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Involvement of a GABAergic Mechanism in the Pharmacologic Action of Phenytoin

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CHWEH, A. Y., E. A. SWINYARD AND H. H. WOLF. *Involvement of a GABAergic mechanism in the pharmacologic action of phenytoin.* PHARMACOL BIOCHEM BEHAV **24**(5) 1301–1304, 1986.—*In vivo* interactions between phenytoin (PHT) and baclofen (a $GABA_B$ receptor agonist) or PHT and progabide (a $GABA_B$ receptor agonist) were investigated by the rotorod minimal neurotoxicity test and maximal electroshock seizure (MES) test. The combination of PHT and baclofen produced an additive effect by the rotorod test, whereas the combination of PHT and progabide elicited a supra-additive (synergistic) effect. The median minimal neurotoxic dose of baclofen augmented the anti-MES activity of PHT. The combination of PHT and progabide induced a supra-additive effect by the MES test. These results imply that $GABA_B$ receptors are involved in both the minimal neurotoxicity and anti-MES activity of PHT.

GABA Maximal electroshock Phenytoin Seizure

PHENYTOIN (PHT) is the drug of choice for simple and complex partial seizures as well as generalized tonic-clonic seizures [1]. In animals, PHT is very effective against seizures induced by maximal electroshock [14]. However, the cellular mechanisms of action of PHT remain uncertain. Numerous studies show that PHT inhibits Na^+ and Ca^{++} fluxes [4, 12, 13, 16, 17], modifies Na^+, K^+-transport [8,10], increases brain γ-aminobutyric acid (GABA) level [2,18], and potentiates GABA-mediated postsynaptic inhibition [6,15]. Work by Czuczwar *et al.* [5] indicates that the $GABA_B$ receptor agonist muscimol enhances the anticonvulsant activity of PHT, but the $GABA_B$ receptor agonist baclofen does not influence it. An electrophysiological study [15] suggests that PHT potentiation of postsynaptic GABA function may be related to the neurotoxicity of PHT but it is not linked to the anticonvulsant action of PHT. Therefore, the present study was undertaken to explore further the GABA involvement in the minimal neurotoxicity and anticonvulsant activity of PHT.

METHOD

Male albino mice (CF No. 1 strain, 20 to 30 g wt., Charles River Breeding Laboratories, Wilmington, MA) were used for all studies herein. PHT was suspended in 0.9% sodium chloride solution, whereas baclofen and progabide were suspended in 30% aqueous polyethylene glycol 400 and 1% aqueous Tween 80, respectively. All drugs were administered intraperitoneally in a volume of 0.01 ml/g body weight. The minimal neurotoxicity and anticonvulsant tests were conducted at the previously determined time of peak effect for each substance.

Drug Combination

Drug combination studies were performed by the method described by Weaver *et al.* [25]. PHT, baclofen, and progabide are all effective by the rotorod neurotoxicity test. Therefore, when PHT was used in combination with either baclofen or progabide, each drug was given in such proportions (doses) that the sum of the individual effectiveness equals the desired level of response (in studies reported herein, 50%). The doses employed were determined by means of the following equation: ED_x of drug A + (ED_{50} of drug B − ED_x of drug B) = ED_{50} of the combination (where x = any selected level of response). PHT and progabide were effective by the maximal electroshock seizure (MES) test. Thus, the two drugs were combined as described above for the anticonvulsant test. Baclofen was ineffective by the MES test; thus, the median minimal neurotoxic dose (TD_{50}) and $^1/_2TD_{50}$ of baclofen were used when combined with PHT. The response observed was described as either additive or supra-additive (synergistic, *i.e.*, potentiates).

Minimal Neurotoxicity Studies

Minimal neurotoxicity was measured by the rotorod test [7,22]. When a normal mouse is placed on a knurled rod (2.5 cm diameter) that rotates at a speed of 6 rpm, the mouse can maintain its position for long periods of time. Neurological deficit is indicated by inability of the mouse to maintain its equilibrium for 1 min on this rotating rod in each of 3 trials. To determine the median minimal neurotoxic dose (TD_{50}), groups of eight mice each were tested with various doses of the drug until at least four points were established between the limits of 100% toxicity and 0% toxicity.

