pentobarbital (200 μM) and alphaxalone (3 μM). These anesthetics showed greater efficacies in combination with propofol than with loreclezole. These results suggest that, unlike diazepam, loreclezole and propofol may activate the receptor-associated Cl^- channel in the absence of GABA. Furthermore, the difference in the pharmacological profiles of loreclezole and propofol may result from their different effectiveness in activating the receptor Cl^- channel directly. BIOCHEM PHARMACOL 51;11:1527–1534, 1996.

KEY WORDS. loreclezole; propofol; diazepam; $GABA_A$ receptor; Cl^- channel

Loreclezole (R 72 063), (Z)-1-[2-chloro-2-(2,4-dichlorophenyl)ethenyl]-1H-1,2,4-triazole, is a broad-spectrum anticonvulsant that is effective against tonic-clonic convulsions and generalized absence seizures [1, 2]. Although its anticonvulsant profile is similar to that of GABA†ergic drugs, the pharmacology of loreclezole resembles that of barbiturates more than that of benzodiazepines [3]. Molecular biology studies have shown that loreclezole recognizes a novel specific site on β subunits of the $GABA_A$ receptor complex [4, 5]; a single amino acid residue that confers sensitivity on loreclezole is present in β_2 and β_3 subunits, but not in the β_1 subunit. Thus, only $GABA_A$ receptors that contain β_2 or β_3 subunits are activated by loreclezole. The β subunit has also been shown to be required for the direct activation of the GABA-coupled Cl^- channel by the general anesthetic propofol [6], and different β-subunit isoforms confer different sensitivity on the positive modulatory action on $GABA_A$ receptors by nonvolatile anesthetics [7]. On the other hand, it is yet to be clarified as to whether or not these two compounds share the same recognition site on this subunit. However, this suggests that loreclezole and propofol represent prototypes of drugs with specificity for allosteric sites on the β subunit but are endowed with a different range of pharmacological effects.

Based on these premises, we have compared biochemically the efficacies and potencies, with regard to the enhancement of $GABA_A$ receptor function, of loreclezole, propofol, and diazepam, an anticonvulsant, anxiolytic and, at high doses, hypnotic benzodiazepine. Thus, by comparing the effects of drugs that act on the $GABA_A$ receptor complex at different sites and display different pharmacological profiles, it may be possible to gain insight into the

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† *Abbreviations:* GABA, γ-aminobutyric acid; [^{35}S]TBPS, t-[^{35}S]butylbicyclophosphorothionate.

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molecular events associated with the facilitatory effects of these drugs on GABAergic transmission in mediating anticonvulsant and anxiolytic vs hypnotic and anesthetic effects. We, thus, measured the effects of loreclezole, propofol, and diazepam on the binding of [^{3}H]muscimol, [^{3}H]flunitrazepam, and [^{35}S]TBPS to membranes from rat cerebral cortex. These three radioligands interact with different recognition sites on the $GABA_A$ receptor: in particular, flunitrazepam interacts with the benzodiazepine receptor, and its action is strongly dependent upon the α subunit isoform and requires the γ subunit to express its full pharmacological effect [8–10]. Finally, [^{35}S]TBPS binds to a site located in the GABA-gated chloride channel [11]. We studied the effects of loreclezole, propofol, and diazepam either alone or in combination with GABA or pentobarbital or alphaxalone, two general anesthetics that enhance $GABA_A$ receptor function and have been shown to directly activate the coupled Cl^- channel in the absence of GABA [12–14].

MATERIALS AND METHODS

Chemicals

Loreclezole was a gift of Janssen Research Foundation (Beerse, Belgium); propofol was kindly provided by Ici-Pharma (Milan, Italy); diazepam was obtained from FIS (Vicenza, Italy); and [^{3}H]muscimol (specific activity, 20.0 Ci/mmol), [^{3}H]flunitrazepam (74.1 Ci/mmol), and [^{35}S]TBPS (70 to 100 Ci/mmol) were from DuPont Biotechnology Systems (Milan, Italy). Other drugs and materials were obtained from commercial sources.

Animals

Male Sprague-Dawley CD rats (Charles River, Como, Italy) with body masses of 200 to 225 g were maintained under a 12-hr light, 12-hr dark cycle at a temperature of 23 ± 2°C and 65% humidity, with water and standard laboratory food *ad lib.* The animals were killed in the middle of the light period. The brains were rapidly removed and the cerebral cortex dissected out and used to prepare membranes for measurement of [^{3}H]muscimol binding, [^{3}H]flunitrazepam binding, and [^{35}S]TBPS binding.

