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Brain Amines and Spontaneous Epileptic Seizures in the Mongolian Gerbil

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COX, B. AND P. LOMAX. *Brain amines and spontaneous epileptic seizures in the Mongolian gerbil.* PHARMAC. BIOCHEM BEHAV. 4(3) 263–267, 1976. – The seizure sensitive (WJL/UC) strain of Mongolian gerbils has been used to investigate the effects of drugs that modify central monoamine activity on spontaneous epileptiform seizures. Increasing central norepinephrine and dopamine levels significantly reduced the severity of the seizures and the importance of dopamine was further demonstrated by the protection from seizures after administration of the dopaminergic agonist apomorphine. These data indicate that a search for differences in regional levels, or turnover rates, of these amines between seizure sensitive and seizure resistant strains of gerbils might be profitable in determining the genetic deficit underlying the seizure phenomenon in these animals.

Seizures Gerbils Epilepsy Norepinephrine Dopamine Serotonin Apomorphine

THE development of a colony of selectively bred (WJL/UC strain) Mongolian gerbils (*Meriones unguiculatus*) exhibiting spontaneous epileptic seizures, and the use of animals from the colony as a model for the study of the epilepsies, have been described in previous publications from these laboratories [12, 17, 19, 20]. The epileptic nature of the seizures, defined as spontaneous, recurrent, self-sustaining paroxysmal discharges of the central nervous system [32], has been demonstrated in studies relating the motor activity to electroencephalographic (EEG) records [18]. It has also been demonstrated that the seizures can be prevented, or reduced in frequency, by common [17] and atypical [8,31] anticonvulsants.

The role of abnormalities of central neurotransmitters in the pathophysiology of epilepsy has been extensively discussed (see reviews in [13]) but remains to be determined. In preliminary studies, in which seizure sensitive gerbils were compared with seizure resistant animals, no strain differences in whole brain serotonin (5-HT) levels or striatal dopamine uptake could be demonstrated, although low parietal lobe and thalamic γ-aminobutyric acid (GABA) levels were found in seizure sensitive animals [16]. Whether the last finding indicates that disinhibition of synaptically related cortical and subcortical structures may be important in the etiology of the seizures requires further investigation. Brain 5-HT concentrations, dopamine uptake and GABA levels were measured simply because these methods were available in unrelated projects in our laboratories. The estimation of regional levels, or turnover rates, of brain amines is a considerable undertaking, especially with the limited number of gerbils available. Therefore, it was felt that it might be better to attempt to obtain indirect evidence of likely etiological candidates before proceeding to such analyses.

In the present study the technique of testing for modification of the seizure propensity has been refined and further standardized over earlier methods and used to investigate the effects on the seizures of drugs known to modify brain amine function.

METHOD

Adult gerbils of either sex weighing 65–95 g from the seizure sensitive (WJL/UC) strain were used in all experiments. Since we wished to be able to detect either increases or decreases in seizure severity groups of animals were selected which exhibited mean seizure scores of median severity (Grade 3), although the range was from 1–5. The maintenance and feeding schedules were as described previously [16].

Animals were tested for seizure severity prior to the start of the experiments. Thereafter, further testing following various treatments took place such that no single gerbil was tested at less than 7 day intertrial intervals, thus, ensuring that none would be refractory at the time of testing. The seizure scoring system has been described in detail elsewhere [16,20]. Briefly, the seizures are classified: grade 0, no seizure; grade 1, animal moving with vibrissae and pinnae twitching; grade 2, motor arrest with twitching of the vibrissae and pinnae; grade 3, motor arrest with myoclonic jerks; grade 4, clonic–tonic seizure; grade 5, clonic–tonic seizure with body rollover; grade 6, seizure progressing to death.

