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Anticonvulsants and the Metabolism of Separated Mammalian Cerebral Tissues

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Anticonvulsants can prevent the occurrence in the brain of living animals of events associated with convulsive activity. These events include new patterns of electrical activity and a group of metabolic changes. The metabolic changes include the depletion of labile phosphates and increase in energy-yielding reactions which are associated with enhanced activity in several excitable tissues. When increase in respiration in cerebral tissues is induced by electrical pulses of certain characteristics, it can be antagonized by several anticonvulsants (Forda & McIlwain, 1953). Also, some anticonvulsants affect acetylcholine metabolism by cerebral tissues (McLennan & Elliott, 1951).

These observations have given assurance that anticonvulsants can have direct cerebral effects rather than that they act primarily elsewhere in the body (a matter previously in doubt; see, for example, Staple, 1951). They leave unknown, however, the primary point of action of the drugs, for many mechanisms can be envisaged for interference with response to applied pulses and with acetylcholine metabolism. Present studies were directed to finding such a point of action for clinically valuable anticonvulsants, several of which are related in structure (Toman, 1949).

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Fig. 1 includes the formulæ of substances studied in the present work and illustrates this point.

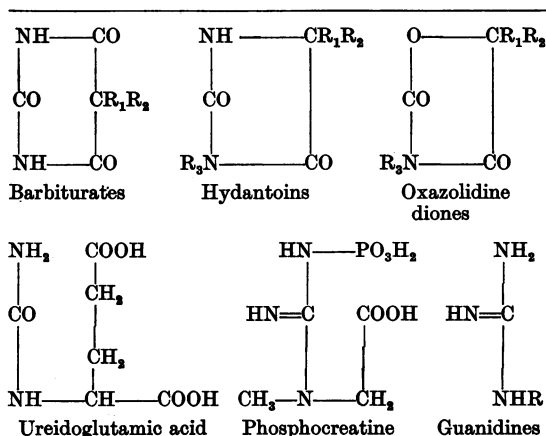


Fig. 1. Some compounds examined. Barbiturates: phenobarbitone, R_1 = ethyl, R_2 = phenyl; butobarbitone, R_1 = ethyl, R_2 = *n*-butyl. Hydantoin: R_1 = R_2 = phenyl and R_2 = H, diphenylhydantoin; R_1 = ethyl, R_2 = phenyl, R_2 = methyl, methoin. Oxazolidinediones: R_1 , R_2 and R_3 = Me, trimethadione.

$R = -(\text{CH}_2)_{10}\text{NH} \cdot \text{C}(\text{NH})\text{NH}_2$,
decamethylenediguanidine (synthalin).

EXPERIMENTAL

Materials

Tissues were mainly the grey matter from the cerebral hemispheres of rats and guinea pigs, prepared and studied as described by McIlwain (1951*a, b*) and McIlwain, Buchel & Cheshire (1951), where details of experimental solutions are given. Salines employed in the present work contained Ca salts at 2.6 mM.

Drugs were of the standards of the British Pharmacopoeia (1953) or the British Pharmaceutical Codex. Trimethadione was obtained from Abbott Laboratories, diphenylhydantoin from British Drug Houses Ltd., butobarbitone from May and Baker Ltd. and 5-ethyl-3-methyl-5-phenylhydantoin (methoin) from Sandoz. The molarities of the drugs recorded express their concentrations in the incubating medium surrounding the slice in the vessel. This is achieved, unless otherwise stated, by dissolving the substance in a suitable portion of the saline used in other vessels of the same experiment. In most cases 3.5 ml. of this solution were pipetted into vessels which later received 40–80 mg. of tissue. A different arrangement is described for some of the experiments of Table 1 and of Fig. 2.

