Pharmacology Biochemistry & Behavior, Vol. 29, pp. 781–783. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Specificity Within the $GABA_A$ Receptor Supramolecular Complex: A Consideration of the New ω_1-Receptor Selective Imidazopyridine Hypnotic Zolpidem

K. G. LLOYD AND B. ZIVKOVIC

Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 92220 Bagneux, France

LLOYD, K. G. AND B. ZIVKOVIC. *Specificity within the $GABA_A$ receptor supramolecular complex: A consideration of the new ω_1-receptor selective imidazopyridine hypnotic zolpidem.* PHARMACOL BIOCHEM BEHAV **29**(4) 781–783, 1988.—The relative contribution of different recognition sites within the $GABA_A$ receptor supramolecular complex (GRSC) to the pharmacological effects of anxiolytic and hypnotic drugs is unknown. The development of the ω_1 (ex BZ_1) specific hypnotic zolpidem allows a more direct approach to the problem. In contrast to many benzodiazepine hypnotic/anxiolytics (e.g., flunitrazepam, diazepam), zolpidem shows a specificity for GABAergic function, e.g., selectively reversing isoniazide-induced seizures. Furthermore, zolpidem produces a highly specific hypnotic action as compared to myorelaxant or amnesic effects (ratio of ED_{50}'s >4.0 for zolpidem; <1 for flunitrazepam). Zolpidem exerts its action within the GRSC as it enhances ^{35}S-TBPS binding, as do mixed ω_1/ω_2 compounds or GABA agonists. Both the *in vivo* and *in vitro* actions of zolpidem are reversed by flumazenil and the enhanced ^{35}S-TBPS binding is also bicuculline-sensitive. Thus, ω_1 recognition site stimulation (e.g., by zolpidem) is sufficient to produce potent pharmacological effects and modulation of the $GABA_A$ receptor-gated chloride ionophore.

Zolpidem Hypnotic ω_1 Recognition site $GABA_A$ Receptor supramolecular complex ^{35}S-TBPS binding Benzodiazepine recognition sites Flunitrazepam Anticonvulsant Memory deficits Myorelaxation Ataxia

IT is generally accepted that many clinically useful hypnotics and anxiolytics exert their activity by enhancing the function of the $GABA_A$ receptor-mediated chloride ionophore. The benzodiazepine hypnotics and anxiolytics (e.g., diazepam, flunitrazepam, midazolam) act at recognition sites within the $GABA_A$ receptor-chloride ionophore supramolecular complex (GRSC), enhancing the efficiency of GABA in opening the chloride ionophore. Other classes (e.g., imidazopyridines, pyrazoloquinolines) of anxiolytic and hypnotic compounds also act at these specific recognition sites. However, hypnotic barbiturates act at a physically distinct recognition site within the GRSC which appears to be more closely linked to the chloride ionophore than to the GABA receptor [2].

The specific recognition sites for benzodiazepines, pyrazoloquinolines and imidazopyridines within the GRSC exist as subclasses originally termed as BZ_1 and BZ_2 sites, and are now designated as ω_1 and ω_2 recognition sites in recognition of the diverse classes active at these sites [5]. Although the distribution and pharmacological profiles of these sites differ, a major unresolved question is the relative importance of each subset for different actions of compounds active at these sites (e.g., hypnotic, anxiolytic, myorelaxant, anticonvulsant). Furthermore, it would be most useful to know how a binding profile could be used to predict relative clinical activities.

The development of the hypnotic zolpidem, a selective compound for ω_1 receptors [1, 3, 5], has made it possible to approach these questions more directly. Furthermore, using this compound the relationship of ω_1 recognition sites to GABA receptor function in the GRSC can be studied, both *in vivo* and *in vitro*.

METHOD

Animals

For *in vivo* experiments male albino, Swiss mice of 18–20 g body weight were used (CD, Charles River, France). The mice were fasted overnight prior to the experiment.

Anticonvulsant Activity

Convulsive challenges were administered 30 min after drug pretreatment with the exception of isoniazid which was administered concomitantly. In the pentylenetetrazole- and

TABLE 1
RELATIVE ANTICONVULSANT ACTIVITIES OF ZOLPIDEM AND BENZODIAZEPINES IN THE MOUSE

	Relative Anticonvulsant Activity (ED_{50}; INH=100)			
	Isoniazid	Pentetrazole	Electroshock	Strychnine
Zolpidem	100 (1.1 mg/kg)	819	827	909
Diazepam	100 (1 mg/kg)	30	200	60
Flunitrazepam	100 (0.1 mg/kg)	40	140	—

Absolute activity (ED_{50}) in the isoniazid test is in parentheses.

electroshock-induced convulsions the presence of tonic extensions of hind legs were the end points measured [3]. In the isoniazid and strychnine models the onset of convulsions and protection against death, respectively, were measured [7].