TABLE 1

MINIMAL NEUROTOXICITY AND ANTICONVULSANT POTENCY OF PHENYTOIN, BACLOFEN, AND PROGABIDE IN MICE

Substance	Time of Test (min)	TD_{50} (mg/kg)	MES ED_{50} (mg/kg)
Phenytoin	120, 120	64.2 (57.9–73.0) [13.4 ± 4.22]	9.50 (8.10–10.4) [13.7 ± 3.89]
Baclofen	60, 60	13.9 (12.3–15.8) [14.0 ± 3.82]	No activity up to 40
Progabide	15, 30	603 (401–919) [3.81 ± 1.19]	334 (307–352) [24.5 ± 9.70]

( ) 95% confidence interval.
[ ] slope of regression line ± S.E.

TABLE 2

THE EFFECT OF COMBINATIONS OF PHENYTOIN AND BACLOFEN OR PHENYTOIN AND PROGABIDE ON MINIMAL NEUROTOXICITY IN MICE

Combinations	Dose (mg/kg)	No. Animals Tested	Expected No. Animals Toxic	Actual No. Animals Toxic
Phenytoin, TD_{25}	56.0			
+ Baclofen, TD_{50}–TD_{25}	1.40	24	12	11
Baclofen, TD_{25}	12.5			
+ Phenytoin, TD_{50}–TD_{25}	8.20	24	12	10
Phenytoin, TD_{25}	56.0			
+ Progabide, TD_{50}–TD_{25}	203	24	12	24*
Progabide, TD_{25}	400			
+ Phenytoin, TD_{50}–TD_{25}	8.20	24	12	18*

*Significantly different ($p<0.01$) from expected number.

The TD_{50} and the 95% confidence interval were calculated by means of a computer program based on probit analysis [9].

Anticonvulsant Studies

Anticonvulsant activity was determined by the maximal electroshock (50 mA, 60 Hz, 0.2 sec, corneal electrodes) seizure test [22]. Abolition of hindlimb tonic extension was taken as the endpoint. To determine the anticonvulsant potency (MES ED_{50}), groups of eight mice each were tested with various doses of the drug until at least four points were established between the limits of 100% protection and 0% protection. The MES ED_{50} and the 95% confidence interval were calculated by means of a computer program based on probit analysis [9].

Statistical Analysis

The significance of the differences between expected values and observed values was calculated by the χ^2 test.

RESULTS

Minimal Neurotoxicity and Anticonvulsant Potency

The minimal neurotoxicity and anticonvulsant potency of the three compounds are shown in Table 1. It may be seen from the table that baclofen was the most toxic (TD_{50}: 13.9 mg/kg) and progabide the least toxic (TD_{50}: 603 mg/kg). Phenytoin had a TD_{50} of 64.2 mg/kg. With respect to anticonvulsant activity as measured by the MES test, PHT and progabide were effective in nontoxic doses (ED_{50}s: 9.5 and 334 mg/kg, respectively); whereas baclofen was ineffective

TABLE 3

THE EFFECT OF COMBINATIONS OF PHENYTOIN AND BACLOFEN OR PHENYTOIN AND PROGABIDE ON MAXIMAL ELECTROSHOCK SEIZURE ACTIVITY IN MICE

Combinations	Dose (mg/kg)	No. Animals Tested	Expected No. Animals Protected	Actual No. Animals Protected
Phenytoin, ED_{50}	9.50			
+ Baclofen, $^1/_2TD_{25}$	6.95	24	12	14
Phenytoin, ED_{50}	9.50			
+ Baclofen, TD_{50}	13.9	24	12	20*
Phenytoin, ED_{25}	8.40			
+ Progabide, ED_{50}–ED_{25}	14	24	12	21*
Progabide, ED_{25}	320			
+ Phenytoin, ED_{50}–ED_{25}	1.10	24	12	21*

*Significantly different ($p<0.01$) from expected number.

even in toxic doses (up to 40 mg/kg). It should also be noted that the slopes of the rotorod dose-response curves for baclofen and progabide and the MES dose-response curve for progabide are parallel with those for PHT (p values, 0.12). (Parallelism of phenytoin and progabide toxicity and MES regression lines was determined by means of a computer program based on the method described by Litchfield, Jr. and Wilcoxon, *J Pharmacol Exp Ther* **96:** 99–113, 1949.)

