Pharmacological Evaluation of Seizures Induced by Electrical Stimulation of the Hippocampus

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Abstract □ A method to induce minimal seizures in unrestrained rats *via* bipolar electrodes implanted in the right dorsal hippocampus has been described. The threshold for such seizures is reproducible, stable over time, and elevated by trimethadione and high doses of diphenylhydantoin. Propranolol and pronethalol also raise seizure threshold, but MJ1999 and D(−)- and L(+)-INPEA are ineffective. The adrenergic agents do not seem to alter seizure threshold by their ability to block β-receptors.

Keyphrases □ Hippocampal stimulation—bipolar electrodes □ Convulsive seizures—electrical stimulation, hippocampus □ Electrically produced seizures—convulsive threshold □ Anticonvulsants effect—electrically produced seizures

Several techniques to produce minimal seizures experimentally have been reported (1). Some involve the application of electric current *via* corneal electrodes to restrained animals, while others employ such chemicals as hexafluorodiethylether and metrazol. The former method is less than ideal, since brief restraint lowers seizure threshold (2), while the latter methods are subject to criticism of possible drug–drug interaction when used in drug studies designed to elucidate seizure mechanisms.

One objective of this study was to develop a method to induce minimal seizures electrically in unrestrained rats. Ideally, such seizures would be easily induced, stable, and reproducible over a long time.

A second objective of this study was to determine whether such seizures would respond to anticonvulsants such as diphenylhydantoin and trimethadione in a manner similar to that observed with other experimentally induced seizures (3–5).

At present, the mechanisms responsible for seizure expression are still not fully known. However, there is much evidence indicating an involvement of catecholamines. For example, Schlesinger *et al.* (6) and Scudder *et al.* (7) reported that mice with higher than normal susceptibility to seizures had lower catecholamine levels. Other studies have shown that reserpine, tetrabenazine, and more selective catecholamine depletors increased seizure susceptibility; while treatment with the catecholamine precursor, L-dopa, in the presence of iproniazid inhibited seizure activity (8–10). In general, the existing evidence seems to relate low catecholamine levels with a high susceptibility to seizure expression.

Some earlier experiments in this laboratory (11) demonstrated that β-adrenergic blocking agents, *e.g.*, propranolol and pronethalol, elevated thresholds in mice to low-frequency electroshock (l. f. ES). In addition, pronethalol protected susceptible mice from audiogenic seizures. Thus, a third objective was to pursue further the effect of selected β-adrenergic blocking agents on convulsive activity by evaluating their ability to modify threshold for electrically induced hippocampal seizures.

EXPERIMENTAL

The drugs studied were diphenylhydantoin, trimethadione, and the adrenergic agents propranolol [1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride], pronethalol [D,L-1-(2′-naphthyl)-2-isopropylaminoethanol hydrochloride], D(−)- and L(+)-INPEA [D(−)- and L(+)-1-(4′-nitrophenyl)-2-isopropylaminoethanol hydrochloride], and MJ1999 [4′-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide]. Except for L(+)-INPEA, all these compounds are well-established peripheral β-adrenergic blocking agents (12).

The experimental animals employed were male Wistar albino rats (200–300 g.).[1] Under pentobarbital anesthesia (45 mg./kg.), these rats were stereotaxically implanted with stainless steel (0.3-mm. diameter) bipolar electrodes (MS 303-018″-312″-SS-010″)[2] in the right dorsal hippocampus (2.59 mm. lateral to saggital zero; 3.80 mm. anterior to frontal zero, and 2.50 mm. below the brain surface) according to the rat atlas of Konig and Klippel (13). The hippocampus was chosen because of its low threshold to seizure expression.

Following surgery, the rats were housed individually in plastic cages (16 × 25 × 25 cm.) with free access to food and water. At least 1 week later, each rat was transferred to a testing chamber (30 × 30 × 50 cm.) equipped with a oneway mirror. The implanted electrodes were then connected to a Grass S4 stimulator in series with a capacitor (to maintain constant waveform), a timer key (set for 6 sec.), and an external resistor (100 ohm). A current passing through the resistor was measured by an oscilloscope and reflected that passing through the electrode. With the key opened, a resistor box was placed in series with the stimulator to measure rat resistance.

