Pharmacology Biochemistry & Behavior, Vol. 23, pp. 661–670, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Electroencephalographic Investigations in Rabbits of Drugs Acting at GABA-Benzodiazepine-Barbiturate/Picrotoxin Receptors Complex

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MASSOTTI, M. *Electroencephalographic investigations in rabbits of drugs acting at GABA-benzodiazepine-barbiturate/picrotoxin receptors complex.* PHARMACOL BIOCHEM BEHAV **23**(4) 661–670, 1985.—This paper describes the EEG profiles, observed in rabbits, of drugs which affect GABA synaptic activity by acting at GBB complex. Drugs which enhance GABA synaptic activity induce sedation associated with EEG synchronization. However, muscimol, THIP, GHB and baclofen induce signs of CNS stimulation (light tremors of the forelimbs, chewing, light nystagmus and hyperpnea) associated with EEG spikes. Signs of light stimulation (chewing and jerks of the head) also occur after BDZs and barbiturates, and are associated with the presence of 12–24 and 20–25 Hz waves, respectively. Drugs which reduce GABA synaptic activity (bicuculline, inverse BDZ agonists, PTZ, picrotoxin and Ro 5-3663) induce three dose-dependent stages of EEG changes: trains of slow waves, trains of spike-and-wave complexes and paroxysmal activity in the rostral encephalic structures without apparent changes of the electrical activity in the spinal cord. The first two stages are associated with a behavioral state of alert and the third stage with tonico-clonic convulsions. Among the inverse BDZ agonists, DMCM and β-CCM elicit all three stages, whereas FG 7142 and β-CCE induce only the first two and CGS 8216 only the first. The BDZ antagonists Ro 15-1788 and Ro 15-3505 (0.2–30 mg/kg IV) do not significantly affect the EEG pattern. However, they selectively inhibit the effects of diazepam and of the inverse BDZ agonists. In both cases, the inhibition is observed with doses as low as 0.2 mg/kg IV and leads to an EEG desynchronization. The possible involvement of the modifications of GABA synaptic activity in the etiology of both *petit mal* and *grand mal* epilepsies is discussed.

EEG GABA Benzodiazepines Barbiturates Picrotoxin

PSYCHOPHARMACOLOGICAL studies in laboratory animals have clearly established that drugs which facilitate GABA-mediated transmission at synaptic level share similar anxiolytic, sedative, hypnotic and anticonvulsant effects. Conversely, drugs which reduce GABA synaptic activity elicit convulsions and display effects opposite to those of the anxiolytics in several conflict tests. The final effect of either facilitation or reduction is independent of the mechanism by which the drug influences GABA synaptic activity (e.g., turnover, release, uptake or regulation of postsynaptic events), see [33].

In the past, EEG studies have indicated that distinct neuronal substrates trigger the sedative-hypnotic effects of BDZs and barbiturates. BDZs inhibit the proprioceptive function, whereas barbiturates inhibit all brain structures which maintain vigilance [55,58]. In addition, it has been suggested that different levels of the neuraxis are involved in the convulsant effects of picrotoxin, PTZ and bicuculline on the one hand, and strychnine on the other [3, 22, 34, 35].

During the last decade, it has been shown that BDZs facilitate GABA transmission [12,26] by binding at specific recognition sites [5,40] coupled to GABA postsynaptic receptors [61]. Subsequently, a growing attention has been focussed on the events at the GABA postsynaptic junction.

Abbreviations Used:

BDZ	benzodiazepine
β-CCE	ethyl-β-carboline-3-carboxylate
β-CCM	methyl-β-carboline-3-carboxylate
CGS 8216	2-phenylpyrazolo (4,3-C) quinolin-3 (5H)-one
CGS 9896	2-(4-chlorophenyl) pyrazolo (4,3-C) quinolin-3 (5H)-one
CNS	central nervous system
DMCM	methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate
FG7142	N-methyl-β-carboline-3-carboxamide
GABA	γ-aminobutyric acid
GBB	GABA-BD2-barbiturate/picrotoxin receptors
GHB	γ-hydroxybutyric acid
PrCC	propyl-β-carboline-3-carboxylate
Ro 15-1788	ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4-H-imidazo (1,5a)-(1,4)-benzodiazepine-3-carboxylate
Ro 15-3505	ethyl-7-chloro-5,6-dihydro-5-methyl-6-oxo-4-H-imidazo (1,5)-benzodiazepine-3-carboxylate
Ro 5-3663	1,3-dihydro-5-methyl-2-H-1,4-benzodiazepine-2-one
THIP	4, 5, 6, 7-tetrahydroisoxazolo (5,4-C) pyridine-3-ol
PTZ	pentamethylenetetrazol

TABLE 1

EEG CHANGES DUE TO DRUGS WHICH ENHANCE GABA SYNAPTIC ACTIVITY BY ACTING AT THE GBB COMPLEX

Site	Drug	Doses (mg/kg IV) which induce EEG changes: Onset (min)	Duration (hours)	Spindles + 12–24 Hz activity	Synchronization	Spikes	Flattening
(A) $GABA_A$	Muscimol	1–5	0.5–3	—	0.5–1.0	1.0–4.0	—
	THIP	5–10	0.5–1	—	0.5–3.0	3.0–6.0	—
	GHB	10–30	0.5–4	—	250–1000	1000–2000	—
$GABA_B$	Baclofen	1–5	0.5–3	—	1–5	5–10	10
(B) BDZ	Diazepam	0.25	0.5–3	0.2–5.0	5–20	—	
	CGS 9896	0.25	0.1–0.5	0.5–3.0*		—	
(C) barbiturate	Na pentobarbital	0.25	0.5–3	5–15	15–25	—	25

The doses are expressed in mg/kg IV; *no higher doses were tested.

