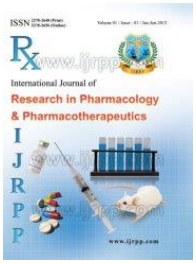
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.8 | Issue 3 | Jul - Sep - 2019***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Anticonvulsant effects of diazepam and nitrazepam in mice

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ABSTRACT

Introduction

The term epilepsy is derived from the Greek word *epilam-banein*, meaning to attack or seize. People once thought that epileptic individuals were being visited by demons or gods. However, in 400 B.C., the early physician Hippocrates suggested that epilepsy was a disorder of the brain—and he was right.

Materials and Methods

In the laboratory procedures have been developed in which epileptic activity is closely approximated. The procedures used in this study are Electrical Methods and Chemical Methods. In the present studies all the anticonvulsants were administered intraperitoneally and their peak effect was determined prior to the evaluation of their potencies against electroseizures and chemical seizures.

Result

The peak anticonvulsant effect of Diazepam and Nitrazepam is 2 hours after intraperitoneal injection whereas for Phenobarbitone it was stretched from 2nd hour to 3rd hour.

Conclusion

The time of peak effect of phenobarbitone in mice on intraperitoneal administration was ranging from 2nd hour to 3rd hour whereas for diazepam and nitrazepam it was 2nd hour. 0.5 mg/kg of nitrazepam was equipotent to 60 mg/kg of phenobarbitone which in turn was roughly equivalent to 0.5 mg/kg of diazepam.

Keywords: Epilepsy, Phenobarbitone, Diazepam, Nitrazepam, Electrical Methods, Chemical Methods

INTRODUCTION

Epilepsy is a chronic medical disorder or condition, usually resulting in unpredictable, unprovoked recurrent seizures that affect a variety of mental and physical functions. [1] It is one of the most common neurological diseases, affecting about 50 million people worldwide. [2] Epilepsy was one of the first brain disorders to be described. [3] It was mentioned in ancient Babylon more than 3,000 years ago.[3] Through the ages, the strange behavior caused by some seizures has led to the creation of numerous superstitions and prejudices.

The term epilepsy is derived from the Greek word *epilam-banein*, meaning to attack or seize. People once thought that epileptic individuals were being visited by demons or gods. However, in 400 B.C., the early physician

Hippocrates suggested that epilepsy was a disorder of the brain—and he was right. [3]

A person is considered to have epilepsy when two or more unprovoked seizures occur that can't be explained by a medical condition such as fever or substance withdrawal. Seizures can be the result of a family tendency toward the disease, or they can occur after a brain injury, but the cause of epilepsy is largely unknown. [4] Epileptic seizures are manifested by an abnormal, excessive, and hypersynchronous electrical discharge of neurons in the brain. [4]

Each distinct form of epilepsy has its own natural history and response to treatment. [4] This diversity probably reflects the many different underlying causes of epilepsy and the variety of epilepsy syndromes in which the clinical and pathological characteristics are distinctive and suggest a specific underlying etiologic mechanism. [5]

There are many kinds of seizures, each with characteristic behavioral changes and electrophysiological disturbances that can usually be detected in scalp electroencephalographic (EEG) recordings. [4] A seizure is a transient epileptic event, indicating a disturbance in brain function. [4] Having a single seizure does not necessarily mean that a person has epilepsy. [4, 5] Ten percent of adults experience a seizure sometime during their lifetime.[1]Seizures can last from a few seconds to a few minutes. Patients and health care professionals do not always recognize the signs or symptoms, which can include convulsions, a loss of consciousness, blank staring, lip smacking, or jerking movements of the arms and legs. [1] A seizure has a clear beginning, middle, and end.

DRUGS

Phenobarbital

Free barbituric acid and its 5-metyl and 5.5-di-methyl derivatives are devoid of hypnotic or antiepileptic activity. 5-methyl-5-ethyl compound is only weakly hypnotic and the 5.5-diethyl compound (Barbital) is hypnotic but not anticonvulsant. [6] The substitution of a phenyl for an ethyl group in barbital is the basis for the specific antiepileptic properties of phenobarbital, but a second phenyl group on C5 diminishes anticonvulsant activity.

