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The Pharmacology of Seizures Induced by Sensitization with Low Intensity Brain Stimulation

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BABINGTON, R. G. AND P. W. WEDEKING. *The pharmacology of seizures induced by sensitization with low-intensity brain stimulation.* PHARMAC. BIOCHEM. BEHAV. 1(4) 461–467, 1973.–Daily low-intensity electrical stimulation of selected brain sites causes seizures to gradually develop. Because the limbic area is particularly susceptible to both the sensitization phenomenon and psychotropic drugs, we evaluated several classes of centrally-active agents for activity against these seizures. For comparative purposes, seizures elicited from the amygdala and a nonlimbic site, the sensorimotor cortex, were tested. Rats with chronically-implanted electrodes were stimulated for one min each day with a 50-μA, 60-Hz current. Initially, no overt effects occurred; but eventually seizures were established. Once the seizures stabilized, drug effects on seizure duration were measured. Antidepressive drugs were more potent in suppressing amygdaloid-elicited seizures than cortically-evoked seizures. Both antianxiety and antiepileptic drugs exerted nonselective blockade but the antianxiety agents were extremely potent in comparison to the antiepileptics. Representative neuroleptic drugs failed to block the seizures, even at doses completely debilitating the animals. Stimulants prolonged the seizures but the activity was weak. Thus, the low-intensity brain stimulation (LIBS) procedure has proved to be not only an interesting phenomenon, but one that responds differentially to various classes of CNS drugs.

Sensitization Seizures Amygdala Neocortex Anxiolytics Antidepressives Antiepileptics Neuroleptics Stimulants

IN 1967, Goddard reported that rats would latently develop seizures when various areas of the brain were sensitized by daily stimulation with low-intensity electrical current. Subsequently, Goddard *et al.* [6] published a more thorough investigation of the phenomenon, which they termed the "kindling effect".

The Goddard group examined primarily the physiological and anatomical bases of the kindling effect, but we were attracted to the procedure because limbic system sites were most susceptible to the conditioning of seizures. In conjunction with the evidence that the limbic system is particularly affected by CNS-active drugs [7,12], the predisposition of limbic sites to sensitization suggested that the seizure response might provide a sensitive method for detecting drugs with psychotropic activity.

This paper presents the neuropharmacologic effects of representative drugs from various classes of centrally-active agents on rats sensitized to seizure by low-intensity brain stimulation (LIBS) of the amygdala or a susceptible nonlimbic site, the sensorimotor cortex.

METHOD

Approximately 300 adult female Holtzman rats were implanted with a stainless steel bipolar electrode (Plastic Products MS 303) in the centromedial amygdala (AP = −0.4; L = 5.0; V = 9.0; Pellegrino and Cushman [10]) or epidurally on the sensorimotor cortex.

Beginning 10 days postoperatively, each animal was stimulated electrically once daily for 1 min with a 50-μA, 60-Hz constant current sine wave (Bio-Medical Electronics Model LS 112 stimulator). With the exception of a few of the cortically-implanted rats, none of the animals exhibited seizures during the first few stimulation sessions. After 8–10 days, bilateral, clonic seizures began to develop during the stimulation period. Once sensitization had been established, stimulation was continued only until the animal began to show signs of seizure, whereupon the current was shut off and the duration of the seizure was measured. Within 4 weeks, sensitization progressed so that according to individual animals, 5–7 sec of stimulation elicited seizures that lasted from 45–75 sec. On drug day, two control measurements were made 15 min apart. If the durations of the seizures did not vary by more than 10 percent, the pair of control values was averaged to serve as the baseline value. The test drug was then administered intraperitoneally as a solution in saline or a suspension in saline-Tween 80.

