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Comparison of Anticonvulsive Properties of Eboracin and Phenytoin in Mice

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ALEXANDER, G J , R B ALEXANDER, L. M KOPELOFF AND N CHATTERJIE *Comparison of anticonvulsive properties of eboracin and phenytoin in mice* PHARMACOL BIOCHEM BEHAV 22(1) 53–55, 1985 —The *in vivo* effects of phenytoin (diphenylhydantoin, Dilantin) and the experimental anticonvulsant, eboracin, a substituted indenopyrrole, were compared in mice Pretreatment with varying dosages of either agent followed by challenge with the chemoconvulsant pentylenetetrazol (Metrazol) indicated that eboracin provided slightly less protection against seizures than phenytoin and was much less toxic Intermediate doses of either agent led to a form of clonic status epilepticus which persisted for an average of 18 min in phenytoin-treated and 58 min in eboracin-treated mice. Pretreatment with higher or lower doses did not lead to these manifestations Animals in which this syndrome had been induced should be of value in studies of the chemistry and physiology of the clonic state

Eboracin Phenytoin Pentylenetetrazol Seizures Status epilepticus Myoclonus, prolonged

THE recently described heterocyclic anticonvulsant, eboracin, was found to protect mice against convulsive seizures produced by pentylenetetrazol (Metrazol), electroshock or auditory stimulation [1, 2, 5]. In animals pretreated with eboracin at dosage levels below those needed for full protection, Metrazol elicited myoclonic manifestations characterized by acute flailing of extremities. These persisted for 30–120 min without spread to the tonic phase. A somewhat similar but less intense syndrome was observed after Metrazol in mice pretreated with phenytoin (Dilantin, Parke-Davis). We now present Metrazol-seizure data obtained in mice pretreated with eboracin and phenytoin under matched conditions

METHOD

Male albino mice, 20–30 g, from our own colony, derived from the Swiss-Webster strain, were housed 6–10 per cage and maintained on Teklad Chow and tap water ad lib.

Eboracin was synthesized by one of us (N.C.) by condensation of methyl-3-crotonate with ninhydrin [1,2] in a procedure described earlier [6]. The sodium salt of phenytoin (diphenylhydantoin) was purchased from K and K Laboratories, Plainview, NY. Both compounds were dispersed in 1% aqueous Tween-20 vehicle. The Tween vehicle served as placebo. Pentylenetetrazol (Metrazol) was purchased from the Knoll Pharmaceutical Company, Orange, NJ, as a 10% solution and diluted 1.30 with deionized water. All solutions were injected intraperitoneally

After injection with eboracin (15–300 mg/kg) or phenytoin (10–50 mg/kg) or placebo (10 ml/kg), 120 animals were observed for 90 min and then challenged with Metrazol, 67 mg/kg. The duration of specific phases of individual Metrazol-induced seizures was recorded. Toxic effects of higher doses of eboracin (300–2,000 mg/kg) and phenytoin (100–350 mg/kg), without Metrazol, were determined in 100 additional mice. Lethality was based on deaths within 72 hr.

Doses which decreased the incidence of maximal Metrazol seizures by 50% (ED_{50}), doses which led to post-Metrazol prolonged flailing in 50% of the subjects (PF_{50}) and doses which led to 50% mortality (LD_{50}) were calculated by regression analysis. Statistical validity of differences between control and treated groups was determined with a Student *t* test for unpaired samples. All calculations were performed on an APPLE II microcomputer.

RESULTS

Treatment with either eboracin (15–300 mg/kg) or phenytoin (10–50 mg/kg) did not produce any observable changes in behavior within 90 min after treatment. Mice pretreated with either eboracin or phenytoin and challenged 90 min later with 67 mg/kg Metrazol responded with fewer maximal clonic-tonic seizures than placebo-pretreated controls (Table 1). When increasing doses of eboracin and phenytoin were tested, it was found that complete protection against tonus was reached at 150 mg/kg eboracin and complete elimination of all seizure manifestations at 300 mg/kg. Corresponding values with phenytoin were 30 and 50 mg/kg, respectively.

