Brit. J. Pharmacol. (1959), **14**, 415.

DRUG ANTAGONISM AND AUDIOGENIC SEIZURES IN MICE

BY

C. W. M. WILSON*

From La Laboratoire de Physiologie Acoustique, Institut National de la Recherche Agronomique. Jouy-en-Josas, Seine et Oise, France

(RECEIVED FEBRUARY 13, 1959)

The occurrence of audiogenic seizures in mice has been used as the basis of a method for measuring the antagonism between sodium thiopentone and bemegride or leptazol. Thiopentone in doses of 8 and 16 mg./kg. protected all the mice against the occurrence of audiogenic seizures. Bemegride and leptazol were administered in doses having molecular concentrations which bore a simple relationship to the doses of thiopentone. Bemegride was four times as powerful as leptazol in antagonizing the protective effect of thiopentone, and comparison of their regression lines showed that both analeptics acted in the same way. The antagonistic effect of bemegride occurred with doses less than its normal convulsant dose, but the doses of leptazol needed to antagonize thiopentone were within the convulsant range, and it increased the severity of any audiogenic seizures.

The susceptibility of rats and mice to audiogenic seizures has been known for a considerable time, and has been fully described (Smith, 1941 ; Frings and Frings, 1953). The psychological and physiological factors which influence the occurrence of these seizures have also been extensively investigated. The audiogenic seizures have been compared with human epilepsy (Lindsley, Finger, and Henry, 1942) and with leptazol convulsions (Maier and Sacks, 1941). Like seizures induced by electric shock, audiogenic seizures are produced by physical stimuli, and lend themselves easily to inhibition by hypnotic and anticonvulsant drugs. There are quantitative differences in the power of these drugs to suppress the seizures, and Lehmann, Halpern, and Busnel, (1957) and Busnel, Lehmann, and Busnel (1958) have suggested sites at which the drugs might act.

Previous investigators have studied the actions of convulsant or anticonvulsant drugs individually on the production of audiogenic seizures. In the present experiments the ease and certainty with which audiogenic seizures can be produced in susceptible mice have been used as the basis of a method for measuring the antagonism between anticonvulsant and convulsant drugs. The occurrence of an audiogenic seizure has been used to determine whether the action of an anticonvulsant drug, which inhibits the production of a seizure, has been overcome by the action of an antagonist Thiopentone has been used as the anticonvulsant, and leptazol and bemegride have been used as its antagonists. Leptazol and bemegride themselves have convulsant actions, but, by comparing their convulsant doses with the doses necessary to antagonize the action of thiopentone, it was possible to differentiate these two effects. In order to make these dose comparisons, the drugs have been administered in doses which bore simple relationships to their molecular weights.

METHODS

Male and female mice (18 to 35 g. body weight) were derived from the strain of Swiss albino mice selected by Frings and Frings (1953) in the U.S.A. These mice respond to a certain kind of intense auditory stimulation by seizures of a clonic-tonic type, named audiogenic seizures (Frings and Frings, 1952).

Audiogenic seizures were produced by an auditory stimulus of 10 kc./sec. with an intensity of 100 decibels emitted close to the mice. The mice were tested individually (Lehmann, 1956). The audiogenic seizure started after a short latent period of 5 to 40 sec. and was indicated by flushing of the tail and exophthalmos, immediately followed by considerable agitation during which the mouse ran rapidly round the cage. Generalized clonic convulsions then occurred, which gradually disappeared or were followed by a catatonic seizure, during which the mouse became stiff and breathing ceased. Breathing

*Present address: Department of Pharmacology and General Therapeutics, University of Liverpool.

generally recommenced spontaneously in about 30 sec. and the mouse recovered. Occasionally artificial ventilation was necessary, but it was uncommon for death to follow the seizure. This sequence of events lasted about 1 min., and the mouse remained resistant to further auditory stimuli for a period of 6 to 12 hr. In assessing the results, an audiogenic seizure was defined as a seizure in which clonic or clonic-tonic phases occurred. The phases of vasodilatation, exophthalmos, and running, when they occurred alone, were not classified as audiogenic seizures. The ability of the mice to develop seizures was tested 24 hr. before the administration of the drugs, during the period of action of the drugs, and 24 to 48 hr. after administration. Any animals in which audiogenic seizures did not occur during the experiments done before and after the 24 hr. test period were excluded from the results. The experiments were always performed in the same laboratory, the temperature of which varied between 19.8 and 21.0°.