Methods

Glutamic acid. Glutamic acid in cerebral tissues was determined by procedures similar to those of Stern, Eggleston, Hems & Krebs (1949). After incubation, slices of about 120 mg. were drained briefly from fluid, dropped into 2.5 ml. 0.4 N-HCl in a homogenizer tube already cooled in an ice bath, and suspended in the acid with the homogenizer pestle. The fluid from the experimental vessels was also made 0.4 N with respect to HCl. The acid solutions were left in a refrigerator until immediately before analysis, which was carried out manometrically using the decarboxylase of *Clostridium welchii* SR12 (Gale, 1945; Krebs, 1948). To the cerebral extract, 0.2 ml. 3 M acetate buffer of pH 4.9 and 0.5 ml. 2 N-NaOH were added; pH was checked and found to be 4.9, and 2 ml. of this solution was used for the determination. The fluid from metabolic experiments was similarly prepared. It was found that the cerebral extracts and the drugs employed did not interfere with the determination of glutamic acid and that, with the batches of organisms prepared, 1 mole of both glutamine and glutamic acid yielded 1 mole of CO₂.

Lactic acid. This was determined by the method of Barker & Summerson (1941) in samples removed from the vessels at the end of the experimental period. In aerobic experiments, adequate aeration of the tissue was maintained until the removal of the samples, which were pipetted into 4% (w/v) CuSO₄·5H₂O.

Inorganic and creatine phosphates. In the experiments of Fig. 2, slices of cerebral cortex weighing approximately 100 mg., held in electrode holders (Heald, McIlwain & Nelligan, 1954), were placed in beakers containing 5 ml. saline. The beakers were kept at 37° in a water bath. Through a narrow polythene tube oxygen was bubbled continuously to aerate and circulate the saline. To some slices, electrical pulses were applied at chosen periods. At the end of the required period the holders, one by one, were quickly removed from the beakers, the slice was released into 4 ml. ice-cold 10% trichloroacetic acid and ground. The trichloroacetic acid mixtures were centrifuged and

neutralized with NaOH; 0.5 ml. 10% w/v, CaCl₂ saturated with Ca(OH)₂ was added, and precipitation of the inorganic phosphate was allowed to proceed for 10 min. The suspensions were then centrifuged strongly and the supernatants used for creatine phosphate estimation. The Berenblum & Chain (1938) method modified by Long (1943) was applied in estimating both fractions.

Respiration. Respiration and anaerobic formation of acid were measured manometrically by conventional methods except when applying electrical pulses when electrode vessels with grid electrodes were used (Ayres & McIlwain, 1953).

Electrical pulses. Electrical pulses and currents were derived from and applied to the tissue by the apparatus described by Ayres & McIlwain (1953). Condenser pulses were alternating; the potential quoted is the peak of an individual pulse. With alternating current, the potential quoted is the virtual voltage. An experiment usually comprised six vessels containing slices of cerebral cortex from the same experimental animal. When electrical pulses were to be applied to any vessels of an experiment, all slices, whether or not they were to receive the pulses, were between similar electrodes. The variation in the degree of response among different individual electrodes has been determined and is not large (Ayres & McIlwain, 1953), but to eliminate this factor experiments were arranged in pairs in which vessels with and without added drug (or other agent studied) were exchanged. When a standard deviation is quoted, it refers to all results regarded individually, but in assessing the significance of the effect of an added agent the grouping of the experiments has been taken into account. Thus in deriving *P* values there is compared a series of averages of results in two vessels without the agent, with a series of averages in the same two vessels with the agent.

RESULTS

Glutamic acid

Glutamic acid is concerned with ion transport and with the metabolism of ammonia in cerebral tissues; both these are relevant to convulsive processes and glutamic acid can affect the electroshock threshold of experimental animals in certain situations (Davenport & Davenport, 1948; Richter & Dawson, 1948). Some aspects of the metabolism of glutamic acid which were examined in the present experiments are recorded in Table 1. Assimilation of the acid by cerebral tissues was followed in the presence and absence of anticonvulsants, for assimilation is associated with changes in potassium salts. It was not, however, affected by quite high concentrations of trimethadione or of methyl-ethylphenylhydantoin. Relatively low concentrations of glutamic acid were employed in these experiments, and in some the tissue was extracted before maximal assimilation had been reached in order to give scope for action of the added substances.

