Short Communication

Pyruvate Carboxylase Activity in Primary Cultures of Astrocytes and Neurons

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Abstract: The activity of the pyruvate carboxylase was determined in brains of newborn and adult mice as well as primary cultures of astrocytes, of cerebral cortex neurons, and of cerebellar granule cells. The activity was found to be 0.25 ± 0.14, 1.24 ± 0.07, and 1.75 ± 0.13 nmol · min^{-1} · mg^{-1} protein in, respectively, neonatal brain, adult brain, and astrocytes. Neither of the two types of neurons showed any detectable enzyme activity (i.e., < 0.05 nmol · min^{-1} · mg^{-1}). It is therefore concluded that pyruvate carboxylase is an astrocytic enzyme. Key Words: Neurons—Astrocytes—Pyruvate carboxylase. Yu A. C. H. et al. Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. *J. Neurochem.* 41, 1484–1487 (1983).

The pyruvate carboxylase (EC 6.4.1.1) that catalyzes the formation of oxaloacetate from pyruvate, and thus leads to a net synthesis of a tricarboxylic acid (TCA) cycle constituent, is present in brain at a moderately high activity (Keech and Utter, 1963; Salganicoff and Koeppe, 1968; Ballard et al., 1970). Because the brain contains different metabolic compartments, which at least partly correspond to different cell types (e.g., Balazs et al., 1973), it is of importance to know whether the pyruvate carboxylase is restricted to certain cell types, and if so, to which cells. This question cannot be unequivocally answered from literature data. On the basis of studies of metabolic compartmentation, which showed that carbon dioxide fixation occurs in a "small," presumably glial, metabolic pool (Berl and Clarke, 1969; Balazs et al., 1973) it seems safe to conclude that the enzyme is present in glial cells. However, it might not be confined to these cells, as Cheng et al. (1978) found evidence for carbon dioxide fixation also in a "large," presumably neuronal, compartment. Immunohistochemical studies by Shank et al. (1981) have shown an astrocytic localization of the pyruvate carboxylase (and suggested that the enzyme may be confined to these cells), but Salganicoff and Koeppe (1968) have, on the other hand, reported that much of the pyruvate carboxylase activity in brain is found in synaptosomal fractions.

On this background, we found it worthwhile to study the localization of the pyruvate carboxylase in primary cultures of astrocytes or of neurons. Such cultures seem to constitute excellent models for their *in vivo* counterparts (astrocytes, see Hertz et al., 1982; neurons, see Materials and Methods). They were used to obtain quantitative measurements of the actual activity of the pyruvate carboxylase in astrocytes and in two different types of neurons, i.e., a cerebral cortical, mainly γ-aminobutyric acid (GABA)ergic neuronal population, and a population highly enriched in cerebellar granule cells, a presumably glutamatergic neuron.

MATERIALS AND METHODS

Cell cultures

Astrocytes. Cultures of astrocytes were prepared as described by Hertz et al. (1982). The parts of the cerebral hemispheres above the lateral ventricles were dissected out of the brains of newborn Swiss mice and, in general, grown for 2 weeks in tissue culture medium [modified Eagle's Minimum Essential Medium (MEM); Hertz et al. (1982)] with horse serum or newborn calf serum and subsequently for another week in the additional presence of 0.25 mM dibutyryl cyclic AMP, a procedure known to evoke a pronounced morphological differentiation of the cells.

Abbreviations used: Acetyl-CoA, Acetyl-coenzyme A; GABA, γ-Aminobutyric acid; GAD, Glutamic acid decarboxylase; MEM, Minimal essential medium; TCA cycle, Tricarboxylic acid cycle.

Cortical neurons. Cortical neurons were cultured from cerebral cortices of 15-day-old mouse embryos, in principle as described by Dichter (1978), but with several minor modifications. The meninges were removed and the hemispheres cut into small cubes and trypsinized for 2 min in 0.2% trypsin in Puck's solution at room temperature. After inhibition of the trypsin by addition of modified MEM with 20% horse serum, the tissue was triturated with a Pasteur pipette and centrifuged for 2 min at 900 × *g*. The pellet was resuspended in a similar medium without serum and with 30 (instead of 7.5) m*M* glucose. A cell suspension corresponding to one-and-one-half brains per dish was seeded in 60-mm Falcon plastic tissue culture dishes that had been coated with polylysine by exposure overnight to 12.5 μg/ml of polylysine in water. After 15 min of incubation at 37°C, unattached cells (nonneuronal cells) were removed together with the medium, which was replaced with a similar, fresh medium containing 5% horse serum. After 3 days of culturing, cytosine arabinoside was added to a final concentration of 40 μ*M*, which led to the disappearance of most astrocytes (Dichter, 1978). The cultures were refed 24 h later with fresh medium (with 5% serum) without the mitotic inhibitor and used for uptake experiments at day 14 without further change of medium. Such cultures appear to contain mainly GABAergic neurons and to reach maximum functional development [characterized by a high glutamic acid decarboxylase (GAD) activity, an intense GABA uptake, and a potassium-induced GABA release] after 11–14 days in culture (for references, see Hertz et al., 1983).