In this environment, the rat was able to move about freely. After 5-min. adaptation in the experimental box, the rat was subjected to a series of electrical stimulations of increasing intensities until a minimal seizure was seen.

The stimulus consisted of a train of 0.2-msec. biphasic pulses, 6 sec. in duration with a frequency of 60 c.p.s. A 2-min. interval separated each stimulus. To minimize the total number of stimuli administered to any rat, the following schedule was employed. A starting current intensity of 150 μamp. was increased in steps of 50 μamp. At intensities of 600 μamp., the increment was raised by a factor of 4; above 1000 μamp., it was raised 10 times the original value. The maximum current intensity administered was 3000 μamp.

A seizure was defined as the presence of readily observed jaw chopping and/or myoclonic jerks, and the current intensity that just produced such symptoms was defined as the seizure threshold. In drug studies, rats that did not exhibit seizures with the maximum current were assigned seizure thresholds of 3000 μamp. for statistical computations.

The rats were eventually sacrified with pentobarbital and perfused with saline, followed by 10% formalin, *via* the right ventricle. Serial collodion sections (30 μ) were stained by the technique of Kluver and Barrera (14) and microscopically examined to localize the electrode tract as well as to evaluate possible tissue damage.

All drug injections were made intraperitoneally. Control animals received the drug vehicle.

To determine appropriate doses and the duration of drug activity, pilot studies were conducted based on drug-induced neurotoxicity.

[1] Greenacres Laboratories, Amelia, Ohio.
[2] Plastic Products Co., Roanoke, Va.

