Pharmacology Biochemistry & Behavior, Vol. 29, pp. 785–790. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Modifications of Brain Electrical Activity After Activation of the Benzodiazepine Receptor Types in Rats and Rabbits

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LONGO, V. G., M. MASSOTTI, D. DEMEDICI AND A. VALERIO. *Modifications of brain electrical activity after activation of the benzodiazepine receptor types in rats and rabbits.* PHARMACOL BIOCHEM BEHAV **29**(4) 785–790, 1988.—The present study reports a comparative electroencephalographic (EEG) study of drugs belonging to different chemical classes which share the property to bind at benzodiazepine (BDZ) recognition sites. The EEG patterns are recorded from the neocortex of rats and rabbits as well as from dorsal hippocampus and red nucleus in rabbits after intravenous administration of diazepam (0.1–10 mg/kg), clonazepam (0.02–2.5 mg/kg), zopiclone (0.3–3 mg/kg), flunitrazepam (0.03–2.5 mg/kg), CGS 9896 (0.1–3 mg/kg), zolpidem (0.1–3 mg/kg) and Cl 218,872 (0.1–10 mg/kg). The most relevant differences are observed at the level of the neocortex. All drugs induced appearance of 7–12 Hz spindle bursts. On the contrary, the presence of 15–30 Hz waves (defined β-like activity) mainly occurs after diazepam, clonazepam and zopiclone. Scarce β-like activity is present after CGS 9896, zolpidem and Cl 218,872. According to the selectivity of these drugs for the various types of BDZ receptor, one can speculate that activation of BDZ_2 is relevant for the appearance of the β-like activity. Flunitrazepam, diazepam, and zolpidem increase the amplitude of the red nucleus waves. Such an effect is less marked after zopiclone and CGS 9896, whereas is almost absent after clonazepam and Cl 218,872. A reduction of the frequency is observed after flunitrazepam, diazepam, clonazepam, CGS 9896 and zolpidem, whereas it is almost absent after zopiclone and Cl 218,872. Finally, all drugs induce a reduction of the amplitude of the hippocampal theta rhythms, whereas after diazepam, flunitrazepam, zolpidem and CGS 9896 a slowing of the record also occurs.

EEG Rats Rabbits Cerebral cortex Hippocampus Red nucleus Diazepam
Flunitrazepam Clonazepam Zolpidem Zopiclone CGS 9896 Cl 218,872

THE major selectivity and safety of benzodiazepines (BDZ) as anxiolytics and hypnotics over barbiturates has suggested that these drugs affect specific biochemical and physiological functions involved in the control of mood and vigilance. Through electroencephalographic (EEG) studies it has been possible to conclude that the central depressant effects of barbiturates is triggered by an inhibition of all brain structures that maintain vigilance, whereas BDZ influence the proprioceptive function [21,24]. Biochemical studies have clearly established that BDZ receptor activation in the central nervous system (CNS) leads to an enhancement of γ-aminobutyric (GABA) receptor function [10]. In spite of these neurochemical findings, their correlation with the behavioral outcomes are still largely incomplete.

The discovery of two types of receptors in neurons—termed BDZ_1 and BDZ_2 [25]—has suggested that each might subserve distinct pharmacological effects of the BDZ agonists (*namely*, muscle relaxant, anticonvulsant, sedative and anticonflict effects). The presence of a third type, also located in peripheral tissues and defined peripheral-type (BDZ_p), has been described after the first report on the existence of receptors for BDZ [3,4]. The functional relevance of each receptor type still remains unknown and the possibility exists that their distribution within CNS is solely relevant in the distinct action of the BDZ agonists. Therefore, it appears interesting to compare the EEG profile of ligands with different selectivity for the distinct types of BDZ receptors.