Each rat was repeatedly challenged at 15 min intervals after injection with the same length of stimulation needed

to produce the control seizures. The data reported represent the results obtained at 30 min after dosing, excepting chlorpromazine and haloperidol where 60-min results are reported. The duration of the postdrug seizures was expressed as a percent of baseline duration. The results obtained from groups of 7 to 9 rats each were averaged to determine each point on the dose–response curve. Whenever possible, the same group of rats were used for the various doses of a single drug. Occasionally, however, animals died or pulled the electrode out; and these were replaced with a new animal. An estimation of the median effective dose (ED_{50}), regression, and parallelism for drugs altering seizure durations were determined by linear regression analysis [2].

The following drugs were tested: amitriptyline hydrochloride (Elavil®, Merck Sharpe & Dohme); nortriptyline hydrochloride (Aventyl®, Lilly); imipramine hydrochloride (Tofranil®, Geigy); chlordiazepoxide hydrochloride (Librium®, Roche); diazepam (Valium®, Roche); oxazepam (Serax®, Wyeth); meprobamate; chlorpromazine (Thorazine®, Smith Kline & French); haloperidol (Haldol®, McNeil); pentobarbital sodium (Nembutal®, Abbott); diphenylhydantoin sodium (Dilantin®, Parke Davis); phenobarbital sodium (Luminal®, Winthrop); phenacemide (Phenurone®, Abbott); *d*-amphetamine sulfate (Dexedrine®, Smith Kline & French); methylphenidate hydrochloride (Ritalin®, Ciba); and chlorpheniramine maleate (Chlor-Trimeton®, Schering).

RESULTS

The pattern of the seizures in our animals was identical with the pattern described for the kindling effect by Goddard *et al.* [6]. Ambulatory activity ceased 5–7 sec after the start of stimulation and immediately prior to the onset of gross seizures. Next, the animals reared back on their hind limbs and tail, and bilateral, clonic forelimb seizures began. Facial contractions and foaming at the mouth were noted in most animals. There was frequent loss of balance during the seizure episode, but the rats invariably returned to the rearing position. Tonic seizures never occurred. There was an abrupt cessation of the seizure, whereupon the animals returned their forelimbs to the floor, then proceeded with normal searching behavior. For several minutes following termination of a seizure, the rats were extremely hyperirritable, leaping violently in response to tactile or auditory stimuli. Spontaneous convulsions were never observed.

Repeated testing at 15-min intervals over a period of 60–90 min proved that for each animal the phenomenon was remarkedly consistent, both in time of stimulation required to trigger the seizure and the duration of each successive seizure. In addition, that consistency was retained from trial to trial even though the animals were tested only once a week after the drug experiments were started.

The effects of three antidepressive agents on the duration of amygdaloid- and cortical-induced LIBS seizures are illustrated in Fig. 1. As can be seen, amitriptyline was quite potent in inhibiting LIBS seizures elicited from the amygdala. Cortically-induced seizures were also suppressed, but at higher dose levels. Similarly, Fig. 1 shows that nortriptyline and imipramine were more active in suppressing amygdaloid, as opposed to cortical, LIBS seizures. However, neither was as potent against amygdaloid-induced

