THE ACTIVITY OF PYRUVATE DEHYDROGENASE IN RAT BRAIN DURING POSTNATAL DEVELOPMENT

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1. Introduction

Hawkins et al. [1] were the first to demonstrate, by arterio–venous blood difference measurements, that the brains of suckling rats show a net uptake of glucose, acetoacetate and D-β-hydroxybutyrate and a net output of lactate. In fed adult rats net uptake by the brain of glucose only occurs and there is a negligible net output of lactate [1, 2]. Furthermore, the amount of glucose utilized by infant rat brain (expressed as μmol/min per g fresh wt.) is considerably less than the amount metabolized by adult rat brain [3]. Changes in the rates of utilization of glucose and ketone-bodies by the brain during development are likely to be a consequence of changes in activities of certain key enzymes. There have been several investigations of the three main enzymes involved in the conversion of D-β-hydroxybutyrate and acetoacetate to acetyl-CoA in rat brain from birth to adulthood [4–7]. There appears to be no such report on the key enzyme forming acetyl-CoA from glucose, namely pyruvate dehydrogenase and it is the purpose of the present paper to present the result of such a study.

2. Materials and methods

2.1. *Animals and tissue preparation*

Rats of the Porton strain were used. The day of birth was taken as day 0 and adult rats were 8 weeks of age. Animals 2 to 30 days old were killed by dropping them into Arcton 12 (dichlorofluoromethane) cooled with liquid N_2. The brains were dissected frozen, weighed and homogenized in 4 vol of 20 mM potassium phosphate buffer, pH 7.0, containing 40% (v/v) glycerol [8], using an Ultraturrax homogenizer. The supratentorial portion of adult rat brain was removed by the rapid blowing and freezing technique [9] and homogenized as just described.

2.2. *Enzyme assay*

Pyruvate dehydrogenase activity was measured by modifying previously described procedures [8, 10, 11]. Part of a freshly prepared brain homogenate was used immediately for assay of the enzyme and another portion was incubated with 10 mM $MgCl_2$ for 30 min at 37°C to fully activate the enzyme [8, 10]. The assays were carried out at 37°C in sealed conical flasks having centre-wells and shaken continuously. The complete assay mixture contained, in a volume of 1.0 ml in the flask, 48 mM phosphate buffer pH 8.0, 9 mM dithiothreitol, 6 mM NAD^+, 2 mM thiamine pyrophosphate, 0.1 mM coenzyme-A (CoA-SH), 2 mM $MgCl_2$, 125 μg lactate dehydrogenase, 5 μg phosphotransacetylase and brain homogenate. Omissions of CoA-SH and $MgCl_2$ were made where appropriate. Flasks were warmed for 2 min at 37°C, the reaction was started by the addition of 0.1 ml sodium [1–^{14}C]pyruvate (20 mM and 1.4 μCi per ml) and was stopped after a further 4 min incubation by the addition of 0.2 ml 6 N H_2SO_4. Hyamine hydroxide, 0.2 ml, was injected into the centre-well and the $^{14}CO_2$ was collected during a further incubation of 40 min. Contents of the centre-well were transferred to counting vials containing 10 ml Instagel

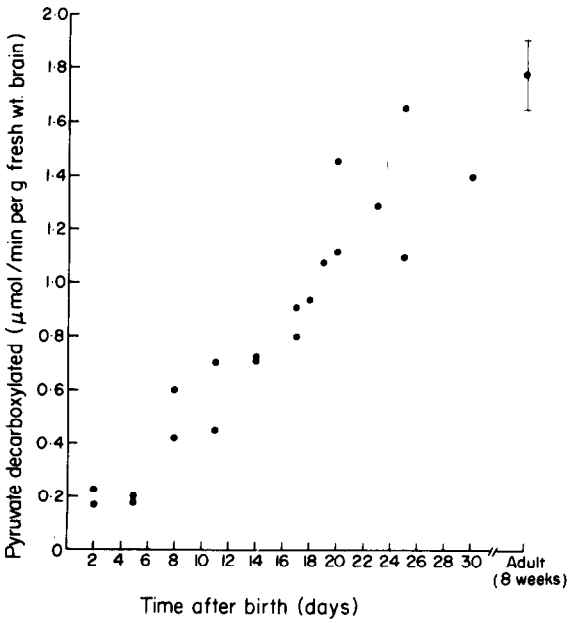

Fig. 1. Total activity of pyruvate dehydrogenase in rat brain. The preparation of the brain homogenates and the enzyme assay were as given in Materials and methods. Homogenates were incubated for 30 min at 37°C in the presence of 10 mM $MgCl_2$; CoA-SH was omitted from the medium of control flasks. All the values are for individual rats except the adult value which is the mean of 5 animals ± S.E.M. (vertical bars).