[^{3}H]Muscimol Binding Assay

Fresh cerebral cortices were homogenized in 10 volumes of ice-cold water with a Polytron PT 10 (setting 5 for 20 sec) and the homogenate centrifuged for 10 min at 48,000 × *g* and 0°C. The pellet was washed once by resuspension and centrifugation in 10 vol. of 10 mM potassium phosphate (pH 7.4) containing 100 mM KCl. The membranes were stored at −20°C until use 1 to 15 days later On the day of the experiment, the membranes were thawed and centrifuged. The pellet was washed 3 additional times by resuspension and centrifugation in ice-cold buffer. The membranes were finally resuspended in 50 vol. of the same buffer and 350 μL of the resulting suspension (250 to 300 μg of protein) added to plastic minivials. Drugs were dissolved in DMSO and serial dilutions were prepared in the same solvent, 5 μL portions of which were added to the incubation mixture. Nonspecific binding was determined in the presence of 100 μM GABA. Incubations were started by the addition of 5 nM [^{3}H]muscimol, and terminated 30 min later by centrifugation at 48,000 × *g* for 10 min. The supernatant was discarded and the pellet gently washed twice with 4 mL of ice-cold distilled water and, then, resuspended in 3 mL of scintillation fluid (Atomlight; DuPont Biotechnology Systems, Boston, MA, U.S.A.). Membrane-associated radioactivity was determined with a scintillation counter (Packard).

[^{3}H]Flunitrazepam Binding Assay

Crude synaptic membranes were prepared as described by Prince and Simmonds [15] with minor modifications. Cerebral cortices were homogenized in 20 vol. of ice-cold wash buffer [5 mM Tris-HCl (pH 7.4), 1 mM EDTA] with a Teflon™ pestle and glass homogenizer, and the homogenate was centrifuged at 1000 × *g* for 10 min. The supernatant was carefully removed and centrifuged for 20 min at 48000 × *g*, and the resulting pellet washed (resuspended and centrifuged) 3 times in 20 vol. of ice-cold wash buffer, with the freeze-thaw step included between each centrifugation. The final pellet was resuspended in wash buffer and frozen at −20°C until required. On the day of the assay, the membranes were thawed and centrifuged for 20 min at 48,000 × *g*. The pellet was resuspended in 20 vol. of 1 mM Tris-citrate (pH 7.4), 200 μL portions of which (150 to 300 μg of protein) were preincubated for 10 min at 37°C in a total vol. of 1 mL containing various concentrations of drugs (dissolved in DMSO as described above) and 200 mM NaCl. Then, the binding reaction was initiated by the addition of 0.5 nM [^{3}H]flunitrazepam and was terminated after 60 min at 0°C by rapid filtration through a glass-fiber filter (GF/B; Whatman, Clifton, NJ, U.S.A.) in a filtration manifold (model M-24; Brandel, Gaithersburg, MD, U.S.A.). The filters were washed with 2 4-mL portions of ice-cold wash buffer and then dissolved in 3 mL of scintillation fluid (Atomlight). Filter-bound radioactivity was determined by liquid scintillation spectroscopy. Nonspecific binding was determined in the presence of 5 μM diazepam and represented ~10% of total binding.

[^{35}S]TBPS Binding

[^{35}S]TBPS binding was measured in two different membrane preparations from rat cerebral cortex: washed membranes devoid of endogenous GABA and unwashed membranes rich in endogenous GABA. The use of such preparations was suggested by the observation that the modulation of [^{35}S]TBPS binding by different ligands of the $GABA_A$ receptor complex is strictly dependent on the absence or presence of endogenous GABA [11, 16]. Thus,

washed and unwashed membranes represent two different biochemical experimental models.

