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Effects of Fatty Acids, Ketone Bodies, Lactate and Pyruvate on Glucose Utilization by Guinea-Pig Cerebral Cortex Slices

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1. Starvation did not affect the rates of glucose utilization or lactate formation by guinea-pig cerebral cortex slices. 2. Palmitate (1mM), butyrate (5mM) or acetoacetate (5mM) did not affect glucose utilization or lactate formation by cerebral cortex slices from guinea pigs starved for 48hr. 3. DL-β-Hydroxybutyrate (10mM) increased the formation of lactate without affecting glucose utilization by cerebral cortex slices from guinea pigs starved for 48hr. This implies that β-hydroxybutyrate decreased the rate of glucose oxidation. 4. Metabolism of added ketone bodies can account for 20–40% of observed rates of oxygen consumption. 5. Lactate or pyruvate (5mM) decreased the rates of glucose utilization by guinea-pig cerebral cortex slices.

There is evidence that fatty acids (Volk, Millington & Weinhouse, 1952; Vignais, Gallagher & Zabin, 1958; Beattie & Basford, 1966; Allweis, Landau, Abeles & Magnes, 1966) and ketone bodies (Jowett & Quastel, 1935; Mann, Tennenbaum & Quastel, 1938) can be oxidized in brain. However, it is not known whether ketone bodies or fatty acids can decrease the requirement for glucose as a respiratory fuel for brain. Therefore the effects of these substances on glucose utilization by cerebral cortex slices were investigated.

The effects of lactate and pyruvate on glucose oxidation were also tested as both of these compounds can support the same rates of oxygen consumption in cerebral cortex slices as glucose (i.e. they are oxidized at the same rate as glucose) (Elliott, Greig & Benoy, 1937). A preliminary report on the effects of pyruvate on glucose metabolism has appeared (O'Neill, Simon & Shreeve, 1963).

MATERIALS AND METHODS

Materials. Adult guinea pigs (M.R.C. Hartley strain, albino), wt. 450–600g., of both sexes, fed with diet 18 [Herbert C. Styles, (Bewdley) Ltd.] were used. Starved animals had their food removed at 10 a.m. and were used 48hr. later.

Sodium pyruvate, NAD and $NADH_2$ were obtained from Boehringer Corp. (London) Ltd. DL-Carnitine hydrochloride and palmitic acid were obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks. Albumin (fraction V from bovine plasma) was obtained from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, and was freed from fatty acids and used to form a complex with palmitic acid according to Garland, Newsholme & Randle (1964). L-Lactic acid was prepared by Mr T. Gascoyne using the method of Krebs (1961).

Lactate dehydrogenase and peroxidase were obtained from Boehringer Corp. (London) Ltd. β-Hydroxybutyrate dehydrogenase, prepared as described by Williamson, Mellanby & Krebs (1962), was provided by Mr D. H. Williamson. Glucose oxidase was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and diazyme (Takamine) from Miles Chemical Co., Clifton, N.J., U.S.A. Carbonic anhydrase was prepared from ox erythrocytes according to Roughton (1934). The side arms of all flasks (including manometer flasks) were treated with Silicone Repelcote (Hopkin and Williams Ltd., Essex) to permit quantitative transfer to 0·1ml. quantities from side arms to the incubation mixtures.

Methods. All incubations were performed on guinea-pig cerebral cortex slices cut as described by Deutsch (1936) and incubated in bicarbonate saline (Krebs & Henseleit, 1932) containing 5mM-glucose. The ratio of fluid to tissue was 15–20:1 (w/v).