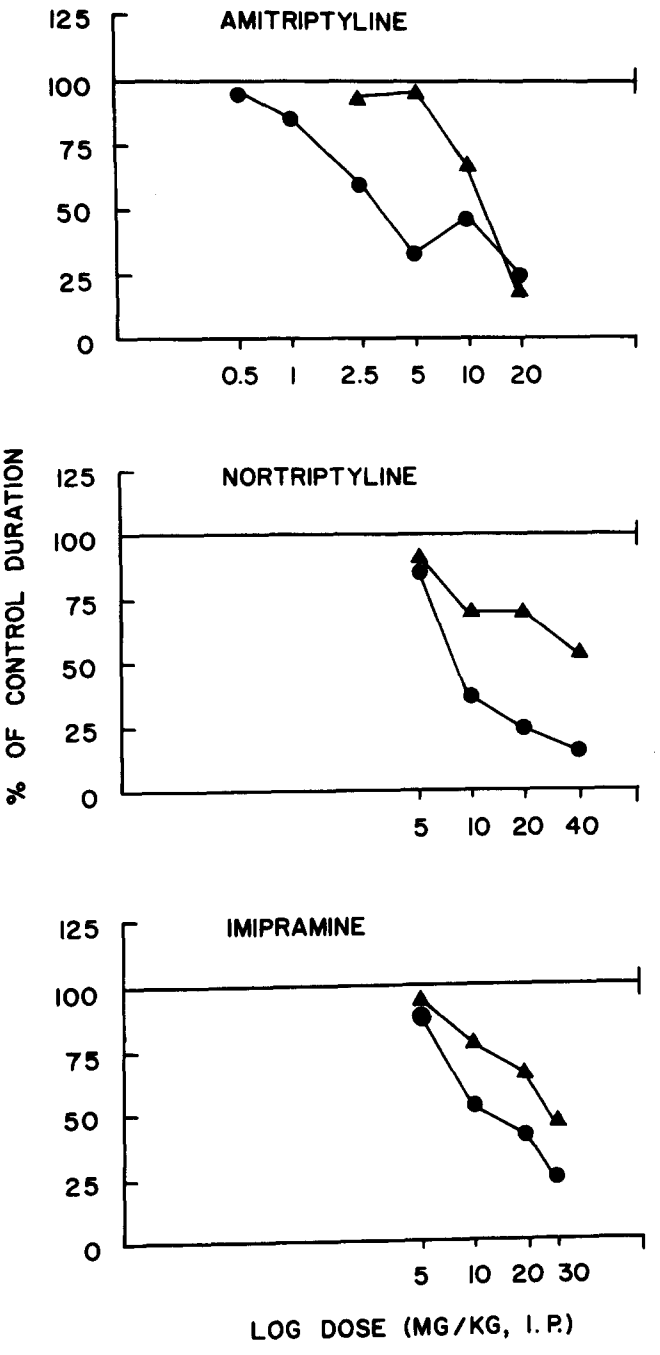

FIG. 1. Effects of three antidepressive agents on the duration of LIBS seizures. Seizures were elicited from the amygdala (●) or neocortex (▲).

seizures as amitriptyline; and the separation of the activity curves was least manifest with imipramine. With all three antidepressives, the difference in the slope of the amygdaloid and cortical dose–response curves was significantly different ($p<0.05$). None of the animals receiving an antidepressive exhibited overt signs of CNS depression at any of the dose levels tested.

Figure 2 presents the activity curves against LIBS seizures of four antianxiety drugs: chlordiazepoxide, diazepam, oxazepam, and meprobamate. A characteristic