After these experiments less glutamic acid (plus glutamine) was recovered than had initially been added. The acid 'metabolized' (Table 1) was

presumably oxidized or bound to the tissue, but the quantity so changed was not appreciably affected by the drugs. Experiments comparable to those of Table 1 were carried out with glutamine as substrate, but again the drugs were without action.

Uramidoglutamic acid has proved important in aspects of glutamic acid and ammonia metabolism (Grisolia & Cohen, 1951) though not in the brain.

(Elliott & Henderson, 1949). These findings and the structural relationship indicated in Fig. 1 prompted study of the possible interaction of guanidines with anticonvulsants. Stimulation of the respiration of cerebral cortex, noted by Dickens (1939) in bicarbonate-buffered media, was confirmed but was not always found in phosphate-buffered media, and lactic acid formation in these media was also less.

Table 1. *Summary of processes found unaffected by anticonvulsants*

Experiments *A* were with guinea pig cerebral cortex in phosphate-buffered salines. Glutamic acid was added at 6 mM; without this addition, the tissue contained very little glutamic acid. Experiments *B* employed vessels containing about 0.4 g. of guinea pig cerebral cortex in 15 ml. glycylglycine-buffered salines; glutamic acid was at 20 mM. Rat cerebral cortex behaved similarly. Some of experiments *C* and *D*, giving the higher values quoted, were with rat cerebral cortex in bicarbonate-buffered salines; others were with the rat or guinea-pig tissue in phosphate buffers.

Measurement (G, glucose as substrate; GA, glutamic acid)	Typical values ($u = \mu\text{moles/g.}$)	Anticonvulsants examined (mM)
A. Glutamic acid content, G + GA	22-30 u	Methoin, 3.3 Trimethadione, 3.3 and 10
Glutamic acid metabolized, G + GA	5-10 $u/\text{hr.}$	
B. Respiration, G	63-67 $u/\text{hr.}$	Methoin, 0.06, 0.1 Trimethadione, 3.3, 13.3
Respiration, GA	72-81 $u/\text{hr.}$	
Inorganic phosphate content, G	3.3-3.6 u	Diphenylhydantoin, 0.06, 0.1
Inorganic phosphate content, GA	4.8-5.3 u	
Creatine phosphate content, G	1.2-1.5 u	
Creatine phosphate content, GA	0.3 u	
C. Respiration, G, stimulated by:		Trimethadione, 1, 2
Guanidine, 0.2, 0.8 and 1 mM	63-122 $u/\text{hr.}$	
Synthalin, 5 and 8 μM	74-105 $u/\text{hr.}$	
KCl, 50 mM	116 $u/\text{hr.}$	
2:4-Dinitrophenol, 50 μM	130 $u/\text{hr.}$	
D. Lactic acid formation, G, stimulated by:		Diphenylhydantoin, 0.1
Guanidine, 0.2, 0.8 and 1 mM	60-170 $u/\text{expt.}$	
Synthalin, 5 and 8 μM	60-120 $u/\text{expt.}$	
KCl, 50 mM	60-68 $u/\text{expt.}$	
2:4-Dinitrophenol, 50 μM	100-120 $u/\text{expt.}$	

Because of structural relationships with anticonvulsants (Fig. 1) its possible breakdown by cerebral slices and suspensions was examined by determining ammonia and glutamic acid after incubation in the presence of uramidoglutamic acid. Changes found were small and unaffected by anticonvulsants.

Previous experiments (see also below) have shown that trimethadione and diphenylhydantoin are without appreciable effect on the respiration of cerebral tissues as ordinarily measured with glucose as substrate. This was found to be the case also when glutamic acid served as oxidizable substrate (Table 1).

Anticonvulsants and aerobic respiration and glycolysis stimulated by added substances

Guanidines. Guanidine itself and also polymethylenediguandines increase cerebral respiration and glycolysis (Dickens, 1939). Guanidine can cause convulsions through action at the neuromuscular junction (Minot, 1931). Its blood level was believed to be increased in epilepsy (Murray & Hoffman, 1940), though this is probably not so

Trimethadione and (where examined) diphenylhydantoin were without effect on respiration and lactic acid formation in the presence of the guanidines (Table 1). Concentrations of guanidines were chosen which were minimal for bringing about appreciable increases in respiration and glycolysis, and the concentrations of anticonvulsants were equal to or higher than those effective therapeutically. This enabled concentrations of trimethadione to be examined which were equal to or ten times higher (mole/mole) than guanidine and up to 400 times higher than the diguanidine. In addition to the values quoted in Table 1, the guanidines were observed increasingly to inhibit oxygen uptake and CO_2 output after the first hour of such experiments. The anticonvulsants were without effect on the course or extent of such inhibition.