Undesirable Effects

The effect of drugs on muscle strength and ataxia were evaluated as described by Depoortere *et al.* [3], whereas the memory impairment effects were assessed by the method reported by Sanger *et al.* [6].

^{35}S-TBPS Binding

^{35}S-t-Butylbicyclophosphorothionate (TPBS) binding was performed on extensively washed and refrozen membranes from rat cerebral cortex as described by Honoré and Drejer [4]. TBPS (90 Ci/mmol, NEN) at a concentration of 1 nM (or 0.05–100 nM for Scatchard plots) was incubated together with 500 μl membranes (0.45 mg protein) in Tris citrate buffer (50 mM, pH 7.1 containing 1 M NaCl), the study compound or its solvent. Under these conditions 99% association is obtained within 60 min.

After a 90 min incubation period at 25°C, the mixture was diluted with 10 ml ice-cold buffer then filtered rapidly on Whatman GF/C glass filters, and the radioactivity on the filter determined. Non-specific binding is that not displaced by 10 μM picrotoxinin, and was normally <10%.

RESULTS

Zolpidem antagonizes the convulsions induced by inhibition of GABA synthesis by isoniazid, in a manner similar to that of other compounds active within the GRSC (e.g., ED_{50}, mg/kg, IP: zolpidem=1.1; diazepam=1.0; midazolam=1.0; flunitrazepam=0.1). However, the maximum effect (increased latency to onset of seizures) was much greater with zolpidem (+280%) than for other compounds acting at the hypnotic/anxiolytic drug recognition site (range of 185 to 210% increase for midazolam, triazolam, flunitrazepam, diazepam and clonazepam).

Another evident difference within the spectrum of the anticonvulsant activities of these compounds is the high degree of specificity of zolpidem (BZ_1) for GABA-deficit-induced convulsions (by isoniazid) as compared to flunitrazepam or diazepam (non-selective). As shown in Table 1, zolpidem is 8–9 times more active in the isoniazid test than against convulsions produced by pentetrazole, electroshock or strychnine. In contrast, the anti-isoniazid activity of diazepam and flunitrazepam is situated well within the range of their other anticonvulsant activities.

Similarly, zolpidem (ω_1) exerts a much greater specificity for its therapeutic action (hypnotic) than exists for the non-selective compounds diazepam and flunitrazepam. The ratio of the ED_{50}'s for ataxia (rotarod + muscle strength tests) vs. hypnotic or anxiolytic action, for zolpidem is 4.54, whereas for flunitrazepam this ratio is 0.8 and for diazepam it is 3.3. In terms of induction of memory impairment, the ratios are zolpidem=4.0; flunitrazepam=0.25; diazepam=0.83. This results in the following rank ordering of activities:

Zolpidem: *Hypnosis* > Anxiolytic > Anticonvulsant >> Ataxia; Flunitrazepam: Ataxia ≥ Anticonvulsant > *Hypnosis* = Anxiolytic; Diazepam: Anticonvulsant = *Anxiolytic* = Ataxia > Sedation.

These spectra of activity and the specificities of action are so different that one must ask if this indicates that novel ω_1 specific compounds (e.g., zolpidem) have a differential action within the GRSC as compared to classical benzodiazepines (e.g., diazepam, flunitrazepam) which do not differentiate very well between ω_1 and ω_2 receptors.

The binding of TBPS to the GRSC chloride ionophore of rat cerebral cortex membranes has been used to try to answer this question. Under the present experimental conditions (well-washed membranes in the presence of NaCl) most agonists at the hypnotic/anxiolytic sites of the GRSC induce an initial enhancement of TBPS binding at concentrations similar to their activity at either ^{3}H-diazepam or ^{3}H-$GABA_A$ recognition sites (e.g., flunitrazepam, clonazepam, alprazolam, oxazolam, GABA, THIP, SL 75.102). At high concentrations, TBPS is displaced from its bindings site.

In contrast, zolpidem only enhances TBPS binding, even at extremely high concentrations (5×10^{-4} M). As shown in Table 2, the EC_{50} for the enhancing effect of zolpidem and flunitrazepam are both very similar to their IC_{50}'s for displacement of ^{3}H-diazepam binding (60 nM for zolpidem, 4 nM for flunitrazepam) [1,3]. This enhancement of TBPS binding is due to activation of the specific hypnotic/anxiolytic site within the GRSC as it is competitively inhibited by the benzodiazepine antagonist flumazenil. Furthermore, this activity is mediated at least partially by the $GABA_A$ receptor as bicuculline significantly reduces the maximum response

TABLE 2

EFFECT OF FLUMAZENIL AND BICUCULLINE ON THE ACTION OF ZOLPIDEM AND FLUNITRAZEPAM ON ^{35}S-TBPS BINDING TO RAT CEREBRAL CORTEX MEMBRANES

		Enhancement of TBPS Binding		
Compound	Parameter	Compound Alone	Plus Flumazenil (10^{-5} M)	Plus Bicuculline (10^{-3} M)
Zolpidem	MEC (nM)	50	50000	1000
	EC_{50} (nM)	84	176000	400
	Max Eff (%)	36	20*	23*
Flunitrazepam	MEC (nM)	5	10000	400
	EC_{50} (nM)	8	20000	30
	Max Eff (%)	41	20*	28*

*$p<0.01$ vs. compound alone.
MEC=minimal effective concentration.
EC_{50}=concentration at half-maximal effect.

and displaces the dose-response curve to the right (although less effectively than with flumazenil).

