Competition among oxidizable substrates in brains of young and adult rats

Dissociated cells

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The rates of conversion of D-(–)-3-hydroxy[3-¹⁴C]butyrate, [3-¹⁴C]acetoacetate, [6-¹⁴C]glucose and [U-¹⁴C]glutamine into ¹⁴CO₂ were measured in the presence and absence of alternative oxidizable substrates in intact dissociated cells from the brains of young and adult rats. When unlabelled glutamine was added to [6-¹⁴C]glucose or unlabelled glucose was added to [U-¹⁴C]glutamine, the rate of ¹⁴CO₂ production was decreased in both young and adult rats. The rate of oxidation of 3-hydroxy[3-¹⁴C]butyrate was also decreased by the addition of unlabelled glutamine in both age groups, but in the reverse situation, i.e. unlabelled 3-hydroxybutyrate added to [U-¹⁴C]glutamine, only the brain cells from young rats were affected. No significant effects were seen when glutamine and acetoacetate were combined. The addition of either of the two ketone bodies to [6-¹⁴C]glucose markedly lowered the rate of ¹⁴CO₂ production in young rats, but in the adult only 3-hydroxybutyrate was effective and the magnitude of decrease in the rate of [6-¹⁴C]glucose oxidation was much lower than in young animals. Unlabelled glucose decreased the rate of [3-¹⁴C]acetoacetate oxidation to a minor extent in brain cells from both age groups; when added to 3-hydroxy[3-¹⁴C]butyrate, glucose had no effect in young rats and greatly enhanced ¹⁴CO₂ production in adult brain cells. Many of these patterns of substrate interaction in dissociated brain cells differ from those in whole homogenates; they may be a function of the plasma membranes and the role of a carrier-mediated transport system or a reflection of a difference in the population of cell types or subcellular organelles in these two preparations.

Studies on the metabolic characteristics of brain have led to the concept of compartmentation of metabolic activities (see Balazs & Cremer, 1973; Berl *et al.*, 1975). At least three compartments (glial cells, neuronal perikarya and nerve endings) have been recognized (Nicklas *et al.*, 1979), each of which has its own resident mitochondria to utilize a variety of substrates for energy production. We have examined the effects of adding alternative substrates on the rate of oxidation of several ¹⁴C-labelled compounds by whole homogenates with intact mitochondria (Roeder *et al.*, 1984). The results revealed a complex age-dependent pattern of interaction in which certain substrates apparently competed with each other, whereas enhanced rates of ¹⁴CO₂ production were found with others. Since we have also reported (Tildon *et al.*, 1983)

that the intact brain cell membrane may play a role in the regulation of alternative substrate utilization, the present series of studies evaluated the interactive effects of oxidizable substrates in preparations of intact, dissociated cells from brain of young and adult rats. The brain cells were obtained from the contralateral hemispheres of the brains of the same animals used for studies on substrate competition in brain whole homogenates (Roeder *et al.*, 1984). A preliminary report of these data has been presented (Roeder & Tildon, 1983).

Materials and methods

Materials and animals

Materials and sources of supply, and animals, were the same as those used in similar studies on whole homogenates of brain (Roeder *et al.*, 1984).

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Preparation of tissue

The brains were removed and placed in Petri dishes containing 0.9% NaCl. Two brain preparations were used as previously reported (Tildon *et al.*, 1983); one hemisphere was used to prepare a whole homogenate and the contralateral hemisphere was used to prepare dissociated brain cells by the following procedure, modified from the method of Wiesmann *et al.* (1975) and Siegrist *et al.* (1981). The tissue was minced into small (1 mm³) pieces, transferred to a 50 ml centrifuge tube, and incubated in 20 ml of 0.2% trypsin solution for 20 min at 37°C. The supernatant fraction was removed; the cells were washed three times with 0.9% NaCl, and were further dissociated by gentle trituration in a 10 ml wide-mouth pipette. The suspension was centrifuged at 100g for 5 min and the supernatant was discarded. The appropriate volume of 0.9% NaCl was added to the pelleted cells, large clumps were broken up, and the mixture was filtered through nylon screening cloth or lens paper (pore size 0.33 µm). Estimates of the integrity of cell membranes in these preparations were made on the basis of both exclusion of Trypan Blue dye and release of lactate dehydrogenase. Both tests indicated that 95% or more of the cells were intact. These freshly prepared cells were used immediately.