Potassium chloride and 2:4-dinitrophenol. These alter the respiration and glycolysis of separated cerebral tissues (Dickens & Greville, 1935; Ashford & Dixon, 1935), and also their inorganic and creatine phosphates (McIlwain & Gore, 1951; Gore & McIlwain, 1952) in the fashion in which

convulsants change these characteristics in the brain *in vivo*. Concentrations of the agents which were minimal for near-maximal change in respiration and glycolysis were examined and their effects found to be unaltered by trimethadione and diphenylhydantoin (Table 1).

Anticonvulsants and the aerobic metabolism of electrically stimulated tissues

Respiration and glycolysis. Tables 1 and 2 show trimethadione, methoin and diphenylhydantoin to be without effect on the normal respiration and glycolysis of guinea pig cerebral cortex with glucose as substrate. The concentrations employed are estimated to be effective in anticonvulsant action

electrical pulses. This conclusion must be qualified by the observation that the increase in lactic acid formation in the present experiments has been smaller and more variable than that in respiration. During experiments with 0.1 mm diphenylhydantoin the increase in respiration with currents of 2000 cyc./sec. in the absence of drug was $63 \pm 5\%$ of the unstimulated value, and with the drug was $9 \pm 2\%$ (2% and similar values are standard deviations derived from groups of experiments). Corresponding values for lactic acid formation were $18 \pm 20\%$ and $23 \pm 25\%$. With 0.4 mm diphenylhydantoin values were $29 \pm 10\%$ without and $38 \pm 18\%$ with the drug, and with trimethadione $36 \pm 17\%$ and $21 \pm 12\%$. As indicated in

Table 2. *Anticonvulsants on respiration and lactic acid formation during application of sine-wave alternating currents*

Each experiment usually involved six vessels with slices of cerebral cortex from the same animal in the electrodes *H* of Ayres & McIlwain (1953) in vessels *A* of McIlwain (1951b). No current was applied to two vessels, a.c. at 50 cyc./sec. (1.5v) to two and a.c. at 2000 cyc./sec. (3.5v) to the other two. Rates of metabolism are followed by their standard deviation; the *P* values refer to the differences between vessels with and without the added substances.

Added substance (mm)	No. of expts. in group	Respiration \pm s.d. (μ mole O ₂ /g./hr.)			Lactic acid formation \pm s.d. (μ mole/g./expt.)		
		Without addition	With addition	<i>P</i>	Without addition	With addition	<i>P</i>
Vessels without applied currents							
Diphenylhydantoin (0.1)	7	55 \pm 3	56 \pm 3	0.8	72 \pm 12	65 \pm 13	0.7
Diphenylhydantoin (0.4)	4	60 \pm 3	60 \pm 2	0.9	45 \pm 4	48 \pm 15	0.9
Trimethadione (1)	8	63 \pm 3	63 \pm 4	0.9	58 \pm 16	58 \pm 19	0.9
Vessels with 50 cyc./sec. a.c.							
Diphenylhydantoin (0.1)	4	82 \pm 8	75 \pm 8	0.7	88 \pm 20	82 \pm 15	0.1
Diphenylhydantoin (0.4)	4	86 \pm 3	79 \pm 6	0.9	72 \pm 10	85 \pm 2	0.3
Trimethadione (1)	9	88 \pm 7	86 \pm 6	0.7	88 \pm 17	86 \pm 6	0.7
Vessels with 2000 cyc./sec. a.c.							
Diphenylhydantoin (0.1)	4	90 \pm 3	60 \pm 1	0.001	85 \pm 15	80 \pm 16	0.9
Diphenylhydantoin (0.4)	4	82 \pm 7	63 \pm 2	0.02	58 \pm 4	66 \pm 8	0.5
Trimethadione (1)	10	95 \pm 3	80 \pm 4	0.01	79 \pm 10	70 \pm 7	0.5