Binding studies indicate that at this level a morphofunctional complex exists, which regulates the opening of the GABA receptor-associated chloride channel. It includes distinct binding sites for GABA, BDZ and barbiturates, which interact allosterically with each other [33, 46, 47, 50, 59, 60].

This report is a comprehensive review of early and more recent work done in our laboratory, concerning the modifications of brain electrogenesis in rabbits after administration of drugs acting at the level of the GBB complex.

METHOD

Male rabbits weighing 2.0–2.5 kg were used. In acute preparations, six screw electrodes were implanted over the skull under local anesthesia (2% xylocaine) at the level of the anterior and posterior sensorimotor and of optic cortices of both hemispheres. Strictly speaking, this type of record is the electrocorticogram; for brevity it will be referred to as EEG. In one group of animals deep bipolar concentric electrodes were also placed at the level of the red nucleus and of the hippocampus using a technique described elsewhere [34]. At the end of the experiments, histological examinations were performed in order to determine the location of the deep electrodes. In a limited number of animals, electrodes were also implanted over the 3rd and 4th lumbar vertebra under slight ether anesthesia, in order to record the electrical activity at the level of the lumbar enlargement of the spinal cord.

Each session commenced with a one hour pre-drug period. After the injection of the training drug(s), the EEG was continuously recorded until it returned to the pre-drug pattern. All drugs were injected by slow intravenous route.

During the recording session, the animals were partially restrained. In addition, when the recording from the spinal cord was performed, the animals were curarized (gallamine 5 mg/kg IV) in order to prevent artifacts, and maintained under artificial respiration.

Measurements of the arterial blood pressure were carried out in a separate group of experiments using a catheter inserted into the carotid artery.

RESULTS

GABA Receptor Ligands

Agonists. Muscimol [42], THIP and isoguvacine [19] bind as agonists at bicuculline-sensitive GABA receptors, located at the GABA postsynaptic junction, termed $GABA_A$. Baclofen binds at bicuculline-insensitive GABA receptors, termed $GABA_B$ [4,28]. GHB occurs normally in mammalian brain as a GABA metabolite. On the other hand, when administered to laboratory animals it induces a hypnotic effect allegedly by forming GABA in the brain [32].

Data from the literature show that muscimol, GHB and baclofen induce identical EEG changes both in rats [23, 24, 56] and rabbits [56]. In the latter, two main dose-dependent stages of EEG changes can be observed. The first is characterized by cortical synchronization made up of 1–3 Hz waves. In the second stage, high voltage spikes are also recorded (Table 1A).

In Fig. 1 are reported the typical EEG patterns observed after doses of 1 and 6 mg/kg of THIP. The cortical changes are similar to those observed after GHB and muscimol. Hippocampal theta rhythm is replaced by slow waves and scattered spike-like waves. On the contrary, no change of electrical activity can be recorded from the red nucleus. From the behavioral point of view, the animals show sedation with head drop. However, signs of slight excitation are also noticed. They consist of light tremors of the forelimbs, chewing, light nystagmus and hyperpnea. Such behavioral changes are also noticed after muscimol, GHB and baclofen [56].

Unlike muscimol, THIP, and GHB, after large doses of baclofen (10 mg/kg and higher), the hypersynchronous EEG record is replaced by an almost isoelectrical pattern (Table 1A), with scattered bursts of polymorphic waves. This, however, does not seem to be an effect peculiar to the $GABA_B$ agonist, since it also occurs in the rat after large doses of muscimol and GHB [56].

Isoguvacine (0.4–15 mg/kg) fails to induce EEG changes. This seems to be consistent with the reported poor capability of the drug to cross the blood-brain barrier [19]. Due to a limited supply of this compound, it was not possible to study it in full detail.

Antagonists. Bicuculline is a $GABA_A$ receptor antagonist [14] which induces EEG and behavioral seizures. As previously reported [22,53], in rabbits the drug elicits EEG changes characteristic enough to be divided into three dose-dependent stages: first trains of slow waves (2–4 Hz; 300–400 μV) in the optic cortex (0.02–0.04 mg/kg), followed by the appearance of trains of spike-and-wave complexes (4–6 Hz; 300–400 μV) in the sensorimotor cortex (0.05–0.09 mg/kg),