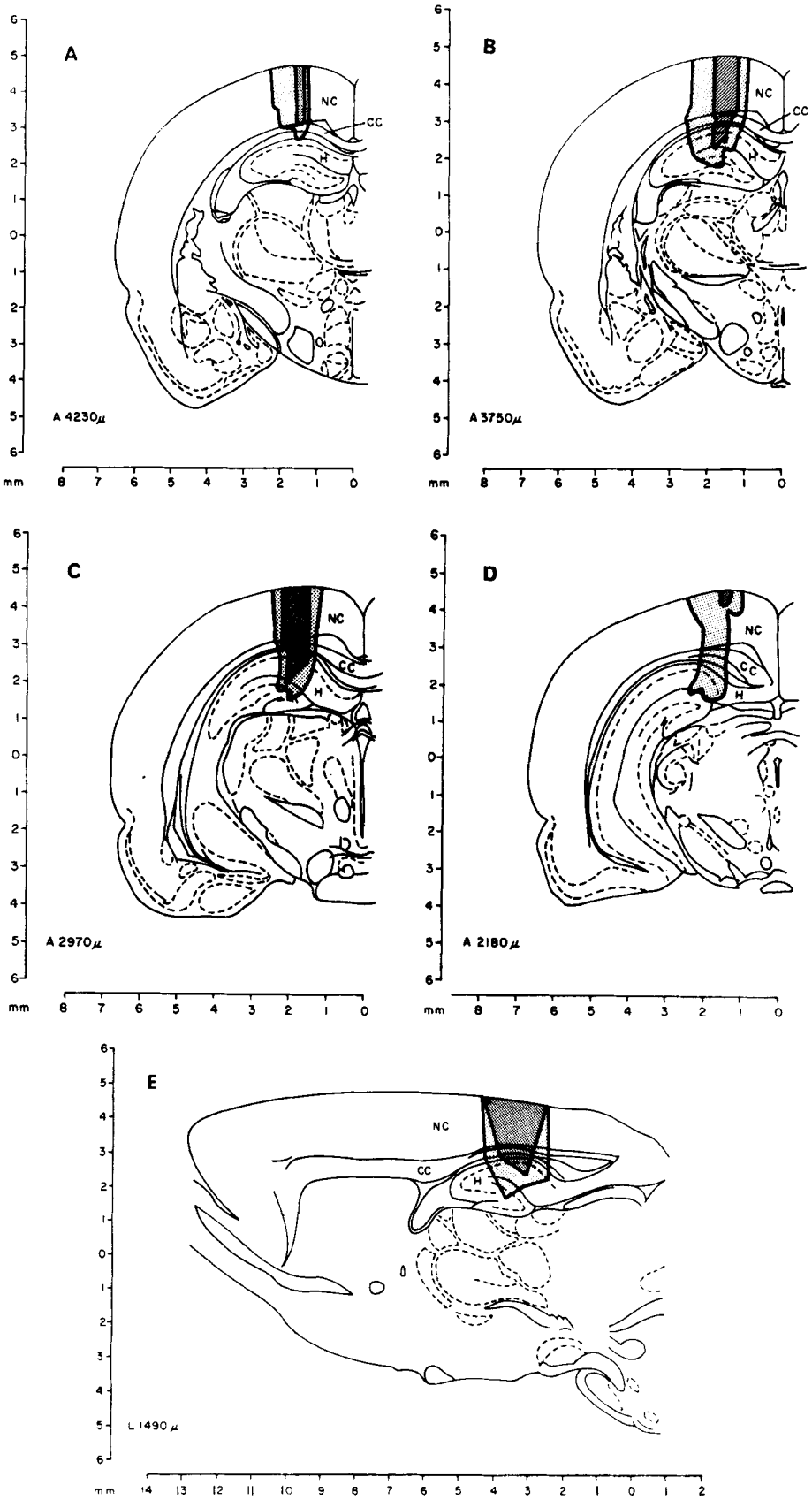

Figure 1—*Minimal (▒) and maximal (:::) extent of the lesions projected on frontal sections (A–D) and saggital section (E) at the stated distances from frontal and saggital zeroes, respectively. Key: NC, neocortex; CC, corpus callosum; and H, hippocampus.*

Table I—Stability of Rat Resistance

Min. between Test	Mean Resistance (kohm) Initial	Final	Difference Mean
2	12.51	11.93	0.58[a]
15	13.05	13.62	0.57[a]
20	12.80	12.94	0.14
30	12.91	14.09	1.18[a]
1440	13.39	13.61	0.22[a]

[a] $p < 0.05$.

This was defined as the failure of a rat to stay on a rotating rod (6 r.p.m.) for 1 min., given three trials. Drug-induced neurotoxicity in 50% of the rats (TD_{50}) with 95% confidence limits was calculated using the method of Litchfield and Wilcoxon (15).

In the evaluation of drug effects on seizure threshold, groups of 6–10 rats (average of 8) with a predetermined stable seizure threshold (SST) were employed. Half of these received the drug and the other half received the drug vehicle. Seizure threshold after drug treatment (DST) and control seizure threshold (CST) were determined at the time of peak neurotoxic effect of the drug. A crossover design with a 7-day interval was employed, so each rat served as its own control. Threshold ratios, *i.e.*, DST/SST and CST/SST, with 95% confidence limits were calculated by the method described by Goldstein (16).

RESULTS AND DISCUSSION

The lesion produced by electrode implantation was reconstructed with the aid of the atlas of Konig and Klippel (13) and is shown in Fig. 1. Tissue damage extended from about 2.2 to 4.2 mm. anterior to frontal zero and from 1.1 to 2.5 mm. lateral–saggital zero. Only the neocortex, the corpus callosum, and the dorsal hippocampus in this region were affected. In all the animals studied, the electrode tip lay in the right dorsal hippocampus between 3.7 and 3.8 mm. anterior to frontal zero, but it was 1.1–2.0 mm. lateral to saggital zero. This could be due to the different strain of rat used in this study compared to those employed for the construction of the Konig and Klippel atlas.

The stability of rat resistance over time was evaluated; these data, presented in Table I, were analyzed by a paired comparison *t* test.