At intermediate dosages of either compound a *status*-like myoclonic syndrome of prolonged flailing of extremities (PF) appeared after Metrazol challenge As the number of animals

TABLE 1

RESPONSE TO METRAZOL IN MICE PRETREATED WITH EBORACIN OR PHENYTOIN

Eboracin					Phenytoin				
Dose mg/kg	n	PF %	Tonus %	Deaths %	Dose mg/kg	n	PF %	Tonus %	Deaths %
0	10	0	100	50	0	10	0	100	50
15	10	0	60	30	10	4	0	100	50
30	10	0	40	20	20	10	0	50	50
75	10	60	20	20	30	10	70	0	50
150	10	100	0	20	40	10	100	0	0
300	10	0	0	0	50	10	0	0	0

PF=prolonged flailing syndrome, Metrazol, 67 mg/kg, administered 90 min after pretreatment with eboracin or phenytoin Flailing or tonic seizures began 3–15 min after Metrazol injection

exhibiting maximal clonic-tonic seizures diminished, the proportion responding with the new syndrome increased, peaking at 150 mg/kg eboracin and 40 mg/kg phenytoin, and decreasing at higher doses. At peak response, the symptomatology of the myoclonic state was similar after both anticonvulsants except that after eboracin the PF motor movements were stronger, more frequent, more extensive and more than three times longer in duration (Fig. 1). The use of higher dosages of either drug did not intensify or prolong the PF manifestations but rather decreased their incidence In all cases, flailing animals showed a loss of muscle tone and increased perspiration in proportion to the severity and duration of the symptoms Phenobarbital, in small doses, administered 2 min after onset, abolished all flailing manifestations.

The ED_{50} protective dose against maximal Metrazol seizures was found to be 28 mg/kg for eboracin and 22 mg/kg for phenytoin (Table 2) The death rates at these concentrations were 20% and 50%, respectively, the latter value similar to that obtained in the control group The dose for induction of the prolonged myoclonic state in 50% of the subjects (PF_{50}) was calculated to be 70 mg/kg for eboracin and 28 mg/kg for phenytoin However, it required 4.3 times as much eboracin (300 mg/kg) and only 1 8 times as much phenytoin (50 mg/kg) to completely prevent the PF manifestations

Toxicity of the two anticonvulsants alone, without Metrazol, was observed after treatment with high doses (>300 mg/kg eboracin; >50 mg/kg phenytoin) Doses of eboracin between 300–500 mg/kg, and phenytoin between 50–100 mg/kg, were toxic but not lethal, and significantly decreased spontaneous motility and reaction to handling Higher doses led to total immobility, labored breathing, excessive perspiration, loss of body tone and, finally, death LD_{50} of eboracin was 830 mg/kg, of phenytoin 250 mg/kg (Fig 2) A calculation of the therapeutic effectiveness of each compound against maximal Metrazol seizures divided by its corresponding LD_{50} indicated that the safety factor for eboracin was almost three times greater than that for phenytoin (Table 2) The safety factor against the PF syndrome also favored eboracin over phenytoin

DISCUSSION

Pretreatment with phenytoin (Dilantin) can at times lead to a prolongation of the clonic phase of seizures induced by electroshock [8] In the case of seizures induced by Metrazol

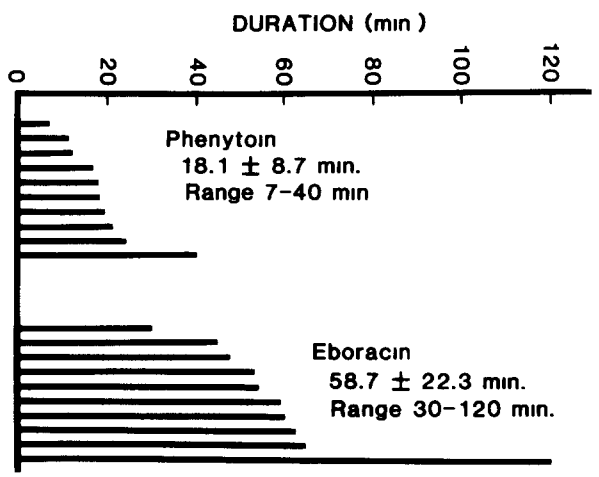

FIG 1 Duration of the myoclonic state induced by Metrazol in individual mice pretreated with eboracin or phenytoin

or picrotoxin, phenytoin inhibited the tonic phase without reduction in clonic activity These findings implied that phenytoin did not alter convulsive thresholds but prevented the progression to the repetitive hyperexcitability of the tonic phase Thus, it blocked spread without affecting initiation of seizures In our experiments, pretreatment of mice with phenytoin followed by challenge with Metrazol led to enhanced clonic activity and inhibition of tonus Pretreatment with the experimental anticonvulsant, eboracin, followed by Metrazol, also resulted in prolonged flailing (PF) and inhibition of tonus [1,2]