Among these drugs, large interest has been devoted to the triazolopyridazine derivative Cl 218,872, first compound described as a selective ligand for BDZ_1 receptor type [15]. A similar selectivity has been reported for the imidazopyridine derivative zolpidem [17] and, with a lesser degree, the pyrazoloquinoline derivative CGS 9896 [2]. Among the most known BDZ derivatives, it has been established that diazepam and flunitrazepam are full agonists [10] which bind indifferently at the three types of receptors [25], whereas

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TABLE 1

OCCURRENCE OF CHANGES IN THE ELECTROCORTICAL ACTIVITY AFTER AGONISTS OF BDZ RECEPTORS IN RATS AND RABBITS

	Rabbits			Rats		
	N	Spindles	β-Like	N	Spindles	β-Like
Diazepam	12	+++	+++	10	+++	+++
Clonazepam	16	+++	+++	10	+++	+++
Zopiclone	4	+++	+++			
Flunitrazepam	11	+++	++	10	+++	++
CGS 9896	6	+++	+	6	+++	+
Zolpidem	7	+++	+			
Cl 218,872	16	++	+	12	++	+

The symbols indicate the percent incidence of spindles during the synchronization and of β-like activity during desynchronization.
+++=more than 60%; ++=20–60%; +=less than 20%.
N=number of animals.

clonazepam selectively binds to BDZ_1 and BDZ_2 receptors only [7,25]. Finally, the cyclopyrrolone derivative, zopiclone, shows an atypical profile. Although it binds at BDZ_1 and BDZ_2 receptor types, without any binding capacity at BDZ_p, the atypical modulation by GABA of its binding would suggest a specific recognition site different from those of BDZ (see for details [12]).

In this study an attempt has been made to classify these drugs on the basis of changes of the electrical activity in the cortex of rats and rabbits, as well as in the hippocampus and red nucleus of rabbits.

METHOD

Male mongrel rabbits weighing 2.0–2.5 kg were used. Surgical procedures were performed under local anesthesia (2% xylocaine). Six screw electrodes were implanted over the skull at the level of sensorimotor (anterior and posterior) and optic cortices of both hemispheres. Deep concentric electrodes were also implanted unilaterally at the level of the dorsal hippocampus and of the red nucleus according to the method described by Longo [16]. In some animals bipolar electrodes were positioned over the neck to allow the recording of the electromyogram (EMG). A pediatric infusion set was inserted into the lateral vein of the ear to allow the injection of the drugs. Two-three hours after the end of the surgery, the rabbits were tested for EEG studies. During the experimental session, the animals were partially restrained according to the method described by Longo [16], and wound edges were infiltrated periodically with xylocaine.

Male Sprague-Dawley rats weighing 200–250 g were used. Surgical procedures were performed under Na pentobarbital (40 mg/kg IV) anesthesia. A chronic indwelling cannula was inserted into the external jugular vein to allow intravenous injection of the drugs. Then 4 stainless steel electrodes were implanted over the skull at the level of sensorimotor and optic cortices of both hemispheres. Twenty-four hours later the animals were tested for EEG studies.

The EEG was recorded continuously, beginning 1 hr before the drug injection, until it returned to the predrug pattern. Each animal received only one treatment with single dose.

Diazepam, flunitrazepam, clonazepam, zolpidem, CGS 9896 and zopiclone were dissolved with two drops of 12 N HCl. The volume was then adjusted with saline. Cl 218,872 was dissolved in polyethylene glycol 300. Preliminary experiments showed that this solvent does not influence brain electrical activity up to 2 ml/kg IV. All drugs were injected by slow (1 ml/kg) intravenous route.