Over time intervals ranging from 2 to 1440 min., rat resistance varied significantly in all but one test. These results emphasize the importance of taking the instantaneous rat resistance into consideration in any measurement of current intensity passing through the brain. Therefore, in all subsequent studies, rat resistance was measured during every stimulus, and the actual current intensity passing through the electrodes was thus recorded.

The stability of hippocampal seizure threshold over time is presented in Fig. 2. The vertical bars denote 95% confidence limits, and any point where a bar does not cross 1.0 indicates a significant change in seizure threshold. These data indicate that hippocampal seizure threshold remained essentially stable over time intervals ranging from 10 to 60 min. between determinations. Additional studies with injection of saline between tests also produced no apparent change in threshold over the same time intervals.

The results obtained in the neurotoxicity studies are represented in Table II. The time of peak effect varied from 10 min. for trimethadione to 60 min. for diphenylhydantoin. The remaining compounds exhibited peak activity between 15 and 30 min. As would be expected from previous studies (4, 5), the neurotoxicity potency of trimethadione was quite low compared to that of the other drugs tested. Complete toxicity studies for MJ1999 were not conducted, due to the limited amount of drug available and its relative nontoxicity. No toxic effect was seen with 8×10^{-1} mmoles/kg. The choice of 30 min. as the time of peak activity for this agent was based on observable gross central nervous system depression and on previous work (17).

Table II—Neurotoxicity of Drugs

Drug	Time of Peak Effect, min.	$TD_{50} \times 10^{-1}$, mmoles/kg.
Diphenylhydantoin	60	8.30 (5.90–11.60)[a]
Trimethadione	10	32.80 (29.50–33.40)
Propranolol	15	1.11 (1.04–1.18)
Pronethalol	15	1.23 (0.99–1.52)
MJ1999	30	>8.0
D(−)-INPEA	20	2.06 (1.35–3.15)
L(+)-INPEA	20	2.08 (1.51–2.87)

[a] Figures in parentheses represent 95% confidence limits.

The effect of diphenylhydantoin on seizure threshold is demonstrated in Fig. 3. Control solution did not alter seizure threshold significantly in this or any subsequent studies. At doses of 0.5 and 1.0×10^{-1} mmoles/kg., diphenylhydantoin did not have a significant effect; a higher dose of 2.5×10^{-1} mmoles/kg. produced a slight but significant elevation of threshold. These results agree with those reported for this compound on other types of experimentally induced minimal seizures, since low doses have no effect on thresholds for minimal electroshock, metrazol, and l.f. ES seizures, while higher doses have been shown to increase l.f. ES seizure threshold (4, 5, 18). Diphenylhydantoin also raises the convulsive threshold of the motor cortex in the monkey (19).

Several workers who measured electrical afterdischarges evoked by stimulation of specific brain areas in monkeys, cats, and rabbits (19–21) generally observed an increase in threshold and shorter discharge duration in the motor cortex, hippocampus, thalamus, and septal area following diphenylhydantoin treatment. However, the compound has been reported to produce no effect on cortical or hippocampal afterdischarges in the rabbit (22), although transcortical spread of abnormal activity from chronic epileptogenic focus in the visual cortex (rabbit) can be suppressed by this agent (23). In view of the wide variation in the animal species employed, the dose, the route of drug administration, the electrical stimulus, and the brain regions stimulated, it is difficult to correlate these data with the present findings. However, diphenylhydantoin is known to have a nonspecific stabilizing action on excitable membranes (24). This is thought to result from a more efficient extrusion of sodium ions from brain cells, probably by stimulation of the metabolic sodium pump (25–27). This activity may account for the slight elevation of seizure threshold induced by the high dose of diphenylhydantoin.