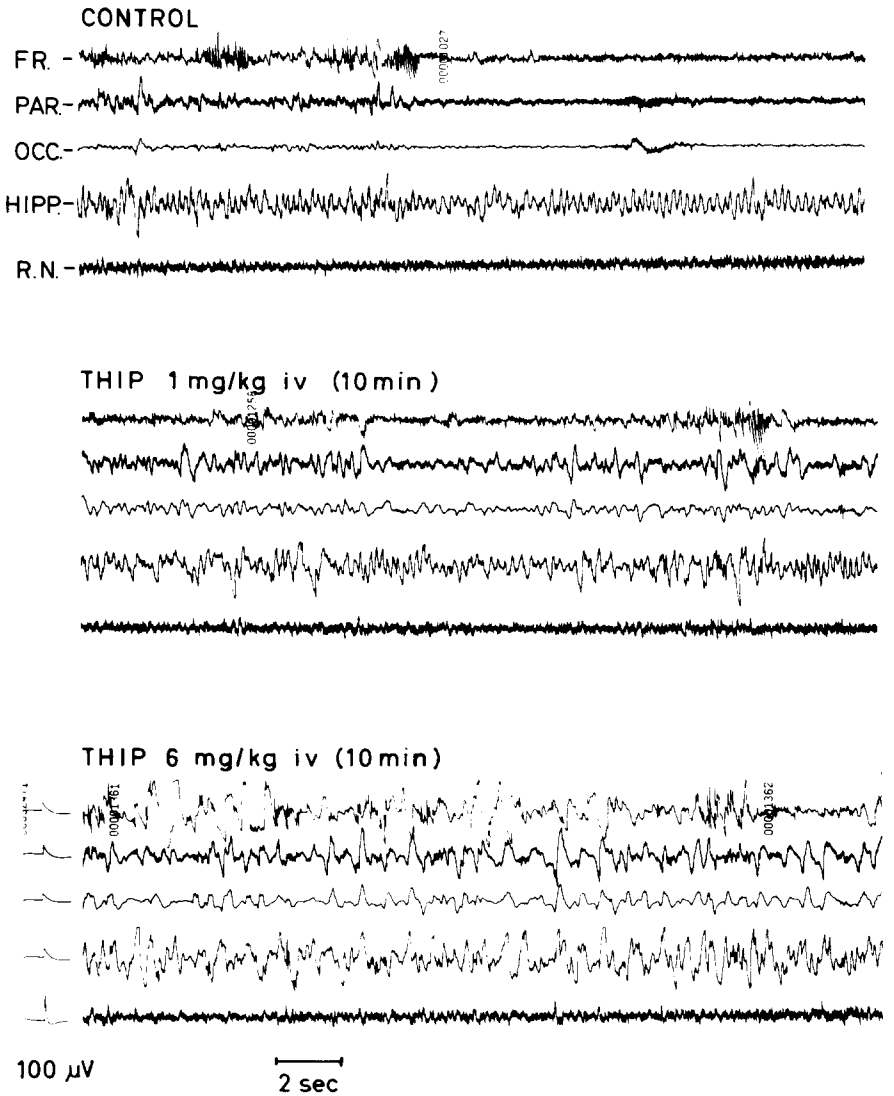

FIG. 1. EEG effects of THIP in rabbits. The upper record shows the pattern observed just before administration of the drug. The middle record shows the synchronization pattern observed mainly at the level of the parietal and occipital leads 10 min after administration of 1 mg/kg of THIP. Note the slowing of the hippocampal pattern with absence of theta rhythm. The lower pattern is recorded 2 hours after administration of the previous dose and shows the effect observed 10 min after the dose of 6 mg/kg of THIP. Note the synchronized pattern with the presence of scattered spikes at the level of cortical and hippocampal leads, as well as the absence of changes at the level of the red nucleus. Leads: FR=anterior sensorimotor cortex; PAR=posterior sensorimotor cortex; OCC=optic cortex; HIPP=hippocampus; RN=left red nucleus.

and finally generalized seizures (0.1 mg/kg and higher), see Table 2A.

The first two stages are superimposed on a desynchronized pattern in the cortical leads, accompanied by an increased hippocampal theta rhythm. No changes of the record can be noticed at the level of the red nucleus. From the behavioral point of view, a slight state of alertness and hyperreactivity are noticed. The third stage consists of EEG seizures which appear first in the cortical areas then spread to the subcortical areas. No change of electrical activity can be recorded from the spinal cord.

The EEG effects of bicuculline have been compared with those observed after the administration of isoniazid. The latter reduces GABA synthesis and content in the brain by inhibiting the enzyme glutamic acid decarboxylase [36]. Within 10–40 min, the drug (100–500 mg/kg) elicits tonico-clonic convulsions associated with paroxysmal EEG activity. This effect lasts 15–100 min, then a flattened record occurs, followed by the death of the animals. Unlike bicuculline, isoniazid does not induce significant EEG changes either during the period that precedes the appearance of the seizures or at the subconvulsant doses (10–70 mg/kg).

TABLE 2

EEG CHANGES DUE TO DRUGS WHICH REDUCE GABA TRANSMISSION BY ACTING AT THE GBB COMPLEX IN RABBITS

Site	Drugs	Onset (min)	Duration (min)	Doses (mg/kg IV) which induce EEG changes: Slow Waves (optic cortex)	Spike- and Wave complex (sensorimotor cortex)	"Grand-mal" seizures
(A) GABA	Bicuculline	0.25–0.5	10–20	0.02–0.04	0.05–0.09	>0.1
(B) BDZ	β-CCM	0.5–1.0	15–30	0.25–1.20	1.2–2.00	>2.0
	DMCM	0.25–2.0	10–30	0.40–1.00	(0.8–1.20)*	>1.0
	β-CCE	0.5–1.0	10–25	0.20–0.50	0.50–2.00	—
	FG 7142	1–5	30–40	2.00–10.0	10.0–20.0**	—
	CGS 8216	0.5–1.0	20–30	5.00–20.0	—	
(C) Picrotoxin	Picrotoxin	10–20	15–30	0.4–0.6	0.7–0.9	> 1
	PTZ	0.25–1	10–20	4–7	8–15	>20
	Ro 5-3663	0.25–1	10–15	2–3	4–6	> 7

*At this range of doses the effect occurs occasionally.
**This effect occurs in 50% of the animals.

BDZ Receptor Ligands

Based on biochemical [6, 7, 9, 18, 29, 33, 37, 38, 39, 41, 45, 57] and behavioral [10, 13, 31, 33] findings, three categories of exogenous ligands of BDZ receptors have been identified. They are termed agonists, antagonists and inverse agonists [27]. Their pharmacological effects seem to be related to the ability to increase, not affect and decrease GABA receptor activity, respectively (see [33]).

Agonists. Diazepam is widely used in laboratory studies as a prototype of the anxiolytic 1,4-BDZs. A few sec after the administration, the drug produces EEG changes characteristic enough to be classified into two dose-dependent stages (Table 1B). In the first, diazepam (0.2–5 mg/kg) elicits a lengthening of the spindle bursts (7–12 Hz; more than 300 μV) more sustained in the frontal cortex (0.2–1.0 mg/kg), which increase both in the incidence and amplitude then spread to the parietal cortex (1–5 mg/kg). These spindles alternate with periods of desynchronization after low doses and with periods of 12–24 Hz (100 μV) waves after higher doses. The second stage is observed with doses of 5–20 mg/kg and consists of the immediate appearance of EEG synchronization followed, 10–15 min later, by the occurrence of the pattern described in the first stage. At the level of the subcortical areas, a slowing of electrical activity with disappearance of the theta rhythm is observed in the hippocampus, whereas an increase of voltage and a decrease of frequency are noticed in the red nucleus (Fig. 4; [17]). De Trujillo *et al.* [17] reported an increased voltage of the 25–30 Hz waves at the level of the cerebellar vermis.