in vivo (Ford & McIlwain, 1953). The concentrations do, however, inhibit respiration when it is increased by passage of brief condenser pulses or sine-wave alternating currents at 2000 cyc./sec. (Tables 2 and 3). A comparable degree of respiratory stimulation at 50 cyc./sec. was little if at all affected by the drugs. This selective effect of the drugs is more clearly demonstrated by the experiments of Table 2 than by earlier experiments reported previously (Ford & McIlwain, 1953). When similar experiments were carried out with aminotrihydroxymethylmethane or with glycylglycine as buffer, the drug acted in the same way as in phosphate buffer.

There is no evidence from the experiments of Table 2 that lactic acid production is affected by the drugs either in presence or absence of applied

Table 2, none of these differences give *P* values lower than 0.5. When, however, in earlier experiments respiratory response to impulses was similarly lower and variable (Ford & McIlwain, 1953) an effect of the drugs could nevertheless be seen: 1 mm trimethadione lowered the response from 30 to 1% with a *P* value of 0.01. It appears, therefore, that lactic acid formation is significantly less susceptible to the drugs than is respiration.

Inorganic and creatine phosphates. The levels of these substances in cerebral tissues were examined not only because they change greatly during convulsive activity, but also because several anticonvulsants can be regarded as structurally related to phosphocreatine (Fig. 1). With tissues studied by conventional methods in absence of applied pulses, the anticonvulsants were without effect on the

levels of the two phosphates (Table 1). This was the case both when normal levels of the phosphates were maintained with glucose as substrate, and also when, with glutamic acid, creatine phosphate was much lower and inorganic phosphate higher.

Pulses applied to separated cerebral tissues with glucose as substrate normally change the levels of respiration, glycolysis, inorganic phosphate and

creatine phosphate (McIlwain & Gore, 1951; Kratzing, 1953; Heald, 1954). As the anticonvulsants were noted above to affect the respiratory but not the glycolytic response to the pulses, their effects on the phosphates of the tissue exposed to pulses were of especial interest. No significant effects were found, however, on either phosphate. Many of the results are shown in Fig. 2. This gives the course of change in phosphates resulting from the application of alternating current at 2000 cyc./sec.; that is a type of pulse whose respiratory effects are susceptible to the anticonvulsants. However, neither the rate of fall of phosphocreatine during the pulses nor its rate of recovery afterwards is altered by trimethadione.

Threshold. Applied currents have little or no effect on respiration of cerebral tissues until a threshold potential gradient is exceeded, when response increases relatively rapidly with increase in potential (McIlwain, 1951b; 1954). This is expressed by the voltage/response curve of Fig. 3. An added substance could have many effects on such a voltage/response curve, but Fig. 3 shows that the effect of the anticonvulsant employed is a relatively simple one of increasing the threshold for response. Response can still reach the value obtained in absence of drug, but a greater potential is required. This effect of trimethadione is compared in Fig. 3 with that of a barbiturate. The action of the two is seen to differ; further analysis of this is deferred.

Interrupted currents. Respiratory response to a period of electrical stimulation can persist for periods of a few seconds after pulses cease (McIlwain, 1954). Certain anticonvulsants can depress the after-discharge produced in peripheral nerve under some conditions of repetitive stimulation (Toman, 1949). To see whether comparable phenomena occur in the present systems, the experiments of Table 3 were carried out. These indicate no greater sensitivity to the anticonvulsants to be induced by the different types of interrupted currents employed.

Specificity of the respiratory effect of anticonvulsants

Several substances of structure similar to the compounds of Table 1 are not anticonvulsant (Merritt & Putnam, 1938; Toman & Goodman, 1948). Some of these are compared in Table 4 with an established anticonvulsant, in their effects on the respiratory response to sine-wave alternating currents of 2000 cyc./sec. None of the related substances without anticonvulsant action had any metabolic effect. The metabolic response was also unaffected by ethylenediaminetetraacetic acid.

