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Involvement of a GABAergic Mechanism in the Anticonvulsant Effect of Pentobarbital Against Maximal Electroshock-induced Seizures in Rats

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RASTOGI, S K AND M. K. TICKU *Involvement of a GABAergic mechanism in the anticonvulsant effect of pentobarbital against maximal electroshock-induced seizures in rats* PHARMACOL BIOCHEM BEHAV 22(1) 141–146, 1985.—The interaction between pentobarbital and other modulators of GABAergic transmission (diazepam, ethanol and progabide) was investigated on maximal electroshcok seizures and on the loss of righting reflexes in rats. Pentobarbital, diazepam and ethanol produced a dose-dependent protection against electroshock seizures, with pentobarbital being more potent (3- and 50-times) than diazepam and ethanol Progabide neither provided protection nor caused loss of righting reflex. Subprotective doses of pentobarbital and diazepam, together or when combined with a single ineffective dose of ethanol or progabide, caused protection against seizures and loss of righting reflex for variable durations, while ethanol and progabide combination did not provide protection The protective effect of diazepam was antagonized by RO15-1788, picrotoxin and bicuculline pretreatments The antagonism of pentobarbital protection by a specific GABA receptor antagonist, bicuculline suggests involvement of the GABAergic system in the anticonvulsant effect of pentobarbital These results indicate that, like diazepam, the anticonvulsant effect of pentobarbital appears to be mediated through a GABAergic mechanism

Maximal electroshock seizures Anticonvulsants Modulators GABA receptor complex Pentobarbital Benzodiazepines Agonists/antagonists

CHANGES in the functional status of GABAergic neurons have been implicated in a variety of experimental seizures, as well as in the mechanism of action of various antiepileptic agents There is evidence that the anticonvulsant activity of benzodiazepines is primarily mediated by an enhancement of ongoing GABAergic activity [11,30]. Various biochemical, electrophysiological and histochemical studies support the notion that benzodiazepine receptors are associated with GABAergic synapses [2, 3, 5, 12, 16]. It is widely recognized that barbiturates also facilitate GABAergic transmission and have a pharamcological profile similar to benzodiazepines [10, 14, 15, 19] However, the exact relationship of this facilitatory effect to the anticonvulsant effect is far from clear. Hence, it was thought worthwhile to elucidate the mechanism of anticonvulsant effect of pentobarbital on the controversial (but most widely accepted) animal model of epilepsy, the maximal electroshock seizures pattern test

Recently, Sackeim *et al.* [17] have hypothesized that convulsant properties of maximal electroshock seizures are likely to be mediated via GABAergic mechanisms. Further, the doses of barbiturates used to prevent seizure patterns are not significantly different from those effective against chemoconvulsants, which are GABA antagonists, whereas benzodiazepines require considerably higher doses to prevent electroshock seizures than chemically-induced convulsions [4].

In order to establish a direct functional link between the anticonvulsant action, benzodiazepine receptors and GABAergic transmission, the present study compared the anticonvulsant effect of pentobarbital alone and in combination with known modulators of GABAergic transmission on the maximal electroshock seizure (tonic-clonic) pattern test The interaction of pentobarbital was also studied after pretreatment with antagonists of GABA and benzodiazepine receptors. Also, an attempt was made to correlate the time-course of the anti-convulsant and hypnotic effects in the drugs

METHOD

Male Sprague-Dawley rats, weighing 100–120 g, were used. The animals were kept under conditions of constant temperature (25°C), a 12 hour light-dark cycle (light on at

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TABLE 1

PROTOCOL FOR DRUG ADMINISTRATION

Dose-Response of Individual Drugs (mg/kg or g/kg* IP)	Drug-Interactions (mg/kg or g/kg* IP)	Agonist-Antagonist Interaction (mg/kg)
Pentobarbital, 10, 20, 30, 40	Pentobarbital 5, 10, 20 + Diazepam (40) + *Ethanol (0 5) + *Progabide (1200)	Pentobarbital 40 + Bicuculline (2 0) +Picrotoxin (5 0) + RO15-1788 (50, 100, 200)
Diazepam 20, 40, 80, 120	Diazepam 20, 40, 80 + Pentobarbital (10) + *Ethanol (0 5) + Progabide (1200)	Diazepam 120 + Bicuculline (2 0) + Picrotoxin (5 0) + RO15-1788 (50, 100, 200)
Progabide 400, 800, 1200	0	0
*Ethanol 0 5, 1, 2, 4,	0	0