(Packard Instruments). Checks were made to ensure that the rate of reaction was linear with time and proportional to the amount of brain homogenate added.

2.3. *Chemicals and enzymes*

Thiamin pyrophosphate chloride, CoA-SH, and sodium pyruvate were obtained from Sigma Chemical Corp. NAD^+, lactate dehydrogenase and phosphotransacetylase were from Boehringer Mannheim and sodium [1–^{14}C]pyruvate (sp. radioactivity 13.1 mCi/mmol) was from The Radiochemical Centre, Amersham.

3. Results

Pyruvate dehydrogenase exists in an 'active' and an 'inactive' form in brain [12] as well as in other tissues. Conversion of the 'inactive', phosphorylated form to the 'active', dephosphorylated form occurs during incubation of tissue homogenates in 10 mM Mg^{2+} [8, 10]. Assessment of the portion of the two forms present in a tissue in situ can be made by comparing the activities of the enzyme in an homogenate freshly prepared from rapidly frozen tissue with an homogenate incubated with Mg^{2+}. The two forms of the enzyme have been measured in brain homogenates prepared from rats between the ages of 2 days and 8 weeks (fig. 1 and table 1). The rate of the fully activated enzyme increased abruptly between 8 and 25 days of age, which was in keeping with data on many other enzymes in developing rat brain [13–15]. The value for adult rats was about ten times greater than that for the 2 and 5 day-old animals when expressed per gramme of wet tissue (fig. 1). Since the water content of rat brain decreases during post-natal maturation and the protein content increases [16], the rate of the fully activated form of pyruvate dehydrogenase has also been expressed relative to protein weight (fig. 2). There is about a three-fold increase in the specific activity of the enzyme during maturation of the brain.

Table 1
Pyruvate dehydrogenase activity in fresh brain homogenates.

Age of rat (days)	Enzyme activity (μmol/min per g wet wt.) Without Mg^{2+}		With Mg^{2+}	
2	0.16	(80)	0.18	(90)
5	0.20	(100)	0.22	(110)
8	0.17	(35)	0.32	(63)
11	0.18	(30)	0.41	(68)
14	0.42	(58)	0.65	(90)
17	0.30	(35)	0.39	(45)
19	0.59	(54)	0.90	(82)
20	0.56	(43)	1.04	(80)
23	0.84	(65)	0.92	(71)
25	0.63	(48)	1.08	(83)
30	1.0	(73)	0.90	(65)
Adult (frozen)	0.62 ± 0.08	(35)	1.00 ± 0.06	(56)
Adult (room temp.)	1.27	(71)	–	

Fresh tissue homogenates from rapidly frozen brains were used. The assay medium was as described in Materials and methods except that in one set of flasks Mg^{2+} was omitted. The animals were the same as those used in fig. 1 except for two adult rats which were decapitated, the brains were left for 2 min at room temperature and were then homogenized. The numbers in parentheses are the percentages of the total enzyme activity given in fig. 1.

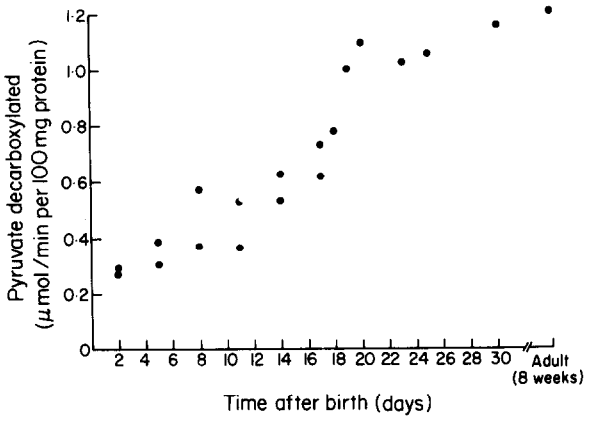

Fig. 2. Total activity of pyruvate dehydrogenase in rat brain. The homogenates were the same as those used in fig. 1.

