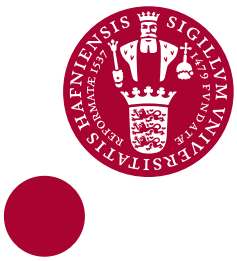

In vivo concentration of lactate in the brain of consious rats before and during seizures: A new ultra-rapid technique for the freeze-sampling of brain tissue

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Published in:
Journal of Neurochemistry

Publication date:
1973

Document version
Publisher's PDF, also known as Version of record

Journal of Neurochemistry, 1973, Vol. 21, pp. 1345 to 1348. Pergamon Press. Printed in Great Britain.

SHORT COMMUNICATION

In vivo concentration of lactate in the brain of conscious rats before and during seizures: a new ultra-rapid technique for the freeze-sampling of brain tissue

(*Received* 21 *May* 1973. *Accepted* 2 *July* 1973)

INCREASED cerebral metabolic activity during epileptic seizures accompanied by excess brain lactate formation has been amply demonstrated in animal experiments, where no control of gas tensions and acid-base parameters in the arterial blood was exercised (KLEIN and OLSEN, 1947; GURDJIAN *et al.*, 1947; KING *et al.*, 1967). However, in a series of studies both in experimental animals and in man, no convincing evidence of cerebral lactate acidosis during seizure activity was found when sufficient oxygenation of the arterial blood was assured during controlled ventilation and oxygen breathing (PLUM *et al.*, 1968; BERESFORD *et al.*, 1969; COLLINS *et al.*, 1970). In these studies, an increased cerebral blood flow was demonstrated, and as the cerebral hyperaemia observed during epileptic seizures has been assumed to be, at least in part, due to tissue lactic acidosis, the failure to find excess lactate in hyperaemic brains during seizure activity casts doubt upon a key argument in the theory of metabolic flow regulation. In a recent study employing relaxed, ventilated patients receiving electroconvulsive therapy, an increased output of lactate from brain together with an increased cerebral blood flow during seizures was demonstrated (BRODERSEN *et al.*, 1973).

Such different findings all obtained in situations of sufficient oxygen supply make the unsolved problem of lactic acid production in brain tissue during seizures a crucial one. Since the steady state concentration of lactate in brain changes very rapidly after interruption of blood supply to the tissue (LOWRY *et al.*, 1964), estimations of *in vivo* concentrations requires a sampling method that reduces the time from biopsy to cessation of metabolic activity to a minimum. In the present study an ultra-rapid freeze-sampling method, originally developed by QUISTORFF (1972) for rat liver studies, was used. This technique, which allowed removal and freeze-clamping of about 20 per cent of the rat brain in 0·12 s, was used in an investigation of brain lactic acid levels during electroshock seizures in immobolized, artificially ventilated rats.

MATERIALS AND METHODS

The experiments were performed with male Wistar strain albino rats (Møllegård, Denmark) weighing 200 g. The animals were fasted for 12 h prior to the experiments with free access to water. In halothane anaesthesia, induced and maintained with a halothane–oxygen gas mixture delivered from a calibrated halothane vaporizer (Fluotec, Keighley, England), the animals were tracheotomized. They were then immobolized with D-tubocurarine 0·2 mg/kg, and artifically ventilated with a rodent respirator preset to give an arterial carbon dioxide tension (P_aCO_2) of 35–45 mm Hg. A polyethylene catheter inserted into the femoral artery was used for anaerobic sampling of arterial blood. After local infiltration analgesia of the incised tissues with 1 % lidocaine, anaesthesia was discontinued, and the animals were ventilated with pure oxygen for the last 15 min of the experiments. Only when the difference between two consecutive measurements of P_aCO_2 was less than 10 per cent, were the animals considered to be in a respiratory steady state. The rectal temperature, measured with a mercury thermometer, was kept close to 37°C by means of intermittent heating of the animal with a lamp.

A 50 Hz alternating sine wave current, 50 V (effective), 50 mA, electroshock stimulus lasting 0·9 s was delivered via electrodes placed bilaterally in the external ears of the animals. In the control rats, tissue sampling was performed as soon as a respiratory steady state was attained, in the electroshocked rats this was exactly 5 s after the electrical stimulus was delivered.