From the behavioral point of view, the animals show head drop, sedation, myorelaxation and short-lasting periods of chewing and jerks of the head.

CGS 9896 is a pyrazoloquinoline derivative which binds with high affinity at central BDZ receptors and is endowed with anxiolytic effects [33,48]. As shown in Table 1B, the drug (0.5–3.0 mg/kg) induces the first stage of EEG changes, similar to that reported after diazepam. Changes of the electrical activity also occur in the hippocampus and red nucleus, which are similar to those described after diazepam. Due to a limited supply of the compound, it was not possible to study it in full detail.

Antagonists. This group includes the imidazobenzodiazepine derivatives Ro 15-1788 [2, 27, 30, 49] and Ro 15-3505 [27], as well as the β-carboline derivative PrCC [62]. There are data indicating that under certain experimental conditions, Ro 15-1788 can display partial agonist [16, 25, 43, 52, 63] or inverse agonist [11,20] effects. In general, the two imidazobenzodiazepines (0.2–30 mg/kg) do not significantly modify the EEG recording. In several experiments, however, when the pre-drug pattern shows a preponderant synchronization, the two antagonists (5 mg/kg and higher) increase the periods of desynchronization. Such an effect lasts 10 and 20 min after Ro 15-1788 and Ro 15-3505, respectively. Much lower doses (as low as 0.2 mg/kg) are required to antagonize the EEG modifications induced by diazepam (see below).

PrCC (0.2–12 mg/kg) does not influence the EEG pattern. Higher doses produce a slowing of the EEG record associated with a decrease of the blood pressure down to 40 mm Hg.

Inverse BDZ agonists. This group of drugs includes several β-carboline derivatives, which on the basis of their biochemical [6, 9, 29, 33, 39, 49] and pharmacological [10, 13, 20, 31, 33, 44] profiles can be divided into two subgroups. One includes DMCM and β-CCM, which are endowed with convulsant effects, and another includes β-CCE and FG 7142, which exhibit proconvulsant effects only. These drugs, furthermore, share effects opposite to those of BDZs in several schedule-controlled tests, which suggest a possible anxiogenic effect (see [33]). The latter has been described in humans after administration of FG 7142 [8].

β-CCM elicits three dose-dependent stages of EEG changes similar to those already described after administration of bicuculline (Table 2B), namely, trains of slow waves in the associative cortex, followed by trains of spike-and-

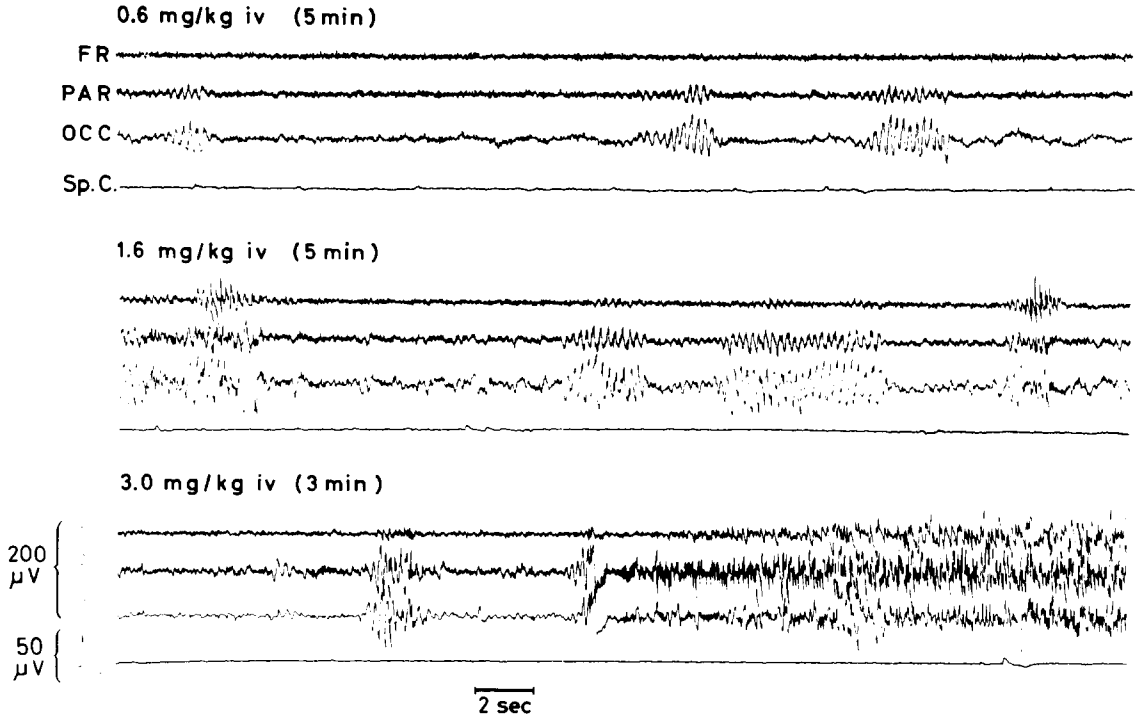

FIG. 2. EEG effects of β-CCM in rabbits. The upper record shows the trains of slow waves that occur at the level of the optic cortex 5 min after administration of β-CCM (0.6 mg/kg). The middle record shows the trains of spike-and-wave complexes at the level of the sensorimotor cortex and the slow waves at the level of the optic cortex observed 5 min after administration of the drug (1.6 mg/kg). The lower record shows the cortical EEG seizures observed 3 min after administration of a dose of 3 mg/kg of the drug. Note the absence of changes at the level of the spinal cord. Leads: as reported in Fig. 1, plus SpC=Lumbar enlargement of the spinal cord.

wave complexes in the sensorimotor cortex and finally by EEG *grand mal* seizures. The typical patterns of the three EEG stages observed after β-CCM are reported in Fig. 2. Also in this case, no change of electrical activity can be recorded from the spinal cord.

