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Metabolism and Metabolic Response to Electrical Pulses in White Matter from the Central Nervous System

BY B. M. BOLLARD AND H. MCILWAIN

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

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The greater part of electrophysiological and metabolic work on the peripheral nervous system has been carried out with medullated fibres, as these preponderate in convenient samples such as the sciatic nerve. On the other hand, most observations on the central nervous system are with grey matter, probably because this preponderates in the cerebral cortex and is most active metabolically. Present observations aim at linking these two types of study by examining cerebral white matter. An opportunity likely to be afforded by cerebral white matter rather than by grey is the possibility of observing phenomena related to transmission along fibre tracts some millimetres in length. For electrical stimulation in directions related to fibre tracts sheets of white matter were desired, and in small animals these were found most conveniently in the subcortical white matter, with which most of the present studies have accordingly been made. Parts of the brain stem, medulla and thalamus have also been included.

A prerequisite was to know which substrates would maintain respiration, the level of phosphocreatine and the respiratory response to electrical pulses in the separated tissue, especially as peripheral nerve is suspected of differing from the cerebral cortex in this respect. A variety of different substrates have therefore been studied with white matter in the way in which grey matter had previously been examined (Kratzing, 1953).

EXPERIMENTAL

Media and tissues. Media were those employed by Kratzing (1953), all containing 2.75 mM-CaCl₂ and glycylglycine as the main buffer at pH 7.4. Tissues were prepared from the brain of guinea pigs as sheets by cutting with a blade and guide (McIlwain, 1951), grey matter 0.35 mm. and

white matter 0.4 mm. in thickness. Subcortical white matter was obtained by exposing the lateral ventricle, placing the guide upon it and cutting and floating the slice to a dish of medium. It was here inspected and any portions of grey matter were cut off. Normally, however, it contained little grey matter, and white matter was left on the uncut portion of the hemisphere. This uncut portion was inverted and the slices of grey matter were cut from it. Medulla was taken from the ventral surface of the lower part of the brain just posterior to the pons; two successive slices parallel to the outer surface were cut and were visibly different from each other in structure. In a few observations with thalamus and midbrain (at the level of the anterior colliculi), these were chopped in the machine of McIlwain & Buddle (1953) at 0.35 mm. intervals. Sciatic nerve, also from the guinea pig, was freed from its outer sheath and adherent fat.

In a given experiment, tissues from two or three different parts were compared in their behaviour towards a chosen substrate. From one animal were obtained six or eight specimens of cerebral cortex, and two specimens of the other parts named above. In most experiments tissues from one guinea pig were prepared in the order: subcortical white matter, grey matter of the cerebral cortex, and medulla; these specimens were trimmed to rectangles about 40–50 mg. in weight which fitted grid electrodes *H* used in vessels *A* (Ayres & McIlwain, 1953). All specimens of these tissues were placed in electrodes whether or not they were to receive pulses. Electrical condenser pulses were from the generator of Ayres & McIlwain (1953), at 100/sec., peak potential 10 v, and time constant 0.4 msec.

Determinations. Respiration was determined manometrically by conventional procedures. At the end of experiments 1 ml. of the fluid was taken for determination of lactic acid according to Barker & Summerson (1941).

In the experiments in which tissue content of phosphates was to be determined (Table 3), the procedure after making manometric measurements was as follows. A vessel was removed from the thermostat, its electrode holder carrying the tissue was rapidly removed to a shallow dish of medium and opened, and the tissue lifted on a mounted bent wire; it was drained from medium for 1 sec. against the dish,

transferred to 1 ml. of 5% (w/v) trichloroacetic acid (in a centrifuge tube kept in ice-water) and ground with a plunger. This was done rapidly so that the tissue was ground 20 sec. after removing the vessel from the thermostat. A duplicate slice was then removed in the same way and added to the same tube. Other tissues were successively placed in other tubes. The plungers were washed into the tubes with 1 ml. of ice-cold trichloroacetic acid, the contents of the tubes mixed and after 5 min. centrifuged at -5° . Determination of inorganic and phosphocreatine phosphate was completed as described by Heald (1954).