Similar data obtained with trimethadione are presented in Fig. 4. All four doses raised threshold significantly, and an apparent dose–response relationship was seen. The efficacy of trimethadione

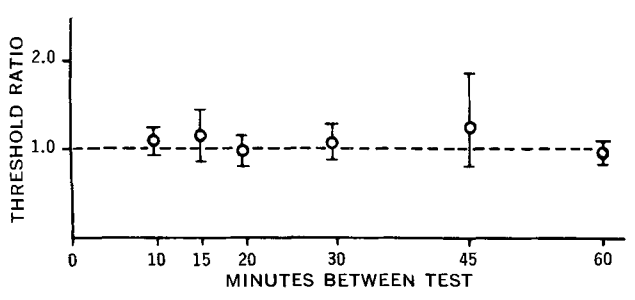

Figure 2—*Stability of hippocampal seizure threshold over time. Threshold ratio was determined by dividing the final seizure threshold by the initial seizure threshold* (n = 6).

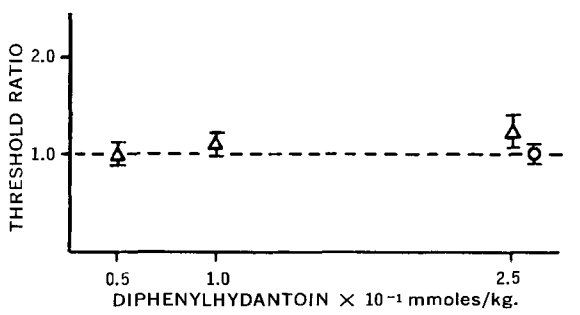

Figure 3—*Effect of diphenylhydantoin on hippocampal seizure threshold* (n = 6). *Key:* ○, *propylene glycol; and* △, *diphenylhydantoin.*

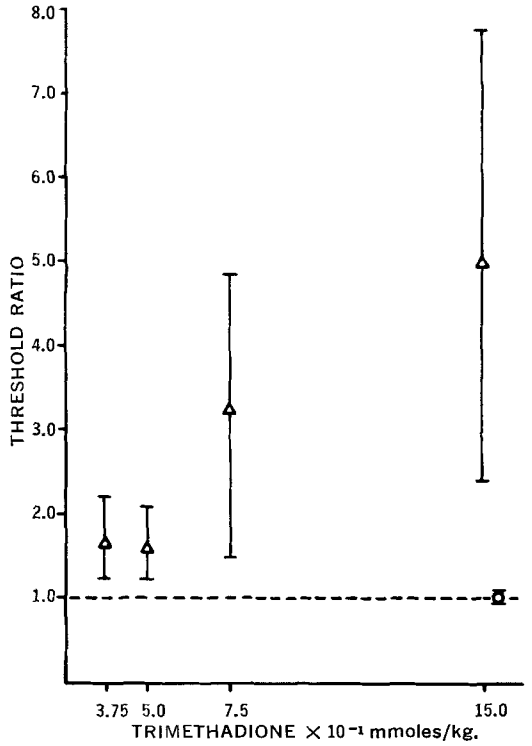

Figure 4—*Effect of trimethadione on hippocampal seizure threshold* (n = *10*). *Key:* ○, *propylene glycol; and* △, *trimethadione.*

in this procedure was further emphasized by the absence of seizures in several animals at the maximum stimulus employed. Again, these data reflect anticipated results, because trimethadione is known to elevate minimal seizure threshold as measured by other techniques (4, 5).

Schallek and Kuehn (20) found trimethadione to be superior to diphenylhydantoin in increasing seizure thresholds at cortical and other brain sites. Furthermore, thresholds to electrical afterdischarges in the motor cortex and the thalamus are elevated, while the duration of these afterdischarges is reduced in several species subsequent to treatment with the drug (19–22). Morrell *et al.* (23) observed that trimethadione not only suppresses chronic epileptogenic foci but also depresses the projection of seizure activity from cortical foci to the thalamus and to the contralateral side. This