Cerebral cortices were homogenized with a Polytron PT 10 (setting 5 for 20 sec) in 50 vol. of ice-cold 50 mM Tri-citrate buffer (pH 7.4 at 25°C) containing 100 mM NaCl. The homogenate was centrifuged at 20000 × *g* for 20 min and the pellet resuspended in 50 vol. of 50 mM Tris-citrate buffer to constitute the unwashed membrane preparation. For the experiments performed with well-washed membranes, the pellet from the first centrifugation was washed (suspended and centrifuged) 4 times in 50 vol. of 50 mM Tris-citrate buffer containing NaCl, and once in the same buffer without NaCl. The final pellet was suspended in an equal volume of 50 mM Tris-citrate buffer and frozen overnight. The frozen membranes were then thawed, centrifuged at 20000 × *g* for 20 min, and reconstituted in 50 vol. of 50 mM Tris-citrate buffer for the binding assay. [^{35}S]TBPS binding was determined in a final volume of 1 mL, consisting of 400 μL of membrane preparation (300 to 400 μg of protein), 100 μL of 2 nM [^{35}S]TBPS, 100 μL of 0.2 M NaCl, 5 μL of drug or solvent, and 395 μL of 50 mM Tris-citrate buffer. Reactions were initiated at 25°C by the addition of membranes and were terminated 90 min later by rapid filtration through glass-fiber filters. The filters were rinsed twice with 4 mL of ice-cold 50 mM Tris-citrate buffer in a filtration manifold. Radioactivity bound to the filter was quantitated by liquid scintillation spectroscopy. Nonspecific binding was defined as binding in the presence of 100 μM picrotoxin and represented ~10% of total binding.

Protein concentrations were determined by the method of Lowry *et al.* [17] with bovine serum albumin as standard.

Statistics

Biochemical data are expressed as means ± SEM and were analyzed by ANOVA followed by Scheffe's test. A *P* value of <0.05 was considered statistically significant.

RESULTS

[^{3}H]Muscimol Binding

Loreclezole enhanced specific [^{3}H]muscimol binding to washed rat cerebral cortical membranes in a concentration-dependent manner (Fig. 1); the effect was statistically significant at concentrations of ≥3 μM and maximal (172 ± 18%) at 100 μM. Consistent with previous studies [18], propofol also increased [^{3}H]muscimol binding in a concentration-dependent manner (maximal enhancement; propofol, 166.7 ± 5% at 100 μM). Thus, loreclezole was more potent than propofol with regard to enhancement of [^{3}H]muscimol binding. On the other hand, the intrinsic efficacy of loreclezole and propofol was similar.

The combination of loreclezole with a fixed concentration of propofol (1 or 10 μM) resulted in additive effects on [^{3}H]muscimol binding even at the highest concentration (100 μM) (Table 1). Similar results were obtained using

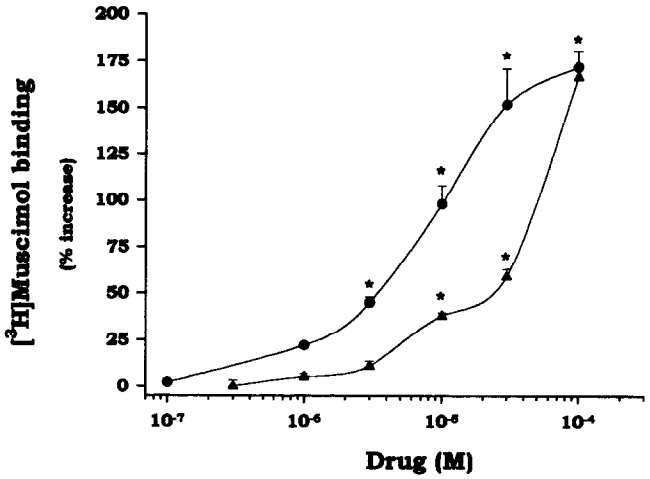

FIG. 1. Effects of loreclezole (●) and propofol (▲) on specific [^{3}H]muscimol binding to washed rat cerebral cortical membranes. Binding was measured in the presence of 5 nM [^{3}H]muscimol. Data are means ± SEM of 4 separate experiments and are expressed as percentage increase over control (solvent). *P < 0.01 vs control (ANOVA followed by Scheffe's test).

[^{3}H]GABA as radioligand (data not shown).