With separated cerebral tissues lactate and pyruvate are often similar to glucose as added substrate, and this was found to be the case also in

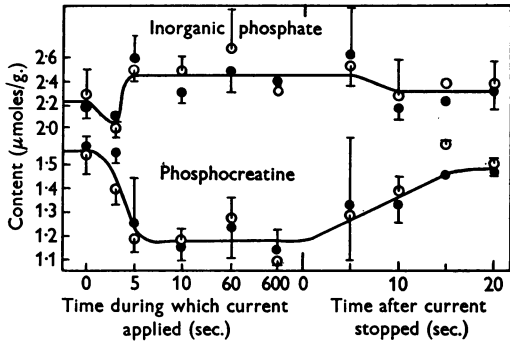


Fig. 2. The course of change in inorganic phosphate and phosphocreatine following application of sine-wave a.c. of 2000 cyc./sec. and 3.5 v. Points give mean values for four to eight slices, and lines extend from the points for distances equal to the standard deviation. Open points: no drug; black points: with 1 mm trimethadione. In the right-hand part of the graph, the tissues fixed some seconds after current had stopped had previously all been exposed to the current for 10 sec.

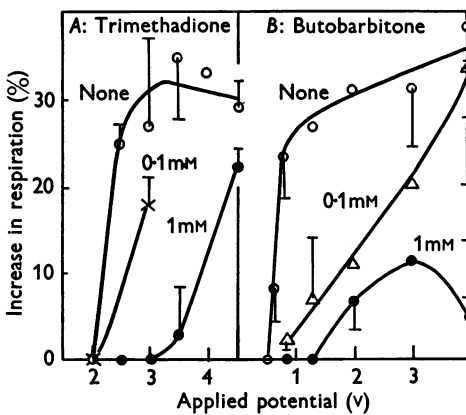


Fig. 3. Response/voltage curves for guinea pig cerebral tissues with and without a barbiturate and anticonvulsant. *A*, Tissue exposed to sine-wave a.c. of 2000 cyc./sec. and the different voltages quoted; *B*, tissue exposed to sine-wave a.c. of 50 cyc./sec. Points give mean values for several slices, and where sufficient data are available lines extend from the points for distances equal to the standard deviation.

Table 3. *Diphenylhydantoin on effects of continuous and interrupted currents*

Guinea pig cerebral tissues were used in electrodes *H*. Respiratory rates were determined during an initial period of 40 min. without applied current, and a subsequent one of 40 min. with current. In different vessels the current was continuous and interrupted; experiments were run in pairs in which the types of current applied to a given vessel were interchanged. Sine-wave current was of 2000 cyc./sec. and 3.5 v; condenser pulses were of 10 v peak potential and a time-constant of 0.4 msec. Diphenylhydantoin at 0.1 mm was present in half the vessels, throughout the experiment.

Current applied	No. of expts. in group	Respiration \pm s.d. (μ moles O_2 /g./hr.)		Lactic acid formation (μ moles/g./expt.)	
		Without drug	Change with drug	Without drug	Change with drug
None	4	56 \pm 2	-4 \pm 2	35 \pm 3	+2 \pm 5
Sine-wave a.c.; continuous	4	92 \pm 9	-27 \pm 3	53 \pm 7	-2 \pm 8
Sine-wave a.c.; on, 1 sec.; off, 1 sec.	4	92 \pm 12	-22 \pm 10	50 \pm 3	-2 \pm 5
None	8	60 \pm 2	-4 \pm 4	37 \pm 3	1 \pm 4
Condenser pulses; continuous	8	118 \pm 6	-34 \pm 3	70 \pm 3	-2 \pm 12
Condenser pulses; on, 0.1 sec.; off, 0.44 sec.	4	117 \pm 4	-33 \pm 3	73 \pm 2	-7 \pm 9

Table 4. *Anticonvulsants and related substances on respiration with different substrates*

Current was sine-wave, alternating, of 2000 cyc./sec. at 3.5 v. Substrates were at 10 mm except lactate which was at 33 mm. Statistical analyses of the results, grouping values according to the vessels employed, showed only diphenylhydantoin and trimethadione of the added substances to have significant effect.