In initial studies of drugs modifying seizures results of treatment were expressed in terms of the degree of protection from seizures "% animals seizing" [17], irrespective of the severity of the seizure. Later it was found that greater sensitivity could be obtained by recording the change in

mean seizure scores of a group of animals following treatment [8,31]. Because of the limited number of animals available which exhibited seizures in the median (Grade 3) range, various methods for precipitating seizures were tested. Seizures can be induced in the sensitive strain by simply transferring the gerbil (single handling) from its home cage to a novel environment [16]. The following triple handling technique was eventually adopted for the present study: (1) remove animal from home cage, weigh and place in novel environment (top of a laboratory cart) for 2.5 min; (2) handle for 20 sec, replace in novel environment for 2.5 min; and (3) suspend by tail above top of cart for 2.5 min, replace in novel environment. If the animal had not developed a seizure during this period (approximately 8 min) of testing it was scored Grade 0. The seizure score was given equal weight, regardless of its stage of induction. This protocol resulted in more consistent seizure induction and allowed the demonstration of drug effects with smaller groups of animals.

Motor activity was measured within 2 min of the animal returning to pre-ictal behavior, following the seizure, using the tremor analyzer described by Silverman and Jenden [29]. This device converts mechanical movements into an electrical analogue which was passed thorugh a 20-24.6 Hz bandpass filter, rectified and integrated over 15 sec epochs into a 25 mV peak output for potentiometric recording.

For the administration of drugs the gerbils were anesthetized, by flooding the cage with $N_2 0/0_2$ (80:20 mixture, 10 l/min flow), weighed, injected intraperitoneally and returned to the cage which was then flushed with 100% 0_2. Animals revived within 15 sec. This procedure produced sufficient anesthesia to allow handling without precipitating seizures. Drugs were injected between 06.00 and 07.00 hr and the animals were tested at appropriate time periods afterwards (for time intervals see text); the same weekday (Monday) was used for testing in order to maintain a fixed feeding schedule.

Drugs were dissolved either in sterile NaCl (0.9%) solution or 0.1 N HCl (threoDOPS) for injection with the concentration adjusted so as to allow an injection volume of 1 ml/kg. Control vehicle injections were made in all experimental procedures using the same animals – thus, in every drug treatment group each animal acted as its own control.

Data were analyzed for significance using the nonparametric Mann-Whitney U test.

RESULTS

The effect of the two handling procedures on mean seizure scores was tested on 4 groups of 8 seizure sensitive gerbils. As seen in Table 1, the triple handling method gave more consistent mean scores than single handling. This consistency also held up when a single group of animals was tested at weekly intervals (Table 2). That the test is sensitive to standard anticonvulsants is demonstrated in Table 3: acute administration of phenobarbital (20 mg/kg IP) or diphenylhydantoin (25 mg/kg IP), 1–2 hr prior to testing, significantly reduced the severity of the seizures. These data confirm the validity of the method for testing for modification of seizure behavior.

Drugs that deplete brain biogenic amines were tested: Groups of animals were injected with diethyldithiocarbamate (500 mg/kg IP 4 hr) (DDC), or p-chloroamphetamine (3.5 mg/kg IP 48 hr) (PCA), or α-methylmetatyrosine (250 mg/kg IP 16 hr) (AMMT). These compounds, which deplete norepinephrine (DDC), 5-HT (PCA), and dopamine plus norepinephrine (AMMT) were given in doses, and at time intervals prior to testing (indicated after dose) reported to be effective in other rodents [5, 7, 21]. From the results presented in Table 3 it is seen that depletion of norepinephrine (DDC) and norepinephrine plus dopamine (AMMT) significantly reduced the seizures and spontaneous motor activity whereas lowering 5-HT (PCA) was without effect on these parameters.

Since DDC may increase brain dopamine activity [5, 6, 22] the effect on the seizure scores of the dopamine receptor agonist apomorphine was investigated. The peak behavioral effect of apomorphine is at 30 min [9]. As seen in Table 3, apomorphine (1 mg/kg IP) significantly reduces the seizure severity; the effect of this dose has declined at 60 min. A dose of 5 mg/kg was no more effective in preventing seizures although motor activity was more depressed. A dose of 0.2 mg/kg (tested at 20 min) caused some reduction in both seizures and motor activity but in neither case to a significant level.