Rapid freezing of tissue in situ is thought to prevent the post-mortem activation of pyruvate dehydrogenase by a phosphatase enzyme, and to give a measure of that portion of pyruvate dehydrogenase present in vivo in the 'active' form [8]. Since the phosphatase is activated by Mg^{2+} it is difficult to decide whether any Mg^{2+} should be added to the assay medium when measuring the 'active' form of pyruvate dehydrogenase in freshly prepared tissue homogenates. Therefore, in table 1 rates have been given for enzyme activity assayed in the absence and in the presence of 2 mM Mg^{2+}. Other authors have used concentrations of Mg^{2+} of 0.5 to 2 mM [8, 10, 11]. From the age of 8 days onwards the rate of the 'active' form of the enzyme was about 40% of that of the fully activated form and about 70% when assayed with 2 mM Mg^{2+} (table 1 and fig. 1).

Our value for the total activity of pyruvate dehydrogenase in adult rat brain is in agreement with that found by others [12]. When the brain was removed at room temperature the rate of the 'active' form was about double that of rapidly frozen brain tissue (table 1). These data provide further evidence that the enzyme exists in two forms in the brain in situ and that post-mortem activation can occur.

4. Discussion

The main purpose of this study was to compare the rate of pyruvate dehydrogenase activity, as determined in vitro, with the rate of glucose oxidation measured in vivo in young and adult rat brains. In the normal, conscious adult rat the rate of glucose utilization is about 0.65 μmol/min per g brain [2, 3, 17]. Since there is no net output of lactate from the brain [2] the pyruvate formed from glucose must be oxidized via pyruvate dehydrogenase at a rate of about 1.3 μmol/min per g brain. The rate of pyruvate dehydrogenase activity measured in rapidly frozen tissue in the absence of Mg^{2+} was only half this value but in the presence of Mg^{2+} the rate approached the in vivo value and the fully activated enzyme rate was somewhat in excess.

In suckling rats pyruvate oxidation in the brain competes with ketone-body oxidation. The rate of the latter is proportional to the concentration of ketone-bodies in the blood [1]. The activities of the three enzymes of the initial reactions of ketone-body metabolism, D-β-hydroxybutyrate dehydrogenase, 3-oxoacid CoA-transferase and acetoacetyl-CoA thiolase (mitochondrial) increase in the brain during the first 3 to 4 weeks after birth and then, following weaning the activities decrease [5, 7]. For each enzyme the activity in adult brain is several times less than that attained in the 3 to 4 weeks-old rat brain. This fall in enzyme activity after weaning is in complete contrast to the continued increase in activity of pyruvate dehydrogenase.

Both acetoacetyl-CoA thiolase and pyruvate dehydrogenase require CoA-SH to form acetyl-CoA; the K_M value for CoA-SH is similar for each enzyme in brain [7, 12, 18]. Within mitochondria there is competition between these two enzymes for a common substrate. The ratio of the maximal activities of the two enzymes in the brain changes with the age of the rat. For example in the 20 day-old rat the ratio of acetoacetyl-CoA thiolase to pyruvate dehydrogenase is approximately 4.5 whereas in the adult the ratio is about 1.2 (fig. 1 and ref. [7]). A similar change in the ratio with age also occurs for 3-oxoacid CoA-transferase [5] and pyruvate dehydrogenase. The higher ratios in the suckling rat brain seem to be a major contributory factor to the finding that, for a given con-

centration of acetoacetate in the blood, the young rat brain metabolizes about thee times more of this substrate than does the brain of the adult [1].

The total activity of pyruvate dehydrogenase in the brain is considerably less than that reported for the other enzymes of the glycolytic pathway, as measured in vitro [19]. It would seem that at all ages rat brain has a limited capacity to decarboxylate pyruvate. This is probably true also of brain of other species [18]. When ketone-bodies are utilized by the brain of either young suckling rats or fasted adults there is a net output of lactate from the brain to venous blood [1]. This implies that pyruvate dehydrogenase is functioning more slowly than preceding steps in the glycolytic pathway and, due to the high activity of lactate dehydrogenase [13], excess pyruvate produced in the brain is converted to lactate, which passes into the blood. The low activities found in vitro for both forms of pyruvate dehydrogenase in rat brain make it likely that this enzyme can be finely controlled in vivo and not only by the concentration of its substrate, pyruvate.

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