Diazepam and nitrazepam

Neurological experiments show that Benzodiazepine derivatives have less direct depressant effect on the reticular activity system than other hypnotics and this probably accounts for the clinical observation that patients are readily awakened from sleep produced by Nitrazepam. The main site of action appears to be on structures in the limbic system, the region of the brain concerned with the experience of emotion and anxiety. Reverberating neuronal activity in the limbic system associated with anxiety can activate the brain stem reticular formation and then maintain wakefulness. [7]

The Benzodiazepines are believed to suppress the ability of the limbic system to activate the reticular formation and are therefore of particular value in insomnia due to anxiety. Nitrazepam disturbs the balance of REM and NREM sleep, as do the barbiturates, but there is evidence that they do so to a considerably less extent than the barbiturates. [8] Dependence on Nitrazepam after prolonged use occurs, but may be less pronounced than with the barbiturates.

The Benzodiazepines do not appear to interact with monoamines, acetylcholine or other possible central neurohumoral transmitters to any significant effect. [9] Nitrazepam is a benzodiazepine derivate and is chemically 1,3-dihydro-7 nitro-5-phenyl 1-2H1, 4-Benzodiazepine-2-one. Nitrazepam has hypnogenic, muscle relaxing and anxiolytic properties. [10]

MATERIALS AND METHODS

In the laboratory procedures have been developed in which epileptic activity is closely approximated. It has been found that this is possible by the use of both physical and chemical stimulation. The approach used in this study will be to discuss the various types of stimuli used to cause or evoke convulsive activity in order to permit the investigation of anticonvulsant activity of test compounds.

Electrical methods

The electrical methods of producing seizures are grouped as: [11]

1. Maximal Electro-shock seizure pattern (M.E.S.)
2. Minimal Electro-shock seizure threshold (M.E.T)
3. Psychomotor seizure Test (P.S.T)

Mice weighing about 20 gm were given free access to both food and water. All drugs were administered intraperitoneally. Each drug was tested for its effect at the peak of activity. Minimal electroshock threshold test involved the administration of single shocks of 6 to 9 ml. It was repeated every 48 hours until the threshold had been established and did not vary more than 3 per cent during successive trials. A threshold seizure in mice consisted of at least 7 seconds of facial, lower jaw or fore limb clonus without loss of the righting reflex. When threshold was determined, the mice were given the drug and shocked with a 20 per cent larger than the threshold. Complete protection from the seizure was used as the end point. [12]

Unidirectional rectangular pulses of 0.2 msec. duration, at 6 Hz for 3 seconds intervals, were administered by means of corneal electrodes. The mouse first appeared to have been stunned. The posture was awkward, but upright. The fore limbs were often crossed and the hind limbs were spread wide apart. The tail was frequently held practically vertical. The stunning was occasionally preceded by a few seconds of running with a rolling gait. The face and fore limb movements resembled "purposeful" automatism. Not infrequently, the mouse stood almost erect on its hind limbs while exhibiting the automatic behaviour. Catatonia was often present, duration of seizures varied from 10 to 75 seconds. At the end of the seizures the animal rather suddenly resumed normal locomotion and exploratory behaviour. A drop of normal saline was placed on each eye to assure good contact between cornea and electrode. [13] The current was delivered and the animal was immediately released. The current used was 32 milli ampes i.e. 4 times threshold. The end point used was protection against seizure i.e. animal walked away normally within 7 seconds after the end of the stimulation.

Maximal electroshock seizure pattern of Toman, Swinyard and Goodman (1946) has been chosen for the present assay of anticonvulsant activity in mice.

Maximal electroshock seizure pattern

Healthy mice weighing between 18-22 grams were used. They were allowed free access to food and water before subjecting them for electrical convulsions. As starvation modifies electroshock seizure pattern (Swinyard, 1952), [14] convulsions were given with convulsometer of 'Techno' vis cormeal electrodes. Electrodes were dipped in normal saline and were retained by clamps. Current was regulated for maximal electroshock seizure but time was kept constant at 0.3 seconds. Mouse was held through its neck while the current delivered through corneal electrodes. A convulsion was positive if mouse extended both fore and hind limbs and it ws considered otherwise if it did not extend its hind limbs. The electrodes were removed and the animal was transferred to a separate cage. Convulsions were produced in these animals once in 24 hours for a period of 4 days.