In view of the protection afforded by apomorphine the dopamine antagonist pimozide was tested. Pimozide (0.5, 0.1 and 0.05 mg/kg IP) was injected 3 hr [1] prior to seizure induction in an initial group of 8 gerbils selected from a group of 16, each dose at 7 day inter-trial intervals. Although each dose reduced motor activity (to 48%, 66%

TABLE 1

EFFECTS OF DIFFERENT TESTING PROCEDURES ON MEAN SEIZURE SCORES

Group*	Number of Animals	Mean seizure score†(± S.E.M.) Single handling‡	Triple handling‡
A	8	3.8±0.6	3.25±0.6
B	8	3.0±0.7	3.25±0.6
C	8	1.8±0.6	3.0 ±0.7
D	8	3.1±0.8	3.0 ±0.7

*All animals in each group were tested on same day by same procedure. Interval between testing was 7 days.
†See text for scoring system.
‡See text for testing procedures.

TABLE 2

REPRODUCIBILITY OF TRIPLE HANDLING* TESTING PROCEDURE IN ANIMALS RANDOMLY SELECTED FROM A GROUP OF 16 SEIZURE SENSITIVE GERBILS

Day of testing	Number of animals‡	Mean seizure score† (±S.E.M.)
1	16	3.0±0.6
8	15	3.0±0.6
15	12	2.8±0.8
22	12	2.9±0.6

*See text for testing procedure.
†See text for scoring system.
‡Group size reduced due to use of some animals in other studies.

TABLE 3

EFFECTS OF DRUGS ON MEAN SEIZURE SCORE AND MOTOR ACTIVITY IN SEIZURE SENSITIVE GERBILS

Treatment† (dose)	Number of animals	Mean seizure score (±S.E.M.)	Motor activity (% control)
0.9% NaCl (1 ml/kg IP)	16	3.0 ±0.6	100
Phenobarbital (20 mg/kg IP)	8	0.38±0.38†	166
Diphenylhydantoin (25 mg/kg IP)	7	0.86±0.40†	78
Diethyldithiocarbamate (500 mg/kg IP)	9	1.1 ±0.48†	30†
p-Cl-amphetamine (3.5 mg/kg IP)	8	2.8 ±0.80	78
α-methylmetatyrosine (250 mg/kg IP)	11	1.3 ±0.30†	28†
DL-threodihydroxy-phenylserine (50 mg/kg IP)	7	1.1 ±0.60†	60
Diethyldithiocarbamate (500 mg/kg IP) + DL-threodihydroxy-phenylserine (50 mg/kg IP)	7	0.3 ±0.28†	35†
Apomorphine (1 mg/kg IP)	14	0.8 ±0.45†	41†

*For treatment schedule see text.
†Significantly different ($p<0.05$) from vehicle injected controls.

and 55% respectively) there was no significant effect on the mean seizure scores. Although the mean scores were not significantly different pimozide did, however, lead to a marked change in the character of the seizures and all of the animals were scored Grades 2–3 (mean 2.7) whereas the initial range covered all grades from 0–5 (mean 3.0). The animals initially showing low scores had more severe seizures and the Grade 5 seizures were attentuated. Also, the general course of the seizures was shorter in onset and duration.

An effort to separate further the roles of norepinephrine and dopamine was made using a combination of DDC and DL-threodihydroxyphenylserine (50 mg/kg IP) (threoDOPS) administered 4 hr and 1 hr, respectively, before testing for seizures. The rationale behind this treatment is that DDC will increase brain dopamine activity but the concomitant fall in norepinephrine will be prevented both biochemically [11] and functionally [10] by direct conversion of threoDOPS to norepinephrine by decarboxylation. The data in Table 3 indicate that lowering norepinephrine and increasing dopamine activity (DDC) and increasing norepinephrine levels (threoDOPS) decreases both the seizure severity and spontaneous motor activity. However, the greatest seizure protection was afforded by combined DDC and threoDOPS pretreatment – raising dopamine activity without depleting norepinephrine.

DISCUSSION

There are no reports, to date, of the effects of enzyme inhibitors, or other pharmacological tools, on brain amines in the gerbil. Studies of neuroamine metabolism in this species have, however, been carried out [12, 14, 16, 33] which reveal that brain concentrations of acetylcholine, norepinephrine, dopamine and 5-HT are similar to those in rats and mice. Also, identical changes in brain enzyme activity occur when gerbils and rats are exposed to differential environments [23]. Thus, it is likely that the effects on brain amines, of the drugs used in the present study, will be qualitatively and quantitatively similar to those in the rat (which species has been most extensively investigated).

