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CONVULSIVE AGENTS AND THE PHOSPHATES OF BRAIN EXAMINED *IN VITRO*

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Many agents which change the functional activity of the brain *in vivo* change also its concentration of inorganic and labile phosphates (for references see McIlwain, 1950). Under suitable conditions, the concentrations of these phosphates can be maintained in sections separated from mammalian brain *in vitro* (McIlwain, Buchel, and Cheshire, 1951). Such sections therefore give a means of examining any direct actions of different agents on the metabolism of central nervous tissues without secondary—e.g., endocrine or vasomotor—effects from the rest of the body. Examination of narcotics in this way has already been reported (Buchel and McIlwain, 1950).

During convulsions caused by a variety of agents the inorganic phosphate of the brain rises and creatine phosphate and in some cases adenosine polyphosphates fall. This has been demonstrated for pentamethylene tetrazole (cardiazol ; leptazol ; metrazol) in dogs (Stone, Webster, and Gurdjian, 1945) and cats (Klein and Olsen, 1947) ; for electrical stimulation in cats (Klein and Olsen, 1947) and rats (Dawson and Richter, 1950) and also for caffeine, bromocamphor, theophylline with ethylenediamine, and nikethamide (Klein and Olsen, 1947).

In the present paper we describe our study of the phosphates of brain metabolizing *in vitro* in the presence of pentamethylenetetrazole, picrotoxin, caffeine, and strychnine and also while subject to varying potential gradients which were intended to simulate conditions during electrically induced convulsions. The effects of these agents on respiration and sometimes on glycolysis have also been observed. In most experiments glucose was used as substrate, but some with glutamic acid as substrate are included.

EXPERIMENTAL

For studying the action of added substances.—The methods and salines used were based on those described by McIlwain, Buchel, and Cheshire (1951) and Buchel and McIlwain (1950). An experiment commonly employed three large manometric vessels of about 100 ml. with sidearms and centre wells, together with a thermobarometer. Two or three guinea-pigs or rats were decapitated, the cerebral cortex separated and sliced, and the slices distributed so that each experimental vessel received a representative sample of about 0.4 g. fresh weight. The vessels each contained a total of 15 ml. saline, of which 0.1–1.5 ml. was in some experiments used to contain an added substance in a

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sidearm, and mixed with the rest during an experiment. The vessels were equilibrated with O_2 at 37° and their O_2 uptake followed each 5 minutes during the experimental period, commonly (see Tables) of 30 minutes.

At the end of this time the contents of the flasks were tipped one at a time into a porcelain filter funnel with a perforated disc, so collecting the slices, which were allowed to drain for about 10 seconds. The funnel was used as a sieve, without any filtering medium. The slices were then picked up with a mounted, bent wire, drained for a few seconds on the side of the funnel and dropped into 1.5 ml. of 10 per cent (w/v) trichloroacetic acid in a homogenizer tube already cooled in ice and weighed. The tissue was immediately homogenized and later the tube was wiped dry outside and reweighed to give the wet weight of tissue used in the experiment. Meanwhile the contents of the second and third flasks were treated in the same way. As the tissue was weighed at the end of the experiment, small detached fragments of the slices could be ignored and allowed to pass the filter. On the other hand, the tissue is known to swell during such experiments; this was allowed for as described by McIlwain, Buchel, and Cheshire (1951). Inorganic and creatine phosphates were then determined in the trichloroacetic filtrate after Ca-ethanol separation, also as described by McIlwain, Buchel, and Cheshire (1951).

For studying the action of electrical changes.—The methods of McIlwain and Gore (1951) were used. These depended on passing varying currents (described in the Table concerned) between electrodes in the above media close to slices of brain cortex. Respiration was measured concomitantly. At the end of the experiment the slices were removed individually and rapidly from their holders and extracted as described above.

RESULTS

Large differences have been found in the biochemical effects of different convulsive agents on separated cerebral cortex. Two extreme cases are quoted in detail in Table I.

