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Turnover of succinyl-CoA:3-oxoacid CoA-transferase in glioma and neuroblastoma cells

Specific influence of acetoacetate in neuroblastoma cells

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The specific activity of succinyl-CoA:3-oxo-acid CoA-transferase (3-oxoacid CoA-transferase, EC 2.8.3.5) increases significantly during growth in culture in both mouse neuroblastoma N2a and rat glioma C6 cells. To investigate the mechanism(s) responsible for this, antibody specific for rat brain 3-oxoacid CoA-transferase was raised in rabbits. Immunotitrations of 3-oxoacid CoA-transferase from neuroblastoma and glioma cells on days 3 and 7 of growth after subculture showed that the ratio of 3-oxoacid CoA-transferase activity to immunoprecipitable enzyme protein remained constant, indicating that differences in specific activity of the enzyme at these times in both cell types reflect differences in concentration of enzyme protein. In glioma cells, the relative rate of 3-oxoacid CoA-transferase synthesis was about 0.04–0.05% throughout 9 days in culture. In contrast, the relative rate of synthesis of 3-oxoacid CoA-transferase in neuroblastoma cells was about 0.07–0.08% on days 3, 5 and 7 after subculture, but fell to 0.052% on day 9. The degradation rates of total cellular protein ($t_{\frac{1}{2}} = 28$ h) and 3-oxoacid CoA-transferase ($t_{\frac{1}{2}} = 46$–50 h) were similar in both cell lines. The rise in specific activity of the enzyme in both cell lines from days 3 to 7 without a significant increase in the relative rate of synthesis reflects a slow approach to steady-state conditions for the enzyme secondary to its slow degradation. Differences in 3-oxoacid CoA-transferase specific activity between the two cell lines are apparently due to a difference of about 60% in relative rates of enzyme synthesis. The presence of 0.5 mM-acetoacetate in the medium significantly increased the specific activity of 3-oxoacid CoA-transferase in neuroblastoma cells during the early exponential growth phase. This treatment increased the relative rate of synthesis of 3-oxoacid CoA-transferase by 23% ($P < 0.025$) in these cells on day 3, suggesting that substrate-mediated induction of enzyme synthesis is a mechanism of regulation of 3-oxoacid CoA-transferase.

Acetoacetate and D-3-hydroxybutyrate are important substrates for cerebral metabolism in the developing rat (for reviews see Robinson & Williamson, 1980; Sokoloff *et al.*, 1977). Compared with adult rats, suckling rats demonstrate higher rates of both cerebral uptake (Moore *et al.*, 1976) and oxidation (Hawkins *et al.*, 1971) of ketone bodies. Activities of ketone-body-metabolizing enzymes are elevated 3–5-fold in brains of suckling rats (Page *et al.*, 1971; Tildon *et al.*, 1971). The post-weaning decline in the activity of these enzymes is unique among mitochondrial oxidative enzymes of rat brain. An apparent association between the circulating concentrations of ketone bodies and the pre- and post-natal development of ketone-body-metabolizing enzymes in rat brain has emerged from several studies. The mechanism(s) responsible for these alterations in enzyme activity are not delineated. In a previous study we documented the specific differences which exist in the capacity of two established cell lines, namely neuroblastoma C1300 (N2a) and glioma (C6) cells, to metabolize ketone bodies (Patel *et al.*, 1981). The specific activity of succinyl-CoA:3-oxoacid

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CoA-transferase (3-oxoacid CoA-transferase, EC 2.8.3.5) in both cell lines increased markedly during the exponential growth phase, and was approx. 2-fold higher in neuroblastoma cells than in glioma cells. Also, the addition of acetoacetate, but not DL-3-hydroxybutyrate, increased the specific activity of 3-oxoacid CoA-transferase in neuroblastoma cells, but not in glioma cells. In the present study, we describe differences in the turnover rates of 3-oxoacid CoA-transferase in these two neural cell lines during growth after subculture and the influence of acetoacetate on the relative rate of synthesis of the enzyme in neuroblastoma cells. A preliminary report of this work has been presented (Patel *et al.*, 1984).