Convulsions were given starting with 24 milli ampes and gradually increasing it to as high as 45 milli ampes and gradually increasing it to as high as 45 milli ampes. Mice showing hind limb tonic extensor component at 24 milli ampes and above were used in the experiments and various groups were separated according to the milli ampes on which they got convulsions. Majority got convulsions on 45 milli ampes. Males were more susceptible for convulsions than females and mice weighing more than 20 grams usually survived the shock better.

Chemical

Convulsions of clonic type are produced by intraperitoneal injection of metrazol, administered to groups of mice. The convulsions begin with transient, intermittent myoclonic jerks without loss of posture, the hind limbs are spread and the tail is erect. In 3-10 minutes the convulsions became generalised and clonic, associated with loss of posture. [10] A threshold Metrazol convulsion is one which persists for at least 5 seconds.

All the analeptics are capable of producing generalised convulsions in sufficient doses, excitability of the CNS reflects a balance between excitatory and inhibitory influences that is normally maintained within relatively narrow limits. The drugs can increase excitability either by blocking inhibition or by enhancing excitation. Strychnine and picrotoxin increase the level of neuronal excitability by selectively blocking inhibition in the CNS and others presumably act by enhancing excitation. Nerve impulses are normally confined to appropriate pathways by inhibitory influences. When inhibition

is block by strychnine ongoing neuronal activity is enhanced and sensory stimuli produce exaggerated reflex effects. (Goodman and Gilman 6th edition). [7]

Seizures can also be induced by injecting chemicals as strychnine, picrotixin, thujon in olive oil, 20% camphor in seasome oil, cocaine, ephedrine, caffeine etc. but chiefly metrazol is employed for induction of chemical seizures and in the present studies also only metrazol has been used for this purpose.

Pentylene Tetrazol: (Pentamethylenetetrazol is a synthetic compound)

It is a convulsant that is widely used as a laboratory tool for screening anticonvulsant drugs. Although the basic mechanism by which this compound produces seizures is unknown, many investigations have suggested that Pentylenetetrazol alters membrane ionic permeabilities. [14]

Administration of anticonvulsant drugs

All the anticonvulsant drugs tested were administered by intraperitoneal route with the help of a tuberculin syringe in a fixed volume.

An assistant held the mouse by the skin at the neck posteriorly with the other hand stretched the hind limbs. The required volume of drug was measured by tuberculin syringe and was injected intraperitoneally. The animal was freed after the injection and transferred to a separate cage. The time was noted.

Groups of mice of either sex five in each were administered with anticonvulsant drugs by intraperitoneally in three different doses to each group taking into account the same factors as for maximal electroshock seizure. Ten minutes before the anticipated peak effect of the anticonvulsant drug, metrazol was injected subcutaneously, 80mg/kg. The number of mice protected from the convulsions within a period of one hour was noted. Such five experiments were performed for each x drug and the results obtained are tabulated in Table C.

Convulsions from metrazol are of clonic type. Clonic spasm persisting for atleast 5 seconds was considered as positive. Usually mice get twitching on and off after injecting metrazol which were not taken into account.

In control group when metrazol was injected the animal became very active. They got clonic type of convulsions within 3 to 10 minutes. They closed their eyes suddenly and bent their necks, jerks were first seen in the fore limbs, spreading through the whole body and the animals jumped in the cage. There was cent percent mortality.

Animals under the influence of anticonvulsants were drowny, quiet and became active after injection of metrazol. Their hair erected and unprotected animals got convulsions within 10-30 minutes after injection. The protected animals either tried to eat or cleaned their faces with their fore limbs. Some were still drowny. In some cases they got nly one bout of convulsions whereas in others convulsions were seen periodically. After the convulsions the mice laid completely relaxed, respiration became rapid and in some mice froth could be seen at their mouths. After a period of postictal relaxation some got another bout of convulsions. Few animals survived and few animals died.