Initial consideration of the data in Table 3 suggests that depletion of norepinephrine is the major factor in reducing seizure severity. As noted above, however, DDC not only depletes norepinephrine (due to inhibition of dopamine-β-hydroxylase) but concurrently increases dopamine activity. AMMT lowers both norepinephrine and dopamine levels but some caution is necessary in interpreting the effects of this compound; not only is AMMT extremely toxic at this dose (there is profound depression of motor activity at 16 hr) in the gerbil (80% of the animals died within 7 days, probably of inanition) but examination of the data revealed that the low mean seizure score was due to ratings of 0 or 2 grade seizures only. Since the drug has to be injected 16 hr prior to testing it is entirely possible that spontaneous seizures could have been unobserved so that the animals were refractory when tested (it was not feasible to observe the gerbils throughout the 16 hr interval). Thus, if the AMMT effect is non-specific an entirely different interpretation of the results might emerge – namely, that it is the increase in activity of catecholamines, particularly

dopamine, that is mainly responsible for ameliorating the severity of the seizures.

It should be noted that motor activity is used in these studies as a measure of nonspecific, mainly toxic, effects of the compounds. In previous investigations [8, 17, 31] it has been demonstrated that there is no correlation between the effects of drugs on spontaneous motor activity and seizure protection (e.g. compare phenobarbital and diphenylhydantoin in Table 3).

The concept of inhibition of seizures by dopaminergic mechanisms is supported by the effect of apomorphine, a dopamine agonist. The time course for seizure protection correlates well with that for the stereotyped behavior seen in the rat [9] which is mediated specifically at dopamine receptors. The significance of the change in the character of the seizures, following administration of the dopamine receptor blocking agent pimozide, is obscure. It is not, however, surprising that dopamine antagonism fails to potentiate the seizures if lowered activity of the amine is the underlying deficit in the WJL/UC strain. Certainly, pimozide does not induce seizures in the seizure resistant strain; or any other species.

Elevating brain norepinephrine levels with threoDOPS significantly reduced the seizure scores and moderately depressed spontaneous motor activity (Table 3). Since both depleting (DDC) and raising (threoDOPS) norepinephrine levels had similar effects on the seizures it is likely that other factors account for one of these findings: the most significant difference in the two experimental situations is the rise in brain dopamine accompanying the fall in norepinephrine after DDC. The combination of DDC and threoDOPS – which would tend to maintain normal norepinephrine levels while raising dopamine turnover – supports this suggestion; such combined treatment proved to be the most effective in suppressing the seizure activity (Table 3) while the reduction of motor activity was similar to that after DDC alone.

Most of the previous studies of the role of monamines in seizure activity have been carried out with the audiogenic mouse model of epilepsy. There is considerable, and fairly uniform, evidence from such investigations that raising the activity of 5-HT, norepinephrine or dopamine will protect these animals from seizures [3, 4, 15, 24–28]. Diminished seizure intensity following electroconvulsive shock has been reported in rats in which central dopamine activity was enhanced [30]. Recently it has been shown that dopamine agonists (apomorphine, ergocomine, bronocryptine) diminish the severity and character of reflex epilepsy in audiogenic mice and reduce myoclonic jerks induced photically in the baboon, *Papio papio* [2]. Whether the responses to manipulation of brain amines in such experiments, and the present study, are purely pharmacological effects or represent a correction of an underlying genetic neurochemical defect in these seizure sensitive species remains to be determined. It should also be borne in mind that the biochemical effects may be causing modification of effector pathways leading to the behavioral manifestations of the seizures rather than altering the underlying epileptic diathesis. This last possibility, although unlikely, should be amenable to resolution in the gerbil since the animal is suitable for chronic EEG recording and the behavioral manifestations have been correlated with associated changes in EEG patterns [18].

The present findings, although essentially exploratory, do suggest that the catecholamines, particularly dopamine, may be of importance in the genesis of seizures in the WJL/UC strain of gerbils. This conclusion encourages the search for differences in the regional distribution, or turnover rates, of these amines between the seizure sensitive and seizure resistant strains.

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