RESULTS

Effects of BDZ Agonists on the Electrocortical Activity in Rats and Rabbits

Rabbits. As shown in Table 1, the IV administration of diazepam (0.1–10 mg/kg), flunitrazepam (0.03–3 mg/kg), clonazepam (0.02–2.5 mg/kg) and zopiclone (0.3–3 mg/kg) elicits the appearance of repeated 7–12 spindle bursts (200–300 Hz) alternated with 15–30 Hz, 50–100 μV waves (thereafter defined β-like activity). Each spindle burst lasts 2–7 sec, whereas during control period it lasted 1–2 sec. Only zopiclone fails to modify the duration of each spindle burst. The β-like activity is associated with head up and slight increase of the attentiveness, whereas the presence of spindle bursts is associated with ptosis of the eyelids and decrease of the muscle tonus. With the low doses, the sedative EEG pattern disappears after external vibroacoustical or tactile stimuli and is replaced by either β-like activity or desynchronized pattern (11–14 Hz, 30–50 μV waves). With the largest doses, occasionally, bursts of 2–4 Hz waves can be observed, associated with head drop. Neither EEG nor behavioral responses to vibroacoustical and tactile stimuli are observed.

Typical changes of the electrocortical pattern following the injection of flunitrazepam can be observed in Figs. 1 and 2. The drug induces a slightly higher incidence of synchronization and lower β-like activity in respect to diazepam, clonazepam and zopiclone (Table 1).

CGS 9896 (0.1–3 mg/kg IV), zolpidem (0.1–3 mg/kg IV) and with a lesser incidence Cl 218,872 (0.1–10 mg/kg IV) also elicit the appearance of spindle bursts. In contrast, only occasionally β-like activity and behavioral activation can be observed (Table 1), whereas the bursts of 2–4 Hz waves are markedly increased. The EEG response to external stimuli is evident after Cl 218,872 and CGS 9896, whereas it is reduced after zolpidem.

Rats. As shown in Table 1, in freely moving rats EEG manifestations similar to those already reported in rabbits