RESULTS

Respiration and glycolysis by different parts of the brain

In the absence of added substrate, subcortical white matter of the guinea pig respired at a lower rate than did grey matter (Fig. 1). The rate fell with time, reaching about half its original value in 2 hr.; this is a smaller fall than in grey matter. Glucose prevented this fall and also gave higher initial values, of about $28 \mu\text{moles of O}_2/\text{g./hr.}$ Comparable values were found in medulla, whereas the respiratory rates of thalamus and midbrain approximated to those of the cerebral cortex (Table 1). Sciatic nerve respired much more slowly. With glucose as substrate, accumulation of lactic acid occurred with each tissue. Values are included in Table 1, and with cerebral cortex, averaging some $25 \mu\text{moles of lactate/g./hr.}$ during an experiment lasting for

Fig. 1. Respiration of slices from guinea-pig brain. ●, Grey matter of the cerebral cortex; ○, subcortical white matter. Curves: (a) with 10 mM-glucose as substrate; (b) with no substrate. Points are mean values from several experiments.

Table 1. *Respiration and formation of lactic acid by different parts of the brain, and by sciatic nerve*

Approx. 50 mg. of tissue was incubated in 3.5 ml. of oxygenated phosphate media with the additions described below. Respiratory rates were derived from graphs such as those of Fig. 1, and are followed by standard deviations.

Tissue	Substrate concn. (mM)	No. of expts.	Respiratory rate (μ moles of O_2 /g./hr.) for period (min.)			Lactic acid formed (μ moles/g./expt. of 135 min.)
			0-40	40-80	80-120	
Guinea-pig tissues						
Cerebral cortex	None	{ 4	49 $\pm$ 8	28 $\pm$ 3	19 $\pm$ 4	—
Subcortical white matter		{ 6	31 $\pm$ 2	23 $\pm$ 4	17 $\pm$ 5	6 $\pm$ 2
Medulla		{ 6	22 $\pm$ 5	18 $\pm$ 4	17 $\pm$ 3	6 $\pm$ 2
Cerebral cortex	Glucose (10)	{ 6	59 $\pm$ 5	59	59	70 $\pm$ 3
Subcortical white matter		{ 6	29 $\pm$ 6	29	29	52 $\pm$ 8
Medulla		{ 6	33 $\pm$ 5	33	33	53 $\pm$ 8
Thalamus		{ 6	54 $\pm$ 4	54	56	43 $\pm$ 3†
Midbrain		{ 6	60 $\pm$ 4	60	59	44 $\pm$ 4†
Sciatic nerve		{ 4	8 $\pm$ 1	8	9	6 $\pm$ 1†
Subcortical white matter	Citrate (20)	{ 4	31 $\pm$ 6	26 $\pm$ 2	16 $\pm$ 2	8 $\pm$ 4
Medulla		{ 4	25 $\pm$ 5	20 $\pm$ 3	14 $\pm$ 3	4 $\pm$ 1
Rabbit tissues						
Cerebral cortex	Glucose (10)	{ 7	47 $\pm$ 6*	47*	—	31 $\pm$ 4
Subcortical white matter		{ 4	16 $\pm$ 2*	16*	—	24 $\pm$ 3
Human tissues						
Cerebral cortex	Glucose (10)	{ 6	41 $\pm$ 5	42	40	22 $\pm$ 6
Subcortical white matter		{ 5	25 $\pm$ 6	25	24	21 $\pm$ 5

* Values during periods of 50 min.; the lactic acid results refer to periods of 115 min.

† Lactic acid during 150 min.

3 hr., are comparable with previous observations; during this period the rate of formation falls (McIlwain & Grinyer, 1950). Accumulation of lactate with subcortical white matter and medulla was only a little less than with cerebral cortex; sciatic nerve, again, was much less active.