The time obtained at which the maximum number of mice were protected indicated the time of peak anticonvulsant effect Table A.

The anticonvulsant drugs were tested subsequently with electric and chemical seizures at the time of peak effect in different does.

Determination of percentage protection with three different doses

Three groups mice of either sex were taken. Their threshold for maximal electroshock seizure was determined as above.

Each group was given a separate dose. One group was given a dose at which lowest number of mice were protected, another was given a dose at which highest number was protected and the third dose was chosen between these two extremes and was administered to a third group noting the protection rate. [12] Maximal electroshock seizures were given at the time of the previously established peak effect and the protection was noted in each group. Such experiments were done for each dose and results obtained of these experiments were tabulated in Table B.

RESULTS

The comparative times of peak anticonvulsant effect of phenobarbitone sodium, diszepam and nitrazepam was established in mice by intraperitoneal injection as shown in table A and table 1, 2 and 3.

It may be seen that the time of peak effect was from 2nd hour to 3rd hour in case phenobarbitone and 2 hours for diszepam and nitrazepam. In table 1, 2, 3 are shown details of determining the peak effect for each drug and results of such experiments were shown collectively in Table-A. It is obvious that the peak effect may vary from drug to drug and the date emphasize the importance of testing any anticonvulsant drug at its time of peak effect.

The anticonvulsant potencies of these drugs were determined by maximal electroshock seizures and metrazol seizures at the time of their respective peak anticonvulsant effects. The anticonvulsant potencies as measured by the metrazol in mice for each drug are shown in table 4, 5, 6. Table B showing the percentage of protection of mice against maximal electroshock seizures with anti convulsant drugs. It can be seen from the results that nitrazepam is equipotent of phenobarbitone. However it has to be confirmed by testing more elaborately in some other species as well, hence for the time being we can give the equal status for the nitrazepam and phenobarbitone. Diazepam stands last in order. With the above results one can tentatively draw the conclusion that against the electroshock seizures in mice the effect of 0.5 mg/kg of nitrazepam is equivalent to 60 mg/kg of phenobarbitone and diazepam is roughly equivalent at 0.5 mg/kg of body weight.

Therefore N = P = D
0.5 = 60 = 0.5
1 = 120 =1

Where N stands for nitrazepam and P stands for Phenobarbitone and D stands for diazepam their ratio of potencies are shown above. It may be seen from table B diazepam is 35% less potent than nitrazepam and phenobarbitone.

The comparative anticonvulsant potencies as measured by metrazol seizures are shown in table C. It can be seen again nitrazepam and phenobarbitone are effective than diazepam against metrazol seizures. There is equal percentage of protection i.e. 85% with nitrazepam and phenobarbitone with diazepam it was only 65%.

While using metrazol in producing convulsions in mice (80 mg/kg) under phenobarbitone nitrazepam and diazepam certain number of mice died. The details are given in Table C. It can be seen nitrazepam is equally effective with that of phenobarbitone and percentage of mortality is less than diazepam.

Table 1: Showing the time of peak effect of phenobarbitone in mice.

Mice	MES	Dose in mg/kg	15 mts	1 hr	3 hr	5 hr
1	**45 mA 0.3 sec**	**60**	**+**	**+**	**--**	**-**
2	”	**60**	**-**	**-**	**-**	**-**
3	”	**60**	**+**	**-**	**-**	**+**
4	”	**60**	**+**	**+**	**-**	**+**
5	”	**60**	**-**	**-**	**-**	**+**
			½ hr	**2 hr**	**4 hr**	**6 hr**
1	”	**60**	**+**	**-**	**-**	**-**
2	”	**60**	**-**	**-**	**-**	**+**
3	”	**60**	**-**	**-**	**+**	**+**
4	”	**60**	**+**	**-**	**-**	**+**
5	”	**60**	**-**	**-**	**+**	**+**

+ Convulsions **- Protection**

Peak effect 2nd hour to 3rd hour

Table 2: Showing the time of peak effect of diazepam

Mice No	MES	Dose in Mg/kg	15 mts	1 hr	3rd hr	5th hr
1	45 mA 0.3 sec	0.5	+	+	+	+
2	”	0.5	+	+	-	-
3	”	0.5	+	-	+	+