Substrate	Added agent (mm)	No. of observations	Average increase in respiratory rate with current (% of rate without current)	
			Without added agent	With added agent
Glucose	Trimethadione, 1	4	32	3
Glucose	Allantoin, 1	5	23	28
Glucose	Hydantoin, 1	5	23	21
Glucose	Phenylacetic acid, 10	5	26	24
Glucose	Ethylenediaminetetraacetic acid, 1	4	32	35
Glucose	Ethylenediaminetetraacetic acid, 0.1	4	32	28
Glucose	<i>s</i> -Diphenylurea, 1	9	23	20
Lactate	Diphenylhydantoin, 1	6	26	1
Pyruvate	Diphenylhydantoin, 1	6	48	1

relation to the effect of diphenylhydantoin (Table 4). Almost complete inhibition of the metabolic response to sine-wave currents of 2000 cyc./sec. was observed with each substrate.

Anticonvulsants and the anaerobic glycolysis of cerebral tissues

Electrical pulses have been found to decrease the anaerobic glycolysis of sections of cerebral tissues (McIlwain, unpublished). Accordingly, the effects of anticonvulsants on this process have been assessed also, determining both the rate of acid formation manometrically by the evolution of carbon dioxide from bicarbonate salines, and also the lactic acid, colorimetrically (Table 5). By both criteria, 1 mm trimethadione and also 0.1 mm diphenylhydantoin antagonized the effect of sine-wave alternating currents of 2000 cyc./sec. Again,

the effect of such currents at 50 cyc./sec. was not affected in the instance examined, that is with trimethadione.

Acetylcholine and eserine

An effect of anticonvulsants on cerebral tissues reported by Tower & Elliott (1953) is to increase or decrease their bound acetylcholine. In seeking features common to this and to the respiratory effects of anticonvulsants reported here, an action of acetylcholine on the respiration of cerebral tissues was sought. Acetylcholine was examined alone and in the presence of eserine sufficient to delay its breakdown by the cholinesterases of the tissue. No significant effect was found in sixteen experiments with condenser pulses of peak potential 5 and 10 v, and time-constant 0.4 msec. and with sine-wave alternating current at 2000 cyc./sec. and of 2 or

Table 5. *Electrical pulses and anticonvulsants on the anaerobic glycolysis of cerebral cortex*

Anticonvulsants used: in *A* and *B*, 1 mm trimethadione; in *C*, 0.1 mm diphenylhydantoin. Guinea pig cerebral tissues were used in electrodes *H*, in glucose-bicarbonate salines. Current was applied throughout the experiments to the vessels indicated.

Current applied (sine-wave a.c.)	Group of expts.; no. of expts. in group	Rate of acid formation (μ moles CO_2 evolved/g. tissue/hr.)		Total lactic acid formed (μ moles/g./expt.)	
		Without drug	With drug	Without drug	With drug
None	<i>A</i> ; 2	105	96	110	119
50 cyc./sec., 3v	<i>A</i> ; 6	50 \pm 7	57 \pm 5	58 \pm 13	53 \pm 13
<i>P</i> value		0.9		0.9	
None	<i>B</i> ; 3	106	115	100	109
2000 cyc./sec., 3.5v	<i>B</i> ; 6	76 \pm 5	89 \pm 4	74 \pm 4	88 \pm 4
<i>P</i> value		0.05		0.02	
None	<i>C</i> ; 2	99	103	99	104
2000 cyc./sec., 4.5v	<i>C</i> ; 4	80 \pm 6	104 \pm 5	85 \pm 9	97 \pm 2
<i>P</i> value		0.01		0.3	

2.5v. The drugs were used at concentrations up to 1 mm. The experiments thus supplement those previously quoted (McIlwain, 1951*a*) which with different pulse types gave the same result.