Effect of Drug Combinations on Minimal Neurotoxicity

The effect of combinations of PHT and baclofen or PHT and progabide on minimal neurotoxicity in mice is summarized in Table 2. Combinations of PHT and baclofen (PHT TD_{25} + baclofen TD_{50}–TD_{25} and baclofen TD_{25} + PHT TD_{50}–TD_{25}) induced toxicity in 11 and 10 mice, respectively, out of 24 animals tested. When PHT was combined with progabide (PHT TD_{25} + progabide TD_{50}–TD_{25} and progabide TD_{25} + PHT TD_{50}–TD_{25}), 24 and 18 mice, respectively, were toxic out of the 24 animals tested.

Effect of Drug Combinations on Maximal Electorshock Seizures

The effect of combinations of PHT and baclofen or PHT and progabide on maximal electroshock seizure activity in mice is shown in Table 3. When PHT (ED_{50}) was administered with $^1/_2TD_{50}$ or TD_{50} of baclofen, 14 and 20 mice, respectively, of the 24 tested were protected from seizures induced by maximal electroshock. Combinations of PHT and progabide (PHT ED_{25} + progabide ED_{50}–ED_{25} and progabide ED_{25} + PHT ED_{50}–ED_{25}) protected 21 and 21, respectively, out of 24 animals.

DISCUSSION

The results presented herein show that PHT, baclofen, and progabide all exhibit neurotoxicity by the rotorod test and that only PHT and progabide effectively prevent MES. Combinations of PHT and baclofen produce an additive effect by the rotorod test, whereas combinations of PHT and progabide elicit a supra-additive (synergistic; *i.e.*, potentiates) effect by this test. With respect to the MES test, *median neurotoxic doses* of baclofen potentiate the anti-MES activity of PHT. PHT and progabide, in *nontoxic* doses, interact synergistically against MES. These observations indicate that interactions between PHT and progabide by both rotorod and MES tests are more profound than those of PHT and baclofen. Thus, it is conceivable that $GABA_a$ receptors may contribute to PHT neurotoxicity and anticonvulsant activity since baclofen is a potent $GABA_b$ receptor agonist [11] and progabide is a $GABA_a$ receptor agonist [3]. This notion is supported by the fact that PHT potentiates postsynaptic GABA function mediated through $GABA_a$ receptors [6,15].

McLean and Macdonald [15] have reported that PHT potentiation of GABA function is related to PHT neurotoxicity rather than to PHT anticonvulsant activity. Their analysis is based on the fact that PHT potentiates GABA function *in vitro* at concentrations of 5 and 10 μg/ml, 2 to 5 times above the range of clinically useful concentrations. Since rodents require approximately 2 to 4 times as much PHT as do humans [12,21], the concentrations (5 to 10 μg/ml) of PHT to potentiate GABA function *in vitro* are effective anticonvulsant concentrations in rodents. In addition, Sironi *et al.* [19] have demonstrated that whole brain therapeutic concentrations of PHT in man and animals are up to 10-fold higher than the responsive cerebrospinal fluid (CSF) levels.

Skerritt and Johnston [20] have demonstrated that PHT does not interact with postsynaptic GABA receptors. This raises a question as to how PHT enhances postsynaptic GABA function. According to Ticku *et al.* [23] PHT interacts with picrotoxin-sensitive barbiturate receptors in the GABA receptor ionophores. Since picrotoxin-sensitive barbiturate receptors are associated with GABA receptors-coupled chloride ionophores [24], an interaction of PHT with these barbiturate receptors could potentiate GABAergic function. This notion is supported by reports that PHT enhances GABA receptor-regulated, chloride-dependent inhibitory postsynaptic potentials [6] and that PHT augments postsynaptic response to ionophoretically applied GABA [15]. These observations suggest that PHT interacts with barbiturate receptors and enhances GABA function via prolonging GABA receptor-regulated chloride conductance.

In summary, the data presented show that baclofen, a $GABA_b$ receptor agonist [11], interacts additively with PHT

by the rotorod toxicity test and augments the anti-MES activity of PHT in the median minimal neurotoxic dose. However, progabide, a $GABA_B$ receptor agonist [3], interacts synergistically with PHT by the rotorod toxicity and MES tests. The latter results indicate that $GABA_B$ receptors contribute to both neurotoxicity and anti-MES activity of PHT.

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