7 00 a m) and had free access to water and food, except during the actual experiments All animals were used only once in the experiments

Maximal Electroshock Seizures

The anticonvulsant activity was studied according to the method of Woodbury and Davenport [31] Tonic seizures of the hind limbs were induced by passing an alternating electrical current (150 mV, 50 pulses/sec, 0 2 sec) via ear clip electrodes Control animals responded with a tonic seizure (characterized by extension of the hind limbs), followed by clonic seizures Protection was defined as the suppression of the hind limb extension beyond 90° [7,20] The animals were not used for assay purposes until they were exposed to 2 or 3 control supramaximal electroshock at 24 hour intervals to establish the reproducibility of their shock seizure pattern Prior to application of ear clip electrodes, a local anesthetic electrode cream was routinely applied to the ear

Effects on Sleeping Time

The loss of righting reflex was taken as a criteria for hypnotic activity. The end-point of the test was the ability of the animals to right themselves within 30 sec when placed on their back on a flat surface

Drug-Interaction Studies

In order to study the interaction between pentobarbital and various modulators of GABAergic transmission, three different doses of pentobarbital (doses that had no or partial anticonvulsant effect) were combined with specific GABA mimetic agents (single dose which, by itself, did not produce protection against electroshock) Similar comparative drug-interaction studies were also done with prototypes of GABA mimetic agents, like diazepam

For the antagonist-agonist interaction study, the 100% protective dose of pentobarbital or diazepam was combined with subconvulsive doses of GABA receptor antagonists, bicuculline or picrotoxin and various doses of RO15-1788, a benzodiazepine antagonist The protocol for drug administration is described in Table 1

Animal and human research has shown that electroshock results in increased seizure threshold [27], which return to baseline within 24 hours following electroshock exposure [28] In order to avoid this effect, different groups of animals pretreated with the same dose were exposed to electric shock at 30, 60, 90 and 120 min, to assess the duration of protection afforded by drug alone or in combination against electroshock-induced seizures

Materials

Pentobarbital sodium and picrotoxin were purchased from Sigma Chemical (St Louis, MO) and bicuculline from Pierce Chemicals (St Louis, MO) Benzodiazepines were a gift from Hoffman-LaRoche (Nutley, NJ) and Progabide from Synthelabo (Paris, France)

Picrotoxin, progabide and RO15-1788 were dissolved in dimethylsulphoxide (DMSO) Bicuculline was dissolved in a few drops of 0 1 M HCl, after which the final volume was made up with 0.9% NaCl Ethanol was given as a 20% (w/v) solution made of 95% ethanol and distilled water Diazepam was dissolved in propylene glycol All drugs were administered intraperitoneally (IP), in a volume of 1 ml/kg body weight, with the exception of ethanol, which was given in a volume of 20 mg/kg body weight All drugs except progabide (60 min before) were injected 30 minutes prior to electroshock exposure The animals were observed for recovery from drug effects every 30 min for 120 min

Data and Statistical Analysis

Experiments evaluating the effects of drugs on the maximal electroshock seizures were analyzed by Fisher Exact Probability Test (one-tailed) for comparing the degree of significance from the control Data are expressed as a frequency of occurrence

Sleep time data are expressed as the mean±S D of the number of animals in each group Statistical analysis was performed using a one-way analysis of variance with a multiple range analysis test, the Student Newman-Keuls procedure A value of $p<0$ 05 was considered statistically significant