DMCM induces only trains of slow waves (0.4–1.0 mg/kg) and *grand mal* seizures (1 mg/kg and higher). In the range of the doses from 0.8 to 1.2 mg/kg, trains of spike-and-wave complexes occur occasionally (Table 2B).

FG 7142 and β-CCE have a different EEG profile, in that they elicit trains of slow waves at doses of 2–10 mg/kg and 0.2–0.5 mg/kg, respectively, and trains of spike-and-wave complexes at doses of 10–20 mg/kg and 0.5–2.0 mg/kg, respectively. Paradoxically, higher doses of the two drugs induce a hypersynchronous cortical pattern, reminiscent of slow wave sleep. At these doses, the animals show muscle relaxation and head drop. Measurements of the arterial blood pressure were performed in order to evidence a possible cardiovascular interference on such an EEG pattern. No significant change was observed with both drugs up to the dose of 35 mg/kg.

CGS 8216 was at first considered a BDZ antagonist [15]. However, biochemical [9,33] and behavioral [21, 31, 33, 48] studies indicate that this drug possesses a weak inverse agonist effect. CGS 8216 (1–4 mg/kg) elicits a desynchronized record in the cortical leads. At higher doses (5–20 mg/kg), occasionally or after external vibroacoustical stimuli, low voltage (200–300 μV) trains of slow waves appear. No change in the gross behavior can be noticed.

Barbiturate/Picrotoxin Receptor Ligands

Barbiturates, picrotoxin, PTZ and Ro 5-3663 initiate their pharmacological effects by interacting at specific binding site(s) located directly at the level of the GABA receptor-associated chloride channel [46, 47, 50, 59, 60].

Agonists. Na pentobarbital is widely used in laboratory studies as a prototype of barbiturates. At doses of 5–15 mg/kg, the drug induces continuous spindling alternated with 20–25 Hz waves at the level of the sensorimotor cortex, associated with a decrease of frequency in the optic cortex. The basic hippocampal theta rhythm becomes irregular. At the level of the red nucleus, an increase of voltage and a decrease of frequency are noticed. Higher doses (15–25 mg/kg) induce uniform high amplitude slow waves (1–3 Hz) in the cortical leads (Table 1C). A further increase of voltage and decrease of frequency in the red nucleus as well as a slowing of the hippocampal waves with scattered spike-like waves are noticed. The highest doses (25–35 mg/kg) produce an almost isoelectrical EEG record with scattered bursts of polymorphic waves. Behaviorally, a dose-dependent sedation and myorelaxation are observed. Stimulatory signs also occur with low doses, similar to those already described after diazepam.

Antagonists. The EEG effects of PTZ, picrotoxin and Ro 5-3663 have been extensively described [34, 35, 53]. They elicit changes in the various brain structures similar to those previously described for bicuculline and inverse BDZ agonists. The ranges of doses which induce trains of slow

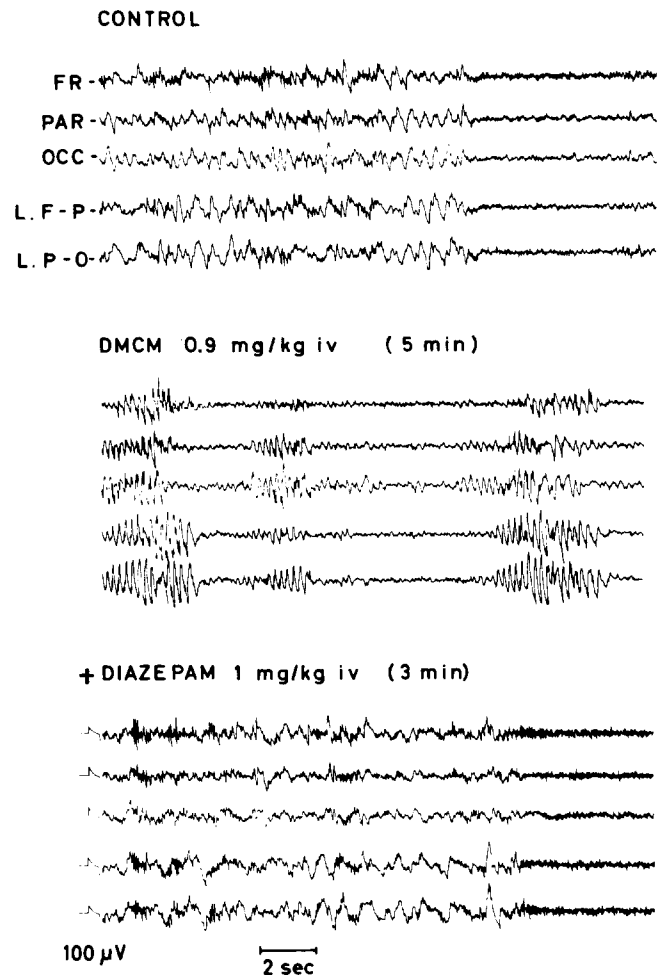

FIG. 3. Effect of diazepam on the EEG changes due to a low dose of DMCM in rabbits. The upper record shows the pattern observed just before the administration of DMCM. The middle record shows the presence of spike-and-wave complexes at the level of the frontal and parietal cortex together with slow waves in the optic cortex 5 min after administration of DMCM (0.9 mg/kg) and just before injection of diazepam. The lower record shows the EEG pattern observed 3 min after injection of diazepam (1 mg/kg): periods of synchronization with the presence of spindles and periods of desynchronization are present. Leads: as in Fig. 1 plus L F-P=left frontoparietal cortex; L P-O=parieto occipital cortex.

waves, trains of spike-and-wave complexes and *grand mal* seizures are reported in Table 2C.