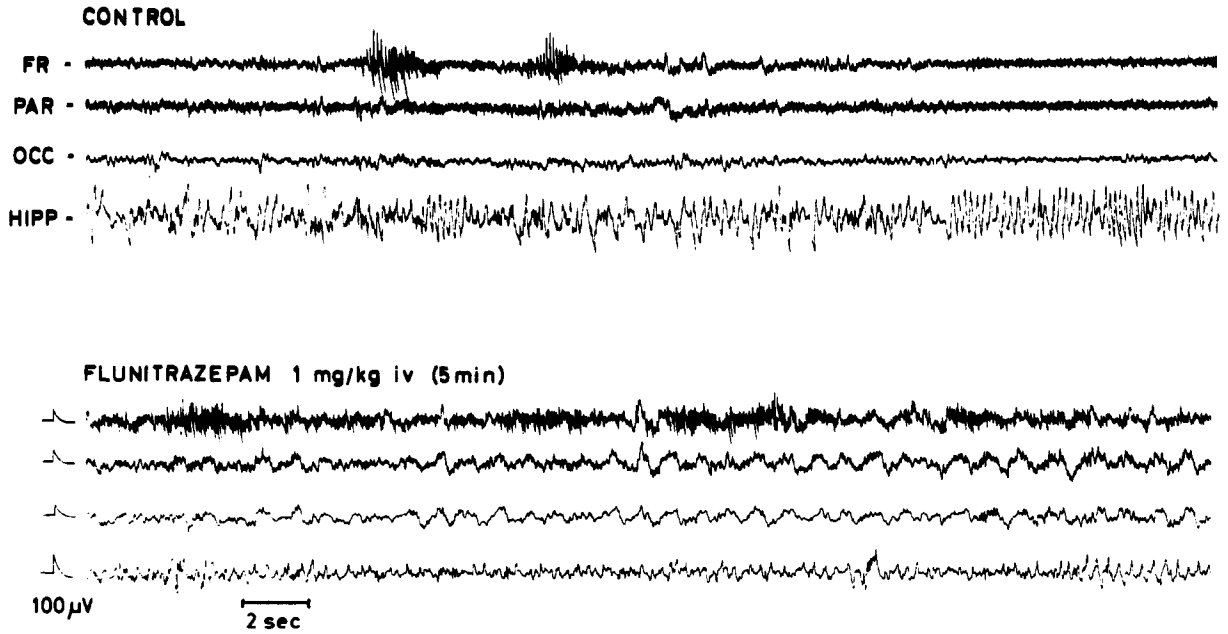

FIG. 1. Modifications of cortical and hippocampal activity induced by flunitrazepam in the rabbit. Control pattern is shown in the upper record. Note that each spindle burst lasts 1–2 sec. In the right side, hippocampal theta rhythms associated with cortical desynchronization can be observed. The dose of 1 mg/kg IV of flunitrazepam (lower record) elicits cortical synchronized pattern mainly characterized by spindles, each lasting 2–4 sec. Hippocampal rhythms are strongly reduced both in amplitude and frequency. Leads: FR=left-right anterior sensorimotor cortices; PAR=left-right posterior sensorimotor cortices; OCC=left-right optic cortices; HIPP=left dorsal hippocampus.

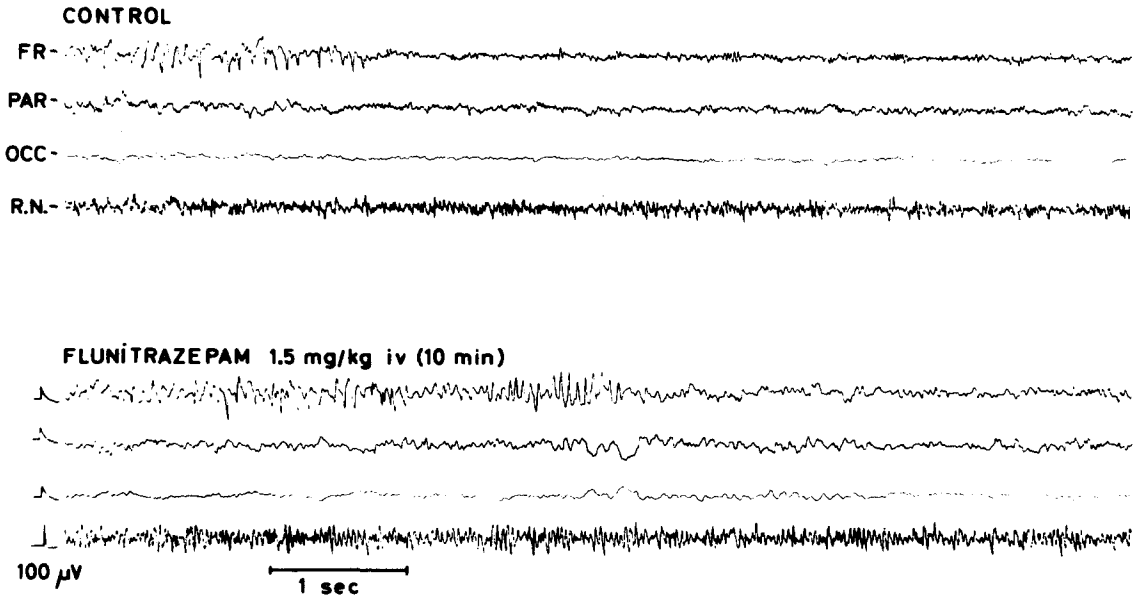

FIG. 2. Modifications induced by flunitrazepam on the red nucleus rhythms in the rabbit. The control pattern is shown in the upper record. The red nucleus waves average 40–50 Hz and 30–50 μV. The administration of 1.5 mg/kg of the drug (lower record) strongly reduces the frequency, to 25–30 Hz, and increases the amplitude to 100–120 μV. Note also the increase in duration of the spindles, as shown in Fig. 1. Leads: FR, PAR and OCC, See Fig. 1. R.N.=right red nucleus.