TABLE I

PENTAMETHYLENE TETRAZOLE AND ELECTRICAL STIMULATION ON PHOSPHATES AND RESPIRATION OF GUINEA-PIG CEREBRAL CORTEX

All experiments quoted were carried out at 37° in glycylglycine-buffered medium with glucose as substrate, and similar results were also obtained in bicarbonate-buffered media. Tissue-wt./ml. = 20–25 mg. Electrical stimulation with a.c. at 2.9V in electrodes D and vessels A′ of McIlwain (1951)

Measurements	Pentamethylene tetrazole			Electrical stimulation	
	Control	10^{-3} M	10^{-2} M	Control	Stimulated
O_2 uptake at 10 min. (μ mol./g.)	14.6	14.5	14.4	9	17
O_2 uptake at 20 min. (μ mol./g.)	25.8	25.6	24.8	20	36
O_2 uptake at 30 min. (μ mol./g.)	37	36.8	36.1	32	52
Final *p*H	7.3	7.3	7.3	7.2	7.2
Inorganic P (μ mol./g.) ..	3.58	3.55	3.50	4.10	4.95
Creatine phosphate (μ mol./g.)	1.38	1.48	1.38	1.16	0.27

Pentamethylene tetrazole was found to have remarkably little effect on the phosphocreatine or inorganic phosphate concentration, even when examined in higher concentrations and after longer periods of action than are effective *in vivo*. The convulsive dose in man or cats would give a maximum concentration of the order

of 10^{-4} M, if it was uniformly distributed, or perhaps 10^{-3} M if it was in the central nervous system only. Concentrations of 10^{-4} to 10^{-2} M were examined *in vitro* with negative results. Respiration also was unchanged; *in vivo*, cerebral respiration increases during convulsions. Both glucose and glutamate (Table II) were used as substrates *in vitro*.

Electrical stimulation in vitro was found to have major effects on phosphates. Table I shows that it could lead to loss of three-quarters of the creatine phosphate, with an approximately equivalent increase in inorganic phosphate. The electrical stimulation used was not greatly above the threshold for the metabolic response. This had been found to be of the order of 1.5 V for sine-wave a.c. currents of 50 cyc./sec. (McIlwain, 1951), with cortex slices in the present apparatus. The experiments of Tables I and II employed currents of these characteristics at 2.7 and 2.9 V. This voltage is on the lower range of those found necessary in stimulating exposed cortex or in stimulating various parts of the brain with electrodes buried in it (for reference see McIlwain, 1951). It was much lower than the voltages used in electro-convulsive therapy, which are, however, not comparable, as they are applied to the outside of the head.

Electrical stimulation in the present experiments led also to increased cerebral respiration, which is found *in vivo* (Schmidt, Kety, and Pennes, 1945).

Picrotoxin, strychnine, and caffeine.—Results with these substances are quoted in Table II. Effects were never obtained comparable to those of electrical stimulation, although quite high concentrations were examined. Thus, with strychnine, Klein and Olsen (1947) found effects on the phosphates *in vivo* with quantities which, if evenly distributed, might yield 3×10^{-6} M of the drug, or 10^{-4} M if it was largely in the central nervous system. Veit (1935) found about $2–4 \times 10^{-5}$ M strychnine in some parts of the central nervous system during its action in cats, dogs, and apes. The present experiments showed no effect on phosphates at 10^{-3} M, but a fall in labile phosphates at 10^{-2} M when glucose was used as substrate. The fall is however likely to be secondary to the change in respiration which is also recorded. In contrast to the effect of strychnine *in vivo*, respiration falls. This was associated with a decrease also in inorganic phosphate, whereas *in vivo* Klein and Olsen (1947) observed a large increase in inorganic phosphate to follow strychnine convulsions. No change in inorganic phosphate was found with glutamate as substrate.

With caffeine the stimulating dose would if immediately distributed give a mean body concentration of the order of 2×10^{-5} M, and the convulsive dose found to affect phosphates, one of about 5×10^{-3} M. We found a small but definite lowering of creatine phosphate with 10^{-2} M caffeine *in vitro*, which appeared to be independent of respiratory change and thus may be to some extent analogous to the change *in vivo*. It did not occur with glutamate as substrate.

The changes in phosphates reported by Klein and Olsen (1947) to occur on administering picrotoxin were not large but were given with doses equivalent to 2×10^{-5} M if rapidly and evenly distributed, or about 10^{-3} M if mainly localized in the central nervous system. In the present experiments 10^{-3} or 10^{-2} M concentrations caused an appreciable lowering of phosphocreatine, which may therefore bear some relationship to the action *in vivo*. This change also occurred with glucose, but not glutamate, as substrate.