The volume of each preparation was adjusted for the appropriate range of protein concentration

(Roeder *et al.*, 1984). Protein contents were determined by the method of Lowry *et al.* (1951).

Oxidation of ¹⁴C-labelled substrates

The method used to measure ¹⁴CO₂ production from the labelled substrates was the same as that described by Roeder *et al.* (1984) except for the concentrations of sucrose (80 mM) and NaCl (103 mM) and the omission of NAD⁺ and ATP. These omissions do not affect the rates of oxidation by intact cells (Tildon *et al.*, 1983).

Statistical analyses

The data were analysed for statistical significance by Student's *t* test.

Results

Table 1 shows the rates of conversion of the various ¹⁴C-labelled substrates into ¹⁴CO₂ by dissociated brain cells from young and adult rats. In the youngest (4–6-day-old) animals, [6-¹⁴C]glucose showed the lowest rate of oxidation and [3-¹⁴C]acetoacetate the highest. Both [6-¹⁴C]glucose and [U-¹⁴C]glutamine oxidation rates increased with age, whereas those for the two ketone bodies {D-(–)-3-hydroxy[3-¹⁴C]butyrate and [3-¹⁴C]acetoacetate} decreased significantly between young animals and adults.

Table 2 shows the effect of adding unlabelled

Table 1. Rates of conversion of ¹⁴C-labelled substrates into ¹⁴CO₂ by dissociated cells from brains of young and adult rats

Results are means ± S.E.M. for the numbers of experiments shown in parentheses. Asterisks indicate significant differences from adult values (*P* ≤ 0.05).

Substrate	Substrate oxidized (nmol/h per mg of protein)		
	Young (4–6 days)	Young (16–20 days)	Adult (90 days or older)
[6- ¹⁴ C]Glucose (2 mM)	1.94 ± 0.12* (14)	2.86 ± 0.43* (5)	4.11 ± 0.27 (10)
[U- ¹⁴ C]Glutamine (2 mM)	7.58 ± 0.59* (11)	14.88 ± 1.80 (6)	13.62 ± 1.03 (11)
3-Hydroxy[3- ¹⁴ C]butyrate (1 mM)	8.31 ± 0.78* (11)		2.81 ± 0.41 (9)
[3- ¹⁴ C]Acetoacetate (1 mM)	15.26 ± 2.41* (10)		2.21 ± 0.62 (7)

Table 2. Effects of added unlabelled substrate on rates of ¹⁴CO₂ production from ¹⁴C-labelled glucose and glutamine in dissociated cells from brains of young and adult rats

Results are means ± S.E.M.; number of rats per group = 5–13 for controls and 5–9 for groups with added unlabelled substrate. Asterisks indicate statistically significant (*P* ≤ 0.05) differences from rates with labelled substrate alone.

		Change from rate of ¹⁴ CO ₂ production with labelled substrate alone (%)		
		Young		Adult
Labelled substrate	Unlabelled substrate	(4–6 days)	(16–20 days)	(90 days or older)
[6- ¹⁴ C]Glucose	Glutamine (1 mM)	–35 ± 4.6*	–24 ± 9.0*	–40 ± 10.4*
	(5 mM)	–44 ± 2.6*	–60 ± 3.1*	–63 ± 1.9*
[U- ¹⁴ C]Glutamine	Glucose (1 mM)	–35 ± 1.8*	–28 ± 7.8*	–32 ± 3.6*
	(5 mM)	–32 ± 3.6*	–34 ± 7.4*	–36 ± 1.5*

glutamine on the rates of oxidation of [6-¹⁴C]-glucose by dissociated brain cells from animals at three different ages. When unlabelled glutamine was added to these preparations there were marked decreases in the amounts of ¹⁴CO₂ produced, and the magnitude of effect was related to the concentration of added glutamine. Furthermore, the decreases in rates of oxidation were seen in all three age groups.

Table 2 also illustrates the effect of glucose on [U-¹⁴C]glutamine oxidation. The addition of unlabelled glucose significantly diminished the rate of glutamine oxidation in all age groups; however, there was little or no difference between 1 mM- and 5 mM-glucose.