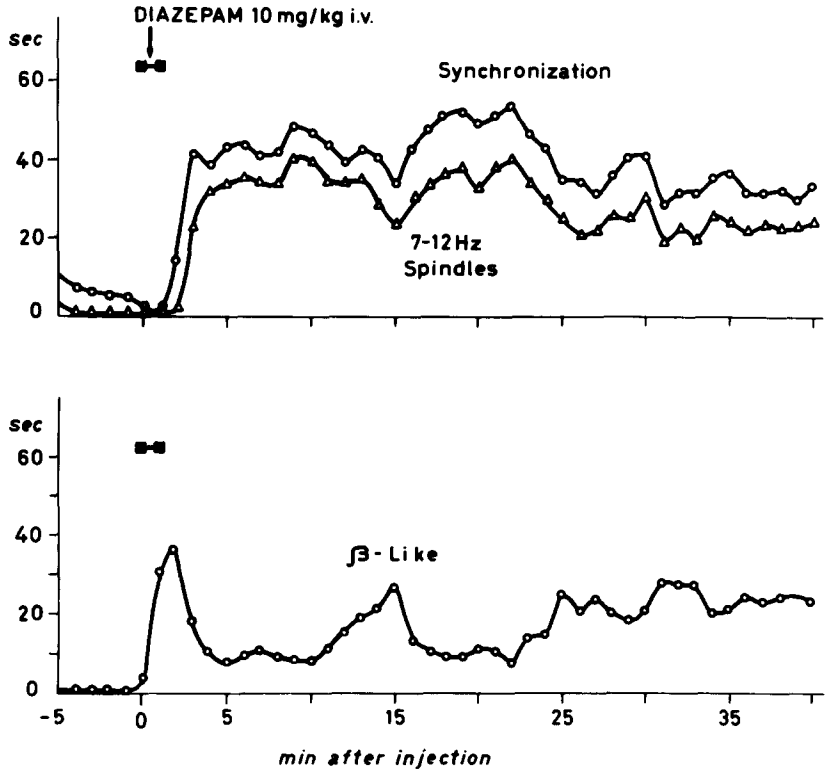

FIG. 3. Time-course of the electrocortical manifestation in the rat after diazepam. The figure depicts the time-wise occurrence of the spindle bursts and of the β-like activity after administration of 10 mg/kg IV of diazepam (arrow). Epochs of 1 min have been evaluated up to 40 min after the injection of the drug (■–■). The periods of synchronized pattern and during this the periods of the spindle bursts as well as the periods of β-like activity have been measured at each epoch. The values are the means of 7 replications. The drug induces a large preponderance of a synchronized pattern largely occupied by the occurrence of the typical spindle bursts (upper diagram). On the contrary, a peak for the β-like activity is noticed in the first min after injection, then it is present in largely lower amount up to the 30th min (lower diagram). After this time almost similar values for the spindle burst and the β-like activity can be observed. Note at the 15th min the increase in β-like activity parallels the decrease of the synchronizing pattern (compare the upper and lower diagrams).

are observed after diazepam (0.1–10 mg/kg IV), flunitrazepam (0.02–2.5 mg/kg IV), clonazepam (0.01–3 mg/kg IV), Cl 218,872 (0.2–10 mg/kg IV) and CGS 9896 (0.1–3 mg/kg IV). Figure 3 shows the time-course of the occurrence of the EEG manifestations after a large dose of diazepam (10 mg/kg IV). The injection of the drug induces the appearance of the β-like activity associated with signs of behavioral excitation (gnawing, running, ear twitches and sometimes wet-dog shakes). In the following two-three min it decreases and is replaced by a synchronized pattern. This is largely characterized by repeated 7–12 Hz spindle bursts associated with signs of behavioral sedation (crouched, eyes open and myorelaxation). Each spindle burst lasts 2–6 sec, whereas in the control record it lasts 0.5–1 sec. This sedative pattern alternated with short-lasting periods of EEG and behavioral activation. In some instances, bursts of 2–4 Hz waves occur associated with either marked sedation (lying down, eyes closed and response to tactile stimuli) or sleep (stretched on the side, no response to tactile stimuli and absence of the righting reflex). Similar time-course effects are observed with clonazepam and, with a lower incidence of the β-like activity, after flunitrazepam.