4	”	0.5	+	+	-	+
5	”	0.5	-	-	-	-
1	”	0.5	+	+	+	+
2	”	0.5	+	-	-	-
3	”	0.5	+	-	+	+
4	”	0.5	+	-	-	+
5	”	0.5	-	-	-	+

+ Convulsions **- Protection**
Maximum Protection 2nd hour

Table 3: Showing the time of peak effect of nitrazepam

Mice No	**MES**	**Dose in mg/kg**	**15 mts**	**1 hr**	**3rd hr**	**5th hr**
1	**45 mA 0.3 sec**	0.5	+	-	-	-
2	”	0.5	+	-	-	+
3	”	0.5	--	-	+	+
4	”	0.5	+	+	-	+
5	”	0.5	-	-	-	-
			½ hr	**2nd hr**	**4th hr**	**6th hr**
1	”	0.5	+	-	-	-
2	”	0.5	+	-	-	+
3	”	0.5	-	-	+	+
4	”	0.5	+	-	+	+
5	”	0.5	-	-	-	-

+ Convulsions **- Protection**
Maximum Protection 2nd hour

The maximum number of mice protected out of 20 tested for each drug, show the time of peak effect which is underlined.

Table a: Showing the time of peak effect of anticonvulsant drugs against mes

Sl No	**Name of the Drug**	**Dose in mg/kg**	**Time in Hours No.of mice protected at the end of**							
			15 mts	½ hr	1 hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
1	Phenobarbitoneq	60	8	9	11	15	15	12	10	5
2	Diazepam	0.5	4	7	10	13	12	9	7	4
3	Nitrazepam	0.5	5	9	12	15	14	12	9	5

Table b: Showing the percentage of protection of mice against mes with anticonvulsant drugs.

Sl. No	**Anticonvulsant Drug**	**Dose in mg/ kg**	**No. of Mice Tested**	**No. of protected**	**Deaths**	**Percentage of protection**	**Percentage of deaths**	**Percentage of unprotected but survived**
1	Phenobarbitone	15	20	5	2	25	10	65
		30	20	9	2	45	10	45
		60	20	19	0	95	0	5
2	Diazepam	0.25	20	3	2	15	10	75
		0.50	20	13	2	65	10	25

		1.00	20	5	15	25	75	0
3	Nitrazepam	0.25	20	6	2	30	10	60
		0.50	20	18	1	90	5	5
		1.00	20	12	8	60	40	0

Table c: Showing the percentage of protection of mice against metrazol seizures with anticonvulsant drugs.

Sl.No	Anticonvulsant Drug	Dose in Mg/kg	No.of mice tested	No. of mice protected	No. of mice died	Percentage of protection	Percentage of death
1	Phenobarbitone	15	20	4	16	20	80
		30	20	7	13	35	65
		60	20	17	3	85	15
2	Diazepam	0.25	20	1	19	5	95
		0.50	20	13	7	65	35
		1.00	20	4	16	20	80
3	Nitrazepam	0.25	20	2	18	10	90
		0.50	20	17	3	85	15
		1.00	20	11	9	55	45

Table 4: Showing the protection of mice against pentylenetetrazol seizures with three different doses of phenobarbitone.

Mice No	Dose of Pheno	Dose of Metrazol	Result
1	15 mg/kg	80 mg/kg	+
2	”	”	+
3	”	”	+
4	”	”	-
5	”	”	+
1	30 mg/kg	80 mg/kg	-
2	”	”	+
3	”	”	+
4	”	”	+
5	”	”	-
1	60 mg/kg	80 mg/kg	-
2	”	”	-
3	”	”	-
4	”	”	-
5	”	”	-

+ Convulsions **- Protection**

Maximum Protection with 60 mg/kg

Table 5: Showing the protection of mice against pentylenetetrazol seizures with three different doses of diazepam