Reactions of the subcortical white matter and medulla to substrates other than glucose were qualitatively similar to those of grey matter (Tables 1 and 2). Thus pyruvate permitted stable respiratory rates comparable with those with glucose; rates with succinate, glutamate and citrate, though initially high, fell in 2 hr. Values with acetate were not significantly different from those in the absence of added substrate.

Electrical pulses applied to the different cerebral tissues, during *in vitro* experiments, in each case caused increase of respiration when glucose was used as substrate. As with grey matter, the increase

was 70–100 % of the original rates. With subcortical white matter and medulla, pyruvate also supported the increase in respiration, which did not, however, occur in the absence of substrate nor with succinate or acetate or glutamate (Table 2; Fig. 2). With glutamate, a small increase of doubtful significance was observed in each case.

Inorganic phosphate and phosphocreatine

Cerebral white matter has been found capable of maintaining defined phosphocreatine concentrations during *in vitro* metabolism (Table 3). In samples both of subcortical white matter and of medulla, the levels of a phosphate derivative of the lability of phosphocreatine, were about two-thirds of those reached in the cerebral cortex, with glucose as substrate. Without substrate, little resynthesis occurred; with glutamate levels again were low in white as in grey matter. Confirmation that the

Table 2. *Respiration and respiratory response to electrical pulses by different tissues from the guinea pig*

Expts. are as in Table 1 and Fig. 2. Values obtained during the application of electrical pulses are in bold type.

Tissue	Substrate concn. (mM)	No. of expts.	Respiratory rate (μ moles of O_2 /g./hr.) for period (min.)			Increase in respiration caused by applied pulses		
			0-40	40-90	90-130	%	<i>P</i>	
Cerebral cortex	None	4	45 $\pm$ 4	36 $\pm$ 7	10 $\pm$ 7	—	—	
	Glucose (10)	8	57 $\pm$ 5	100 $\pm$ 9	57 $\pm$ 5	76	<0.001	
	Succinate (20)	4	76 $\pm$ 6	61 $\pm$ 5	45 $\pm$ 9	—	—	
		4	68 $\pm$ 6	66 $\pm$ 10	45 $\pm$ 10	0	—	
	Glutamate (20)	3	64 $\pm$ 6	58 $\pm$ 6	44 $\pm$ 4	—	—	
		3	73 $\pm$ 9	63 $\pm$ 8	47 $\pm$ 6	0	—	
	Acetate (20)	3	33 $\pm$ 8	16 $\pm$ 8	13 $\pm$ 3	—	—	
		3	38 $\pm$ 6	33 $\pm$ 7	13 $\pm$ 3	0	—	
	Subcortical white matter	None	4	26 $\pm$ 3	19 $\pm$ 2	13 $\pm$ 1	—	—
			4	24 $\pm$ 1	19 $\pm$ 5	12 $\pm$ 4	0	—
Glucose (10)		11	28 $\pm$ 5	50 $\pm$ 4	22 $\pm$ 3	77	<0.001	
Succinate (20)		5	42 $\pm$ 9	36 $\pm$ 3	22 $\pm$ 5	—	—	
		5	36 $\pm$ 4	36 $\pm$ 6	22 $\pm$ 6	2	0.9	
Glutamate (20)		6	34 $\pm$ 6	29 $\pm$ 5	22 $\pm$ 5	—	—	
		6	32 $\pm$ 4	37 $\pm$ 2	26 $\pm$ 5	16	0.2-0.3	
Acetate (20)		3	25 $\pm$ 4	17 $\pm$ 6	12 $\pm$ 6	—	—	
		3	27 $\pm$ 4	19 $\pm$ 7	8 $\pm$ 6	0	—	
Pyruvate (20)		3	28 $\pm$ 1	28 $\pm$ 1	28 $\pm$ 1	—	—	
	3	30 $\pm$ 5	59 $\pm$ 6	30 $\pm$ 5	97	<0.001		
Medulla	None	2	32	20	14	—	—	
		2	28	21	18	0	—	
	Glucose (10)	11	31 $\pm$ 4	51 $\pm$ 5	31 $\pm$ 4	65	<0.001	
	Succinate (20)	5	51 $\pm$ 10	44 $\pm$ 6	35 $\pm$ 12	—	—	
		7	51 $\pm$ 4	46 $\pm$ 6	41 $\pm$ 3	0	—	
	Glutamate (20)	6	27 $\pm$ 3	27 $\pm$ 3	18 $\pm$ 1	—	—	
		6	32 $\pm$ 3	35 $\pm$ 7	30 $\pm$ 3	9	0.5-0.7	
	Pyruvate (20)	3	31 $\pm$ 3	53 $\pm$ 3	31 $\pm$ 3	71	0.01-0.02	
		3	33 $\pm$ 4	33 $\pm$ 4	33 $\pm$ 4	—	—	
	Acetate (20)	3	35 $\pm$ 7	25 $\pm$ 9	15 $\pm$ 4	—	—	
		3	34 $\pm$ 8	25 $\pm$ 5	16 $\pm$ 5	0	—	