In the case of CGS 9896, the period of latency for the appearance of the spindle bursts lasts 3–5 min. In addition, after this drug as well as after Cl 218,872, the reaction to the external stimuli is present and the β-like activity occurs occasionally. The EEG sedative patterns alternate with periods of desynchronization.

Effects of Agonists of BDZ Receptor on the Hippocampal Theta Rhythms in the Rabbit

Basically 4–7 Hz, 300–400 μV waves (theta) can be recorded from the hippocampus in awake conditions. After 0.1 mg/kg IV of flunitrazepam a slowing of the recording with occasional sharp waves can be observed associated with cortical synchronization. Short-lasting periods of theta rhythms can be observed associated with either the presence of a desynchronized cortical pattern or the presence of β-like activity. Higher doses (in this case 1 mg/kg IV) strongly reduce the amplitude and the frequency of the record (Fig. 1). Occasionally, a tendency to reorganize theta rhythms can occur, paralleling the appearance of cortical activation. Periods of flattening can be also observed. The reaction to the external stimuli is absent.

TABLE 2

EFFECTS OF SEVERAL AGONISTS OF BDZ RECEPTORS ON THE HIPPOCAMPAL THETA RHYTHMS IN THE RABBIT

	Theta Disruption	
	N	MED
Flunitrazepam	9	0.03–0.07
Clonazepam	14	0.07–0.15
Zolpidem	5	0.1–0.3
Diazepam	12	0.2–0.4
CGS 9896	6	0.2–0.4
Zopiclone	6	0.5–1.0
Cl 218,872	11	0.5–1.0

MED=Minimal effective doses in mg/kg IV.
N=number of animals.

TABLE 3

EFFECTS OF AGONISTS OF BDZ RECEPTORS ON THE RED NUCLEUS RHYTHMS IN THE RABBIT

	Doses (mg/kg IV)	N	Amplitude (μV)	Frequency (Hz)
Control		27	45	51
Pentobarbital	10	25	209	9
Flunitrazepam	2	6	147	18
Diazepam	5	4	161	29
Zolpidem	3	3	148	36
CGS 9896	6	3	107	37
Zopiclone	2	3	101	45
Clonazepam	2	8	60	39
Cl 218,872	10	6	74	44

N=number of animals.

Within the range of the dose tested, all drugs dose-dependently reduce the amplitude. At the minimal effective doses (Table 2), a slowing of the record can be clearly observed after diazepam, flunitrazepam, zolpidem and CGS 9896. The appearance of theta rhythms upon vibroacoustical stimuli occurs only after clonazepam and Cl 218,872. After diazepam, zopiclone, zolpidem and CGS 9896, this reaction is reduced (theta appears for 1–4 sec under stimulation) and it is not always associated with cortical desynchronization.

Effects of BDZ Agonists on the Red Nucleus Activity in the Rabbit

Basically, 40–50 Hz, 50–60 μV waves are recorded from the red nucleus. It has been reported that barbiturate-like central depressants strongly reduce the frequency and increase the amplitude of the red nucleus electrical activity [13]. Therefore, the effects of the various agonists of BDZ receptor have been compared with those of Na pentobarbital. A large dose of flunitrazepam (1.5 mg/kg IV) increases the amplitude and decreases the frequency of the red nucleus rhythms. These effects are dose-dependent and attain the values of 147 μV and 18 Hz at the dose of 2 mg/kg IV, respectively (Table 3).

As shown in Table 3, diazepam and zolpidem also significantly enhance the amplitude (to 140–150 μV), whereas the effect is less evident with zopiclone and CGS 9896 (to 100–110 μV), and is almost absent after clonazepam and Cl 218,872. A decrease of the frequency can be observed after diazepam (to 29 Hz) and, to a lesser extent, with zolpidem, CGS 9896 and clonazepam (to 36–39 Hz).