In the present report we compared the two agents, phenytoin and eboracin, in terms of their anti-Metrazol activity, lethality and, in particular, their ability to cause the post-Metrazol PF syndrome It became evident that while eboracin was somewhat less anticonvulsant than phenytoin it had a greater margin of safety because of its lower toxicity (Table 2) More eboracin than phenytoin was required for induction of the PF syndrome and also for elimination of all seizure manifestations Once induced, the eboracin/Metrazol PF syndrome was more severe and of much longer duration than that due to phenytoin and Metrazol (58 7 vs 18.1 min) Eboracin-pretreated animals should, therefore, be particularly suitable for studies of the clonic state

TABLE 2

A COMPARISON OF EFFECTS OF EBORACIN AND PHENYTOIN

Effect	Eboracin	Phenytoin
50% protection from Metrazol-induced seizures (ED_{50})	28 mg/kg	22 mg/kg
50% induction of prolonged post-Metrazol flailing (PF_{50})	70 mg/kg	28 mg/kg
Duration of post-Metrazol flailing (Avg±S D)*	58 7 ± 22 3 min	18.1 ± 8 7 min
Toxicity (LD_{50})	830 mg/kg	250 mg/kg
Therapeutic index (ED_{50}/LD_{50})	0 03	0.09
Therapeutic index (ED_{50}/PF_{50})	0 40	0.78

*$p<0$ 01 (Student t test for difference between the two groups)

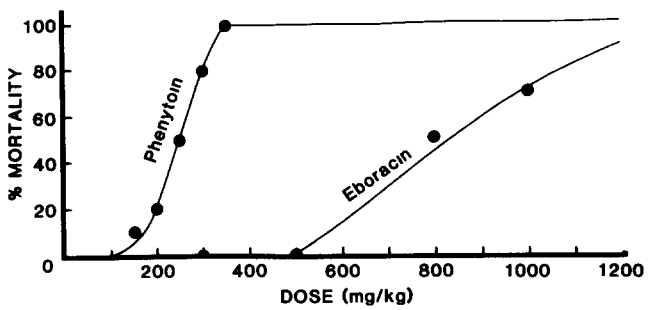

FIG 2 Lethality of eboracin and phenytoin

The mode of action of phenytoin is known to be complex and multifaceted, while that of eboracin is virtually unknown. Our findings suggest that, among possible mechanisms, both compounds may: (a) interfere with neurotransmitters involved in spread of seizures to the tonic phase, thus maintaining the test animal in mid-seizure; (b) react with Metrazol; or (c) compete with Metrazol for active sites. The latter two mechanisms would decrease Metrazol effectiveness and lengthen its availability.

Knowledge of the role of neurotransmitters in initiation and spread of experimental or idiopathic epilepsies is extensive but fragmented. It is known, however, that transmitters or their antagonists can produce symptoms akin to those reported here. We have observed a comparable prolongation of clonic *status epilepticus* in inbred audiosensitive mice pretreated with 6-hydroxydopamine, a norepinephrine antagonist, following a challenge with auditory signals [4]. A similar phenomenon was observed in animals treated with near-toxic doses of the antiarrhytmic drug, mexiletine, which by itself induced a prolonged clonic state accompanied by dryness of skin, dilatation of pupils, labored breathing and decrease in urination [3] A different but perhaps related phenomenon of "limbic *status epilepticus*" was reported in rats after intra-amygdaloid injections of acetylcholine agonists or acetylcholinesterase inhibitors. This phenomenon, said to parallel human temporal lobe epilepsy, was characterized by slow onset, repetitive rearing and sustained clonus of forepaws [7].

In experimental seizures the clonic phase which precedes progression to the clonic-tonic and tonic phases is usually brief, lasting a few seconds. In the present study this clonic component has been greatly extended by means of pretreatment with anticonvulsant agents whose main action is directed against spread of neuronal discharges This technique should provide a convenient model for the study of (a) brain chemistry and physiology during the clonic state, (b) endogeneous and exogeneous factors which enhance or inhibit that state and (c) factors affecting the transition from clonus to the subsequent phases of seizure (i.e., spread to tonus)

Through the use of incremental quantities of eboracin or phenytoin we demonstrated the feasibility of modifying the course of Metrazol-induced seizures: large doses of either agent prevented the convulsive manifestations, but moderate doses exaggerated the intermediate (i.e., clonic) stage by interfering with spread but not with the initiation of seizures. Eboracin induced the prolonged clonic state in mice more effectively and for longer periods than phenytoin.

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