Sodium thiopentone, leptazol, and bemegride were administered by intraperitoneal injection. Dilutions were made in 0.9% NaCl, and the volume injected never exceeded 0.75 ml. Thiopentone was administered at zero time, and the reactions of the mice to the auditory stimuli were tested 30 min. later. When an analeptic was being examined together with the thiopentone, the analeptic was injected 10 min. after the thiopentone, and susceptibility to the stimulus was tested 20 min. later. At each end of the analeptic dose ranges four mice were used, and for the other doses larger groups (up to twelve mice) were tested.

Results

The Protective Action of Thiopentone

Thiopentone in doses of 8 or 16 mg./kg. abolished audiogenic seizures 30 min. after administration in all the mice. Neither of these doses caused loss of consciousness or any obvious signs that they were having a therapeutic effect. Smaller doses had less protective action, and a dose of 1 mg./kg. did not prevent the occurrence of seizures. The protective effect of thiopentone is shown in Fig. 1.

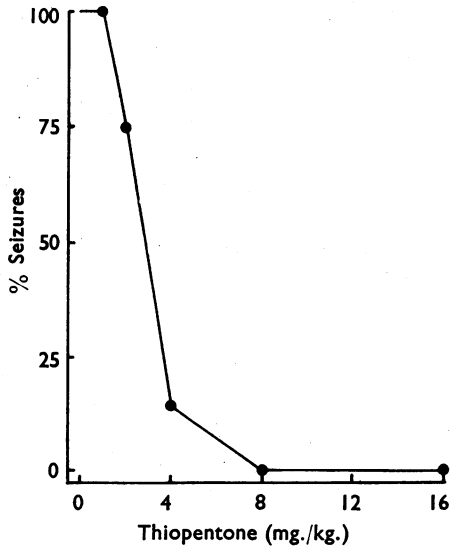

FIG. 1.—The effect of thiopentone in preventing the occurrence of audiogenic seizures in mice.

TABLE I

PERCENTAGE OF MICE HAVING CONVULSIONS WITHIN 10 MIN. OF THE INTRAPERITONEAL INJECTION OF BEMEGRIDE AND OF LEPTAZOL

Drug	Dose (mg./kg.)	No. of Mice	% of Mice having Convulsions	
			Clonic	Tonic
Bemegride	20	2	0	0
	25	6	40	0
	30	5	100	0
	40	4	100	100
Leptazol	40	5	0	0
	50	6	50	17
	60	5	100	50

The Convulsant Effects of Bemegride and Leptazol

The convulsions produced by these drugs were similar. They occurred within 2 to 10 min. of administration, and, with the smaller doses, consisted of intermittent clonic convulsions. With larger doses, the clonic phase ultimately developed into a tonic phase, which was invariably fatal. The doses which caused the convulsions are shown in Table I. The dose of bemegride to cause convulsions in 50% of the mice (CD50) was obtained by interpolation of the line relating log dose and percentage of mice convulsing; it was found to be approximately 25.5 mg./kg. The CD50 of leptazol was 50 mg./kg.