The apparatus used for the brain tissue sampling is shown as a simplified diagram (Fig. 1a). In the following, the letters a–e and the numbers 1–4 refer to Fig. 1a. The machinery consists of a cutting part and a freeze-clamping part, and it is operated automatically by means of compressed air. The cutting is performed by two circular knife-blades (a,a), 17 cm in diameter, having both a rotary motion round their own axis (2) as shown by the arrow and a cutting movement round another axis (1), whereby the knives will pass through corresponding gaps (3,4) in the PVC tube (b,c,d). In this tube the rat is placed when the biopsy is made. A pair of cooling tongs (e,e), when cooled to the

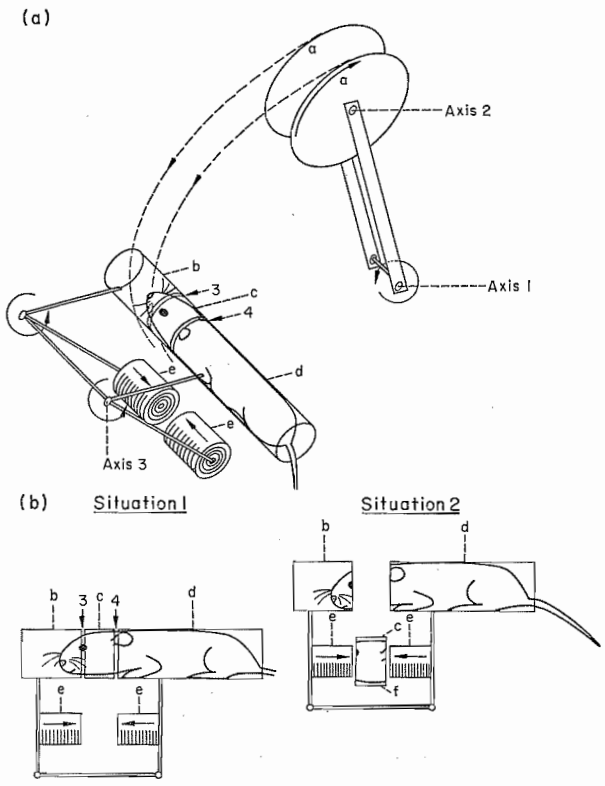

FIG. 1.

temperature of liquid N_2, can be rapidly lifted up on both sides of the section (c) of the tube, making it possible to clamp that part of the skull and brain of the rat which is positioned in this section.

Figure 1b outlines the working principle of the machinery and in the following the letters a–f and numbers 3 and 4 refer to Fig. 1b. In situation 1 the rat is in the correct position in the tube (b,c,d), which means that the eyes are at the first gap (3) and the ears at the second gap (4). When the machinery is activated, the two rotating knife-blades are released and they cut simultaneously through the rat, as seen in Fig. 1a. This cutting lasts about 0·03 s. Both knives will pass through the head of the rat giving a slice 14 mm thick (f), containing a transverse section of the brain. The short part of the tube (c), containing this slice (f), is held in place, while the rest of the rat is removed (situation 2) and in the same movement, the cooling tongs (e,e) are lifted up on both sides of the slice (f). At the very moment the cooling tongs reach this position they are released, and the brain tissue is clamped with great force, 10 kg/cm$_2$, between the aluminium pistons (e,e), precooled in liquid N_2. The direction of the clamping movement is indicated by the arrows drawn on the pistons (Fig. 1). The resulting biopsy is about 3 mm thick. The whole sequence, from cutting to clamping, lasts 0·12 s.

The blood samples were analysed for pH, PCO_2 and PO_2 using microelectrodes operated at 37°C (Radiometer, Copenhagen); pH values were referred to the buffers of the National Bureau of Standards (pH 6·841 and 7·383 at 37°C).

The part of the brain in the biopsies was easily identified by position in the biopsy, colour and macroscopic structure. The separation of brain tissue from other tissues in the biopsies was done under liquid N_2 by means of cooled forceps. The isolated brain tissue was placed in a 7-ml PVC tube containing liquid N_2. As a control of the separation procedure, a small piece of each sample was allowed to thaw and then identified by colour, consistency and macroscopic structure. The weighing procedure was as follows: The N_2 was poured out of the PVC tube and the tube containing the sample was placed on the balance (Mettler HE 20). After 15 s the weight was read and the tissue immediately placed in another tube with liquid N_2. The empty tube was weighed, the difference between the two readings giving the tissue weight. In preliminary experiments, this procedure followed by thawing and reweighing gave identical values. The tissue was then ground to powder in a mortar

under liquid N_2 and homogenized in a Virtis blender (Gardiner, N.Y., U.S.A.) for 1 min at a speed of 45,000 rev./min, adding seven times the tissue weight of 1 M-perchloric acid at 0°C. The homogenate was centrifuged for 20 min at 20,000 g, at 0°C and 1·4 M-KOH–tris was added to the supernatant fluid to a final pH of 6·5. The lactate concentration of this extract was determined by the enzymic method described by HOHORST (1970), with reagents supplied by Boehringer and Sons. The measurements were made at 340 nm on a Beckman DB-GT spectrophometer, internal lactate standards being employed.

