Brit. J. Pharmacol. (1953), **8**, 225.

ANTICONVULSANTS ON ELECTRICALLY STIMULATED METABOLISM OF SEPARATED MAMMALIAN CEREBRAL CORTEX

BY

OLGA FORDA AND H. McILWAIN

From the Biochemical Laboratories, Institute of Psychiatry, (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E.5

(RECEIVED DECEMBER 2, 1952)

Convulsive activity, initiated in the central nervous system of experimental animals by various means, is associated with large biochemical changes. These do not occur when the convulsion is prevented by anticonvulsants. As reported earlier, comparable chemical changes can be brought about in completely separated sections of mammalian cerebral tissues by suitably applied electric potentials (Anguiano and McIlwain, 1951). The present experiments were carried out to find whether the changes induced electrically in the separated tissues were affected by anticonvulsive agents. Such action was found, and further experiments were then carried out to see to what extent the anticonvulsants were distinct from general depressant drugs in their action on the separated, electrically stimulated, cerebral cortex.

The agents studied were chosen to include representatives of the main chemical types of substances which have been used clinically as anticonvulsants, namely: sodium bromide, phenobarbitone, 3:5:5-trimethyloxazolidine-2:4-dione (trimethadione ; Tridione), 5:5-diphenylhydantoin (phenytoin ; Dilantin), and phenylacetylurea (Phenurone). They were compared with the general depressants butobarbitone, chloral hydrate, and urethane.

METHODS AND MATERIALS

Tissue Metabolism.—Guinea-pigs and rats were stunned by a blow on the neck, bled, and the cerebrum removed ; specimens from rabbits were similarly obtained after killing by injecting air into an ear vein. For metabolic experiments, either conical electrode vessels *E* (McIlwain, 1951) were used, or vessels *A* with grid electrodes (Ayres and McIlwain, unpublished ; see Fig. 1). For vessels *E* about 60 mg. of tissue was weighed, chopped (McIlwain and Buddle, 1953), and suspended in saline in the vessel. With grid electrodes, slices 0.35 mm. thick were cut (McIlwain, 1951) and floated into the experimental saline. Slices were trimmed to rectangles about 8×12 mm., drained, weighed (about 35 mg.), fitted to the electrode holders, and these were placed in the vessels already containing saline and absorbing agents, electrical connections tested, the vessels fitted to manometers and shaken at 37° C.

An experiment usually comprised six vessels in which all tissues, whether or not they were subjected to electrical impulses, were between electrodes ; tissues were normally ready for placing at 37° C. 20–30 min. after the death of the animal. Pressure in the vessels was measured each 5 or 10 min. during successive periods, each of which included at least six readings. Experiments normally comprised three or four such periods, and electrical conditions in some of the vessels were changed in successive periods. Salines were those of McIlwain (1951).

Electrical Impulses.—Voltage-time relationships are illustrated in Fig. 1. Condenser pulses were alternating, 100/sec., and of peak voltage and duration

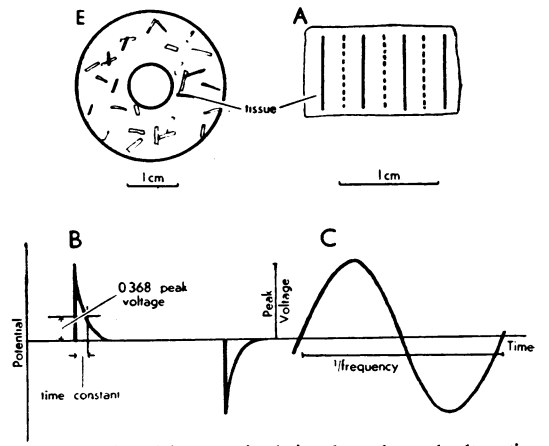

FIG. 1.—Relation of tissue to stimulating electrodes, and voltage-time relationships of impulses employed. A: grid electrodes *A*, the full lines representing wires of a grid connected together above the tissue slices and the dotted lines those also connected together of a grid below the tissue slice; E: concentric electrodes in vessels *E*; B: condenser pulses; C: sine-wave alternating current.

quoted in individual experiments. They were obtained and measured by the instrument described by Ayres and McIlwain (unpublished). Sine-wave alternating current of 50 cyc./sec. was usually derived from mains supply through a transformer; of other frequencies (and also in some experiments of 50 cyc./sec.) it was supplied by a beat-frequency oscillator. The supply voltage and the current through the individual vessels were measured at several times throughout the experiments. The frequency and purity of the sine-wave were determined by displaying the time-voltage relationships with an oscilloscope throughout the experiment.