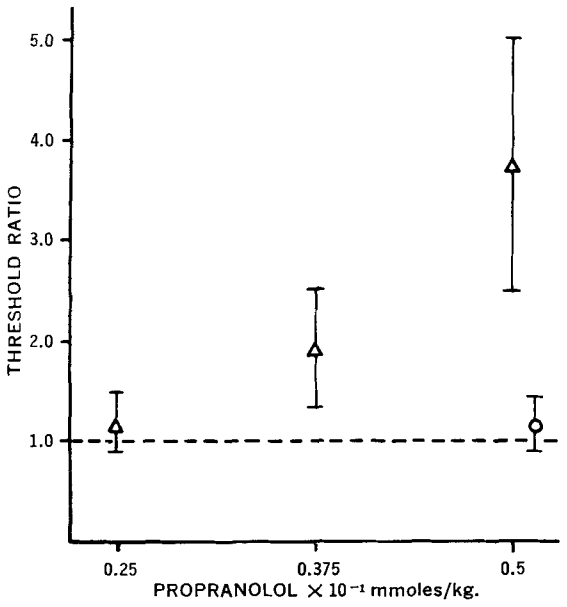

Figure 5—*Effect of propranolol on hippocampal seizure threshold* (n = 9). *Key:* ○, *saline; and* △, *propranolol.*

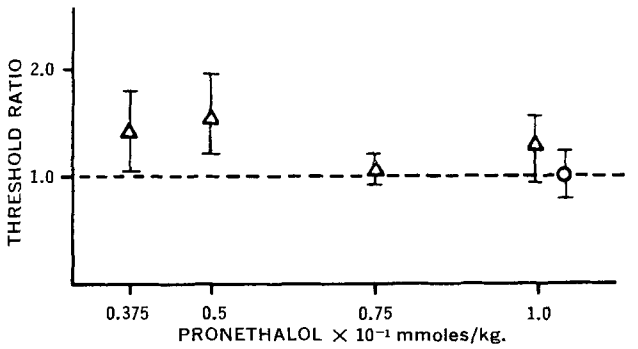

Figure 6—*Effect of pronethalol on hippocampal seizure threshold* (n = *10*). *Key:* ○, *saline; and* △, *pronethalol.*

action may well be due to the ability of the drug to intensify the depression of synaptic transmission following each transmitted volley of impulses (28). Such an action would prevent the build-up and maintenance of an oscillating system between the thalamus and the cortex, postulated to be involved in the precipitation of minimal seizures (26, 29, 30). Thus, the ability of trimethadione to increase seizure threshold in this study may reflect this suppression of showers of impulses at central synapses.

The effects seen with a highly potent β-adrenergic blocking agent, propranolol (31, 32), are illustrated in Fig. 5. At doses higher than 0.25 × 10^{-1} mmoles/kg., it elevated seizure threshold, and again there was an apparent dose–response relationship. An increase in seizure threshold of about 275% was seen with the highest dose employed. At this dose level, several rats did not exhibit seizures even when the maximum stimulus was administered. Since propranolol is known to have central depressant activities, the effect of pronethalol, a less potent β-adrenergic blocking agent with some central stimulant properties, was studied in an attempt to separate effects due to nonspecific central depression from β-blockade. The results are shown in Fig. 6. At low doses, pronethalol increased seizure threshold significantly. A 50% elevation was observed with 0.5 × 10^{-1} mmoles/kg. This is about one-fifth the effect seen with the same dose of propranolol. However, higher doses (in the region of $^1/_2$ TD_{50}) produced no significant alteration of seizure threshold. This could be a reflection of the fact that high doses of pronethalol are known to induce convulsions (33).

Both propranolol and pronethalol have significant local anesthetic properties (34). Thus, the threshold-elevating effects of these two compounds may be related more to this aspect of their activity than to their β-receptor blocking properties. To test this, two blocking agents, MJ1999 and INPEA, reported to be relatively selective as β-adrenergic blocking agents and to possess no local anesthetic action (17, 35), were examined. The results obtained with MJ1999 are seen in Fig. 7. Even with 5 times the effective dose of propranolol, no significant change in seizure threshold was seen.