Oxygen consumption was measured by the method of Warburg & Krippahl (1960) as modified by Gevers & Krebs (1966), and by using manometer cups designed in collaboration with Dr W. Gevers (Fig. 1). This method was used as it permits direct measurement of consumption of O_2 in the presence of bicarbonate buffers; it differs from conventional manometry in the use of a CO_2 buffer with carbonic anhydrase in one compartment of the manometer cup to maintain

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a constant pCO_2 in the gas phase, and any changes in pressure may be attributed to uptake of O_2. The metabolism of carbon substrates was investigated in 25ml. Erlenmeyer flasks fitted with side arms and sealed with rubber stoppers. Flasks were shaken at 120 oscillations/min. in a Gallenkamp metabolic shaker at 38°. Incubations were stopped by cooling to 0° and treating with 0·1 vol. of 60% (w/v) $HClO_4$. The acidified systems were left for at least 15min. to permit equilibration between intra- and extracellular materials, before removing solids by centrifugation; this method was chosen in preference to homogenization of the slices with the acidified medium, which released an inhibitor of the glucose oxidase-assay system. Equilibration between intra- and extra-cellular materials was necessary for accurate measurement of glucose metabolism during the incubation; changes in glucose removal from the incubation medium may not be a satisfactory measure of glucose utilization unless the intracellular concentration of glucose is taken into account.

Glucose was measured in the acidified medium by using the glucose oxidase method of Huggett & Nixon (1957) as modified by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963). Glycogen was measured in the pellet of acidified slices and protein according to Krebs *et al.* (1963). The remaining assays were carried out on samples of the acidified media that had been neutralized with 0·2ml. vol. of 3M-K_2HPO_4. Pyruvate was measured according to Bücher, Czok, Lamprecht & Latzko (1963) and lactate by the method of Hohorst (1963). Acetoacetate and β-hydroxybutyrate were measured according to Williamson *et al.* (1962).

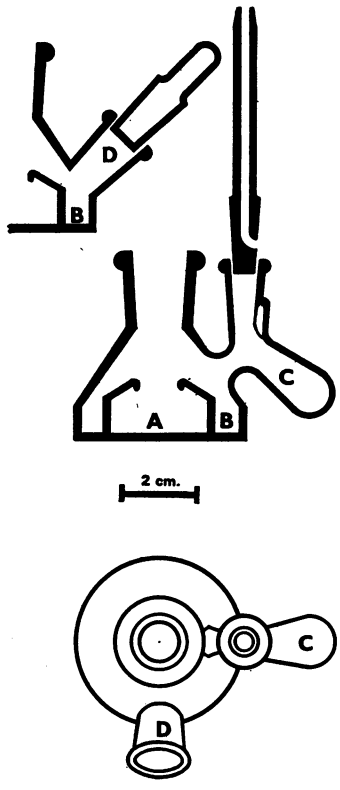

Fig. 1. Manometer cup for measurement of oxygen consumption in bicarbonate salines. This cup was designed in collaboration with Dr W. Gevers for measuring oxygen consumption in the presence of bicarbonate buffers (references in the text). The centre well (A) contains the CO_2 buffer system which maintains the gas phase at a constant pCO_2; the inverted lip of the centre well prevents loss of fluid during shaking. The outer compartment (B) contains the incubation medium, to which additions can be made during the incubation from the side arm (C). The entry port (D), which is stoppered, facilitates addition of the incubation medium and tissue to the flask.

RESULTS

Time-course of glucose metabolism in cerebral cortex slices. The rates of glucose utilization and lactate formation decreased with time, the largest changes occurring during the first 30min. (Table 1). The content of glycogen decreased over the first 30min. (see also McIlwain & Tresize, 1956) and then increased at rates comparable with those reported by Le Baron (1955) and Kleinzeller & Rybova (1957). The measured rates of oxygen consumption were linear with time.

These results indicate that a period of 30min. incubation is sufficient for the cerebral cortex slices to reach a steady state; consequently the effects of ketone bodies, fatty acids, lactate and pyruvate were analysed over the 30–60min. period of incubation. As rates of glycogen metabolism accounted for at most 5% of the glucose metabolized by the slices, and the total tissue content of glycogen was low (1–2μmoles of glucose/g. of fresh tissue), glycogen was not measured under any of the remaining conditions investigated.

Effects of lactate and pyruvate. Glucose utilization by guinea-pig cerebral cortex slices was significantly decreased by the addition of either lactate or pyruvate (both at 5mM), whereas oxygen consumption was not affected (Table 2). In all conditions studied, the oxygen required for complete combustion of measured substrates agreed closely with the observed rates of oxygen consumption.