Interactions Among the Various Ligands of the Three Receptor Sites of the GBB Complex

Considerable evidence has appeared in the literature as to the ability of BDZs to block the convulsant effects of drugs which reduce GABA-mediated transmission. The administration of diazepam (1 mg/kg) counteracts within a few sec the EEG and motor effects elicited by the convulsant and subconvulsant doses of bicuculline, picrotoxin, Ro 5-3663 [53] and the inverse BDZ agonists, such as DMCM (Fig. 3). In all cases, a low voltage record with slow waves and scattered spikes occurs in the following 5–10 min. Then the characteristic EEG pattern induced by the BDZ emerges (see above). On the contrary, diazepam is unable to significantly affect the EEG changes induced by the highest doses of FG 7142 (25 mg/kg) and β-CCE (5 mg/kg).

It has been pointed out that diazepam potentiates the EEG effects of muscimol, GHB and baclofen [56], whereas ethosuximide, trimethadione and n-dipropylacetate antagonize the effect of GHB [23]. As shown in Fig. 6, diazepam (1 mg/kg) also potentiates the EEG effects of THIP. The record induced by the GABA agonist shows a further reduction of the frequency with short-lasting periods of isoelectric pattern.

Ro 15-1788 and Ro 15-3505 counteract the EEG and behavioral effects of diazepam (Fig. 4) and of the inverse BDZ agonists. Within a few sec, doses as low as 0.2 mg/kg of the two antagonists reverse the sedative effect diazepam (up to 20 mg/kg) as well as the convulsant effect of DMCM and β-CCM. In both cases, the EEG patterns are replaced by a desynchronized record lasting 10–15 min after Ro 15-1788 and 20–50 min after Ro 15-3505. In contrast, the two antagonists (0.2–20 mg/kg) fail to affect significantly the EEG and behavioral changes observed after large doses of FG 7142 (25 mg/kg) and β-CCE (5 mg/kg).

In a series of paired experiments, Ro 15-1788 was injected in animals previously challenged with convulsant doses of bicuculline, picrotoxin, PTZ and Ro 5-3663. The antagonist (2 mg/kg), injected 1–2 min after bicuculline (0.1–0.2 mg/kg), counteracts the EEG and behavioral seizures. The record is characterized by slow waves and scattered spikes (Fig. 5), lasting 10–15 min. The spikes are associated with clonic movements of the forelimbs. On the contrary, when the same dose of Ro 15-1788 was injected after the appearance of the EEG and behavioral seizures induced by PTZ (30 mg/kg), picrotoxin (1.5 mg/kg) and Ro 5-3663 (10 mg/kg), a slight but not significant increase of the total seizure time is observed.

In a series of paired experiments, Ro 15-1788 (20 mg/kg) was injected in animals previously challenged with Na pentobarbital (15 mg/kg). While no significant change in the EEG pattern is observed, a slight but not significant increase of the duration of the EEG effect due to pentobarbital is noticed.

Ro 15-1788 (2 mg/kg) fails to affect significantly the EEG changes observed after injection of muscimol (1.5 mg/kg) and isoniazid (50 and 250 mg/kg).

DISCUSSION

In *in vivo* studies, EEG technique offers a valuable tool to the understanding of the central mechanisms involved in the effects of psychotropic drugs. In this paper, based on early and more recent results, an attempt has been made to classify the EEG patterns induced by drugs which affect GABA synaptic activity by acting at the GBB complex.

Drugs which enhance GABA synaptic activity, each acting at GABA, BDZ and barbiturate receptors, all induce general sedation always associated with a certain degree of CNS stimulation. Besides their common synchronizing effect, the EEG pattern shows considerable differences between GABA receptor ligands on the one hand and ligands of BDZ and barbiturate receptors on the other hand. The major differences occur at the level of cortical areas and of the red nucleus. The effect of ligands of BDZ receptors and of barbiturate receptors can be differentiated on the basis of the morphology of the spindle bursts, of the power spectra [54], and of the sensitivity to the selective BDZ antagonists. The EEG effects of diazepam are blocked by Ro 15-1788, whereas those of Na pentobarbital are not.