Similar data obtained with INPEA are presented in Fig. 8. If β-adrenergic blockade was indeed responsible for the elevation of seizure threshold exhibited by propranolol and pronethalol, marked differences between the effects of these two isomers of INPEA would be expected, since only the D(−)-isomer has been reported to be active as a β-adrenergic blocking agent (12). However, both isomers were equally ineffective in changing seizure

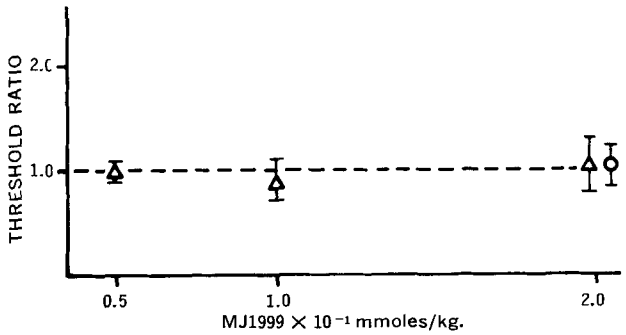

Figure 7—*Effect of MJ1999 on hippocampal seizure threshold* (n = *8*). *Key:* ○, *saline; and* △, *MJ1999.*

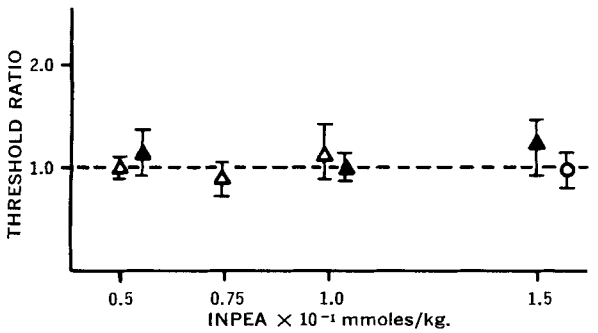

Figure 8—*Effect of INPEA on hippocampal seizure threshold* (n = 9). *Key:* ○, *saline;* △, D(−)-*INPEA; and* ▲, L(+)-*INPEA.*

threshold, even at doses 4 times greater than the effective dose of propranolol.

Thus, although these data do not explain the mechanism whereby propranolol and pronethalol elevate hippocampal seizure threshold, they do provide evidence that the alteration in seizure susceptibility produced by these agents is not causally related to blockade of β-receptors.

CONCLUSION

The threshold of seizures induced by electrical stimulation of the hippocampus in unrestrained rats has been shown to be stable and reproducible over time. Furthermore, it is modified by typical anticonvulsants in a manner similar to that observed with other types of experimentally induced minimal seizures.

Both propranolol and pronethalol increased seizure threshold. These findings are consistent with those seen in earlier studies with l.f. ES and audiogenic seizures (11). However, other β-adrenergic blocking drugs do not have a threshold-elevating effect. Murmann *et al.* (36) have reported essentially similar results employing different seizure-inducing techniques. They found that propranolol and pronethalol reduced susceptibility of animals to maximal metrazol and maximal electroshock seizures, but reported L(+)- and D(−)-INPEA to be ineffective. MJ1999 has also been demonstrated by Chen *et al.* (10) and Lish *et al.* (17) to be incapable of altering both maximal and minimal electroshock seizures.

The results of the latter portion of this study largely substantiate the hypothesis of Leszkovszky and Tardos (37) and Murmann *et al.* (36) that certain β-adrenergic blocking compounds affect seizure expression by mechanisms other than β-blockade.

However, as stated previously, evidence supporting a catecholamine influence on seizure expression exists in the literature. Moreover, α-adrenergic blocking agents, phenoxybenzamine and phentolamine, have been reported to increase seizure susceptibility (10). In view of this, an investigation of the effects of α-adrenergic blocking agents on seizure threshold is currently in progress and should provide useful information.

REFERENCES

(1) J. G. Millichap, *Physiol. Pharmacol.*, **2,** 97(1965).

(2) E. A. Swinyard, N. R. Radhakrishnan, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **138,** 337(1962).

(3) J. E. P. Toman and L. S. Goodman, *Physiol. Rev.*, **28,** 409 (1948).

(4) E. A. Swinyard, W. C. Brown, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106,** 319(1952).