This increase in TBPS binding induced by zolpidem and flunitrazepam is due to an enhanced affinity; however at higher concentrations flunitrazepam also decreases the number of binding sites. The enhanced binding is due to an increase of "specific" binding to the ionophore site as it is completely blocked by picrotoxinin (data not shown).

DISCUSSION

The present results clearly demonstrate that zolpidem, which is selective for ω_1 recognition sites, has an intrinsic activity at the hypnotic/anxiolytic recognition site within the GRSC, resulting in an allosteric activation of chloride channel function (as indicated by TBPS binding) at least partially mediated via $GABA_A$ receptors.

Zolpidem exhibits a more "specific" activity both *in vitro* and *in vivo* than do classical agonists (e.g., benzodiazepines) of the hypnotic/anxiolytic recognition site. First of all, zolpidem is highly specific for ω_1 sites [1, 3, 5]; secondly, it produces only an enhancement of TBPS binding; thirdly, zolpidem is highly selective for hypnotic activity as compared to other activities related to the GRSC; fourthly, within the spectrum of its anticonvulsant activities zolpidem exhibits a high degree of selectivity for GABA-deficit-induced seizures.

The critical question is whether or not the atypical advantageous profile of zolpidem is related to its specificity at the ω_1 site. The weak activity of zolpidem in tests designed to show ataxic and myorelaxant potential is likely due to its negligible activity at ω_2 receptors. Zolpidem does not displace ^{3}H-flunitrazepam (ω_2) binding from spinal cord membranes and exhibits a different neuropharmacological profile on spinal reflexes than do benzodiazepines (Langer, this Symposium). Thus, the spinal mechanisms of ataxia and muscle weakness due to ω_2 site activation are much less relevant to the actions of zolpidem than for benzodiazepines.

The monophasic effect of zolpidem on TBPS binding to rat cerebral cortex membranes is less likely related to its ω_1-specificity. Thus, zopiclone (a cyclopyrrolone hypnotic active at ω_1 and ω_2 sites) like zolpidem only enhances TBPS binding and alpidem, a ω_1-specific anxiolytic imidazopyridine [5], both produce a potent biphasic effect on TBPS binding.

With regards to the specificity of hypnotic action of zolpidem and its apparently high degree of intrinsic activity for GABA-mediated events, it is still premature to speculate as to whether these actions are due to the selectivity at ω_1 receptors or rather to other attributes of the imidazopyridine structure.

REFERENCES

1. Arbilla, S., H. Depoortere, P. George and S. Z. Langer. Pharmacological profile of zolpidem at benzodiazepine receptors and electrocorticogram in rats. *Naunyn Schmiedebergs Arch Pharmacol* **330**: 248–251, 1985.
2. Bowery, N. G. *Actions and Interactions of GABA and Benzodiazepines*. New York: Raven Press, 1984.
3. Depoortere, H., B. Zivkovic, K. G. Lloyd, D. J. Sanger, G. Perrault, S. Z. Langer and G. Bartholini. Zolpidem, a novel non-benzodiazepine hypnotic. I. Neuropharmacological and behavioral effects. *J Pharmacol Exp Ther* **237**: 649–658, 1986.
4. Honoré, T. and J. Drejer. Phenobarbitone enhances [^{35}S] TBPS binding to extensively washed rat cortical membranes. *J Pharm Pharmacol* **37**: 928–929, 1985.
5. Langer, S. Z. and S. Arbilla. Complexities of central and peripheral benzodiazepines receptors: A proposal for a new receptor nomenclature. 5th International Congress of Sleep Research, Copenhagen, 1987.
6. Sanger, D. J., D. Joy and B. Zivkovic. Effects of zolpidem, a new imidazopyridine hypnotic, on the acquisition of conditioned fear in mice. Comparison with triazolam and CL 218,872. *Psychopharmacology (Berlin)* **90**: 207–210, 1986.
7. Worms, P., H. Depoortere, A. Durand, P. L. Morselli, K. G. Lloyd and G. Bartholini. Gamma-aminobutyric acid (GABA) receptor stimulation. I. Neuropharmacological profiles of progabide (SL 76.002) and SL 75.102, with emphasis on their anticonvulsant spectra. *J Pharmacol Exp Ther* **220**: 660–671, 1982.