TABLE 1.—THE CONCENTRATION OF LACTATE IN BRAIN TISSUE FROM THE RAT

Group	Arterial blood pH	PaO_2	$PaCO_2$	Lactate concentration in tissue sample	
Control	7·36	440	40·2	0·702	
	7·45	220	36·5	1·447	
	7·36	260	42·3	1·622	1·08 ± 0·17
	7·39	210	41·2	0·775	
	7·36	410	36·5	1·306	
	7·38	410	35·0	0·633	
Electroshock	7·45	180	36·5	5·832	
	7·35	210	40·1	6·897	
	7·42	400	40·2	7·753	6·65 ± 0·55
	7·35	140	42·3	7·826	
	7·36	365	36·5	4·967	

The acid–base parameters measured before tissue sampling are shown in the first three columns. PCO_2 and PO_2 are expressed as mm Hg. The concentrations of lactate are expressed as μmol/g brain tissue wet wt. The mean values ± S.E.M. are given. Measurements were made 5 s after delivery of electroshock (0·9 s).

RESULTS

The results are given in Table 1. The steady state lactate in brain tissue of unanaesthetized, immobilized, ventilated rats averaged 1·08 μmol/g wet wt. Within the first 5 s of the seizure period, the lactate concentration rose to an average of 6·65 μmol/g wet wt. Using the non-parametric Mann–Whitney test, the difference between controls and electroshock animals was statistically significant, $P<0·01$.

DISCUSSION

The brain lactic acid levels presented in the literature vary considerably. A possible explanation of the reported differences may be found in the variability of sampling methods. A number of reports are based on estimations of lactate after decapitation of the animals, followed by immersion of the heads in liquid N_2. This procedure, used for instance in a 300 g rat, creates a situation of total ischaemia for at least 30 s, where the only source of new energy is the anaerobic production of lactic acid. Thus, values as high as 2–3 μmol/g wet wt. have been measured using immersion techniques (KLEIN and OLSEN, 1947; GURDJIAN *et al.*, 1947). With the quick-freezing technique of plunging the animals into Freon-12 and liquid N_2 introduced by LOWRY *et al.*, (1964) in mice, GATFIELD *et al.* (1966) measured lactate concentrations of 1·50 μmol/g wey wt. in the cortex of unanaesthetized animals, and similar results (1·22 μmol/g wet wt.) were obtained by FOLBERGROVÁ *et al.* (1972), using the freezing front method described by KERR (1935), and further developed by PONTÉN (1966). With the 'freeze-blowing' technique, a lactate concentration as low as 1·23 μmol/g wet wt. has been obtained by VEECH *et al.* (1973). Using the present technique, which is currently the most rapid freeze-sampling method, we found a steady state lactate concentration of 1·08 μmol/g wet wt. in brain tissue of unanaesthetized rats.

In the experimental arrangement described here, the lactate levels cannot be considered the result of artefacts, and the present investigation therefore indicates a maximal lactate production in the rat brain of 5·57 μmol/g wet wt. during the first 5 s of the seizure periods. Recent investigations, in which the freezing front method was employed, have yielded similar results in rats given fluorothyl-induced

seizures (DUFFY *et al.*, 1973). Cerebral lactate acidosis will be associated with a lowering of the pH in the tissue round the arterioles, a factor of major importance in the regulation of cerebral blood flow (SKINHØJ, 1966; INGVAR *et al.*, 1968). Together with the well-known increase in arterial blood pressure during seizures, a cerebral vasodilation may well explain cerebral hyperaemia and the results of the present investigation thus lend support to the metabolic theory as part of the mechanism of functional cerebral hyperaemia during seizures. Our lactate values were similar to those measured by KING *et al.* (1967) using the freezing technique 6 s after electroshock stimulus in unventilated, convulsing mice, which indicates that controlled oxygen breathing during seizures does not prevent production of excess lactate in this situation of the most intense metabolic demand.

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Abbreviation used: $P_a CO_2$, arterial carbon dioxide tension.

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