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Impaired Motor Coordination in Mice Induced by 2-Amino-7-Phosphonoheptanoic Acid (APH), Glutamic Acid Diethyl Ester (GDEE), and Other Compounds

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FREED, W. J. *Impaired motor coordination in mice induced by 2-amino-7-phosphonoheptanoic acid (APH), glutamic acid diethyl ester (GDEE), and other compounds.* PHARMACOL BIOCHEM BEHAV **32**(3) 733–736, 1989.—Impairment of motor coordination by the excitatory amino acid antagonists 2-amino-7-phosphonoheptanoic acid (APH) and glutamic acid diethyl ester (GDEE) was measured and compared to GABA agonists and anticonvulsants and other compounds by the Coughenour inverted screen test. The GABA agonists muscimol and imidazole acetic acid, and the GABA analogue gamma-hydroxybutyric acid were found to produce a marked impairment of motor coordination. The dosages of phenytoin and valproate which impaired motor coordination, on the other hand, were considerably above the dosages which have been reported to inhibit seizures. APH caused motor incoordination at a dosage of 125 mg/kg, and a prolonged motor impairment was present after administration of APH, 250 mg/kg. GDEE did not significantly impair motor coordination in any dosage tested up to 1920 mg/kg. These results further encourage development of more potent GDEE-like compounds as potential anticonvulsants.

Seizures Glutamic acid diethyl ester 2-Amino-7-phosphonoheptanoic acid Excitatory amino acids Glutamic acid Anticonvulsants Motor impairment Toxicity

THERE has recently been a considerable interest in the possible use of excitatory amino acid antagonists as anticonvulsants (5, 7, 11, 12, 17, 23, 25). There appear to be three separate CNS receptors for excitatory amino acids, which can be distinguished by their responses to agonists and antagonists (2, 15, 19, 26). These receptors have been designated AA_1, AA_2, and AA_3 (10). The AA_3 receptor is stimulated by N-methyl-d-aspartic acid and inhibited by 2-amino-5-phosphonovaleric acid and 2-amino-7-phosphonoheptanoic acid (APH). The most selective agonists of the AA_2 receptor are quisqualic acid and α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA). Glutamic acid diethyl ester (GDEE) is a selective antagonist of this receptor. Kainic acid is a selective agonist of the AA_3 receptor.

Both APH and GDEE appear to cross the blood-brain barrier, at least to some degree (3,9). APH blocks or attenuates experimental seizures of several types, including those induced by electroconvulsive shock, pentylenetetrazole, and sensory stimulation (5, 7, 14, 17, 25). Although the spectrum of anticonvulsant activity of GDEE is less broad, this compound has been reported to block seizures induced by ethanol withdrawal, homocysteic acid, increased atmospheric pressure, and quisqualic acid (11, 12, 21, 25). All of the known clinically-useful anticonvulsant drugs cause impaired motor coordination in animal models, usually in dosages somewhat higher than those required to inhibit seizures (13,24), and the degree to which anticonvulsants cause impaired motor coordination is widely used as an indicator of their potential toxicity. Loscher (14) has previously reported that dosages of APH required to block seizures induced in gerbils by air-blast stimulation produced sedation and impaired righting reflexes.

The purpose of the present study was therefore to measure the potency of APH and GDEE in causing impaired motor coordination in mice in order to further evaluate the potential therapeutic value of these agents. The inverted screen test of Coughenour *et al.* (4) was used to measure motor coordination. For purposes of comparison, the effects of two direct GABA agonists (muscimol and imidazole acetic acid), anticonvulsants (phenytoin and valproate), the GABA structural analogue gamma-hydroxybutyric acid, and the GDEE analogue, glutamic acid dimethyl ester, on motor coordination were also measured. Direct GABA agonists were chosen for comparison because of data suggesting interactions between glutamate and GABA receptors (8,18) and because both APH and GDEE act directly on glutamate receptors. Gamma-hydroxybutyric acid was chosen as a GABA structural analogue essentially lacking in anticonvulsant activity [cf. (6)].