Materials and methods

Materials

Eagle's minimal essential medium containing 4-fold increased concentrations of amino acids and vitamins, Eagle's minimum essential medium minus methionine and foetal-calf serum were purchased from Gibco Laboratories (Grand Island, NY, U.S.A.). Acetoacetyl-CoA (lithium salt) was obtained from P-L Biochemicals (Milwaukee, WI, U.S.A.). L-[^{35}S]Methionine was purchased from New England Nuclear (Boston, MA, U.S.A.). Sodium acetoacetate, prepared from ethyl acetoacetate (Krebs *et al.*, 1966), was kindly supplied by Dr. George Reichard, Jr., of Lankenau Hospital (Philadelphia, PA, U.S.A.). All other reagents were of the highest purity commercially available.

Cell culture

Rat glioma C6 and mouse neuroblastoma C1300 (N2a) clones, which were obtained from Dr. Beatrice Garber of the University of Chicago (Chicago, IL, U.S.A.), were maintained in Eagle's minimal essential medium with 4-fold increased concentrations of amino acids and vitamins. The concentration of L-methionine was 0.4mM. The medium was supplemented with 2mM-glutamine, 5mM-glucose, Earle's salts, 10% (v/v) foetal-calf serum, and (per ml of medium) the following: 2.2mg of $NaHCO_3$, 8μg of calcium pantothenate, 100 units of penicillin G and 100μg of streptomycin (complete medium) (Freytag & Utter, 1980; Patel *et al.*, 1981). The glioma C6 and neuroblastoma C1300 (N2a) cells were routinely subcultured at 1×10^5 and 1.5×10^5 cells per dish (area 21cm^2) respectively, on day 7 of the previous culture, and were maintained in a water-jacketed CO_2/air incubator at 37°C (Patel *et al.*, 1981). Both cell lines achieved confluence on day 7 after subculture. To harvest cells, cultures were washed three times with phosphate-buffered saline (Dulbecco & Vogt, 1954), scraped with a rubber policeman in the same buffer, centrifuged, and washed once again before resuspension in the same buffer. Cell suspensions were treated with Triton X-100 (final concn. 1.67%, v/v), and 3-oxoacid CoA-transferase activity (Williamson *et al.*, 1971) and protein (Lowry *et al.*, 1951) were determined in the solubilized extracts. One unit of enzyme activity is defined as 1μmol of acetoacetyl-CoA deacylated/min at 37°C.

Purification of 3-oxoacid CoA-transferase and generation of specific antiserum

3-Oxoacid CoA-transferase was purified to homogeneity by a previously published procedure (Russell & Patel, 1982), with one modification. The starting material was an extract of frozen rat brain rather than a crude mitochondrial fraction. To generate antiserum, New Zealand White rabbits received multiple intradermal injections (Vaitukaitis, 1981) of 100μg of purified enzyme (per rabbit) in Freund's complete adjuvant. Subsequently, intramuscular injections of 50μg of purified enzyme in Freund's incomplete adjuvant were administered periodically to enhance antibody production.

Immunological studies

The washed cells were suspended in 125μl of immunoassay buffer (10mM-potassium phosphate/140mM-sodium phosphate, pH7.4) plus 25μl of 10% Triton X-100 and stored at 4°C for 1h. The extract was then frozen and thawed three times and centrifuged at 12000*g* for 10min at 4°C. 3-Oxoacid CoA-transferase activity (300munits, corresponding to 2μg of enzyme) was immunoprecipitated from the supernatant by using 50μl of rabbit antiserum specific for the rat brain enzyme. Non-radioactive mitochondrial extract of rat brain containing 3-oxoacid CoA-transferase was added to cellular extracts when necessary so that 300munits of enzyme could be precipitated. Quantitative precipitation was confirmed by the absence of enzyme activity in the supernatant after immunoprecipitation. Immunoprecipitates were washed three times with buffer containing 20mM-sodium phosphate, 0.5% Triton X-405 and 0.9% NaCl at pH7.4, with the addition of 10mM unlabelled L-methionine when the cells were labelled with L-[^{35}S]methionine. The washed immunoprecipitates were dissociated for 10min in 2% (v/v) sodium dodecyl sulphate/0.2M-dithiothreitol at 100°C and subjected to gel electrophoresis on a 9%-polyacrylamide slab gel containing 1% sodium dodecyl sulphate and a 6%-polyacrylamide stacking gel. Gels were stained in 0.25% Coomassie Brilliant Blue R-250 and destained by gentle shaking in 50% (v/v) methanol with three changes