Table 3. *Inorganic phosphate and phosphocreatine in guinea-pig cerebral tissue*

Tissues were incubated in electrodes in oxygenated media for 60 min., during which their respiratory rates were measured and found to have values within the limits of those recorded in Tables 1 and 2. Pulses when used were applied for the terminal 30 min. of the experiment. Tissues were then rapidly fixed for phosphate determinations. Values are followed by s.d.

Tissue	Substrate concn. (mm)	No. of expts.	Inorganic phosphate (μmoles/g.)		Phosphocreatine (μmole/g.)	
			No pulses	With pulses	No pulses	With pulses
Cerebral cortex	None	3	6.4 ± 1.8	6.03 ± 0.81	0.33 ± 0.138	0.41 ± 0.09
	Glucose (10)	6	2.84 ± 0.24	3.63 ± 0.18	1.32 ± 0.22	0.66 ± 0.18
	Glutamate (20)	4	4.40 ± 0.62	5.59 ± 0.34	0.49 ± 0.06	0.49 ± 0.08
Subcortical white matter	None	3	5.31 ± 0.99	5.70 ± 0.93	0.37 ± 0.32	0.41 ± 0.04
	Glucose (10)	6	3.91 ± 0.53	4.26 ± 0.15	0.93 ± 0.15	0.68 ± 0.07
	Glutamate (20)	4	5.75 ± 0.79	5.77 ± 0.08	0.52 ± 0.05	0.54 ± 0.01
Medulla	None	3	5.80 ± 0.75	5.10 ± 0.88	0.28 ± 0.1	0.27 ± 0.1
	Glucose (10)	6	3.33 ± 0.44	3.39 ± 0.56	0.85 ± 0.56	0.40 ± 0.17

Fig. 2. Respiration of slices from guinea-pig brain showing the effect of pulses applied at 10 v, 100/sec. and 0.4 msec. time constant, during the period indicated by arrows. Points are mean values from several experiments. Curves (a), cerebral grey matter; values in the ordinates have been increased by 20 μmoles/g. for clarity in the diagram. Curves (b), subcortical white matter. ●, Unstimulated respiration; ○, respiration with pulses.

labile phosphate was indeed phosphocreatine was obtained by the precipitation of its barium salt, and determination in the precipitate of both creatine and labile phosphate. These were present in equimolar amounts (Table 4), when either glucose or glutamate was used as substrate. With these two substrates very different quantities of inorganic phosphate were found in the tissue; with glutamate, levels up to 6 μmoles/g. were found, a phenomenon comparable with that occurring in cerebral grey matter.

Table 4. *Phosphates and creatine in preparations from guinea-pig subcortical white matter*

Slices of white matter were incubated as described in Table 3.