METHOD

Animals

Female Swiss-Webster mice of the NIH general purpose strain were used for the experiment. Each animal was used only once. A

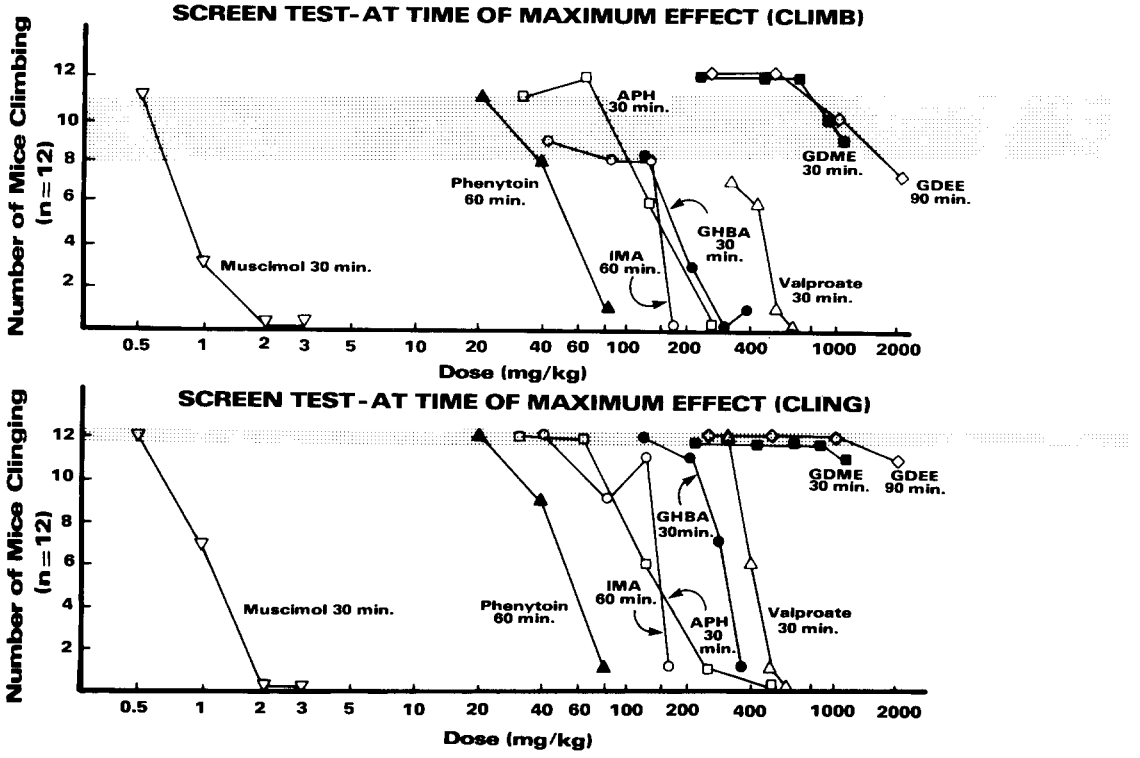

FIG. 1. Dose-response curves for impairment of climbing and clinging responses by various drugs. The dose-response curve is shown for each drug at the time point for which it was most effective. The shaded area (climbing) represents the mean ± S.D. of five groups of 12 saline-injected mice. For the clinging response, the mean of the vehicle-injected mice was 12 mice clinging (all vehicle-injected animals showed the clinging response). By Fisher's exact probabilities test, the incidence of climb failures required for statistical significance was 6 ($p=0.02$). Other p values are: 7 failures: $p=0.005$; 8 failures: $p=0.001$; and 9 or more failures: $p<0.0002$. The number of cling failures required for statistical significance was 2 ($p=0.03$). Other p values are: 3 failures: $p=0.004$; 4 failures: $p=0.0005$; and 5 or more failures: $p<0.0001$. Inverted open triangles represent muscimol, closed triangles represent phenytoin, open circles represent IMA, open squares represent APH, closed circles represent GHBA, open triangles represent valproate, closed squares represent GDME, open diamonds represent GDEE.

total of 540 mice were used in the experiment (a few preliminary runs and repeated or extraneous dosages are not reported).

Drugs and Injections

Imidazole-4-acetic acid HCl (IMA), gamma-hydroxybutyric acid (GABA), L-glutamic acid diethyl ester HCl (GDEE), L-glutamic acid dimethyl ester (GDME), and sodium 5,5-diphenyl-hydantoin (phenytoin) were obtained from Sigma Chemical Company. Muscimol was provided by Smith, Kline, and French Laboratories, Di-propylacetic acid (valproate) was provided by Abbott Laboratories, and 2-amino-7-phosphonoheptanoic acid was provided by the NOVA Pharmaceutical Corp. All compounds were injected IP in a volume of 10 ml/kg. The osmolarity was adjusted to approximately 300 milliosmolar with NaCl, except for the most concentrated solutions of GDEE and GDME, which were hypertonic. Vehicle controls received normal saline.