Effects of the Analeptics and Thiopentone on Audiogenic Seizures

The method, described by Sanford (1958) for cats, in which the relationship between the doses of the hypnotic and analeptic drugs is expressed in terms of molecular ratios, has been employed for the quantitative assessment of drug antagonism in the present experiments. Doses of thiopentone were used which prevented the occurrence of audiogenic seizures in all the mice—namely, 8 and 16 mg./kg. The analeptics were administered in doses which bore simple molecular relationships to these doses of thiopentone. When referring to the doses of the analeptics the term molecular dose

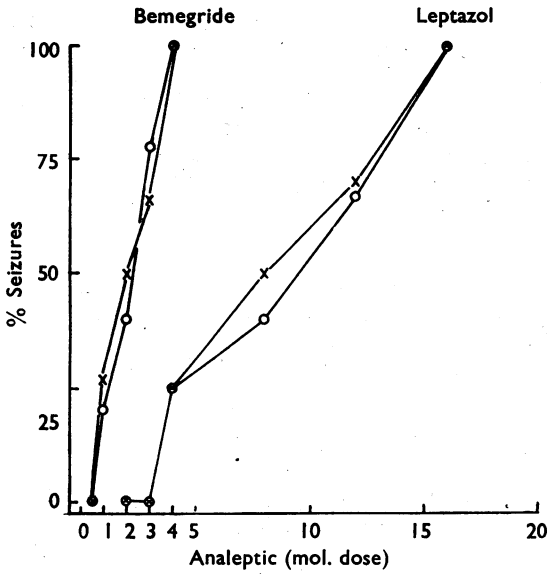

FIG. 2.—The actions of bemegride and leptazol in restoring the susceptibility to audiogenic seizures in mice pretreated with thiopentone. X—X, thiopentone 8 mg./kg. O—O, thiopentone 16 mg./kg.

(mol. dose) has been employed. It indicates the number of molecules of the analeptics administered per single molecule of the hypnotic.

Bemegride.—The administration of bemegride to mice which had received thiopentone in doses of either 8 or 16 mg./kg. caused the reappearance of the susceptibility to audiogenic seizures. Equimolar doses of bemegride were equally effective against the large and the small dose of thiopentone. A 0.5 mol. dose did not cause the reappearance of this susceptibility with either dose of thiopentone, 2 mol. doses caused the reappearance of susceptibility to seizures in 50% of the mice, and 4 mol. doses completely abolished the protective effect of the thiopentone (Fig. 2).

Transformation of the experimental results into probits for each dose of thiopentone, and analysis of the regression lines thus obtained, showed that a linear response/log dose relation existed. A test for parallelism between the two lines demonstrated that they were parallel, and that there was no significant difference between their positions. Therefore the experimental results for the antagonistic action of bemegride towards both doses of thiopentone have been combined and a common regression line has been fitted (Fig. 3).

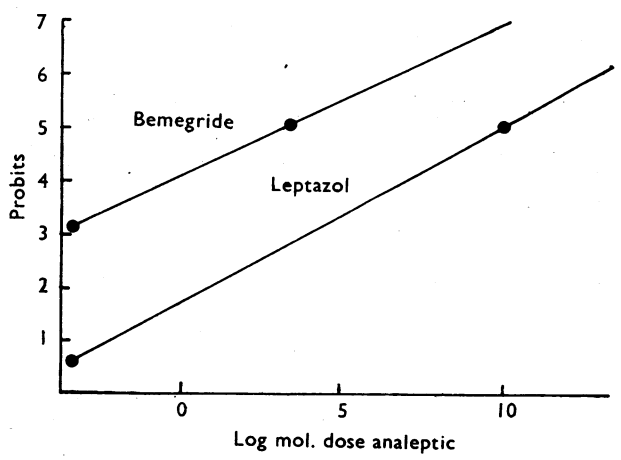

FIG. 3.—Regression lines showing the ability of leptazol and bemegride to restore the susceptibility to audiogenic seizures of mice pretreated with thiopentone. Each line is obtained from the values of the analeptic in the presence of 8 and 16 mg./kg. of thiopentone. Abscissa, log mol. dose. Ordinate, probits of mice showing audiogenic seizures.

TABLE II
COMPARISON OF THE CONVULSANT DOSES (CD50) AND THE AUDIOGENIC SEIZURE DOSES (ASD50) OF BEMEGRIDE AND LEPTAZOL

The ASD50 is the dose of analeptic with which, in the presence of thiopentone, 50% of the animals had audiogenic seizures.