[^{3}H]Flunitrazepam binding

Loreclezole enhanced the specific binding of [^{3}H]flunitrazepam to washed membranes from rat cerebral cortex in a concentration-dependent manner (Fig. 2), with maximal enhancement (53 ± 1%) apparent at a concentration of 30 μM. As expected [15], propofol also increased [^{3}H]flunitrazepam binding; the effect was statistically significant (31 ± 1%) at 30 μM and maximal (45 ± 1%) at 100 μM. Thus, with regard to enhancement of [^{3}H]flunitrazepam binding, loreclezole was, again, more potent than propofol. [^{3}H]Flunitrazepame binding was increased by muscimol and

TABLE 1. Effect of propofol on the loreclezole-induced increase in specific [^{3}H]muscimol binding to washed rat cortical membranes

	[^{3}H]Muscimol binding (% increase)		
		Propofol (μM)	
Loreclezole (μM)	Control	1	10
0 (solvent)	100	109 ± 5.0	133 ± 4.2*
0.1	100 ± 0.9	112 ± 4.5	130 ± 5.6†
1	121 ± 1.6*	133 ± 3.2†	151 ± 5.9†
3	144 ± 5.8*	151 ± 2.0	172 ± 4.0†
10	200 ± 17*	209 ± 1.5	224 ± 3.6‡
100	293 ± 9.0*	299 ± 4.3	316 ± 5.0‡

Binding was measured in the presence of 5 nM [^{3}H]muscimol. Data are means ± SEM of 3 or 4 experiments and are expressed as percent increase over control (solvent).
* P < 0.01, vs control; †P < 0.01, ‡P < 0.05 vs loreclezole (ANOVA followed by Scheffe's test).

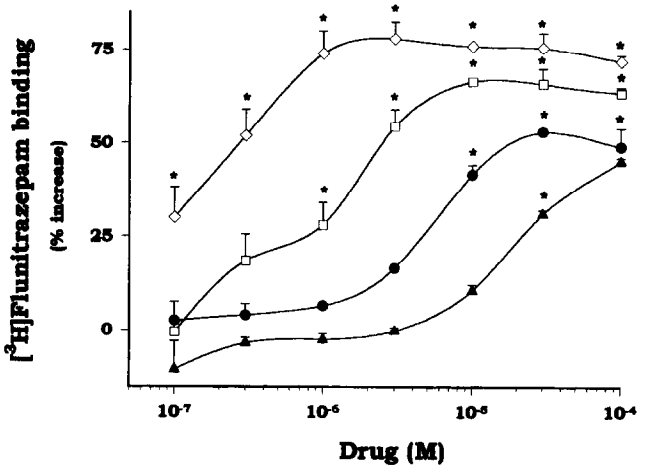

FIG. 2. Effects of loreclezole (●), propofol (▲), GABA (□), and muscimol (◇) on specific [^{3}H]flunitrazepam (0.5 nM) binding to washed rat cerebral cortical membranes. Data are means ± SEM of 4 separate experiments and are expressed as percentage increase over control (solvent). *$P < 0.05$ vs control (ANOVA followed by Scheffe's test).

GABA with potencies and efficacies greater than those of either loreclezole or propofol.

[^{35}S]TBPS Binding

Consistent with previous data [5, 19], loreclezole reduced specific [^{35}S]TBPS binding to unwashed membrane preparations from rat cerebral cortex in a concentration-dependent manner (Fig. 3). The maximal degree of inhibition (98% at 30 μM) was similar to that induced by propofol (95%) and greater than that elicited by diazepam (63%) at the same concentration. The inhibitory effect of loreclezole on [^{35}S]TBPS binding was abolished by bicuculline (10 μM), a specific $GABA_A$ receptor antagonist (data not shown).

To clarify further the molecular basis of the effect of loreclezole on $GABA_A$ receptor function, we compared the effect of this compound with those of propofol and diazepam on [^{35}S]TBPS binding to washed cortical membrane preparations devoid of GABA.

Loreclezole modulated [^{35}S]TBPS binding in a manner that resembled that of propofol, rather than that of diazepam (Fig. 4). Both loreclezole and propofol had a bidirectional effect: at low concentrations (5 to 10 μM), both drugs enhanced the specific binding of [^{35}S]TBPS whereas, at higher concentrations (30 to 100 μM), they elicited an inhibitory effect. The efficacy of propofol with regard to both the increase and decrease in [^{35}S]TBPS binding was greater than that of loreclezole, As expected [20], diazepam increased [^{35}S]TBPS binding less than did propofol, but to a similar extent as that observed with loreclezole. The maximal effect of diazepam (33 ± 5%) was obtained at 0.5 μM; the potency of diazepam was, thus, greater than that of either loreclezole or propofol. Higher concentrations of diazepam failed to increase [^{35}S]TBPS binding further and did not have an inhibitory effect.