Cerebellar granule cells. These neurons were cultured from 7-day-old mice essentially as described by Messer (1977), i.e., following procedures that are in principle similar to those used in the culturing of the cortical neurons (Drejer et al., 1982). The cells were exposed to 0.1% trypsin for 10 min and centrifuged at 2000 × *g*. The modified MEM used during the culturing period contained, in addition to 30 m*M* glucose and 10% newborn calf serum, 24.5 m*M* KCl, 7 μ*M* *p*-aminobenzoic acid, and 100 mU/L insulin. The cells were treated with 5 μ*M* cytosine arabinoside after 4 days and kept in culture for 11–12 days. Similar cultures have been shown to consist of 80–90% granule cells (Currie, 1980) and they constitute in all likelihood a good model system for glutamatergic neurons, showing a relatively high glutamate uptake and a potassium-induced release of glutamate (Yu and Hertz, 1982; Drejer et al., 1982; Gallo et al., 1982).

Pyruvate carboxylase activity

The activity of the pyruvate carboxylase was determined essentially as described by Utter and Keech (1963), using ^{14}C-labeled bicarbonate as the substrate for formation of [^{14}C]oxaloacetic acid. The assay buffer contained a mixture of Tris-HCl (100 m*M;* pH 7.4), magnesium chloride (10 m*M*), pyruvate (20 m*M*), ATP (10 m*M*), acetyl-coenzyme A (acetyl-CoA) (100 μ*M*), and bicarbonate (60 m*M*). Blanks were run in the absence of acetyl-CoA, as pyruvate carboxylase activity is strictly dependent on the presence of this compound and 100 μ*M* acetyl CoA was chosen as the lowest concentration yielding maximum enzyme activity (Keech and Utter, 1963). After incubation of a sonicated cell or tissue preparation [prepared in a solution of Tris-HCl (100 m*M*, pH 7.4) and magnesium chloride (10 m*M*) and containing 1.0–1.5 mg protein/assay] for 5 min at 37°C, the reaction was stopped by addition of ice-cold trichloroacetic acid, and the mixture was subsequently neutralized by addition of sodium hydroxide. To allow liberation of excess bicarbonate by acidification without simultaneous decarboxylation of [^{14}C]oxaloacetic acid, the latter compound was converted to [^{14}C]malate by incubation for 10 min at 37°C in the presence of malate dehydrogenase and NADH. By use of these procedures the decarboxylation of oxaloacetate was negligible (2.9%). Thereafter, the samples were acidified and an excess of nonradioactive malate was added. The samples were allowed to stand at 37°C for 1 h, which led to the liberation of all excess bicarbonate as carbon dioxide. Remaining radioactivity [i.e., mainly malate but possibly also citrate formed by condensation of acetyl-CoA and oxaloacetate (Ballard and Hanson (1967)] in the samples was measured in a liquid scintillation spectrometer, and protein was determined using the conventional Lowry technique (Lowry et al., 1951).

Materials

Swiss mice were used both for preparation of cultures and to provide samples of fresh tissue. Plastic tissue culture flasks or dishes were obtained from NUNC A/S, Denmark or Falcon, Oxnard, CA; Nitex® nylon mesh from L. & S.H. Thompson, Montreal, P.Q., Canada; newborn calf serum from Gibco/Biocult, Scotland; and horse sera from selected horses; dibutyryl cyclic AMP, amino acids, poly-L-lysine, and vitamins from Sigma Chemical, St. Louis, MO; malate dehydrogenase, NADH, ATP, and acetyl-CoA either from Sigma Chemical or from Boehringer-Mannheim, F.R.G.; [^{14}C]-bicarbonate (specific radioactivity 0.1–8.4 mCi/mmol) from New England Nuclear, Boston, MA or Amersham, Oakville, Ontario, Canada. All other chemicals were of the purest grade available from regular commercial sources.