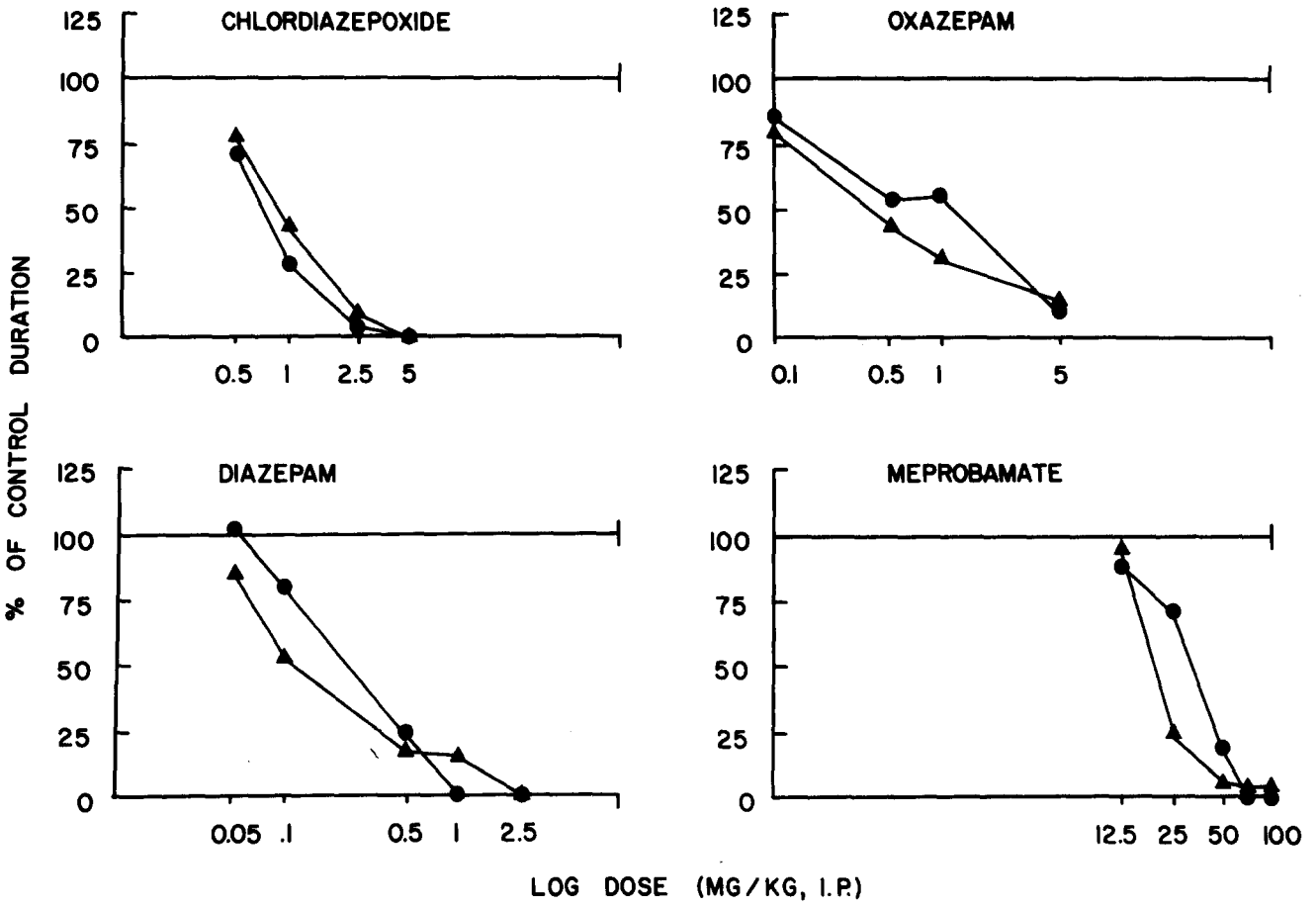

FIG. 2. Effects of four antianxiety agents on the duration of LIBS seizures. Seizures were elicited from the amygdala (•) or neocortex (▲).

common to all four drugs was the marked potency in suppressing LIBS seizures. Although the antianxiety agents produce hypokinesia at effective dose levels in many neuropharmacologic tests in rats, none of the animals were debilitated at doses completely blocking LIBS seizures. In addition, excepting chlordiazepoxide, the activity curves of the antianxiety drugs against cortical LIBS seizures were to the left of the amygdaloid curve; but the separation was nonsignificant in all instances.

Among the drugs that effectively suppressed LIBS seizures, a characteristic of the antiepileptic drugs was juxtaposition of the two dose–response curves. As can be seen in Fig. 3, the amygdaloid and cortical activity for diphenylhydantoin, phenacemide, phenobarbital or pentobarbital was, in each case, virtually indistinguishable. In contrast to the antianxiety drugs, diphenylhydantoin and phenacemide were much less potent in blocking LIBS seizures; and depressed motor activity and ataxia were prominent at the higher dose levels. Pentobarbital and phenobarbital, however, were relatively potent; and with effective doses, gross motor incoordination was not evident.

The two neuroleptics presented in Fig. 4, chlorpromazine and haloperidol, had only slight effects on LIBS seizures at either site. In fact, at the highest doses tested, the rats were severely debilitated, yet they still had seizures when stimulated electrically.