of the solution 30min apart and then with 5% methanol plus 10% acetic acid overnight. The gels were sliced, and after digestion the acrylamide in H_2O_2, the radioactivity in the 3-oxoacid CoA-transferase band was determined in a liquid-scintillation spectrometer.

Antibody characterization

The immunoreactivity of rat glioma C6 and mouse neuroblastoma C1300 (N2a) 3-oxoacid CoA-transferase was quantified by adding increasing amounts of the antiserum to a constant amount of 3-oxoacid CoA-transferase from extracts of glioma and neuroblastoma cells. The mixtures were incubated at 37°C for 1h and then at 4°C overnight. After centrifugation, enzyme activity in the supernatant fractions was measured; 75munits of 3-oxoacid CoA-transferase from both glioma and neuroblastoma cells were quantitatively precipitated by 5μl of antiserum (results not shown). In the experiments reported here, 50μl of antiserum was added per 300munits of enzyme activity. The quantitative nature of the immunoprecipitation of 3-oxoacid CoA-transferase from each cell type was confirmed in a separate experiment. 3-Oxoacid CoA-transferase activity (300munits) in solubilized extracts of cells incubated in medium containing L-[^{35}S]methionine for 24h was immunoprecipitated ($AgAb_1$). To demonstrate that all of the 3-oxoacid CoA-transferase was immunoprecipitated, a second immunoprecipitation ($AbAg_2$) was performed; 300munits of unlabelled 3-oxoacid CoA-transferase contained in a mitochondrial extract of rat brain and an equivalent amount of antiserum were added to the supernatant of the first immunoprecipitate. Immunoprecipitates were subjected to electrophoresis as described above. Each lane was cut into 1.5mm slices and radioactivity contained in each slice was determined.

Relative rates of synthesis of 3-oxoacid CoA-transferase

Cells were labelled in 2ml of methionine-deficient complete medium containing 20μM-L-[^{35}S]-methionine (50μCi) for 2h. The labelling was carried out on days 3, 5, 7 and 9 after subculture of both glioma and neuroblastoma cells. These cells were washed with phosphate-buffered saline containing 10mM-L-methionine (unlabelled) and prepared for immunoprecipitation as described above. Immunoprecipitates were washed twice with the immunoprecipitation wash buffer containing 10mM-L-methionine (unlabelled). The third wash was carried out with the wash buffer devoid of NaCl and L-methionine. The washed immunoprecipitates were subjected to slab-gel electrophoresis, and the radioactivity in the 3-oxoacid CoA-transferase band was determined. To measure the amount of L-[^{35}S]methionine incorporated into total cellular protein, protein was precipitated with trichloroacetic acid from samples of uncentrifuged Triton-X-100-solubilized cells (Mans & Novelli, 1961).

Degradation of 3-oxoacid CoA-transferase

Glioma and neuroblastoma cells were grown in complete medium for 4 days after subculture. On day 5 of subculture the cells were incubated in 2ml of L-methionine-deficient medium containing 20μM-L-[^{35}S]methionine (30μCi). After 24h, the cells were washed, at 37°C, with 5ml of sterile phosphate-buffered saline containing 10mM-methionine (unlabelled). Complete medium (5ml) containing 5mM-L-methionine (unlabelled) was then added. At 6h later (zero time), the medium was replaced with medium of the same composition, to minimize reutilization of labelled L-methionine. Medium in the remaining dishes was replaced every 24h. At zero time and as noted thereafter, the cells were harvested and washed, the enzyme was immunoprecipitated, and radioactivity in 3-oxoacid CoA-transferase and total cellular protein was determined as described above.