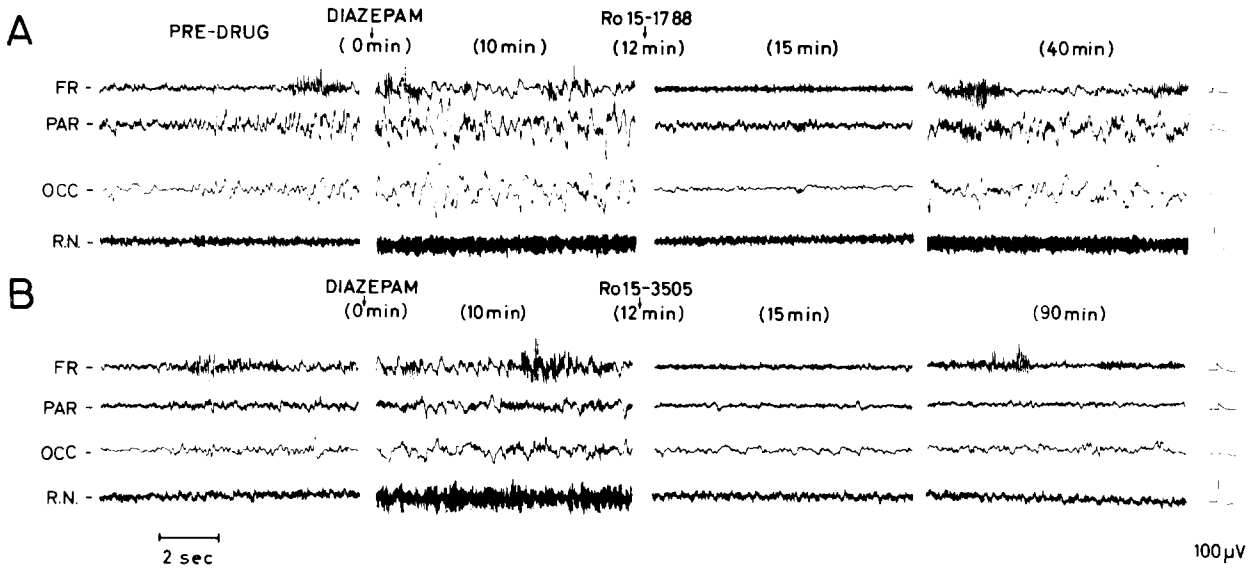

FIG. 4. Reversal by Ro 15-1788 (0.8 mg/kg) and Ro 15-3505 (0.8 mg/kg) of the EEG effects of diazepam (10 mg/kg) in rabbits. The upper recordings (A) show the results obtained in animals challenged with Ro 15-1788. After a period of basal record (Predrug), diazepam is injected at a dose of 10 mg/kg; ten min later (10 min) synchronization and spindles occur in cortical leads, and an increase of voltage is observed in the red nucleus pattern. Ro 15-1788 (0.8 mg/kg) is injected 12 min after diazepam (arrow) and 3 min later (15 min) the record becomes desynchronized at the cortical level, while the voltage of the red nucleus returns to the basal pattern. Twenty-five min later (40 min from diazepam administration) the characteristic pattern of diazepam is observed (cortical synchronization + spindles and increased voltage at the level of the red nucleus). The lower recordings (B) show the results obtained in animals challenged with Ro 15-3505. The schedule of treatments are similar to those reported above. The only difference in respect to the effects found with Ro 15-1788 is that the inhibition of the EEG effect of diazepam is long-lasting and 75 min after injection of Ro 15-3505 (90 min) the patterns at the level of the cortex and of the red nucleus are different from those observed after the benzodiazepine. Leads: as reported in Fig. 1.

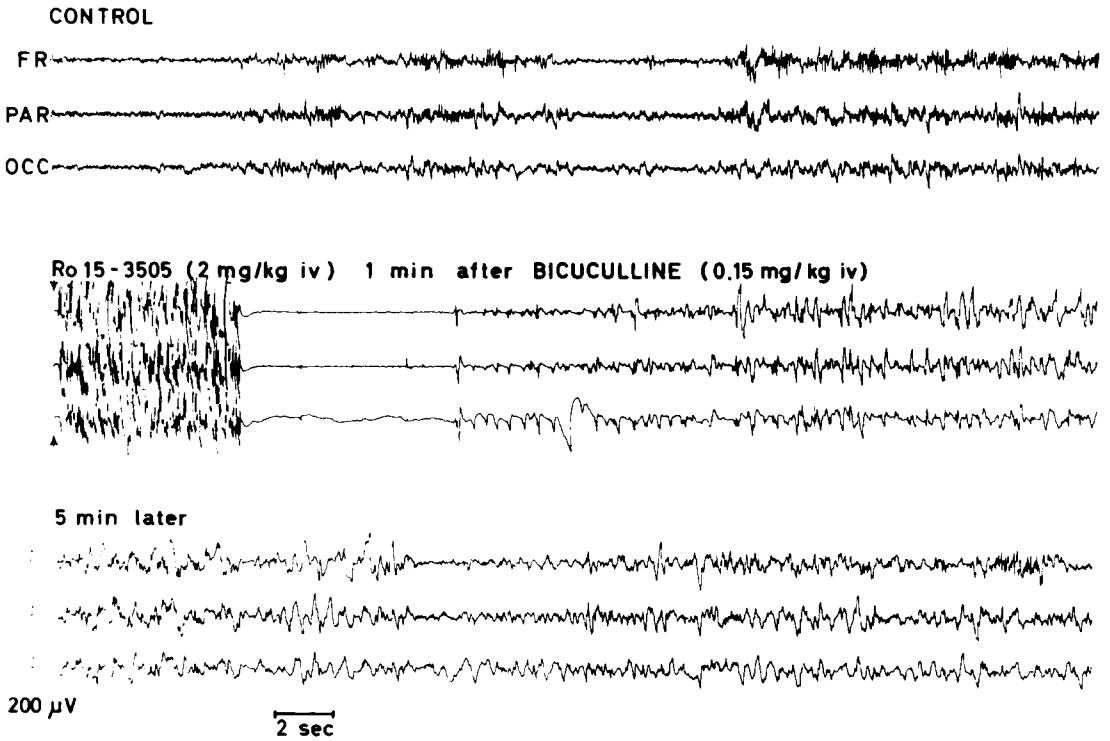

FIG. 5. Reversal by Ro 15-3505 of the EEG seizures induced by bicuculline in rabbits. The upper record shows the EEG pattern recorded just before injection of bicuculline (0.15 mg/kg IV). The middle record shows the pattern observed after administration of a dose of 2 mg/kg of Ro 15-3505 (arrow) in animals previously (1 min) injected with bicuculline. Note the almost immediate inhibition of the EEG seizures followed by a 10 second period of electrical silence, then a record with scattered spikes is present. The lower record shows the pattern observed 5 min later, characterized by slow waves and spikes. Leads: as reported in Fig. 1.