Figure 4 also shows the effects of two drugs that exert stimulatory effects on behavior in rats. *d*-Amphetamine and methylphenidate both prolonged LIBS seizures elicited from either amygdaloid or cortical sites. Unexpectedly, activity in the LIBS test leveled off at the higher dose levels, despite enhanced behavioral stimulation.

An antihistaminic, chlorpheniramine, was tested but the results are not illustrated. At the highest dose tested, 30 mg/kg, there was slight overt depression of motor activity but there was no effect on LIBS seizures.

DISCUSSION

As a neuropharmacologic test system, the LIBS procedure has numerous desirable features, especially the fact that the response is a pathologic entity (cf. [6]), an attribute lacking in most test models. Other attributes include: (a) the seizure duration of each animal is remarkably consistent from trial to trial; (b) the response is easy to measure and has a clear endpoint; (c) the apparatus is simple and inexpensive; (d) the rats can be used repeatedly; (e) both increases and decreases in the duration of seizures can be measured; and (f) the present findings indicate that various types of centrally active agents have differential effects.

The latter point is of particular importance in that a

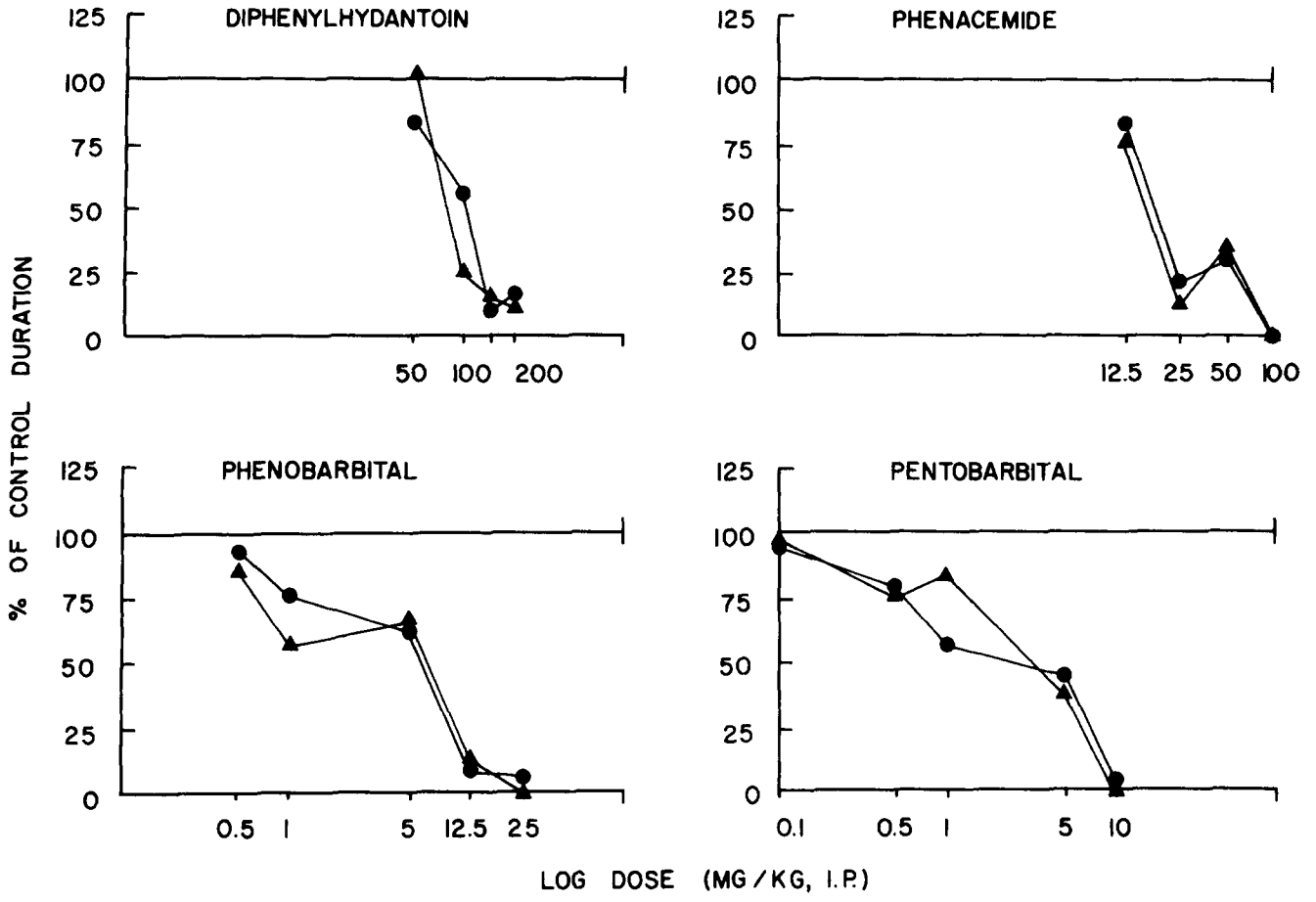

FIG. 3. Effects of four agents with anticonvulsive activity in rats on the duration of LIBS seizures. Seizures were elicited from the amygdala (●) or neocortex (▲).

major objective in the pharmacological testing of psychotropic drugs is to establish distinct mechanisms or sites of action. Our initial assumption that antiepileptic agents would block LIBS seizures was borne out, although effective doses were accompanied by marked debilitation. Those observations were somewhat disconcerting because most antiepileptic drugs are active at non-debilitating doses in experimental models of epilepsy.