We, next, compared the effects on [^{35}S]TBPS binding of loreclezole, propofol, and diazepam added in combination with other drugs (GABA, pentobarbital, and alphaxalone) that enhance the function of the $GABA_A$ receptor. GABA (0.3 μM), which, when added alone, slightly increased [^{35}S]TBPS binding, not only abolished the increase in binding induced by low concentrations (5 to 10 μM) of loreclezole, but actually reversed this effect (20% increase with 10 μM loreclezole alone, but a 34% decrease with added 0.3

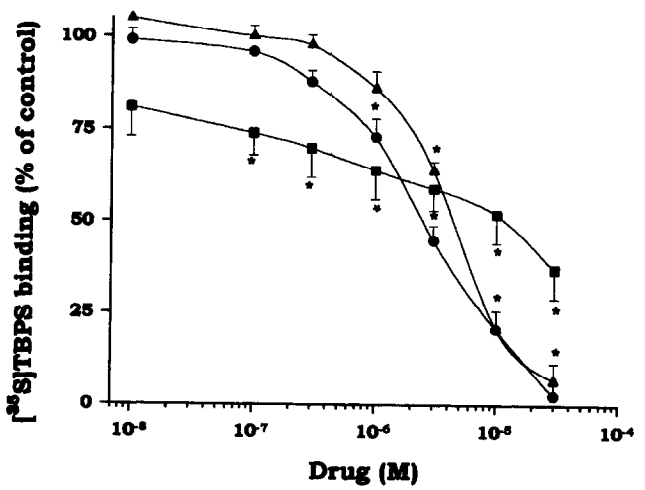

FIG. 3. Effects of loreclezole (●), propofol (▲), and diazepam (■) on specific [^{35}S]TBPS (2 nM) binding to unwashed rat cortical membrane preparations. Data are means ± SEM of 4 separate experiments and are expressed as a percentage of control (solvent). *$P < 0.01$ vs control (ANOVA followed by Scheffe's test).

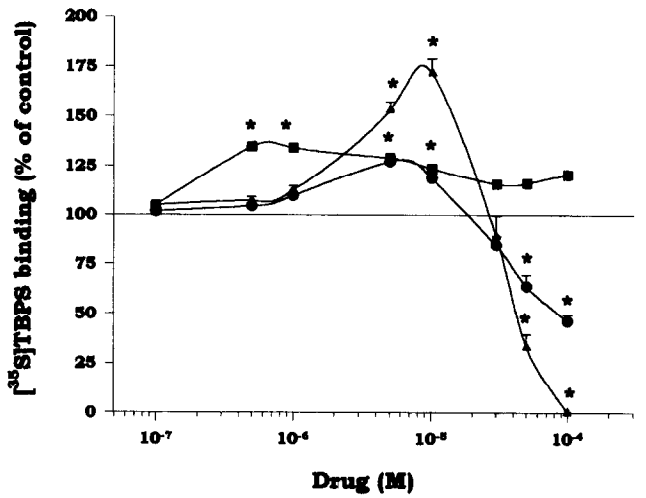

FIG. 4. Effects of loreclezole (●), propofol (▲), and diazepam (■) on specific [^{35}S]TBPS (2 nM) binding to washed membranes from rat cerebral cortex. Data are means ± SEM of 4 separate experiments and are expressed as a percentage of control (solvent). *$P < 0.01$ vs control (ANOVA followed by Scheffe's test).