Effects of fatty acids or starvation of the donor animal. Starvation of guinea pigs for 48hr. had no effect on rates of glucose utilization or lactic acid formation by cerebral cortex slices. Glucose uptake for the two periods of incubation 0–30 and 30–60min. were $20·6 \pm 2·8$ and $10·5 \pm 1·3$ for fed animals and $19·7 \pm 1·4$ and $9·2 \pm 1·5$ for starved animals; lactate formations for the same periods of incubation were $19·3 \pm 1·2$ and $6·5 \pm 1·0$ for fed animals, and $17·2 \pm 4·8$ and $4·7 \pm 1·3$ for starved animals. These values are the means ± S.D. of independent observations on tissue from 12 fed and 12 starved animals.

Similarly, addition of palmitate or butyrate had

Table 1. *Time-course of glucose metabolism in cerebral cortex slices*

Incubations were carried out as described in the text. Results are means ± S.D. Glycogen changes are expressed as μmoles of glucose equivalents.

	No. of observations	Time period (min.) ...	Rates (μmoles/g. of fresh tissue/30 min.) 0–30	30–60	60–90
Glucose uptake	18		21·8 ±2·9	10·4 ±1·2*	6·2 ±1·3*
Lactate formation	18		20·0 ±1·2	5·5 ±2·6*	3·9 ±2·8
Glycogen formation	9		−0·48±0·21	0·27±0·15	0·23±0·18
Oxygen uptake	14		42·1 ±3·8	39·6 ± 2·5	36·8 ±4·3

* ($P < 0·01$), The figure is significantly different from that for the preceding time-interval.

Table 2. *Effects of lactate and pyruvate on glucose metabolism by guinea-pig cerebral cortex slices*

Slices were incubated as described in the text. Lactate and pyruvate (0·15 M solutions of sodium salts) were added from the side arms after incubation for 30 min. (controls were treated with NaCl). Results (means ± S.D. of four independent operations) refer to the 30–60 min. period of incubation. Oxygen equivalents of carbon disappearing were calculated assuming glucose, lactate and pyruvate to be equivalent to 6, 3 and 2·5 moles of oxygen respectively.

Measurements	Additions to incubation ...	Rates (μmoles/g. of fresh tissue/30 min.) None	Lactate (5 mM)	Pyruvate (5 mM)
Glucose uptake		10·3±2·1	7·3±1·1*	6·6± 1·3*
Lactate uptake		−5·4±0·8	5·1±1·6	−14·6± 2·6
Pyruvate uptake		—	−3·3±1·2	20·4± 1·3
Oxygen consumption		43·2±3·8	43·0±4·2	45·1± 1·3
Oxygen required for complete combustion of measured substrates		45·6±6·5	50·8±9·8	46·8±12·3

* ($P < 0·01$), Significance of differences from incubations with no additions.

no effect on glucose uptake or lactic acid formation in slices from guinea pigs starved for 48 hr. (Table 3).

Effects of ketone bodies. Neither acetoacetate nor β-hydroxybutyrate significantly affected glucose utilization by cerebral cortex slices from guinea pigs starved for 48 hr. (Table 4). However, β-hydroxybutyrate caused a statistically significant increase in the formation of lactate from glucose and was found to decrease the net oxidation of glucose (expressed as oxygen equivalents in Table 4) by 23%. Acetoacetate had no statistically significant effect. Utilization of added ketone bodies could account for 20–40% of observed rates of oxygen consumption. Both in the absence and the presence of added ketone bodies, the observed rates of oxygen consumption agreed with those required to account for combustion of substrates removed.

DISCUSSION

The observation that β-hydroxybutyrate can increase the formation of lactate from glucose by cerebral cortex slices may be of physiological significance. Lactate can be converted into glucose in liver and kidney cortex (see Krebs, 1964). Thus the increased formation of lactate from glucose caused by the addition of β-hydroxybutyrate to cerebral cortex slices is equivalent, in the intact animal, to a sparing of glucose. This is of interest in view of suggestions that, under conditions of starvation, glucose synthesis or turnover is not sufficient to account for the requirements of the central nervous system, which must therefore be using an alternative substrate (Mayes, 1962; Cahill *et al.* 1966).