As a result of the awareness that the limbic system was intimately involved with emotionality [8], several investigators have related the therapeutic activity of psychotropic agents to effects on limbic function [7,12]. In a similar vein, we speculated that the LIBS response, also closely tied to the limbic system, might be susceptible to drugs with psychotropic activity. Further pharmacologic examination of the phenomenon subsequently revealed that several classes of psychoactive agents were not only active against LIBS, but showed much greater efficacy than the antiepileptics.

Horovitz [7] has suggested that antidepressives not only affect the limbic system, but selectively suppress the amygdala as their basic mechanism of action. He based his speculations on a series of experiments with antidepressives comparing activity in the amygdala with activity in other limbic structures. In particular, he related the ability of antidepressives to block the muricide response at subneurotoxic dose levels to amygdaloid depression. The present findings strongly support Horovitz's suggestion. Of all the drugs we tested, only the antidepressives demonstrated a significant selectivity for one site over the other; and the specificity was for the amygdala. In fact, we have found [14] that another limbic site, the septum, is much less susceptible to antidepressive agents than the amygdala. Further, as Table 1 indicates, when the dose levels of antidepressives that depressed the duration of LIBS seizures by 50 percent are compared to ED_{50} values for blocking muricide, there is a close relationship between the amygdaloid LIBS and antimuricide values, an association that holds neither for the cortical data nor for any other drug we have studied.

Even though *d*-amphetamine, methylphenidate and chlorpheniramine, like the antidepressives, are quite effective in blocking muricidal behavior, the LIBS findings indicate that the blockade is elicited by a mechanism other than depression of the amygdala. In fact, the behavioral stimulants prolonged the seizures and chlorpheniramine was inactive. To our knowledge, LIBS is the only pharmacologic test utilizing rodents to determine antidepressive activity where the effects of behavioral stimulants and antidepressive drugs are so distinctly separated.