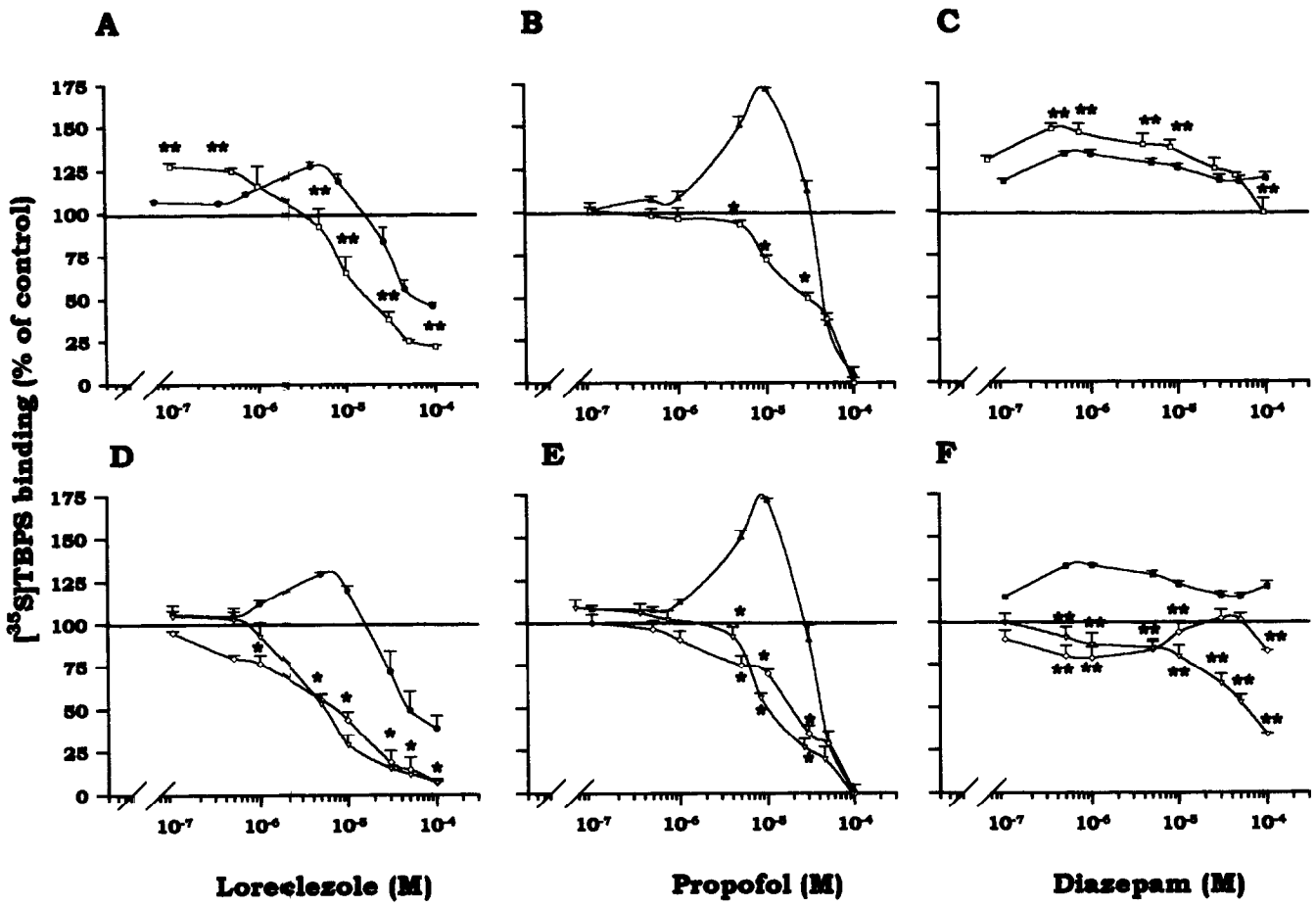

FIG. 5. Effects of 0.3 μM GABA (□) (A to C) and 200 μM pentobarbital (◇) and 3 μM alphaxalone (▽) (D to F) on specific [^{35}S]TBPS (2 nM) binding to washed rat cortical membranes in the presence of various concentrations of loreclezole (●), propofol (▲), or diazepam (■). Data are means ± SEM from 4 separate experiments and are expressed as a percentage of control (solvent). *$P < 0.05$, **$P < 0.01$ vs loreclezole, propofol, or diazepam alone (ANOVA followed by Scheffe's test).

μM GABA) (Fig. 5A). GABA also significantly enhanced the inhibitory effect exerted by higher (30 to 100 μM) concentrations of loreclezole. Consistent with previous data [20–22], GABA abolished and reversed the propofol-induced increase in [^{35}S]TBPS binding (Fig. 5B). In contrast to its effects in combination with loreclezole or propofol, GABA (0.3 μM) did not affect the increase in [^{35}S]TBPS binding induced by diazepam (Fig. 5C).

Like GABA, both pentobarbital (200 μM) and alphaxalone (3 μM), which alone failed to modify [^{35}S]TBPS binding, abolished and reversed the increase in binding induced by loreclezole (Fig. 5D) or propofol (Fig. 5E). Thus, 30 ± 1 and 74 ± 8% maximal increases in binding in the presence of 5 μM loreclezole or 10 μM propofol alone became 44 ± 7 and 30 ± 5% decreases with the addition of pentobarbital and 45 ± 5 and 47 ± 7% decreases in the presence of alphaxalone, respectively. Thus, pentobarbital and alphaxalone reversed the increase in [^{35}S]TBPS binding elicited by propofol (−102% and −116%, respectively) more than that induced by loreclezole (−74% and −76%, respectively), an effect shared by GABA (−98% and −35% for propofol and loreclezole, respectively). Finally, pentobarbital abolished and alphaxalone markedly reversed the effect of diazepam (10 to 100 μM) on [^{35}S]TBPS binding. However, the effect of pentobarbital was markedly less in combination with diazepam (−44% and −38% in combination with 1 or 100 μM diazepam, respectively) than in combination with loreclezole or propofol. In contrast, alphaxalone elicited a much greater effect (−87%) in the presence of 100 μM diazepam, similar to that elicited in the presence of 5 μM loreclezole but less than that when added in combination with 10 μM propofol.