Materials.—Drugs were of the standards of the *British Pharmacopoeia*, the *British Pharmaceutical Codex*, or of *New and Nonofficial Remedies*. They were dissolved in portions of the experimental saline, and these solutions pipetted into some of the experimental vessels. Tissues exposed to the drug were thus in contact with it as soon as they were placed in the vessels. Trimethadione and phenylacetylurea were obtained from Abbott Laboratories; phenobarbitone, chloral hydrate, diphenylhydantoin, and urethane from British Drug Houses; and butobarbitone from May & Baker. Chloral hydrate is presumably acting partly by virtue of breakdown products (Butler, 1949).

RESULTS

Metabolism of Tissues Unstimulated and Stimulated by Condenser Pulses

Of the metabolic changes induced in cerebral tissues by electrical impulses (McIlwain, 1952), those in respiration are of importance in the tissue's main energy-yielding reactions. In stimulated tissues respiration is affected by general depressants at concentrations below those which affect metabolism in unstimulated tissues (McIlwain, 1953). Table I contrasts such an action of butobarbitone with lack of action in trimethadione or sodium bromide. The concentration of butobarbitone is one found to be attained *in vivo* during its action as a depressant; this concentration has a small effect only on the normal respiration of the tissue, but lowers by some 30 or 40% the respiratory effect of applied impulses. Sodium bromide and trimethadione were examined at concentrations about or much higher than those active *in vivo* as anticonvulsants, but had no effect on respiration either with or without application of condenser pulses.

TABLE I

TRIMETHADIONE, A BROMIDE, AND A BARBITURATE ON RESPIRATION WITH AND WITHOUT CONDENSER PULSES

Results quoted are from individual experiments with guinea-pig cerebral cortex in conical vessels *E*, half of which were not subjected to electrical impulses. Condenser pulses, time-constant 0.4 msec., were applied to others during their second period (peak potential, 10 V) and third period (peak potential, 20 V).

Drug (M)	Respiration in Vessels Without Stimulation (μmol. O_2/g./hr.)			Respiration in Vessels With Stimulation (μmol. O_2/g./hr.)		
	0–30 min.	30–60 min.	60–120 min.	0–30 min.	30–60 min.	60–120 min.
Trimethadione, 0 ..	59	60	60	58	98	116
,, 10^{-3} ..	63	61	61	60	97	112
,, 10^{-2} ..	61	61	61	57	94	115
Sodium bromide, 0 ..	60	59	59	59	102	122
,, 2×10^{-2}	61	58	59	62	100	121
Butobarbitone, 0 ..	65	64	65	62	92	111
,, 7×10^{-4}	61	61	60	58	79	84

In addition to the comparison of Table I, trimethadione has been found to be without effect on the unstimulated respiration of guinea-pig and rat cerebral cortex at concentrations up to 1.33×10^{-2} M, with glucose or glutamate as substrate. Similarly negative results with both substrates were obtained with diphenylhydantoin at 10^{-4} M. These findings are not inconsistent with a previous observation (Taylor, Richards, Everett, and Bertcher, 1950) that trimethadione in extremely high concentrations (0.035–0.14 M) may inhibit respiration of mouse brain slices.

Respiration of Tissues Stimulated by Sine-wave Alternating Currents

Respiration of cerebral tissues is increased by sine-wave a.c. of a wide range of frequencies. The frequency stimulating at minimum voltage is about 50–100 cyc./sec., and stimulation is lost below about 5 or above about 5,000 cyc./sec. In the present experiments frequencies both close to and remote from the optimum were examined. Effects of the anticonvulsants were then discovered, and were found to be dependent on the frequency of the impulses applied.