Mice No	Dose of Diazepam	Metrazol	Result
1	0.25 mg/kg	80 mgkg	+
2	”	”	+

3	”	”	+
4	”	”	+
5	”	”	+
1	0.50 mg/kg	80 mg/kg	+
2	”	”	-
3	”	”	-
4	”	”	+
5	”	”	-
1	1.00 mg/kg	80 mg/kg	-
2	”	”	-
3	”	”	-
	No convulsions but 4 of them died		
4	”	”	-
5	”	”	-

+ Convulsions - Protections

Maximum Protection with 0.5 mg/kg

Table 6: Showing the protection of mice against phentylenetetrazol seizures with three different doses of nitrazepam

Mice No	Dose of Nitrazepam	Dose of Metrazol	Result
1	0.25 mgkg	80 mg/kg	+
2	”	”	+
3	”	”	+
4	”	”	-
5	”	”	+
1	0.50 mg/kg	80 mg/kg	-
2	”	”	-
3	”	”	-
4	”	”	-
5	”	”	-
1	1.00 mg/kg	80 mg/kg	-
2	”	”	-
3	”	”	-
	Two of them died but no convulsions		
4	”	”	-
5	”	”	-

+ Convulsions - Protection

Maximum Protection with 0.5 mg/kg

DISCUSSION

The time of peak effect varies not only with each drug but also with the species of animals and with the mode of administration.

For an assay of anticonvulsant drugs by chemical method, a chemical convulsant used for the determination of potencies has to be administered just before the anticipated peak effect.

In the present studies all the anticonvulsants were administered intraperitoneally and their peak effect

was determined prior to the evaluation of their potencies against electroseizures and chemical seizures. [10]

The peak anticonvulsant effect of Diazepam and Nitrazepam is 2 hours after intraperitoneal injection whereas for Phenobarbitone it was stretched from 2nd hour to 3rd hour.

The comparative potency of these drugs as obtained by maximal electroshock in mice can be seen in Table 'B' and 'C'. It is obvious that Nitrazepam can be given fairly a good rank for protection of electro seizures at 0.5 mg/kg dosage level a higher percentage of protection was obtained than with Phenobarbitone and Diazepam. Only 0.5 mg/kg is required for Nitrazepam to be considered equipotent to Pheno barbitone of 60 mg/kg, if not superior to it. Diazepam stands last in order as it appears to be toxic in higher doses than Nitrozepam. The ratio of potency based on results of maximal electroshock will be 0.5 mg/kg of Nitrazepam equivalent to 60 mg/kg of Phenobarbitone which in turn will be roughly equivalent to 0.5 mg/kg of Diazepam.

PHENOBARBITONE	**DIAZEPAM**	**NITRAZEPAM**
60 mg/kg	0.5 mg/kg	0.5 mg/kg
120	:1	: 1

The comparative potency of above anticonvulsants varies against Metrazol seizures. The percentage of protection against Metrazol seizures are recorded in Table 'C'.

PHENOBARBITONE	**DIAZEPAM**	**NITRAZEPAM**
85%	65%	85%

The higher percentage of mortality of mice in chemical seizures with anti convulsant drugs cannot be adequately explained. As it can be seen in Tble 'C' that the mortality is higher with lower dosage than at higher dosage. In this respect Nitrazepam again seems better anticonvulsant against chemical seizures than Diazepam and equipotent with Phenobarbitone.

CONCLUSION

1. Phenobarbitone, the commonly used antiepileptic drug two drugs from benzodiazepine group – diazepam and nitrazepam were tested by maximal electroshock seizure pattern and metrazol seizure threshold at their time of peak effect in mice and their potency was compared with phenobarbitone.
2. The time of peak effect of phenobarbitone in mice on intraperitoneal administration was ranging from 2nd hour to 3rd hour whereas for diazepam and nitrazepam it was 2nd hour.
3. 0.5 mg/kg of nitrazepam was equipotent to 60 mg/kg of phenobarbitone which in turn was roughly equivalent to 0.5 mg/kg of diazepam.
4. Nitrazepam was more effective anticonvulsant drug than diazepam against both MES and metrazol seizures.

Acknowledgement

The authors thank the staff for supporting the educational program.

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