Analeptic	CD50 (mg./kg.)	ASD50	
		8 mg./kg. Thiopentone	16 mg./kg. Thiopentone
Bemegride ..	25·5	9·1	18·2
Leptazol ..	50·0	31·9	63·7

Mice which had received the 4 mol. doses of bemegride in the presence of thiopentone did not appear to be abnormally sensitive to sensory stimuli, and did not show the periodic clonic seizures which occurred when they received bemegride alone in similar doses. In Table II the CD50 of bemegride by itself is compared with the dose which would cause audiogenic seizures in 50% of the mice (ASD50) in the presence of thiopentone. The values of the ASD50 have been expressed as metric doses in Table II. It can be seen that the reappearance of audiogenic seizures in the mice occurred after doses of bemegride smaller than those necessary to produce convulsions when the drug was acting alone.

Leptazol.—Leptazol (2 to 16 mol. doses) also caused the reappearance of susceptibility to audiogenic seizures in mice which had received both 8 and 16 mg./kg. of thiopentone. With 16 mol. doses of leptazol seizures reappeared in all the mice. A 2 mol. dose had no effect, and an 8 mol. dose caused the reappearance of seizures in about half of the mice (Fig. 2). Transformation

of the results into probits showed that linear response/log dose relations existed for each dose of thiopentone, and that the lines corresponded closely. As with bemegride, a common regression line was fitted to the results (Fig. 3).

Mice which had received the 16 mol. dose became restless, and sensitive to external stimuli, but did not develop convulsions spontaneously. When subjected to the auditory stimulus they quickly developed severe seizures of the clonic-tonic type, and these were fatal in 10% of the animals. It was clear that the large dose of leptazol increased the severity of the audiogenic seizures. It can be seen from Table II that a larger dose of leptazol than the CD50 was necessary to antagonize the 16 mg./kg. dose of thiopentone.

Comparison of the Effects of Bemegride and Leptazol

The regression lines for bemegride and leptazol in Fig. 3 were tested and shown to be parallel. The ASD50 of bemegride against both doses of thiopentone was 1.929 mol. doses, and that for leptazol was 7.658 mol. doses, giving an ASD potency ratio of 3.970. By calculation the ASD potency ratio of the two drugs was found to be 4.000 (fiducial limits 2.914 and 5.943, $P=0.05$). Leptazol, besides being less potent than bemegride in antagonizing thiopentone, was an effective antagonist only in doses close to or exceeding its convulsant dose.

Discussion

Several methods have been used to investigate drugs which appear to have antagonistic actions in the central nervous system. The ability of analeptics to prevent the lethal effects of a hypnotic (Maloney, 1931), to restore breathing (Marshall, Walzl, and Le Messurier, 1937), to shorten the sleeping time (Shaw, Simon, Cass, Shulman, Anstee, and Nelson, 1954), and to influence muscular activity in conscious mice (Dews, 1953 ; Brown, 1957), can all be used to measure the effects of drugs on functions attributed to extensive or more circumscribed areas of the central nervous system. The present experiments have examined the antagonistic actions of drugs on the areas of the central nervous system in which convulsions originate. However, it is clear that these areas, which functionally might be fairly circumscribed, display considerable variation in their sensitivity to different convulsant and anticonvulsant drugs (Tripod, Bein, and Meier, 1954). In the present experiments, thiopentone prevented the occurrence of the seizures in doses which had no other noticeable effects on the mice. Mercier (1950) and Gross, Tripod, and Meier (1955) found that phenobarbitone was effective against audiogenic seizures in small doses which caused few other effects, and Humphrey (1942) comments on the very small dose of atropine, in comparison with the lethal dose, which was needed to prevent the occurrence of audiogenic seizures. It is clear from these results that the areas in which convulsions arise are more sensitive than other parts of the brain to barbiturates and other hypnotic drugs. Halpern and Lehmann (1956) explained these results, and their own in which the carbamate of methylpentynol inhibited audiogenic seizures, by suggesting that the seizures are mediated through the reticular formation.