This is shown in the case of trimethadione in Table II. Here the drug at 2×10^{-4} or 10^{-3} M had little effect on respiration stimulated by 50 cyc./sec. a.c., but it largely prevented a.c. at 500 or 2,000 cyc./sec. from having its usual stimulating effect. Butobarbitone, in contrast, was found to depress respiration stimulated by a.c. at all the frequencies examined, and was thus affecting the metabolic sequelae of a.c. impulses in the same fashion as it acted on those from condenser discharges.

Several drugs were therefore examined with cerebral tissues subjected to a.c. impulses at 50, 500, and 2,000 cyc./sec. The experiments were planned with the following considerations in mind.

(1) Sine-wave a.c. of 2,000 cyc./sec. in the present experimental arrangement increased respiration by not more than about 40%. This value was reached at 3 V, and at 4 or 5 V the respiratory response was no more than at 3 V. Impulses at 2,000 cyc./sec.

TABLE II

TRIMETHADIONE AND BUTOBARBITONE ON RESPIRATION STIMULATED BY SINE-WAVE ALTERNATING CURRENT AT DIFFERENT FREQUENCIES

Results quoted are from individual experiments with slices of guinea-pig cerebral cortex, in tissue-holding electrodes, in vessels *A* containing glucose-phosphate saline. Respiration in each vessel was measured during periods of 40–45 min. in which they were successively without applied impulses, and then with impulses at one of the frequencies chosen. The drugs when present were in the salines before the slices were placed in them.

Drug	Respiration (μmol. O_2/g./hr.)					
	At 50 cyc./sec., 1·3 V		At 500 cyc./sec., 3·5 V		At 2,000 cyc./sec., 3·5 V	
	No Impulses	With Impulses	No Impulses	With Impulses	No Impulses	With Impulses
None	60	99	61	78	69	90
Trimethadione, 2×10^{-4}	58	84	60	65	57	60
,, 10^{-3}	66	96	57	60	66	66
Butobarbitone, 10^{-4}	57	60	—	—	63	66
,, 5×10^{-4}	72	72	—	—	57	66

were therefore applied at 3.5 V. Respiration could be increased to a greater extent by a.c. at 50 cyc./sec., but by choosing a suitable voltage between 1.3 and 2 V a degree of stimulation could be obtained which was comparable to that reached by a.c. of 2,000 cyc./sec., and respiration so increased was used as basis for comparison of the lower and higher frequencies.

(2) Comparison of the effects of the two frequencies is most straightforward if it can be made on the same piece of tissue in the same electrode and vessel. This is possible if the tissue is exposed to the two types of impulse successively. When this was done, the effect of impulses during a second period of stimulation was usually found to be less than their effect during the first (Table III). The fall in effect was most in evidence when the high frequency followed the lower; it was often more pronounced with 2,000 cyc./sec. than with the 500 cyc./sec. quoted in Table III, and persisted when unstimulated periods alternated with the stimulated ones. Nevertheless, because application of two frequencies successively to a given vessel obviated differences in tissue-electrode arrangements which were difficult to standardize exactly, it was always carried out, and Table IV quotes results in the two periods separately. Experiments examining the effect of a given substance were usually carried out in groups of at least two in which vessels and electrodes, with and without the drug being studied, were interchanged.

(3) Comparison of the effect of a given stimulus in several experiments has been found most dependable when the change it induced is expressed as a percentage of the rate in absence of stimulus. In this way experimental variations between individual animals and in weighing and handling the tissue are reduced. The magnitude of this variation can be seen in Table III when different specimens of guinea-pig tissues gave initial unstimulated rates of from 60 to 71 μmol./g./hr. Results have been expressed as per cent change in Table IV. In different experiments of Table IV, differences remain in the effect of a given impulse, in the absence of drugs, in different groups of experiments. This is ascribed to differences in handling the tissue, in electrodes, and in animals over the period of 12 months during which the experiments have been in progress. It is for this reason that values for the effect of impulses in the absence of drugs have been quoted together with the corresponding values in the presence of drugs, in each group of experiments.