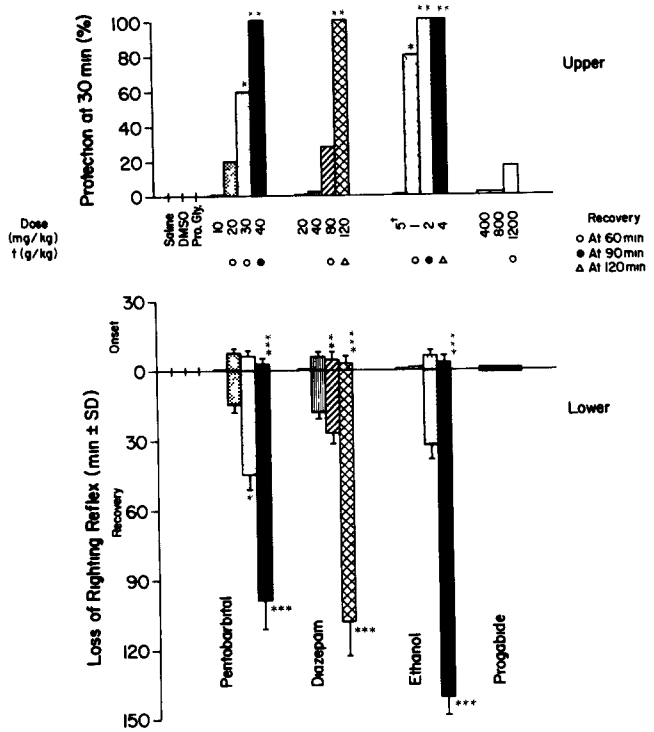

FIG 1 Effects of varying doses of pentobarbital, diazepam, ethanol and progabide on seizures induced by electroshock (upper panel) and loss of righting reflexes (lower panel) in rats Pentobarbital, diazepam and ethanol were administered IP (30 min), progabide (60 min) prior to electroshock The recovery from the drug effect was observed at 60 min (○), 90 min (●) and 120 min (△) Sleep time was recorded as the duration between loss and recovery of the righting reflex The results are shown as the mean±S D of onset and recovery of the righting reflex of 3–5 animals, in minutes $*p<0$ 05, $**p<0$ 001, as compared to vehicle- (saline, dimethyl sulfoxide (DMSO) or propylene glycol) treated group, by Fisher exact probability test (upper panel) $*p<0$ 05, $**p<0$ 01, $***p<0$ 001, as compared to pentobarbital (20 mg/kg) by Student Newman-Keuls procedure (lower panel)