Results and discussion

Postnatal changes in 3-oxoacid CoA-transferase activity in rat brain during normal development reflect changes in the relative rate of synthesis of the enzyme (Haney & Patel, 1983). However, studies *in vivo* are complicated by the heterogeneous nature of the brain, which contains numerous cell types undergoing developmental changes at different times. Since glioma and neuroblastoma cells represent specific cell types, they provide a simplified system for studying the regulation of 3-oxoacid CoA-transferase.

The first step in the analysis of changes in enzyme activity is to determine whether these changes are due to increased amounts of enzyme protein or to activation of existing enzyme by specific modifications. A prerequisite for this experiment is the availability of specific antibody. Therefore, 3-oxoacid CoA-transferase was purified from rat brain to a specific activity of 150 units/mg of protein. The purified enzyme appeared homogeneous on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, as reported previously (Russell & Patel, 1982). The rabbit antisera quantitatively precipitated 3-oxoacid CoA-transferase from extracts of both glioma and neuroblastoma cells which had been radiolabelled with L-[^{35}S]methionine ($AgAb_1$) (Fig. 1). Subsequently, reprecipitation from the supernatant of the $AgAb_1$ after addition of an unlabelled mitochondrial

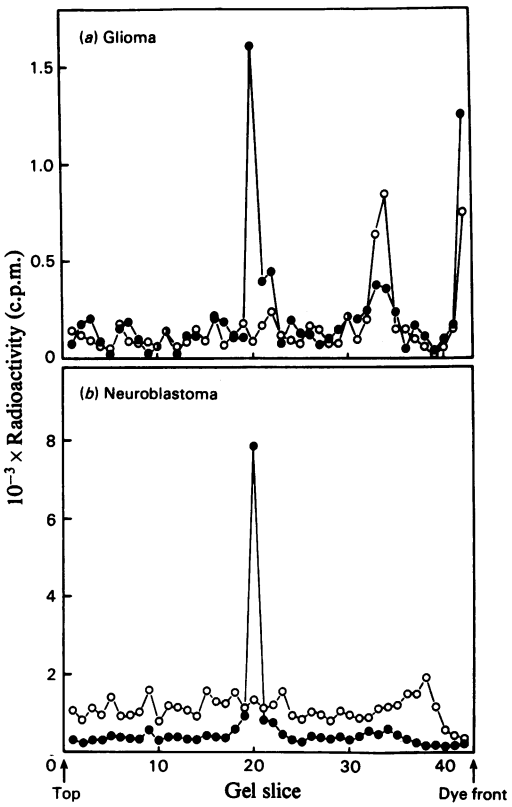

Fig. 1. *Distribution of radioactivity in sodium dodecyl sulphate/polyacrylamide gels of immunoprecipitates of 3-oxoacid CoA-transferase from glioma (a) and neuroblastoma (b) cells*

Extracts of cells radiolabelled with L-[^{35}S]-methionine were incubated with antibody and the immunoprecipitates ($AgAb_1$) were isolated by centrifugation and washed as described in the Materials and methods section. To the supernatant of this immunoprecipitate ($AgAb_1$) unlabelled rat brain mitochondrial extract containing an identical amount of 3-oxoacid CoA-transferase was added, and a second immunoprecipitation ($AgAb_2$) was carried out. Immunoprecipitates were subjected to gel electrophoresis, the gels were sliced, and radioactivity in each slice of the $AgAb_1$ (●) and the $AgAb_2$ (○) was determined.

extract of rat brain, containing the same amount of 3-oxoacid CoA-transferase activity ($AgAb_2$), was performed. The $AgAb_1$ of neuroblastoma cells consisted of only one major radioactive component, which corresponded to 3-oxoacid CoA-transferase. The absence of radioactivity in the $AgAb_2$ confirmed that the initial immunoprecipitation was quantitative (Fig. 1*b*). For glioma cells, the $AgAb_1$ contained two major radioactive components, one of which corresponded to 3-oxoacid CoA-transferase (Fig. 1*a*). The presence of the second radioactive component in the $AgAb_2$ demonstrated that it represented non-specific precipitation. The absence of radioactivity corresponding to 3-oxoacid CoA-transferase in the $AgAb_2$ showed that the enzyme precipitated specifically and quantitatively.