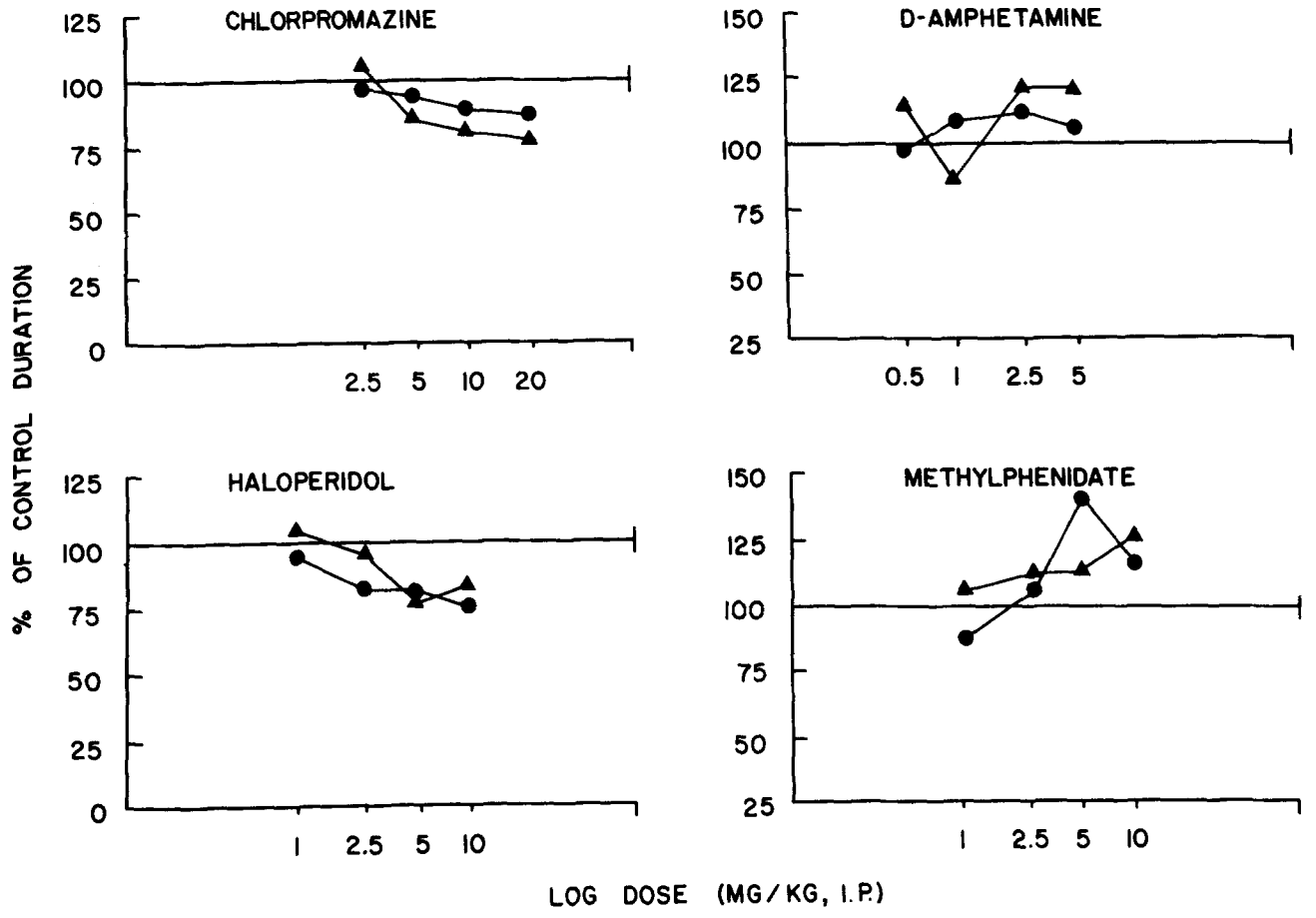

FIG. 4. Effects of four agents causing behavioral depression or stimulation in rats on the duration of LIBS seizures. Seizures were elicited from the amygdala (●) or neocortex (▲).

Rather than a generalized CNS depression, some type of selective depressant activity is apparently necessary for successful blockade of LIBS seizures. For example, at the highest dose tested, the neuroleptics caused complete debilitation of the animals, yet had no effect on the LIBS response. It is possible that the main mechanism of the antidepressives is a strong selective suppression of inhibitory mechanisms. The amygdala is intimately involved with inhibitory functions [1, 3, 9] and a drug with a predilection for inhibition would be expected to exert site-selective effects on substrates such as the amygdala. Disinhibition might account for the stimulatory effects and convulsions produced by high doses of antidepressive agents [13].