The effect of adding ketone bodies to dissociated cells oxidizing [6-¹⁴C]glucose is shown in Table 3. When unlabelled 3-hydroxybutyrate or acetoacetate was added, there were marked decreases in the rates of ¹⁴CO₂ production by brain cells from

the young animals. In the adult, although there was a tendency for similar effects, the magnitude of change was considerably less.

When the effect of adding unlabelled glucose on the rates of oxidation of the two ketone bodies was examined, there were marked differences in the results, depending on the substrate, or the age of the animals. With 3-hydroxy[3-¹⁴C]butyrate as substrate, there was no effect of added glucose in young rats, whereas ¹⁴CO₂ production was enhanced in dissociated cells from adult animals. On the other hand, when acetoacetate was the labelled substrate, the addition of glucose diminished the rate of its oxidation in both young and adult rats, although the magnitude of the effects was not large.

The effects of glutamine on ¹⁴CO₂ produced from the two ketone bodies, 3-hydroxy[3-¹⁴C]butyrate and [3-¹⁴C]acetoacetate, are shown in Table 4. The addition of unlabelled glutamine caused a

Table 3. *Effects of added unlabelled substrate on rates of ¹⁴CO₂ production from ¹⁴C-labelled glucose and ketone bodies in dissociated cells from brains of young and adult rats*

Results are means $\pm$ S.E.M.; number of rats per group = 3-13 for controls and 3-7 for groups with added unlabelled substrate. Asterisks indicate statistically significant ($P \leq 0.05$) differences from rates with labelled substrate alone.

Labelled substrate	Unlabelled substrate	Change from rate of ¹⁴ CO ₂ production with labelled substrate alone (%)	
		Young (5-6 days)	Adult (90 days or older)
[6- ¹⁴ C]Glucose	3-Hydroxybutyrate (1 mM)	-45 $\pm$ 2.9*	-17 $\pm$ 1.0*
	(5 mM)	-59 $\pm$ 4.0*	-21 $\pm$ 3.1*
[6- ¹⁴ C]Glucose	Acetoacetate (1 mM)	-40 $\pm$ 6.1*	-18 $\pm$ 6.8
	(5 mM)	-42 $\pm$ 4.0*	-8 $\pm$ 2.7*
3-Hydroxy[3- ¹⁴ C]butyrate	Glucose (1 mM)	-9 $\pm$ 5.1*	+39 $\pm$ 22.0
	(5 mM)	-9 $\pm$ 5.2	+73 $\pm$ 22.1*
[3- ¹⁴ C]Acetoacetate	Glucose (1 mM)	-17 $\pm$ 4.1*	-18 $\pm$ 0.3*
	(5 mM)	-16 $\pm$ 3.0*	-8 $\pm$ 8.0

Table 4. *Effect of added unlabelled substrate on rates of ¹⁴CO₂ production from ¹⁴C-labelled ketone bodies and glutamine in dissociated cells from brains of young and adult rats*

Results are means $\pm$ S.E.M.; number of rats per group = 6-13 for controls and 3-7 for groups with added unlabelled substrate (except for glutamine added to [3-¹⁴C]acetoacetate in adults, where $n = 2$). Asterisks indicate statistically significant ($P \leq 0.05$) differences from rates with labelled substrates alone.

Labelled substrate	Unlabelled substrate	Change from rate of ¹⁴ CO ₂ production with labelled substrate alone (%)	
		Young (4-6 days)	Adult (90 days or older)
3-Hydroxy[3- ¹⁴ C]butyrate (1 mM)	Glutamine (1 mM)	-14 $\pm$ 2*	-19 $\pm$ 7*
	(5 mM)	-17 $\pm$ 3*	-33 $\pm$ 6*
[3- ¹⁴ C]Acetoacetate (1 mM)	Glutamine (1 mM)	0 $\pm$ 8	-5 (+4, -14)
	(5 mM)	+12 $\pm$ 9	0 (-2, +2)
[U- ¹⁴ C]Glutamine (2 mM)	3-Hydroxybutyrate (1 mM)	-29 $\pm$ 3*	-2 $\pm$ 4
	(5 mM)	-26 $\pm$ 4*	-6 $\pm$ 6
[U- ¹⁴ C]Glutamine (2 mM)	Acetoacetate (1 mM)	-12 $\pm$ 4	0 $\pm$ 13
	(5 mM)	-4 $\pm$ 4	+5 $\pm$ 2

moderate, but significant, decrease in the rate of oxidation of 3-hydroxy[3- 14 C]butyrate in brain cells from both young and adult rats; however, when added to [3- 14 C]acetoacetate, glutamine had no significant effect in either age group. Conversely, when ketone bodies were added to [U- 14 C]glutamine, the only significant effect observed was the decreased 14 CO $_2$ production when 3-hydroxybutyrate was added to brain cells from young animals. Acetoacetate did not alter glutamine oxidation in young rats, and adult animals showed no response to either compound.