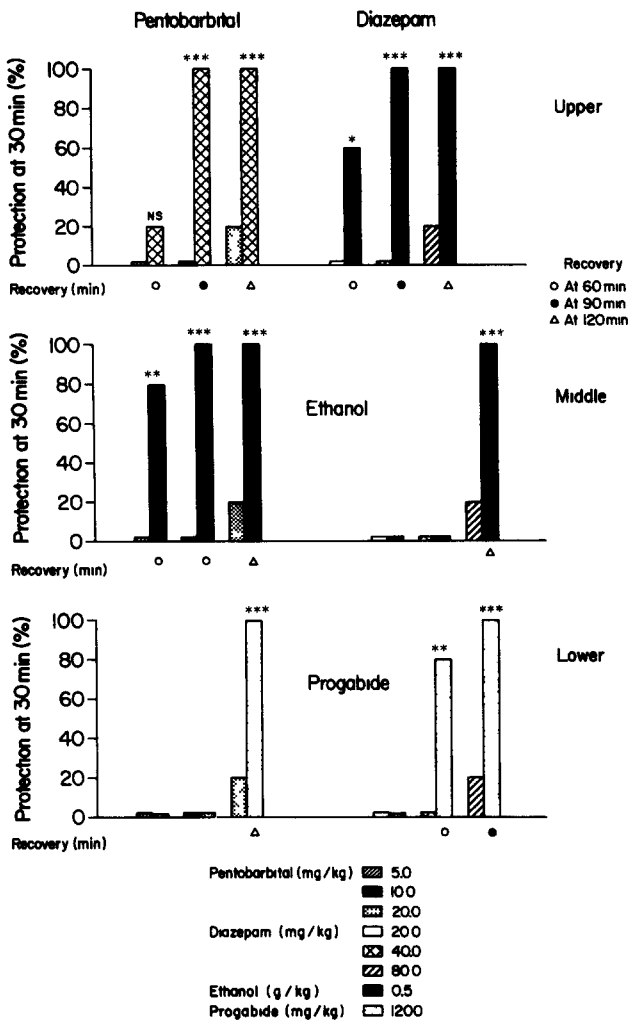

FIG 2 Effects of subprotective doses of pentobarbital (left panel) or diazepam (right panel) in combination with a single subeffective dose of various GABA-mimetic agents on the anticonvulsant effects in rats Pentobarbital (5, 10 and 20 mg/kg) injected IP simultaneously with single dose of diazepam (40 mg/kg, left upper panel), ethanol (0 5 g/kg, left middle panel) and progabide (1200 mg/kg, left lower panel) or different doses of diazepam (20, 40, 80 mg/kg) injected simultaneously with a single dose of pentobarbital (10 mg/kg, right upper panel), ethanol (0 5 g/kg, right middle panel) and progabide (1200 mg/kg, right lower panel) 30 min (except probagide, 60 min) prior to electroshock The results were obtained from 5–7 animals $*p<0$ 05, $**p<0$ 001, $***p<0$ 01, as compared to control values by Fisher exact proability test Note that the anticonvulsant effects of progabide appeared at higher doses of pentobarbital and diazepam Also no satisfactory correlation was observed with the anticonvulsant (Fig 1) and the hypnotic action (this figure) of these drugs

RESULTS

Effects of Pentobarbital, Diazepam and Ethanol on Maximal Electroshock Seizures

The effects of pentobarbital, diazepam and ethanol on maximal electroshock seizures are shown in Fig. 1. Preliminary dose-response studies showed that pentobarbital (20 mg/kg), diazepam (80 mg/kg) and ethanol (1 g/kg), administered IP 30 min prior to electroshock exposure, were approximately equi-effective with regard to their anticonvulsant properties. When given in these doses, ethanol did not cause loss of righting reflex, whereas pentobarbital and diazepam induced sleep for a shorter duration. The recovery of righting reflex was earlier than the recovery of the anticonvulsant effect. In fact, higher doses of ethanol (>2 g/kg), pentobarbital and diazepam (40 and 120 mg/kg, respectively), administered IP 30 min prior to electroshock, caused pronounced anticonvulsant effect for 90, 120 and 150 min duration, respectively, with impairment of righting reflex. In contrast to previous observations in mice [29], progabide (400–1200 mg/kg, 60 min prior to electroshock) did not produce anticonvulsant effect nor cause loss of righting reflex The vehicles used (saline, dimethyl sulphoxide, propylene glycol) did not produce any significant anticonvulsant or hypnotic effects on their own.

Interaction of Pentobarbital or Diazepam with Single Ineffective Doses of Diazepam, Pentobarbital and Progabide

In contrast to hypnotic and anticonvulsant effects induced by higher doses of pentobarbital (Fig 2), combined

treatment of subprotective doses (which had no or minimal anticonvulsant effect), together with subeffective doses of GABAergic modulators (a dose with no or minimal anticonvulsant or hypnotic activity) caused protection against electroshock seizures Figure 2 shows that pentobarbital (5, 10 and 20 mg/kg), combined with diazepam and ethanol (40 mg/kg and 0 5 g/kg, respectively), simultaneously (30 min prior to electroshock) provided 20 and 80% protection against seizures, respectively. These treatments also produced a significant increase in the sleep time (Fig 3) Conversely, GABA agonist progabide, at higher doses (1200 mg/kg, 60 min prior to electroshock), produced anticonvulsant effect only when combined with pentobarbital (20 mg/kg, 30 min before electroshock) The recovery of the anticonvulsant effect was later (120 min) than the recovery of sleep time.

Similarly, subprotective doses of diazepam potentiated the effect of a single subeffective dose of pentobarbital, ethanol and progabide As seen in Fig. 2, diazepam (20, 40 and 80 mg/kg, IP), when combined with pentobarbital (10 mg/kg), progabide (1200 mg/kg) and ethanol (0 5/g/kg), provided significant protection against electroshock seizures Figure 3 shows that the recovery of sleep time with these drugs did not correlate with the recovery of anticonvulsant effects. Control animals were always run with each experiment for comparison

Effects of RO15-1788, Picrotoxin and Bicuculline on Anticonvulsant Effects of Pentobarbital and Diazepam

Administration of the benzodiazepine receptor antagonist, RO15-1788 (50, 100, 200 mg/kg, IP 60 min prior to electroshock), when combined with pentobarbital (40 mg/kg, 30 min prior to electroshock), produced 10, 40 and 100% antagonism of pentobarbital anticonvulsant effects, and the sleep time was also increased (Fig 4)

Administration of the specific GABA receptor antagonist bicuculline (2 0 mg/kg, 60 min prior to electroshock) caused complete antagonism of the anticonvulsant effect of pentobarbital (40 mg/kg) Picrotoxin (5.0 mg/kg, 60 min prior to electroshock) did not protect against the anticonvulsant effect of pentobarbital. Higher doses of picrotoxin were not used, since they produce convulsant activity Moreover, bicuculline and picrotoxin, at the doses listed above, did not affect pentobarbital's sleep time