Procedure

Each drug was studied in a single, separate experiment. Each experiment included 60 mice (or 48 mice in a few cases), and twelve mice were tested at each dosage level. Each experiment usually, therefore, consisted of either five dosage levels of the drug or four dosage levels of the drug plus a vehicle group. The test for GDEE was repeated so that there were 24 mice for each dosage level.

The inverted screen apparatus (4) consists of six 13 cm by 13 cm squares of ¼″ mesh hardware cloth screen. Each square of screen is suspended on a post. A mouse is placed on a screen and the screen is inverted. If the mouse does not fall off the screen, it is scored as a success on the "cling" aspect of the test. If the mouse climbs with all four feet to the top of the screen, it is scored as succeeding on the "climb" aspect of the test. Each test is conducted for 60 sec after inversion of the screen. This test has been used in a number of recent experiments [for example, cf. (1,20)].

Mice were randomly assigned to groups and tested once ("pretest"). The purpose of the pretest was to stabilize the performance of the animals and to insure that most of the mice being tested in any experiment could perform the test. Animals that failed the pretest were *not* discarded. Animals then received drug or saline injections. Animals were retested at 30-min intervals, starting 30 min after drug injection and continuing for 2½ or three hours. Data (incidence of cling or climb failures) were analyzed by Fisher's exact probabilities test, using as a probability value the sum of the probabilities of the observed matrix and all more improbable matrices. ED_{50}'s were determined by Probit analysis.

RESULTS

On the pretest, there were 13 cling failures out of 540 mice tested (failure incidence of 2.4%) and 49 climb failures out of 540

TABLE 1

COMPARISON OF ED_{50}'s FOR IMPAIRMENT OF MOTOR COORDINATION FOUND IN THE PRESENT STUDY WITH MINIMUM DOSAGES OF DRUGS FOUND TO BE PHARMACOLOGICALLY EFFECTIVE AS REPORTED IN THE LITERATURE

	ED_{50}'s for Inhibition of Motor Coordination (mg/kg)*		Approximate Minimum Dosages Inhibiting Seizures†	
Drug	Cling	Climb	Dosage Range (mg/kg)	References
Muscimol	1.1	0.79 (0.62–1.01)	0.34–1.5	(6,13,16,22)
Phenytoin	50 (40–62)	42 (31–66)	2–70	(27)
IMA	130	108	82	(12)
APH	128 (96–161)	114	75–270	(5,7,14)
GHBA	280 (247–318)	144 (105–174)		
Valproate	400 (356–432)	333 (250–380)	40–400	(13)
GDME	—	1276 (1030–?)	848	(11)
GDEE	—	1.99×10^{10} (8010–?)	480–960	(11,12)

*ED_{50}'s determined by Probit analysis. Numbers in parentheses represent 95% fiducial limits. Fiducial limits are not shown where the computer program would not determine limits due to irregularities in the dose-response curve.

†All seizure models are considered together. It is apparent that some of the compounds, such as phenytoin, APH, and valproate, have a broad spectrum of anticonvulsant activity while others, especially imidazole acetic acid and GDME, can antagonize only one or a few types of experimental seizures. GHBA has essentially no anticonvulsant effect but is structurally similar to GABA.

Because the various compounds are not effective in the same seizure models, comparisons between compounds are not intended.

mice (incidence of 9.1%). Of 60 mice that received saline injections, zero mice had cling failures for any test after the pretest and 10 mice had at least one climb failure after the pretest.

The data were similar for the tests from 30 to 90 min, and for each drug the data were analyzed only for the time interval for which the maximum total number of failures was seen. Each of the drugs, with the exception of GDEE and GDME, caused a significant impairment on both the cling and climb aspects of the test (Fig. 1). The impairment induced by each of the drugs was dose-dependent (Fig. 1). In general, all or nearly all mice failed at a dosage approximately twice that which caused a 50% impairment. ED_{50} values for each drug are shown in Table 1.