DISCUSSION

The $GABA_A$ receptor complex is thought to be the major site of action of various anxiolytic, sedative-hypnotic, anticonvulsant, and general anesthetic drugs [23, 24]. Although some of these drugs are known to interact with specific recognition sites on the $GABA_A$ receptor complex, the molecular mechanisms that elicit anxiolytic rather than anticonvulsant, hypnotic, or general anesthetic effects are unclear. In this work, we have characterized the biochemical effects of the anticonvulsant loreclezole and the general

anesthetic propofol at the level of the $GABA_A$ receptor and compared them to the effects of diazepam.

We have shown that loreclezole modulates [^{3}H]muscimol to washed rat cerebral cortical membranes with higher potencies than propofol, but with similar efficacy. Moreover, loreclezole and propofol inhibit [^{35}S]TBPS binding in unwashed membranes from rat cerebral cortex with greater potencies and efficacies than diazepam. The inhibitory effect of loreclezole on [^{35}S]TBPS binding was consistent with the ability of this drug to enhance [^{3}H]muscimol and [^{3}H]flunitrazepam binding. In fact, it is well established that positive allosteric modulators (barbiturates, anesthetics such as propofol, and benzodiazepine) of GABAergic transmission will produce an enhancement of [^{3}H]muscimol binding and a parallel inhibition of [^{35}S]TBPS binding measured in unwashed membrane (i.e. rich in endogenous GABA). On the other hand, negative modulators of GABAergic transmission will induce opposite changes in these two parameters [16, 18].

Moreover, our observation that the combination of loreclezole with propofol resulted in an additive effect on [^{3}H]muscimol binding may suggest that loreclezole interacts with a different site on the $GABA_A$ receptor. This hypothesis is supported by the observation that the modulatory action of loreclezole and propofol, as measured in recombinant $GABA_A$ receptors expressed in oocytes, was additive.* In addition, similar results were obtained by Wafford *et al.* [5] when testing the interaction of loreclezole with compounds acting at the barbiturate and steroid sites on the $GABA_A$ receptor.

Whereas both loreclezole and propofol showed a biphasic effect (stimulation at lower concentrations and inhibition at higher concentrations) on [^{35}S]TBPS binding to washed membranes, diazepam showed only a stimulatory effect on this parameter and failed to cause a decrease even at high concentrations. The different profile displayed by diazepam is probably attributable to the lack of ability of this drug to activate the $GABA_A$ receptor-operated Cl^- channel in the absence of GABA, an effect elicited by both propofol and loreclezole [6, 25, 26]. Consistent with this conclusion, diazepam inhibited [^{35}S]TBPS binding in a concentration-dependent manner in the presence of endogenous GABA (unwashed membranes).

The enhancement of [^{35}S]TBPS binding by low concentrations of loreclezole was observed only in the absence of GABA; in the presence of endogenous GABA (unwashed membranes), the same concentrations of loreclezole inhibited [^{35}S]TBPS binding, an effect shared by propofol. Together with recent observations of Wafford *et al.* [5], these data suggest that, at low concentrations, loreclezole (like diazepam) does not open the $GABA_A$ receptor-coupled Cl^- channel in the absence of GABA. However, the observation that higher concentrations (30 to 100 μM) of loreclezole inhibit [^{35}S]TBPS binding in washed membranes suggests that, at these concentrations, loreclezole (unlike diazepam but similar to propofol) may directly activate the receptor-coupled Cl^- channel. Indeed, at high concentrations (50 to 100 μM), loreclezole has been shown to directly activate human recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes as assessed by voltage-clamp recordings [26]. Thus, in washed membranes (devoid of GABA), high concentrations (30 to 100 μM) of loreclezole inhibited [^{35}S]TBPS binding, just as lower concentrations (1 to 10 μM) did in the presence of endogenous GABA (unwashed membranes).