The main results of this comparison are given in Table IV. This shows the actions of trimethadione on stimulation at different frequencies to be extremely well differentiated. At 10^{-3} or 10^{-4} M it has no effect with 50 cyc./sec. a.c. during its first period, and a small effect only during the second. In contrast, 10^{-3} M completely prevented

TABLE III

TRIMETHADIONE AND BROMIDE ON RESPIRATION STIMULATED BY LOW AND HIGH FREQUENCY ALTERNATING CURRENT

Experimental arrangement: as in Table II, except where indicated otherwise. Values on each horizontal line are from one piece of tissue observed during successive periods of 40 to 45 min., during which it was alternately unstimulated, exposed to impulses at one frequency, unstimulated, and exposed to impulses at the other frequency. The different periods are given in order from left to right, the first always commencing ten minutes after placing vessels at 37° C.

Drug (M)	Species	Respiration (μmol. O_2/g./hr.) with Impulses (cyc./sec.)						
		None	50	None	500 (or *2,000)	None	50	None
None	Guinea-pig	65	105	60	84	—	—	—
Trimethadione 10^{-3}	,,	60	99	60	66	—	—	—
None	,,	—	75	54	69	60	—	—
Trimethadione 10^{-3}	,,	—	75	54	54	54	—	—
None	,,	—	—	69	84	57	81	—
Trimethadione 10^{-3}	,,	—	—	71	68	68	95	—
None	,,	—	—	—	93	63	85	71
Trimethadione 10^{-3}	,,	—	—	—	69	66	93	54
None	,,	66	108	69	96*	—	—	—
Sodium bromide 3×10^{-3}	,,	60	99	63	69*	—	—	—
None	Rat	—	—	108	136*	112	132	—
Trimethadione 10^{-3}	,,	—	—	110	110*	108	150	—
None	Rabbit	—	—	69	93*	75	90	—
Trimethadione 10^{-3}	,,	—	—	72	68*	72	87	—

TABLE IV

SUMMARY OF EFFECTS OF DRUGS ON RESPIRATORY RESPONSE TO SINE-WAVE ALTERNATING CURRENT

Results are derived from experiments arranged as in Table III. Rates there quoted for trimethadione with guinea-pig give two values for the effect of 50 cyc./sec. a.c. as a first period without drug (e.g., (105–65)/65=62%) and two with drug; and the same number at 500 cyc./sec. Several such percentages (number quoted; values in parentheses refer to second period of stimulation) are averaged in the present Table. Those derived from the period of stimulation which follows soonest after the beginning of incubation are quoted first, and those from second periods of stimulation are given second, in parentheses; when sufficient values are available, each is followed by the standard deviation of the group of values. To obtain probability values P, in each experiment the result with drug was subtracted from that without, and the variance of the distribution of these differences in the group of experiments from their mean difference made the basis for determining t; P was read from a table of t.