As compared to saline injections, the incidence of cling failures required for statistical significance was 2/12 ($p = 0.03$). The probability of three or more failures was less than 0.005. The number of climb failures required for statistical significance was 6/12 ($p = 0.02$), and the probability of seven or more failures was less than 0.005. Neither GDEE nor GDME caused a statistically significant incidence of failures at any time interval even at the highest dosages tested, although there was clearly a tendency for a slight impairment at the highest dosages. Practical considerations precluded increasing the dosage of GDEE beyond 1920 mg/kg (8 mmol/kg). The time course of the effects of 250 mg/kg APH and 1920 mg/kg GDEE on the climb aspect of the test are shown in Fig. 2. APH caused an immediate and profound impairment, which partially recovered over the course of the following two

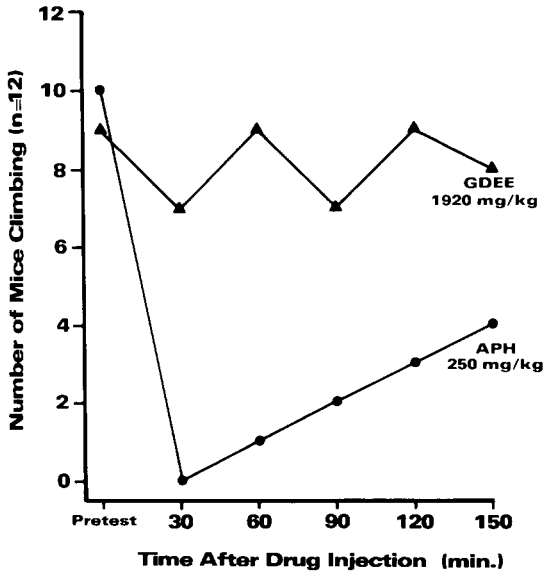

FIG. 2. Time-course of the impairment of the climb response induced by 250 mg/kg of APH and 1920 mg/kg of GDEE. Number of mice with successful climb responses (out of 12 mice tested) are shown for the pretest and five postinjection sessions.

hours. The largest dosage of GDEE caused only a slight impairment with no remarkable time course.

DISCUSSION

Muscimol, imidazole acetic acid, and APH caused impaired motor coordination in the same dosage range that has been reported to inhibit seizures in animals. Loscher (13) has previously reported that muscimol and other experimental GABA agonistic drugs have narrow margins of safety between anticonvulsant dosages and dosages which impair motor coordination. In several studies, IP injections of APH have been found to have anticonvulsant effects in dosages of from 75–180 mg/kg. Although no impairment was induced by 62.5 mg/kg of APH in the present study, 125 mg/kg of APH caused a significant impairment on both the cling and climb aspects of the test, and the impairment caused by 250 mg/kg of APH was both severe and prolonged. The reported anticonvulsant activity and brain concentrations of APH both match the time course of the impaired motor coordination, with a maximum effect after 30 min and a decline between 90 and 180 min (3). The minimum dosages of each drug which caused statistically significant motor impairment in the present experiment are compared with the general range of minimum dosages found to be pharmacologically effective in a variety of seizure models as reported in the literature in Table 1. Because APH and GDEE are efficacious in different seizure models, no direct comparison between the compounds can be made. These data do not, therefore, suggest that APH or related AA_1 antagonists are less likely to serve as clinically-useful anticonvulsants than GDEE-like compounds.

In contrast to muscimol and APH, the two standard anticonvulsants phenytoin and valproate have been reported to have anticonvulsant effects in dosages substantially below those found in inhibit motor coordination in the present experiment. In some experiments and by some measures, even these agents have often been employed as anticonvulsants in experimental studies in dosages near to those found in the present experiment to impair motor coordination. GHBA does not generally have anticonvul-

sant effects, but is structurally similar to GABA. Dosages of GHBA which have been employed in pharmacological studies are nevertheless close to the dosage range which was found to impair motor coordination.

The anticonvulsant effects of GDEE also appear to be somewhat limited. GDEE does not inhibit seizures induced by intracerebral glutamic acid, by audiogenic stimuli, or by pentylenetetrazole (5, 11, 23). A slight inhibition of photically-induced seizures in the baboon by GDEE has been reported (17). On the other hand, this compound does inhibit seizures induced by homocysteic acid, ethanol withdrawal, increased atmosphere pressure, and intracerebral quisqualic acid (11, 12, 21, 25). Significant impairments of motor coordination were not induced in the present experiment even by the highest dosages of this compound that could be administered. Thus, there appears to be a reasonable differential between anticonvulsant and central toxic dosages of GDEE in rodents. Systemic peripheral toxic side effects of GDEE have been noted in the baboon (17). These latter effects may be related to nonspecific systemic effects of GDEE or its metabolites caused by the high dosages of GDEE that are required, rather than to the central effects of this compound. It is possible, therefore, that if more potent analogues of GDEE or other AA_2 antagonists could be developed, the anticonvulsant spectrum of GDEE or similar compounds could be widened. Further consideration of analogues of GDEE as potential anticonvulsants is therefore supported by the present experiment.

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