The differential effects on [^{35}S]TBPS binding to washed membranes elicited by loreclezole and propofol on the one hand, and diazepam on the other, may also be related to the different localization of the sites of action of these drugs. The higher efficacies of loreclezole and propofol with regard to modulation of [^{3}H]muscimol, [^{3}H]flunitrazepam, and [^{35}S]TBPS binding may reflect the abilities of these drugs to directly enhance the function of the GABA-coupled chloride channel, an effect not shared by diazepam. This conclusion is consistent with the pharmacological profiles and efficacies of loreclezole and propofol. In fact, loreclezole has a broad spectrum of anticonvulsant activity and both increases the threshold for seizures and, like phenobarbital, prevents seizure spread, whereas propofol, like pentobarbital, induces general anesthesia and directly activates the $GABA_A$ receptor-coupled Cl^- channel at identical concentrations. Together, these biochemical data are consistent with the pharmacological results of Ashton *et al.* [3], which suggested that loreclezole behaves more like a barbiturate than a benzodiazepine. This pharmacological characterization is further supported by the characterization of the effects on [^{35}S]TBPS binding to washed membranes of loreclezole, propofol, and diazepam in combination with either GABA, pentobarbital, or alphaxalone. Accordingly, GABA, pentobarbital, and alphaxalone, at concentrations that *per se* failed to significantly affect [^{35}S]TBPS binding, not only abolished but reversed the stimulatory effects of low concentrations of loreclezole or propofol and markedly potentiated the decrease in [^{35}S]TBPS binding elicited by higher concentrations of these drugs. In contrast, the same concentration of GABA failed to modify the diazepam-induced increase in [^{35}S]TBPS binding, whereas alphaxalone and pentobarbital abolished the effect of the lowest concentrations (1 to 10 μM) of this drug, and alphaxalone reversed the effect of only the highest concentrations (30 to 100 μM) of diazepam.

Propofol displayed a greater efficacy than loreclezole with regard to the biphasic effect on [^{35}S]TBPS binding to well-washed membranes and, in addition, the combination of propofol with pentobarbital or alphaxalone induced a greater decrease in [^{35}S]TBPS binding than that elicited by the corresponding drug combinations with loreclezole. The extents of these biochemical changes are consistent with the different efficacies of propofol and loreclezole alone with regard to enhancement of $GABA_A$ receptor Cl^- chan-

* Sanna *et al.*, unpublished observation

nel conductance as assessed by voltage-clamp recordings [4, 5, 6, 26]. These biochemical and electrophysiological data are, thus, consistent with the anticonvulsant and general anesthetic actions of loreclezole and propofol, respectively. Moreover, the data overall are also consistent with distinct binding sites for alphaxalone, pentobarbital, propofol, and loreclezole, although these drugs have more similarities than differences. However, it was not possible to differentiate loreclezole and propofol on the basis of their effects on [^{3}H]muscimol, [^{3}H]flunitrazepam, or [^{35}S]TBPS binding in different membrane preparations.

In conclusion, our biochemical studies suggest that the different pharmacological profiles displayed by the two drugs, whose molecular actions are dependent upon the β subunit of the $GABA_A$ receptor complex, are consistent with their different intrinsic efficacies with regard to their ability to activate the receptor-coupled Cl^- channel in the absence of GABA. Thus, the general anesthetic action of propofol is correlated with both its higher potency and greater efficacy in enhancing the opening of the $GABA_A$ receptor Cl^- channel directly and indirectly (in the absence or presence of GABA, respectively). In contrast, the broad-spectrum anticonvulsant action of loreclezole is associated with the ability of this drug both to markedly potentiate the effect of GABA and to exert a weak effect on the receptor-associated Cl^- channel in the absence of GABA. Accordingly, to elicit an anticonvulsant action, it is sufficient to enhance with low efficacy the interaction of GABA with its recognition site without inducing a direct opening of the Cl^- channel, a profile typical of partial agonists of benzodiazepine receptors [27–29]. Finally, the mechanism responsible for the lack of sedative and hypnotic actions of loreclezole remains to be established. Given that this drug, unlike propofol and diazepam, does not interact with $GABA_A$ receptors that contain the β_1 subunit [4, 5, 26], it is possible that this subunit is important for the sedative, hypnotic, and general anesthetic effects of diazepam, propofol, and other allosteric modulators of $GABA_A$ receptors.

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