RESULTS AND DISCUSSION

In the adult mouse brain the pyruvate carboxylase activity was 1.2 nmol/min/mg protein (Table 1), a value that is comparable to that previously reported for rat brain homogenate by Salganicoff and Koeppe (1968) but somewhat lower than that observed in extracts from lyophilized homogenates of rabbit brain (Keech and Utter, 1963) or rat brain (Ballard et al., 1970). In the neonatal brain the activity was several-fold lower.

In 3-week-old cultures of astrocytes the pyruvate carboxylase activity was significantly (p < 0.01) higher than in the adult mouse brain (Table 1). Moreover, it was several times higher than the activity in the neonatal brain or in 1-week-old cultures, indicating that the enzyme develops in spite of the fact that the cultured cells have been removed from their natural environment. This is analogous to what has previously been observed for a number of other enzymes in cultured astrocytes (Schousboe et al., 1980). Contrary to this, pyruvate carboxylase could not be detected in neurons cultured from either cerebral cortex or cerebellum. It might be argued that the absence of any activity of the enzyme in the neurons might be explained by a deficiency in the biochemical development in culture. This is, however, unlikely, as the cultured cells develop a number of other differentiated features, characteristic of mature GABAergic or glutamatergic neurons

TABLE 1. *Activity of pyruvate carboxylase in homogenates of mouse brain and in primary cultures of astrocytes, cerebral cortex neurons, and cerebellar granule cells*

Tissue	Enzyme activity
Mouse brain, adult	1.24 ± 0.07 (4)
Mouse brain, newborn	0.25 ± 0.14 (3)
Astrocytes, 3 weeks old	1.75 ± 0.13 (10)
Astrocytes, 1 week old	0.14 (2)
Cerebral cortex neurons	Not detectable[a] (4)
Cerebellar granule cells	Not detectable[a] (5)

Homogenates were prepared in 100 mM Tris-HCl buffer (pH 7.4) and, following sonication, pyruvate carboxylase activity was determined as the rate of [^{14}C]bicarbonate incorporation into oxaloacetate in the presence of acetyl-CoA, corrected for the corresponding incorporation rate in the absence of acetyl-CoA. Results are expressed as nmol · min^{-1} · mg^{-1} protein ± SEM. The numbers of experiments, i.e., number of different brain or culture homogenates, are indicated in parentheses. In each experiment assays were made at least in duplicate. For further details, see Materials and Methods.

[a] Activity of the enzyme is <0.05 nmol · min^{-1} · mg^{-1}.

(see Materials and Methods). The present studies therefore strongly suggest that the pyruvate carboxylase is an exclusively glial enzyme. This conclusion is further supported by the observation (A. C. H. Yu, A. Bender, and L. Hertz, unpublished experiments) that in our hands synaptosomes show no pyruvate carboxylase activity, a finding that is in discordance with that of Salganicoff and Koeppe (1968), perhaps reflecting the fact that there is a considerable possibility for contamination of synaptosomal preparations with glial cells (Sieghart et al., 1978). The present findings are thus in agreement with the immunohistochemically determined astrocytic localization of the enzyme in cerebellum (Shank et al., 1981) and they support the conclusion from studies on metabolic compartmentation that carbon dioxide formation takes place primarily in the "small" (i.e., glial) compartment (Berl and Clark, 1969; Balazs et al., 1973). However, they give no evidence for the possible additional neuronal localization suggested by the finding by Cheng et al. (1978) of some carbon dioxide fixation in the "large," presumably neuronal, metabolic compartment. This discrepancy might indicate a less than perfect correlation between the different metabolic pools ("large" and "small") and specific cell types (neurons and glial cells).

The absence of pyruvate carboxylase activity in neurons is puzzling. Excited neurons suffer a substantial loss of TCA cycle constituents by release of GABA and, especially, glutamate (Hertz, 1979; Schousboe and Hertz, 1983). Studies of uptake rates for glutamate and glutamine in neurons and in astrocytes and of the actual metabolic fate of glutamate in astrocytes have shown that this deficit cannot be quantitatively compensated for by a transport of glutamine from astrocytes back to neurons (Hertz, 1979; Schousboe, 1981; Schousboe and Hertz, 1983). We have therefore previously suggested the possibility (Hertz, 1979; Hertz et al., 1980) that carbon dioxide fixation, if it occurred in neurons, might contribute to the replenishment of neuronal TCA cycle constituents. The absence of pyruvate carboxylase in neurons observed in the present study annuls this possibility. Other mechanisms such as a transfer of α-ketoglutarate from astrocytes to neurons should therefore be seriously considered (Shank and Campbell, 1981, 1982) and are at present under study using cultured neurons and astrocytes.

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