Bicuculline, picrotoxin and RO15-1788 caused significant antagonism of anticonvulsant and sleep time induced by diazepam (120 mg/kg)

DISCUSSION

The present study was an attempt to establish a direct functional link between the GABAergic system and the anticonvulsant effect of pentobarbital in a maximal electric shock-induced animal model of epilepsy The GABA receptor complex with which various drugs interact is an oligomeric complex with multiple sites It is widely accepted that GABA agonists and bicuculline bind to GABA receptors, benzodiazepine agonists/antagonists bind to the benzodiazepine receptors, whereas picrotoxin and related cage convulsants bind to the picrotoxin site of this complex [16,24]. Recent equilibrium and dissociation studies with [^{35}S]t-butylbicyclophosphorothionate (TBPT) from our laboratory have shown that depressant barbiturates bind to a distinct allosteric (depressant) site, which is coupled to GABA and picrotoxin sites of the benzodiazepine GABA

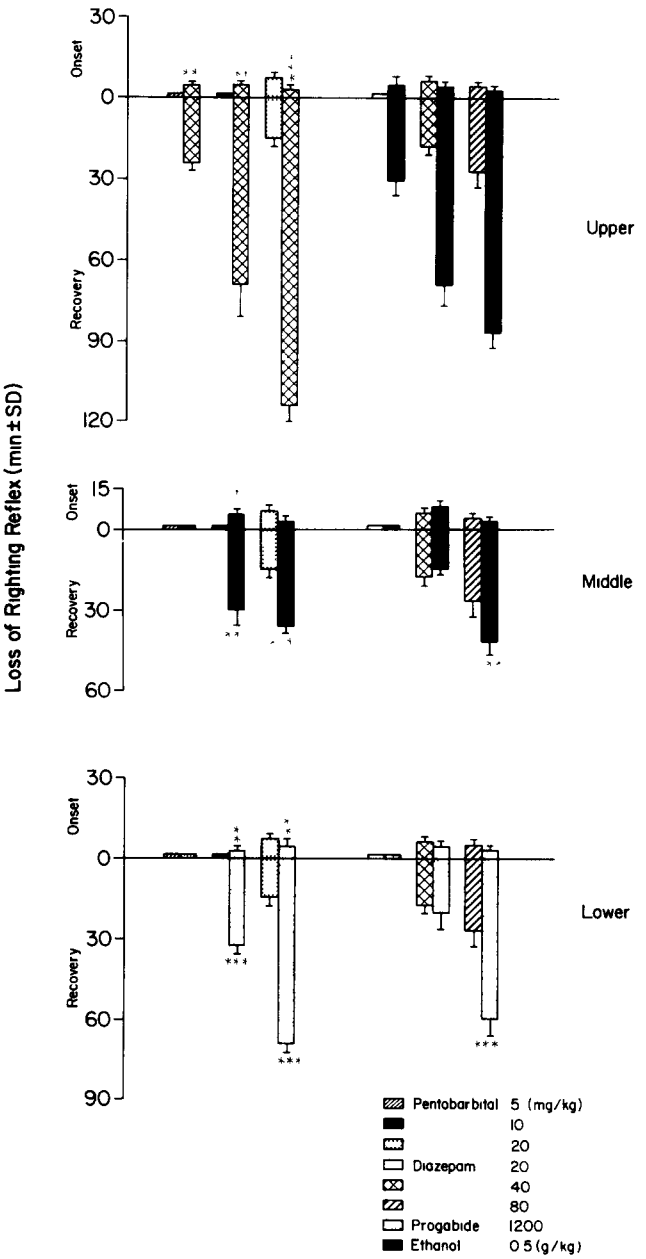

FIG 3 Effects of subprotective doses of pentobarbital (left panel) or diazepam (right panel) in combination with single ineffective dose of various GABA-mimetics on the loss of righting reflex in rats Pentobarbital (5, 10 and 20 mg/kg) was administered simultaneously with single dose of diazepam (40 mg/kg, left upper panel), ethanol (0 5 g/kg, left middle panel) and progabide (1200 mg/kg, left lower panel) or different doses of diazepam (20, 40 and 80 mg/kg) injected with a single dose of pentobarbital (10 mg/kg, right upper panel), ethanol (0 5 g/kg, right middle panel), progabide (1200 mg/kg, right lower panel) administered 30 min prior to pentobarbital or diazepam treatment Sleep time was recorded as the duration between loss and recovery of righting reflex The onset and recovery