DISCUSSION

Although the drugs presently investigated belong to different chemical classes, they all show certain similarities in affecting the electrical activity in cerebral cortex, hippocampus and red nucleus. Differences among them can be noticed for potency and/or efficacy, that allegedly reflect the distinct binding capacity at the various types of BDZ receptors (see the Introduction).

Through the visual inspection of the electrocortical record in rat and rabbit it is possible to differentiate three distinct patterns for these BDZ agonists. They are characterized by: (1) 7–12 Hz spindles, each lasting more than in normal record (*namely*, from 0.5–1 sec in the rat and 1–2 sec in the rabbit to 2–6 sec in both species); (2) 15–30 Hz activity (β-like activity); (3) occasionally, after large doses, bursts of 2–4 Hz waves. The first two features are normally absent in the basal record of mammalian. These EEG changes have been already reported in rats, rabbits [18, 19, 24], monkey [11], cats [14, 20] and humans [22] after BDZ derivatives and after zolpidem in rats [1,6].

On the one hand, the full agonist diazepam [10], very often used as standard reference in pharmacological and clinical studies, induces all the EEG manifestations (see also [18]). The other full agonist flunitrazepam seems to be more potent, but it displays a lower efficacy in inducing the stimulatory component in the neocortex. On the other hand, partial agonists with weak efficacy CGS 9896 [2] and, to a lesser degree, Cl 218,872 [10,15] produce little β-like activity. This effect is also observed after zolpidem. Thus, it seems likely that preponderance of the BDZ_1 over the BDZ_2 activation can be relevant for the reduced formation of the β-like activity and behavioral stimulation.

The red nucleus is a mid-brain center that receives efferents from ipsilateral sensorimotor cortex and contralateral deep cerebellar nuclei [17]. It also participates in the rubro-olivo-cerebellar-rubral loop [8], which seems to be involved in the maintenance of the muscle tonus [23]. In respect to the standard reference pentobarbital, the BDZ receptor agonists possess a lower efficacy in reducing the frequency and in increasing the amplitude of the red nucleus rhythms. These drugs can be, therefore, included within the group of the "barbiturate-type," according to the classification of Gogolak *et al.* [8,9]. The observation that the larger increase in amplitude occurs after diazepam, flunitrazepam and zolpidem, whereas no change is noticed after clonazepam and Cl 218,872 let us speculate that this effect is related to the ability of these drugs to enhance GABA receptor activity. In in vitro studies, a 3-fold increase of their binding affinity is reported in the presence of GABA for diazepam, flunitrazepam and zolpidem [1,13], whereas a limited (20–50%) increase is noticed after clonazepam and Cl 218,872 [13,25].

Changes of the electrical activity in the hippocampus received large attention in the past after the observation that a slowing occurs after the injection of benzodiazepines. This can be clearly differentiated from the effects of the barbiturates under external stimuli and under electrical stimulation of the reticular formation (see [21,24]). In our hands, all drugs tested reduce the amplitude of the hippocampal theta

rhythms, thus suggesting that this phenomenon might be related to activation of BDZ_1 receptor type. Incidentally, this effect does not seem to be influenced by activation of BDZ_2 receptor type. Biochemical studies in rats provided evidence that hippocampus, as well as corpus striatum, nucleus accumbens and hypothalamus, show an equal distribution of BDZ_1 and BDZ_2 receptor types. In contrast, other regions—cerebral cortex, pons, thalamus, bulbus olfactorius and in particular cerebellum—show a high density of BDZ_1 receptor types [5,26].

ACKNOWLEDGEMENTS

A.V. is recipient of a Ente Universitario Lombardia Centrale fellowship. This work was supported in part by contract 86.02039.56 of the National Research Council (Consiglio Nasionale delle Ricerche) of Italy.

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