The anxiolytic drugs were conspicuous as a result of the marked potency they exhibited. This potency is particularly evident when the active doses in LIBS are compared with effective doses in other neuropharmacologic tests using rats, including conditioned avoidance response, septal irritability, operant conditioning and conflict [4, 5, 11, 12]. In the above tests, the active doses ranged from 5–30 mg/kg for chlordiazepoxide, oxazepam and diazepam. In contrast, the benzodiazepines were active in the LIBS test at less than 1 mg/kg.

There was a positive correlation between anticonvulsive activity and the juxtaposition of dose–response curves in LIBS. With both the anxiolytics and antiepileptics there was a tendency for the amygdaloid and cortical plots to lie in close parallel with each other. That the benzodiazepines possess potent anticonvulsive properties is well documented and Gluckman [5] has suggested that the anxiolytic properties of the benzodiazepines are related to their anticonvulsive activity. Interestingly, not only did the activity curves for phenobarbital and pentobarbital juxtapose, but the ED_{50}'s were extremely low values – as might be expected for an anxiolytic. Both barbiturates, in fact, are being used clinically for their anxiety-suppressing activity.

Despite the fact that the pattern of activities were similar, the antianxiety agents were easily distinguished from diphenylhydantoin and phenacemide by the marked disparity in potencies. The differences in the manner by which these drugs exert their anticonvulsive effects might explain the potency differences. Two basic mechanisms by which convulsions might be terminated are: (a) stabilization at the stimulation foci by an elevated threshold or a lessened tendency to repetitive discharge; and, (b) a suppression in the spread of the seizure discharge. Diphenylhydantoin is held to act by the latter mechanism; and it is believed that diphenylhydantoin does not exert effects at specific supraspinal loci but rather influences neuronal processes underlying transmission at numerous CNS sites

TABLE 1

ED_{50}'s FOR VARIOUS CNS-ACTIVE DRUGS AGAINST LIBS SEIZURES AND MURICIDE BEHAVIOR IN RATS

	ED_{50} (mg/kg) for Blocking		
	Amygdaloid LIBS Seizures	Cortical LIBS Seizures	Muricide*
amitriptyline	5.2†	28.5	5.1
nortriptyline	12.0†	>40.0	9.8‡
imipramine	13.5†	26.5	14.8
chlordiazepoxide	1.2	1.2	30.0
diazepam	0.3	0.1	1.9
oxazepam	1.1	0.3	NT
meprobamate	25.9	23.9	200
chlorpromazine	NA	NA	5.5
haloperidol	NA	NA	3.0‡
D-amphetamine	↑	↑	1.5
methylphenidate	↑	↑	33.0
chlorpheniramine	NA	NA	23.0
diphenylhydantoin	109.3	87.1	NT
phenacemide	25.1	23.0	NT
phenobarbital	6.8	7.2	NT
pentobarbital	1.7	2.0	10.0

NA = Not applicable
NT = Not tested
* = From Horovitz [7]
† = Significant difference between amygdaloid and cortical ED_{50}'s ($p<0.05$)
‡ = Unpublished results

[15]. Although less is known regarding the basic ability of the benzodiazepines to prevent seizures, the anxiolytics are extremely effective in protecting against pentylenetetrazol-induced seizures whereas diphenylhydantoin has little or no effect [15]. This observation suggests that the benzodiazepines have a threshold-increasing effect. If so, stabilization of the LIBS foci might be more sensitive to pharmacologic control than suppression of seizure spread.

Although our experience to date indicates that the number of brain sites predisposed to sensitization is limited, it is hoped that neuropharmacologic testing of other susceptible sites will make greater distinctions between drug types feasible. In addition, such information could provide valuable data linking neurologic effects of a drug to specific brain loci as well as unusual profiles of activity leading to new and unique types of CNS-active agents.

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