Drug	Experiments with Current at 50 cyc./sec., 1·3 V or 2* V				Experiments with Current at 500* or 2,000 cyc./sec., 3·5 V			
	No. of Determinations	Increase in Respiration (% of Rate Without Impulses)		P for Difference Between Increase Without Drug and Increase With Drug	No. of Determinations	Increase in Respiration (% of Rate Without Impulses)		P for Difference Between Increase Without Drug and Increase With Drug
		Without Drug	With Drug			Without Drug	With Drug	
Trimethadione, 10^{-4}	4 (4)	36±17 (29±11)	32±14 (10±5)	>0·9 (0·3)	*4 (4)	49±14 (17±6)	24±8 (5±8)	0·2 (0·5)
Trimethadione, 10^{-3}	*6 (4)	49±10 (27±8)	39±19 (22±10)	0·7 (0·5)	*6 (4) 6 —	35±7 (16±2) 36±4 —	3±6 (1±1) 1±1 —	0·001 (0·001) <0·001 —
Diphenylhydantoin, 10^{-4} ..	4 (4)	46±17 (24±7)	32±5 (9±5)	0·5 (0·2)	*5 (4)	73±8 (18±9)	15±7 (−5±1)	0·01 (0·2)
Diphenylhydantoin, 10^{-3} ..	*4 —	49±19 —	45±19 —	0·9 —	4 —	31±3 —	−4±6 —	0·001 —
Phenobarbitone, 10^{-4}	9 —	44±8 —	45±12 —	>0·9 —	*5 (8)	34±33 (14±6)	29±4 (6±7)	>0·9 (0·7)
Phenobarbitone, $3·5\times10^{-4}$ ..	6 (4)	53±21 (10±1)	41±5 (−2±5)	0·7 (0·05)	*4 (5)	33±10 (34±14)	22±9 (1±6)	0·5 (0·05)
Phenobarbitone, 10^{-3}	— (3)	— (39±17)	— (39±10)	— (>0·9)	3 (2)	28±7 (50)	6±4 (10)	0·05 —
Butobarbitone, 10^{-4}	5 (2)	45±6 (25)	21±8 (8)	0·05 —	3 (4)	30±11 (18±9)	13±10 (4±6)	0·3 (0·2)
Butobarbitone, $3·5\times10^{-4}$ ..	4 (2)	34±10 (20)	12±4 (0)	0·01 —	4 (2)	37±3 (13)	7±9 (−3)	0·02 —
Butobarbitone, 10^{-3}	4 (2)	32±5 (14)	8±3 (−16)	0·001 —	2 (4)	23 (24±3)	−15 (−12±11)	— (0·02)
Urethane, 10^{-3}	2 (2)	27 (29)	28 (10)	— —	2 (2)	32 (15)	11 (12)	— —
Urethane, $2·5\times10^{-3}$ ..	2 (2)	48 (25)	35 (0)	— —	2 (2)	35 (14)	13 (−4)	— —
Urethane, 5×10^{-3} ..	2 (2)	38 (24)	14 (11)	— —	2 (2)	26 (8)	8 (0)	— —
Chloral, 10^{-3} ..	5 (7)	29±4 (21±4)	15±6 (17±6)	0·05 (0·5)	7 (5)	24±6 (13±5)	12±4 (−6±6)	0·1 (0·05)

the effect of 2,000 cyc./sec. a.c., and largely prevented that of 500 cyc./sec. At 10^{-4} M the effect of 500 cyc./sec. was halved. Diphenylhydantoin was comparable, with probably a greater effect at 10^{-4} M.

Phenobarbitone, though showing a similar tendency, was not as specific in its action. Its effects were shown in second rather than first stimulated periods, and were seen at 10^{-4} M or 3.5×10^{-4} M at low and high frequencies. Butobarbitone depressed the effects of low and high frequencies at 10^{-4} M. Its effects increased with increasing concentration and were shown in the first stimulated period to much the same extent as in the second. Urethane and chloral behaved similarly; they showed a possible tendency to inhibit more at the higher frequency, but when this was investigated in the case of urethane by examining a range of concentrations, no clearly differential effect was found.

Trimethadione is seen from Table III to be effective in rat and rabbit tissues as well as in those of guinea-pigs. Sodium bromide selectively depressed at 3×10^{-2} M; results with 10^{-2} M were not decisive.

Phenylacetylurea in some experiments acted selectively, but did not yield consistent results. This was considered due to its low solubility, which did not permit a 10^{-4} M solution to be made in the experimental salines.

DISCUSSION

The present investigations are ones in which actions of anticonvulsants directly related to their central effects have been shown in preparations from the brain *in vitro*. They represent a stage in the analysis of anticonvulsant action which makes it likely that the drugs studied affect the brain directly, without chemical interconversion or other intermediation by the rest of the body. Actions of anticonvulsants previously found *in vitro* have been in peripheral nerve, largely from the frog (see references below). Although this system permits many observations not at present possible in separated cerebral tissues, study of it contributes to knowledge of the actions of anticonvulsant

drugs by analogy rather than by analysis of the situation in which their actions as anticonvulsants are shown. Moreover, trimethadione was not found to affect the frog nerve, but was highly effective in the present study.