Substrate	Barium salts insoluble in		
	Water (μmoles of phosphate/g.)	Ethanol (μmole of phosphate/g.) (μmole of creatine/g.)	
Glucose	2.5	0.83	0.87
	2.01	0.83	0.84
	2.4	0.93	0.95
Glutamate	6.0	0.20	0.24
	4.8	0.29	0.31

The phosphates of white matter respiring in the presence of glucose proved susceptible to applied electrical pulses (Table 3). Both in subcortical white matter and in medulla the pulses which increased respiration and glycolysis lowered the level of phosphocreatine and increased that of inorganic phosphate. Comparable changes were not observed when glutamate was present in the place of glucose.

DISCUSSION

In characteristics now measured, different parts of the brain have proved to differ in a quantitative rather than qualitative fashion. Medulla and subcortical white matter showed about half the respiratory rate of cerebral cortex, but all were alike in the proportional increase induced electrically, and in the substrates supporting the response. Glucose and pyruvate were effective and succinate and acetate were not, and the small response with glutamate now observed in white matter has been paralleled in grey matter under some circumstances (Greengard & McIlwain, unpublished; Kratzing, 1956), though it is not exhibited in the present results. The white matter now examined was also

similar to grey in its resynthesis of phosphocreatine *in vitro* when provided with oxygen and glucose; glutamate again was much less effective. Thus with white matter also, circumstances supporting phosphocreatine and respiratory response to pulses are correlated.

That cerebral white matter shows much higher respiratory and glycolytic rates than sciatic nerve is presumably related to the quantity of connective tissue in the peripheral nerve. Relationships between the metabolic activities of grey and of white matter in the brain, as exhibited in the present work or in that of Lowry *et al.* (1954), can largely be explained by regarding white matter as similar to grey, apart from 'dilution' by metabolically less-active lipids of the myelin. Scope remains, however, for many differences in the level of individual enzymes in grey and white matter (see, for example, Naidoo & Pratt, 1951, 1952); data relevant to their allocation to different cell types are discussed by Brierley & McIlwain (1957).

SUMMARY

1. Subcortical white matter of the guinea pig, rabbit and man was capable of respiration and glycolysis in ordinary glucose salines at one-half to two-thirds of the rates found in the cerebral cortex. Under these conditions the guinea-pig subcortical white matter resynthesized phosphocreatine to a level of about 0.9 μ mole/g.

2. In maintaining respiration glucose was replaceable by pyruvate; with succinate and glutamate (but not with acetate and citrate) respiratory rates were greater than in the absence of added

substrate, but decreased with time. Glutamate did not, however, support resynthesis of phosphocreatine.

3. Respiration, glycolysis and phosphocreatine formation maintained by glucose responded to application of electrical pulses to the tissue. Pyruvate also permitted response but other substrates examined were ineffective in this respect.

4. Certain of these characteristics were examined also in tissues from the medulla, thalamus, midbrain and sciatic nerve.

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The Composition of Isolated Cerebral Tissue: Purines

By J. THOMAS*

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

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In previous studies (McIlwain, Thomas & Bell, 1956; Thomas, 1956) it was shown that the composition of isolated cerebral tissues differed appreciably from that *in vivo*, but that the composition *in vitro* could be in part restored by specific additions to fluids in which the tissue was incubated. An attempt has now been made to maintain higher levels of nucleotides in cerebral slices respiring in media fortified with precursors of nucleotides.

* Present address: Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W. 1.

EXPERIMENTAL

Tissues

Rapid fixation of brain. The procedure adopted was that described by Kratzing & Narayanaswami (1953), liquid nitrogen being used. About 500 mg. of frozen powdered brain was weighed in a tube containing 10 ml. of 10% (w/v) trichloroacetic acid and homogenized.

Preparation of tissue and medium for metabolism. Six slices each of about 100 mg. wet wt. were cut from the cerebral cortex of a guinea pig. The slices were suspended in glucose-phosphate medium made according to Rodnight &