The concentration of pyruvate in blood is very low (0·1 mM); thus the observation that 5 mM-pyruvate can decrease glucose utilization by cerebral cortex is not of physiological significance. However, the concentration of lactate in the blood may increase significantly above the normal 1 mM (e.g. during severe exercise); under such conditions, it is suggested that lactate could decrease glucose utilization by the brain. This possibility depends on lactate entry into the brain. Analysis of cerebral tissues from animals injected with lactate has suggested limited penetration of lactate into the brain (Stone, 1938; Klein & Olsen, 1946). However, there does not appear to be any active transport mechanism causing removal of lactate from the

Table 3. *Effects of palmitate and butyrate on glucose uptake and lactate formation by cerebral cortex slices from starved guinea pigs*

Cerebral cortex slices from starved guinea pigs were incubated as described in the text. The fatty acids were present throughout the incubation. Palmitate (1mM) was added as a complex with albumin (2%) and in the presence of DL-carnitine (1mM). Butyrate (5mM) was added as its sodium salt. Controls contained chloride ions in place of palmitate or butyrate.

		Rates (μmoles/g. of fresh tissue/30min.)			
		Glucose uptake		Lactate formation	
Additions	Incubation period (min.) ...	0–30	30–60	0–30	30–60
Control		19·9±3·5	11·9±3·5	18·7±4·4	4·6±2·3
Palmitate (1mM)		22·4±7·3	14·6±4·8	18·1±2·8	8·5±4·0
Control		18·1±1·8	8·1±1·4	14·2±5·3	3·0±1·4
Butyrate (5mM)		18·4±3·5	8·5±2·0	13·3±4·4	3·2±3·5

Table 4. *Effects of ketone bodies on glucose utilization by cerebral cortex slices from starved guinea pigs*

Cerebral cortex slices from guinea pigs starved for 48hr. were incubated as described in the text. Acetoacetate or DL-β-hydroxybutyrate was added to the incubation medium after 30min. (controls were treated with NaCl). Results (means±S.D. of four independent observations) refer to the 30–60min. period of incubation. Oxygen equivalents of carbon disappearing were calculated assuming glucose, lactate, acetoacetate and β-hydroxybutyrate to be equivalent to 6, 3, 4 and 4·5 moles of oxygen respectively.

		Rates (μmoles/g. of fresh tissue/30min.)		
Measurements	Addition to incubation ...	None	Acetoacetate (5mM)	DL-β-Hydroxybutyrate (10mM)
Glucose uptake		9·7±1·3	9·6±0·5	9·4±1·2
Lactate formation		5·6±1·1	6·8±1·3	8·2±1·6*
Glucose oxidized (O_2 equivalents)		41·4±4·5	37·2±4·3	31·8±3·0†
Acetoacetate uptake		—	4·6±2·8	−3·0±0·8
D-β-Hydroxybutyrate uptake		—	−2·3±0·5	6·4±3·2
Removal of ketone bodies (O_2 equivalents)		—	8·0±4·0	16·8±7·2
Oxygen consumption		42·6±2·6	42·8±4·6	46·3±2·1
Oxygen required for complete combustion of measured substrates		41·4±4·5	45·2±7·5	48·6±9·2

* ($0·01 < P < 0·05$) and † ($P < 0·01$), Significance of differences from control incubations.

brain, so that when brain is forming lactate from glucose, the intracerebral concentration of lactate must be higher than extracerebral in the steady state. Observations that intracerebral lactate concentrations are lower than extracerebral must mean either that the system is not in a steady state, or that the brain is utilizing lactate. Thus although these studies suggest that the rate of entry of lactate into the brain may be limited, they do not exclude the possibility that lactate can serve as an alternative fuel for the intact brain.

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