of the righting reflexes are shown as mean±S D of 5–7 animals in minutes $*p<0.05$, $**p<0.01$, $**p<0.001$, as compared to control values of pentobarbital (20 mg/kg, left panel) and diazepam (40 mg/kg, right panel) by a one-way analysis of variance using the Student Newman-Keuls procedure Note that the pentobarbital produced a higher degree of potentiation in combination with GABA modulators, as compared to diazepam

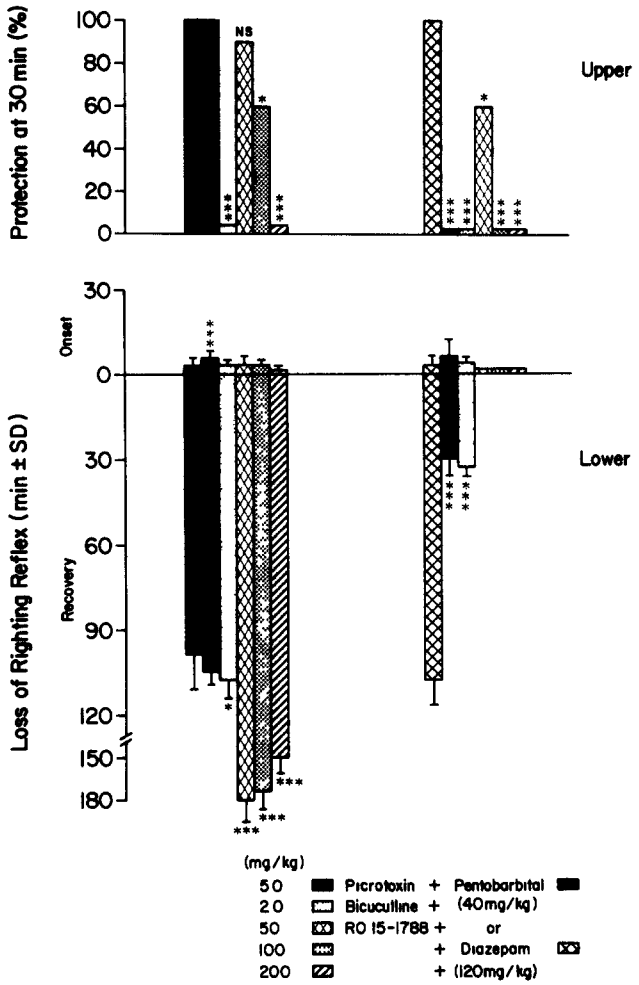

FIG 4. Effects of GABA (picrotoxin and bicuculline) and benzodiazepine (RO15-1788) antagonists on the anticonvulsant (upper panel) or diazepam (right panel) in rats Picrotoxin (5 mg/kg), bicuculline (2 mg/kg) and RO15-1788 (50, 100 and 200 mg/kg) were administered IP 60 min, whereas pentobarbital (40 mg/kg) or diazepam (120 mg/kg) 30 min prior to exposure of electroshock. The duration of onset and recovery of the righting reflexes are shown as mean±S D. of 5–7 animals in minutes Fisher exact proability test shows that the anticonvulsant effect of GABA and benzodiazepine antagonists in combination with pentobarbital (40 mg/kg, left upper panel) or diazepam (120 mg/kg, right upper panel) differ from control rats (i e , pentobarbital or diazepam) $*p<0$ 05, $**p<0$ 001 Sleep-time data reveal that the rats treated with GABA or benzodiazepine antagonists, in combination with pentobarbital (40 mg/kg, lower left panel) or diazepam (120 mg/kg, lower right panel) differ from control (i e , pentobarbital or diazepam) at $*p<0.05$, $**p<0$ 01, as compared by Student Newman-Keuls procedure

receptor-ionophore complex [25,26]. *In vitro* studies have also demonstrated that pentobarbital, while inhibiting the binding of [^{35}S]TBPT [24,26], enhance the binding of [^{3}H]GABA and [^{3}H]-benzodiazepine agonists to brain membranes [8, 11, 18, 21, 22]