TABLE II

VARIOUS CONVULSIVE AGENTS ON PHOSPHATES AND RESPIRATION OF GUINEA-PIG CEREBRAL CORTEX

Experimental arrangement as described in Table I. The changes in italic type represent significant deviations from normal values

Agent and molar concentration	Substrate	Respiration		Inorganic P		Creatine phospnate	
		μ mol./g./hr.	% change from control	μ mol./g.	% change from control	μ mol./g.	% change from control
Pentamethylene tetrazole 10^{-3}	glucose	74	−0.5	3.55	−1	1.48	+7
,, ,, 10^{-2}	glucose	72	−2	3.50	−2	1.38	0
,, ,, 10^{-3}	glutamate	84	0	5.17	−2	0.30	−5
,, ,, 10^{-2}	glutamate	84	0	4.95	−2	0.36	+17
Picrotoxin 10^{-3}	glucose	67 83	−8, +4	3.54, 3.80	*+12*, *+4*	1.10, 0.90	*−29*, *−18*
,, 10^{-2}	glucose	71 79	−3, −3	3.23, 3.43	+3, −6	1.16, 0.86	−25, −23
,, 10^{-3}	glutamate	84	0	5.2	−2	0.33	+4
,, 10^{-2}	glutamate	84	0	5.45	+3	6.30	+3
Strychnine 10^{-3}	glucose	60	*−13*	3.13	*−8*	1.03	−5
,, 10^{-2}	glucose	57	*−18*	2.78	*−18*	0.62	*−43*
,, 10^{-3}	glutamate	66	*−15*	5.6	0	0.43	−4
,, 10^{-2}	glutamate	62	*−21*	5.55	−1	0.44	−3
Caffeine 10^{-3}	glucose	75	−5	3.45	+10	0.93	−8
,, 10^{-2}	glucose	79	0	3.12	−1	0.80	*−20*
,, 10^{-3}	glutamate	80	−4	5.35	+2	0.30	+8
,, 10^{-2}	glutamate	80	−4	5.15	−2	0.30	+7
a.c. 50 cyc./sec., 2.7 V. ..	glucose	144	*+104*	4.52	*+40*	0.52	*−50*

Discussion

It is clear that whereas the biochemical events occurring in the brain during electrically induced convulsions have been imitated *in vitro* in sections of cerebral cortex, this was not so with those associated with pentamethylene tetrazole or strychnine in the present experiments. Results with picrotoxin and caffeine were not sufficiently definite. The similarity in the biochemical events produced by electrical stimulation *in vivo* and *in vitro* extends to respiration, glycolysis, and glucose utilization (McIlwain, Anguiano, and Cheshire, 1951) as well as to the present findings with phosphates. The slices are therefore not precluded by their handling and artificial situation from responding in a fashion typical of convulsive activity.

A possible reason for the differing responses shown by the slices to the agents examined is as follows. The slices appear to show no spontaneous electrical activity comparable to that shown *in vivo*. Indeed, undercutting the cortex in situations which involve much less disturbance than those of the present experiments stops spontaneous electrical activity (Dusser de Barenne and McCulloch, 1941 ; Burns, 1950). Chemical convulsants increase the electrical activity of the intact brain and may do so by lowering the threshold to spread of impulses in the brain. The chemical changes are then understandable as necessary to support the increased electrical activity (see, for example, McIlwain, 1950). However, in a preparation showing no spontaneous activity, such lowering of threshold would be less likely to bring about any increased electrical or metabolic changes. On the other hand, the preparations may, like those of Adrian (1936), Dusser de Barenne and McCulloch (1941), and Burns (1950), remain capable of responding to applied potentials, and hence of showing the metabolic changes reported above. Further examination of the chemical convulsants on separated tissue appears to require their addition during the application of electrical stimuli.

Glutamic acid is one of the few substances other than glucose which are oxidized rapidly by brain slices. The phosphates of the slices respiring with glutamate as substrate did not approximate to the quantities found *in vivo*, as was the case when glucose was used. Nevertheless it was of interest to see whether the chemical convulsants brought about any change in the phosphate levels. Table II shows that little change was caused. This is more likely to be due to the initially low level of creatine phosphate than to any anticonvulsive action of glutamic acid. Glutamic acid is considered in detail from this point of view elsewhere.

Summary

1. Pentamethylene tetrazole and strychnine, whose convulsant action *in vivo* is associated with increased cerebral inorganic phosphate and decreased creatine phosphate, did not affect the phosphates of cerebral cortex slices *in vitro* when added in amounts comparable to those effective in intact animals. Higher concentrations of strychnine altered phosphates, but in a manner suggesting the change to be secondary to a small change in respiration.

2. Applied potential gradients, simulating those causing convulsions, caused changes in the phosphates and in other biochemical characteristics which were closely analogous to those occurring in the brain *in situ* during convulsions.

3. Caffeine and picrotoxin caused relatively small effects on the phosphates *in vitro*, but these may bear some relation to their action *in vivo*.

4. The present findings indicate that the changes in phosphates, respiration, and glycolysis caused in the brain by applied potentials are not mediated by vasomotor or endocrine changes, and they enable suggestions to be made regarding the mode of action of the chemical convulsants studied.

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