DISCUSSION

The metabolic effects observed with anticonvulsants in the present experiments are strictly limited to situations in which metabolism has been influenced by applied electrical pulses, whether this action is to increase or to decrease in level of metabolism. On the other hand, the anticonvulsants do not affect all changes in level of metabolism. Increased respiration is opposed when it results from currents at 2000 cyc./sec. but not when it results from currents at 50 cyc./sec., from increased KCl, from guanidines or from 2:4-dinitrophenol. These agents have in common several effects on the aerobic metabolism of separated cerebral tissues: they increase respiration, decrease phosphocreatine, and increase inorganic phosphate and lactic acid formation. The anticonvulsants, however, affect only respiration. In this they are dissociating the respiratory and glycolytic responses to applied pulses which under many but not all other conditions, are affected or not affected in parallel.

Consequently, it is curious to see that in different circumstances the anticonvulsants can affect glycolysis. Here the action is to tend to preserve the normal level of anaerobic glycolysis which applied pulses decrease. Again, the anticonvulsants oppose the effects of pulses at 2000 cyc./sec. but not at 50 cyc./sec. The sequence of events linking applied pulse and aerobic metabolic response is unknown though mechanisms have been suggested (McIlwain & Gore, 1953). Anaerobically, events

are potentially simpler and further studies are in progress.

That the action of the anticonvulsants is limited to particular pulse-types appears very relevant to their properties as therapeutic agents, for they are not general depressants but repress specifically the type of electrical activity associated with convulsive conditions. Components of this are relatively high in frequency and voltage.

SUMMARY

1. The anticonvulsants diphenylhydantoin, methylethylphenylhydantoin, and trimethylx-azolidinedione were examined for possible effects on several metabolic characteristics of separated cerebral cortex from the guinea pig and rat

2. They were without effect on various aspects of glutamic acid metabolism and on the respiration of the tissues as ordinarily examined with several substrates.

3. They wholly or partly prevented the increase in respiration of cerebral tissues caused by sine-wave alternating currents of 2000 cyc./sec., or by brief condenser pulses. They had no effect on comparable increase in respiration brought about by 50 cyc./sec. a.c., by 50 mm potassium chloride, by 2:4-dinitrophenol, by guanidine or by a decamethylenediguanidine.

4. Respiratory inhibition by the anticonvulsants was comparable when glucose, lactate, or pyruvate was used as substrate. It was not shown by some compounds structurally related to the drugs but without anticonvulsant action.

5. Changes in glycolysis, creatine phosphate and inorganic phosphate which accompanied the respiratory increase susceptible to the anticonvulsants were nevertheless unaffected by them.

6. Anaerobic glycolysis was also unaffected by the anticonvulsants, until its level was altered by currents of 2000 cyc./sec. It then became susceptible; at 50 cyc./sec. no action of the drugs was seen.

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Action of Protoveratrine on the Metabolism of Cerebral Cortex

1. UNSTIMULATED CEREBRAL-CORTEX TISSUE*

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Protoveratrine is one of the most powerful members of an important group of pharmacological agents, the polycyclic ester alkaloids of veratrum. These compounds, especially the mixture of alkaloids known as veratrine, have been the subject of innumerable researches in the physiological and pharmacological laboratory and have proven to be of particular value in the analysis of certain aspects of the physiology of muscle and nerve (Kramer & Acheson, 1946). During the last few years, protoveratrine has become prominent as an agent in the treatment of hypertension.

The central nervous system has been suspected as primarily involved in some effects of veratrum

alkaloids on the circulatory and respiratory systems as well as in the elicitation of convulsive activity which usually precedes and accompanies death due to poisoning by these compounds; but a direct central nervous action has not yet been demonstrated unequivocally. The work of Hill (1933), Schmitt & Gasser (1933) and Schmitt (1936), who found that heat production and oxygen consumption of peripheral nerve are increased by veratrum alkaloids, suggested the possibility that, if protoveratrine has a direct effect on the central nervous system, a similar change in metabolism might occur and be observable in that tissue. As shown in the present and succeeding communications, this is indeed the case. The present communication will deal chiefly with the action of protoveratrine on 'resting', i.e. electrically non-stimulated pieces of cerebral cortex. It will be seen that minute amounts of the alkaloid are

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