Discussion

The rationale for comparing rates of oxidation in intact dissociated brain cells with those in whole homogenates of brain has been documented (Tildon *et al.*, 1983). In extending those results, we have examined the interactions of alternative oxidizable substrates in these two brain preparations. The results in the present paper for dissociated cells show significant differences from those found in whole homogenates (Roeder *et al.*, 1984).

Glutamine and glucose

Glutamine effectively competed with glucose in both whole homogenates (Roeder *et al.*, 1984) and dissociated cells of brain. The addition of glucose decreased the rate of glutamine oxidation in the intact cells, but not in homogenates. The difference in the effect of glucose on glutamine oxidation between the two preparations may reflect a difference in the concentration of glycolytic enzymes, which become diluted in the homogenate.

Although it seems unlikely, it cannot be ruled out that this difference may reflect the difference in the rate of glutamine oxidation by these two preparations; the high rate of glutamine oxidation by homogenates may mask the effects of added unlabelled glucose.

Ketone bodies and glucose

The marked difference in the rates of oxidation of ketone bodies in the absence of added alternative substrates between whole homogenates (Roeder *et al.*, 1984) and intact cells agrees with data reported previously (Tildon *et al.*, 1983). Nevertheless, most of the patterns of interaction between ketone bodies and glucose in dissociated brain cells were very similar to those in homogenates (Roeder *et al.*, 1984). The exception was that, although glucose enhanced the rate of oxidation of both ketone bodies in homogenates from adult rats, an opposite effect (a small decrease) was seen for [3- 14 C]acetoacetate oxidation in the dissociated cells. In other tissue

preparations, such as brain slices (Itoh & Quastel, 1970; Patel & Owen, 1977), the oxidation of both ketone bodies was enhanced by glucose when adult animals were used.

Glutamine and ketone bodies

In a study examining the effects of multiple substrates on oxygen consumption, Lai *et al.* (1977) observed that the addition of 3-hydroxybutyrate stimulated oxygen consumption over that with malate alone in synaptosomal, but not in non-synaptosomal, mitochondria. In our present studies we also found an apparent compartmentation of the interaction of 3-hydroxybutyrate with another oxidizable substrate, in this case glutamine; 3-hydroxybutyrate enhanced the production of 14 CO $_2$ from glutamine in homogenates but not in dissociated cells from young rat brain. Nerve endings may be lost during the preparation of dissociated cells, as reported for similar preparations by Wilkin *et al.* (1976). If so, our findings suggest that the proposed (Roeder *et al.*, 1984) regulation of glutamine oxidation by ketone bodies via depletion of succinyl-CoA and resultant release of inhibition of α -oxoglutarate dehydrogenase activity should occur in synaptosomal and not in free mitochondria. Supporting this proposal is a report that the activity of one of the ketone-body-metabolizing enzymes, 3-oxo-acid CoA-transferase, was considerably higher (about 2-fold) in nerve endings than in free mitochondria from young rat brain (DeVivo *et al.*, 1976). Collectively, these findings also suggest an age-dependent shift in the compartmentation of substrate interaction in the brain. The difference in CoA transferase activity between synaptosomes and free mitochondria reported by DeVivo *et al.* (1976) was found only in young animals, and the synergistic action between 3-hydroxybutyrate and glutamine in our studies was seen in homogenates from young, but not from adult, rats.

Taken as a whole, the results of the present study and that on whole homogenates (Roeder *et al.*, 1984) demonstrate several differences between homogenates and dissociated cells in the effects of alternative substrates on the rates of oxidation of several compounds. These differences may be a function of the plasma membrane and the role of a carrier-mediated transport system, or they may reflect a difference in the population of cell types or subcellular organelles in these two preparations.

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