(5) W. C. Brown, D. O. Schiffman, E. A. Swinyard, and L. S. Goodman, *ibid.*, **107,** 273(1953).

(6) K. Schlesinger, W. Boggan, and D. X. Freedman, *Life Sci.*, **4,** 2345(1965).

(7) C. L. Scudder, A. G. Karczmar, G. M. Everett, J. E. Gibson, and M. Rifkin, *Int. J. Neuropharmacol.*, **5,** 343(1966).

(8) E. H. Jenny, *Fed. Proc.*, **13,** 370(1954).

(9) J. P. Cooke, *Texas J. Sci.*, **13,** 235(1961).

(10) G. Chen, C. R. Ensor, and B. Bohner, *Arch. Int. Pharmacodyn.*, **172,** 183(1968).

(11) P. N. Yeoh and H. H. Wolf, *J. Pharm. Sci.*, **57,** 340(1968).

(12) L. Almirante and W. Murmann, *J. Med. Chem.*, **9,** 650 (1966).

(13) J. F. R. Konig and R. A. Klippel, "The Rat Brain," 1st ed., Williams and Wilkins, Baltimore, Md., 1963.

(14) H. Kluver and E. Barrera, *J. Neuropath. Exp. Neurol.*, **12,** 400(1953).

(15) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96,** 99(1949).

(16) A. Goldstein, "Biostatistics," Macmillan, New York, N. Y., 1964, p. 184.

(17) P. M. Lish, J. H. Weikel, and K. W. Dungan, *J. Pharmacol. Exp. Ther.*, **149,** 161(1965).

(18) H. H. Wolf, E. A. Swinyard, and L. S. Goodman, *J. Pharm. Sci.*, **51,** 74(1962).

(19) J. M. R. Delgado and L. Mihailovic, *Ann. N. Y. Acad. Sci.*, **64,** 644(1956).

(20) W. Schallek and A. Kuehn, *Proc. Soc. Exp. Biol. Med.*, **112,** 813(1963).

(21) R. R. J. Strobos and E. V. Spudis, *Arch. Neurol.*, **2,** 399 (1960).

(22) H. Gangloff and M. Monnier, *Electroencephalog. Clin. Neurophysiol.*, **9,** 43(1957).

(23) F. Morrell, W. Bradley, and M. Ptashne, *Neurology*, **9,** 492(1959).

(24) J. E. P. Toman and J. D. Taylor, *Epilepsia*, **1** (3rd series), 31(1952).

(25) D. M. Woodbury, *J. Pharmacol. Exp. Ther.*, **115,** 74(1955).

(26) J. E. P. Toman, in "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Macmillan, New York, N. Y., 1965, p. 215.

(27) D. W. Esplin and B. Zablocka, *Epilepsia*, **10,** 193(1969).

(28) D. W. Esplin and E. M. Curto, *J. Pharmacol. Exp. Ther.*, **121,** 457(1957).

(29) A. Kreindler, *Progr. Brain Res.*, **19,** 160(1965).

(30) A. Vernadakis and D. M. Woodbury, *Epilepsia*, **10,** 163 (1969).

(31) J. W. Black, A. F. Crother, R. G. Shanks, L. H. Smith, and A. C. Dornhorst, *Lancet*, **I,** 1080(1964).

(32) C. Raper and J. Wale, *Europ. J. Pharmacol.*, **3,** 279(1968).

(33) J. W. Black and J. S. Stephenson, *Lancet*, **II,** 311(1962).

(34) A. Morales-Aguilers and E. M. Vaughan Williams, *Brit. J. Pharmacol.*, **24,** 332(1965).

(35) P. Somani and B. K. B. Lum, *J. Pharmacol. Exp. Ther.*, **147,** 194(1965).

(36) W. Murmann, L. Almirante, and M. Saccani-Guelfi, *J. Pharm. Pharmacol.*, **18,** 317(1966).

(37) G. Leszkovszky and L. Tardos, *ibid.*, **17,** 518(1965).

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