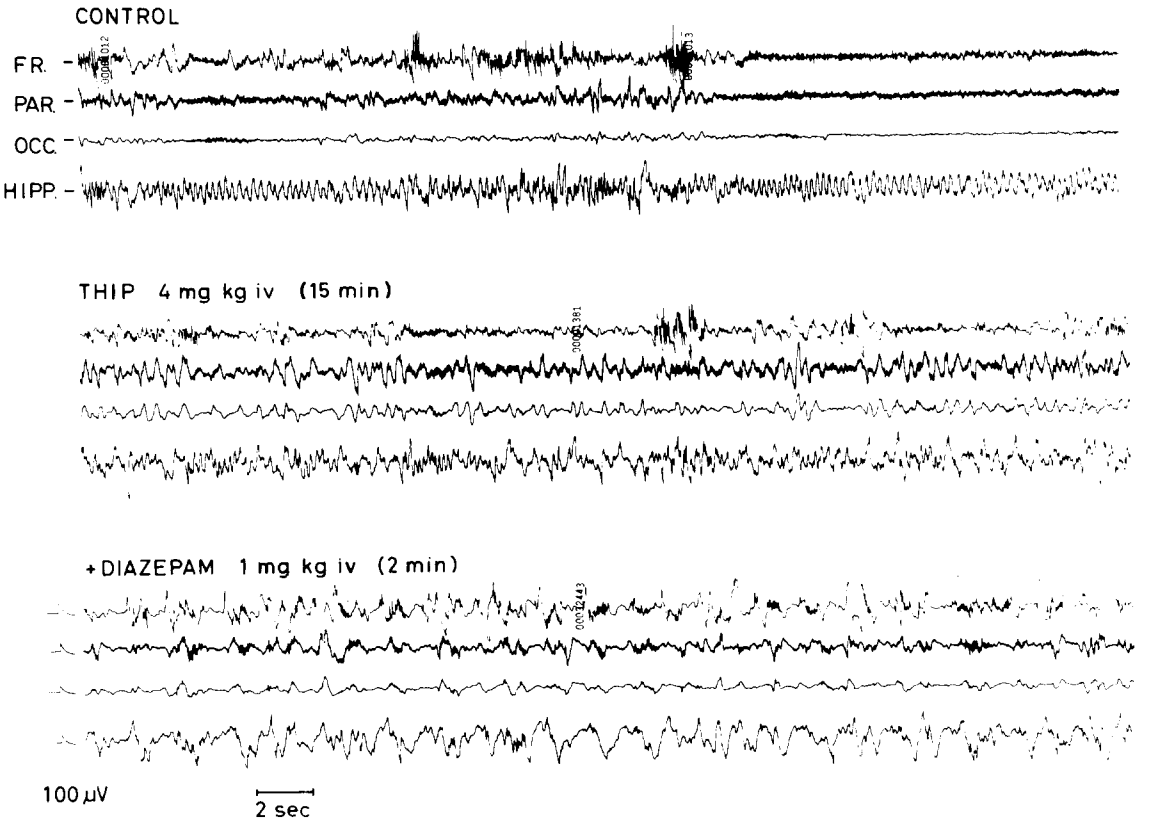

FIG. 6. Potentiation by diazepam of the EEG effect of THIP in rabbits. The upper record shows the pattern observed just before administration of THIP. The middle record shows hypersynchronous activity with the presence of spikes at the level of the cortical and hippocampal leads, obtained 15 min after administration of a dose of 4 mg/kg of THIP and just before the injection of diazepam. The lower record shows the pattern observed 2 min after administration of diazepam (1 mg/kg). Note the periods of flattening more evident in parietal and occipital leads. Leads: as reported in Fig. 1.

It is interesting to note that the EEG patterns observed after muscimol [56], GHB [23, 24, 56] and THIP (present data) resemble those induced by α-chloralose [64]. This leads us to include the GABA receptor agonists in the group of the so called "convulsant anesthetics" (see also [1]). It has been further observed that the cortical hypersynchronous waves associated with spikes observed with these drugs are reminiscent of the generalized non-convulsive epilepsy [24,56]. This possibility is further strengthened by the observation that these EEG effects are worsened by several *anti-grand mal* drugs [23,56] and can be blocked by several *anti-petit mal* drugs [23].

These results would suggest that a direct stimulation of GABA receptors may play a role in the etiology of *petit mal* epilepsy (see also [24,56]).

The EEG profile of drugs which reduce GABA synaptic activity do not distinguish among ligands of each of the three receptors of the GBB complex. They all elicit EEG seizures which can be recorded mainly at the level of the rostral encephalic structures. Therefore, they can be included in the group of the so called "supraspinal convulsants" [3, 34, 35]. On the basis of the sensitivity to the BDZ antagonists, however, one can distinguish ligands of picrotoxin receptor from those of the GABA and BDZ receptors.

It is interesting to note that the ability of the inverse BDZ agonists to progress through the three dose-dependent stages of EEG changes, as found in this study, seems to be correlated with the extent of inhibition of their binding by GABA [9, 29, 51] and barbiturates [9], as observed in *in vitro* studies.

The observation that the effects of compounds which reduce GABA synaptic activity acting at the GBB complex are blocked by *anti-grand mal* drugs might suggest that an inhibition of GABA transmission plays a role in the etiology of *grand mal* epilepsy.

Finally, the present findings show that Ro 15-1788 and Ro 15-3505 fail to affect significantly the EEG pattern and possess a similar potency in inhibiting the EEG effects of both diazepam and inverse BDZ agonists. This, together with the observation that a common EEG pattern emerges, seems to argue against the possibility that the two BDZ antagonists possess an intrinsic activity [16, 20, 25]. This appears to be also confirmed by the failure of Ro 15-1788 to affect the EEG patterns induced by ligands of barbiturate/picrotoxin receptor(s). Although the BDZ antagonist blocks the effect of bicuculline, an EEG pattern emerges which is different from that observed when Ro 15-1788 counteracts the effects of inverse BDZ agonists. The antagonism by the imidazobenzodiazepine of the EEG effects of bicuculline is difficult to explain and requires additional studies using other $GABA_A$ receptor antagonists.

ACKNOWLEDGEMENTS

The author is indebted to Dr. V. G. Longo for the continuous guidance throughout this work. Supported in part by CNR Contract No. 83.02730.56.

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