The analeptics were always given at the same interval after the barbiturate, and in making quantitative comparisons it has been assumed that the analeptics reached their sites of action in the brain in the same ratios as those in which they were injected. Both bemegride and leptazol antagonized the protective action of thiopentone against audiogenic seizures. The occurrence of a seizure in the present experiments has been used as an indication of complete antagonism of the barbiturate by each of the analeptics. Leptazol increased the severity of the audiogenic seizures in doses which corresponded approximately to the convulsant dose, and also to the dose which antagonized the effect of the thiopentone on the site of origin of convulsions. The ability of leptazol to increase the severity of the audiogenic seizures has also been observed by other workers (Maier, Sacks, and Glaser, 1941 ; Karn, Lodowski, and Patton, 1941 ; Chen, Ensor, and Bohner, 1954). The 4 mol. doses of bemegride, both equally effective in antagonizing thiopentone, were less than the convulsant dose of bemegride. Quantitative comparison of the antagonism of the analeptics towards thiopentone showed that bemegride was four times as powerful as leptazol. Bemegride therefore acted as an effective antagonist to thiopentone in a dose which, molecule for molecule, was smaller than the antagonistic dose of leptazol. This dose was also considerably less than the normal convulsant dose.

I should like to express my thanks to Dr. B. N. Halpern for his interest and encouragement throughout these experiments, to Monsieur R. G. Busnel and his staff for their interest, hospitality, and assistance, and to Mr. R. L. Plackett for his guidance and help in the statistical analysis of the results. This work was performed during the tenure in France of a Wellcome Research Travel Grant.

REFERENCES

Brown, B. B. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 677.

Busnel, R. G., Lehmann, A., and Busnel, M. C. (1958). *Sem. Hôp. Paris*, **34**, 749.

Chen, G., Ensor, C. R., and Bohner, B. (1954). *Proc. Soc. exp. Biol., N.Y.*, **86**, 507.

Dews, P. B. (1953). *Brit. J. Pharmacol.*, **8**, 46.

Frings, H., and Frings, M. (1952). *J. acoust. Soc. Amer.*, **24**, 163.

—— —— (1953). *Behaviour*, **5**, 305.

Gross, F., Tripod, J., and Meier, R. (1955). *Schweiz. med. Wschr.*, **85**, 305.

Halpern, B. N., and Lehmann, A. (1956). *C.R. Soc. Biol., Paris*, **150**, 1863.

Humphrey, G. (1942). *J. comp. Psychol.*, **33**, 315.

Karn, H. W., Lodowski, C. H., and Patton, R. A. (1941). Ibid., **32**, 563.

Lehmann, A. (1956). *C.R. Soc. Biol., Paris*, **150**, 1860.

Lehmann, A., Halpern, B. N., and Busnel, R. G. (1957). *J. Physiol. Path. gén.*, **49**, 265.

Lindsley, D. B., Finger, F. W., and Henry, C. E. (1942). *J. Neurophysiol.*, **5**, 185.

Maier, N. R. F., and Sacks, J. (1941). *J. comp. Psychol.*, **32**, 489.

—— —— and Glaser, N. M. (1941). Ibid., **32**, 379.

Maloney, A. H. (1931). *J. Pharmacol. exp. Ther.*, **31**, 267.

Marshall, E. K., Walzl, E. M., and Le Messurier, D. H. (1937). Ibid., **60**, 472.

Mercier, J. (1950). *C.R. Soc. Biol., Paris*, **144**, 117.

Sanford, J. (1958). *Vet. Rec.*, **70**, 592.

Shaw, F. H., Simon, S. E., Cass, N., Shulman, A., Anstee, J. R., and Nelson, E. R. (1954). *Nature, Lond.*, **174**, 402.

Smith, K. U. (1941). *J. comp. Psychol.*, **32**, 311.

Tripod, J., Bein, H. J., and Meier, R. (1954). *Arch. int. Pharmacodyn.*, **96**, 406.