Comparison of effective concentrations is important in an analysis such as the present. With *diphenylhydantoin,* the dose 50% effective in antagonizing experimental electroshock is some 0.15 mmol./kg., the therapeutic blood level in man about 0.05 mM, and this concentration acts on frog sciatic nerve (Merritt and Putnam, 1938; Toman and Goodman, 1948; Toman, 1949); action on cerebral tissues in the present experiments is potent at 0.1 mM. *Trimethadione* is 50% effective therapeutically at about 0.3 mmol./kg.; it acts against experimental electroshock seizures at 2.5 mmol./kg., but is without action on frog sciatic at 1 mM (Lennox, 1945; Goodman, Toman, and Swinyard, 1946; Toman, 1952); in the present experiments it is fully active at 1 mM and partly effective at 0.1 mM. *Phenobarbitone* is effective against experimental electroshock at 0.03–0.15 mmol./kg., on peripheral nerve at 1 mM or less (Merritt and Putnam, 1938; Toman, 1952), and is partially active in the present experiments at 0.35 mM. *Phenylacetylurea* has previously been found of too limited a solubility in saline media for *in vitro* observations (Toman, 1952). Effective blood levels of *bromides* as anticonvulsants in man are 10–15 mM, and in the present experiments sodium bromide acted at 30 mM and doubtfully at 10 mM.

Full consideration of possible mechanisms of the present effects is deferred. The different frequencies of applied impulse may excite the same or different nervous elements. Stimulation of the separated cerebral tissue in phosphate buffer and at high frequencies shows resemblance to the situation of "excessive stimulation" found by Toman and Goodman (1948) to be that in which suppression of electrical phenomena by certain anticonvulsants was observed in peripheral nerve. Such suppression in the central nervous system could be understood to play a part in reducing the excessive discharge seen electroencephalographically in the convulsive disorders in which the drugs are effective.

Summary

1. Respiration of separated cerebral tissues when stimulated by sine-wave alternating current at 500 or 2,000 cyc./sec. became sensitive to anticonvulsant drugs. Trimethadione, diphenylhydantoin, and phenobarbitone were effective at 10^{-4} M, and a bromide at 3×10^{-2} M.

2. These substances in the concentrations quoted (and also trimethadione up to 10^{-2} M; diphenyl hydantoin and phenobarbitone up to at least 10^{-3} M) were without comparable effect on respiration of cerebral tissues in the absence of applied impulses. They were also largely ineffective when a comparable degree of respiratory stimulation was induced by sine-wave alternating currents of 50 cyc./sec., or by brief condenser pulses of 100/sec.

3. All the types of stimuli described made the respiration of cerebral tissues more sensitive to the general depressants butobarbitone, urethane, and chloral hydrate.

We are greatly indebted to the Board of Governors of the Bethlem Royal and Maudsley Hospital for a grant from the Research Fund for purchase of apparatus used in these studies, and also to Dr. D. A. Pond of this hospital for his helpful comments.

References

Anguiano, G., and McIlwain, H. (1951). *Brit. J. Pharmacol.*, **6**, 448.
Butler, T. C. (1949). *J. Pharmacol.*, **95**, 360.
Goodman, L. S., Toman, J. E. P., and Swinyard, E. A. (1946). *Amer. J. Med.*, **1**, 213.
Lennox, W. G. (1945). *J. Amer. med. Ass.*, **129**, 1069.
McIlwain, H. (1951). *Biochem. J.*, **49**, 382.
—— (1952). *Biochem. Soc. Sympos.*, **8**, 27.
—— (1953). *Biochem. J.*, **53**, 403.
—— and Buddle, H. L. (1953). Ibid., **53**, 412.
Merritt, H. H., and Putnam, T. J. (1938). *Arch. Neurol. Psychiat.*, **39**, 1003.
Taylor, J. D., Richards, R. K., Everett, G. M., and Bertcher, E. (1950). *J. Pharmacol.*, **98**, 392.
Toman, J. E. P. (1949). *EEG Clin. Neurophysiol.*, **1**, 33.
—— (1952). *Pharmacol. Rev.*, **4**, 168.
—— and Goodman, L. S. (1948). *Physiol. Rev.*, **28**, 409.