Despite differences in dosages of diazepam and ethanol (more than 3 and 50 times, respectively) in comparison to pentobarbital, a significantly high correlation was found with regard to their anticonvulsant activity These results are consistent with the hypothesis that barbiturates and benzodiazepines may mediate their effects through facilitation of GABAergic transmission. A considerable body of evidence indicates that the mechanism of action of the benzodiazepines is closely associated with GABAergic function [2–5, 16]. The potentiation of GABA-mediated transmission by benzodiazepines at both electrophysiological [4,19] and behavioral levels [2,5] suggest that the benzodiazepines may potentiate the synaptic actions of GABA. Neurophysiological evidence also supports the concept that barbiturates mediate their effects via GABAergic mechanisms [10, 14, 15] Recent receptor binding studies have shown that pentobarbital not only increases GABA and benzodiazepine receptor binding [8, 16, 18, 21, 22, 24, 29], but also potentiates the ability of GABA to enhance benzodiazepine binding [18]. Likewise, ethanol has been shown to facilitate GABAergic transmission [1,13] and to interact with GABA-benzodiazepine receptor complex [23].

Further, evidence that pentobarbital acts by enhancing central GABA-ergic transmission comes from the drug-interaction studies In this report, we show that the combined treatment of acute low doses of pentobarbital (a dose without an anticonvulsant effect), with sub-effective doses of various modulators of GABAergic transmission (diazepam, ethanol and a GABA receptor agonist progabide, which had insignificant effect by themselves) resulted in a prolonged protection against electroshock-induced seizures. These results are also in line with the notion that these depressant and anticonvulsant drugs mediate their effects by facilitating GABA-ergic transmission in the CNS [1–4, 8, 10, 13–19, 21–24]. The most likely result of a generalized enhancement of GABAergic transmission would be an inhibition of neuronal firing throughout the neuroaxis, thus reducing the probability of seizures Progabide is a pro-GABA drug and has been reported to possess anticonvulsant activity [32] The reason for the lack of anticonvulsant effect of progabide in our study is not clear. Differences in species, duration and intensity of current could be some of the possible factors involved in the lack of progabide's action [9,32].

The agonist/antagonist results also support the notion that pentobarbital's anticonvulsant effect could be due to an interaction with GABAergic system through a bicuculline-sensitive mechanism The ability of bicuculline to block the effect of pentobarbital is consistent with the role of GABAergic system in the anticonvulsant action of pentobarbital. The lack of antagonism of pentobarbital's anticonvulsant effect by picrotoxin could be due to the use of sub-convulsive doses of picrotoxin in the present study. One may argue that it is not a fair comparison of picrotoxin and bicuculline doses for the antagonism of anticonvulsant effect, but the limitation with these agents in behavioral experiments is that one cannot push the dose up very much, since the animals develop convulsions and subsequent death

The anticonvulsant effect of diazepam is apparently mediated by the benzodiazepine receptors, since it was antagonized by RO15-1788. The anticonvulsant activity of pentobarbital was also antagonized by RO15-1788, albeit at high doses, suggesting that activation of the benzodiazepine receptors may be necessary for the anticonvulsant effect of pentobarbital. These results are in argeement with *in vitro* binding studies that show pentobarbital does indeed increase the binding of GABA and benzodiazepine agonists to their receptor sites [8, 18, 21, 22].

The hypnotic effect of pentobarbital and other facilitators of GABAergic transmission have been well reported. It is

interesting to note that the time of peak effect for pentobarbital, in lower doses, was different for hypnotic than for seizure protection However, at higher dose, no characteristic distinction could be made The duration of sleep time of drug alone or in combination was less than when seizure protection was maximal (Figs. 2 and 3) GABA antagonists (picrotoxin and bicuculline) did not affect pentobarbital sleep time, however, they caused a significant antagonism of diazepam effects. Conversely, RO15-1788 causes complete antagonism of diazepam sleep time, while pentobarbital's sleep time was potentiated The ability of RO15-1788 to increase pentobarbital's sleep time suggests that it may have a benzodiazepine-like action at high doses The precise mechanism of hypnotic components of pentobarbital is not yet fully understood

In conclusion, our data are in agreement with the general hypothesis that enhancement of GABAergic transmission may be critical for the anticonvulsant action of pentobarbital Furthermore, the results also suggest that hypnotic action probably does not play a role in the anticonvulsant action of pentobarbital

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