To examine whether changes in 3-oxoacid CoA-transferase specific activity during growth in culture are due to changes in the specific catalytic activity of a constant concentration of enzyme or to actual changes in enzyme protein concentration, increasing amounts of extracts from glioma and neuroblastoma cells on days 3 and 7 were incubated with a constant amount of antiserum, and enzyme remaining in the supernatant after immunoprecipitation was assayed. The virtually identical equivalence points for each cell type on days 3 and 7, when differences in 3-oxoacid CoA-transferase specific activity were maximal, indicate that the ratio of catalytic activity to immunological reactivity was unchanged (Fig. 2). This demonstrated that the observed differences in specific activity of 3-oxoacid CoA-transferase were due to changes in concentration of enzyme protein. The similarity in equivalence points between immunotitrations of enzyme from glioma cells, derived from rat, and neuroblastoma cells, derived from mouse, indicates that the activity of the antiserum for enzyme from both cell types is similar.

Since differences in 3-oxoacid CoA-transferase activity are due solely to differences in the amount of enzyme protein, we examined the roles of synthesis and degradation in modulating the activity of the enzyme. In experiments concerning the relative rate of synthesis of the enzyme, both cell lines reached their peak enzyme specific activity by day 7 (Fig. 3*b*). However, the increase in the specific activity of the enzyme in glioma cells from days 3 to 7 was less striking in the present study than in our previous report (Patel *et al.*, 1981). The incorporation of [^{35}S]methionine into 3-oxoacid CoA-transferase (Fig. 3*c*) and total cellular protein (Fig. 3*d*) during a 2h labelling period increased steadily in glioma cells during the experimental period, whereas a decline in these values was observed in neuroblastoma cells after 5 and 7 days respectively. The relative rate of enzyme synthesis remained essentially unchanged in glioma cells (Fig. 3*e*), whereas a 37% decrease was observed in neuroblastoma cells between days 7 and 9. The mean relative rate of enzyme synthesis during the first 7 days was approx. 60% higher in neuroblastoma cells than in glioma cells, which corresponds to the difference in maximal 3-oxoacid CoA-transferase specific activity between the two cell lines.

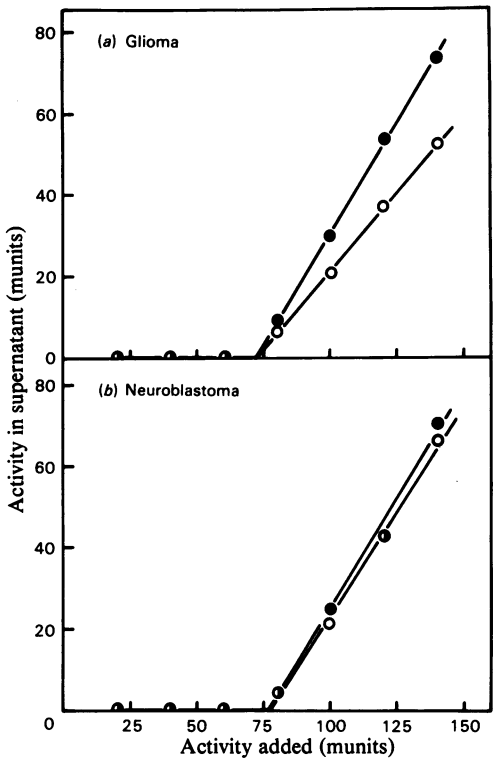

Fig. 2. *Immunotitrations of 3-oxoacid CoA-transferase from glioma and neuroblastoma cells on days 3 (○) and 7 (●) of growth in culture*

Cellular extracts containing different amounts of 3-oxoacid CoA-transferase activity were incubated with 5 μl of antiserum and enzyme activity remaining in the supernatant after immunoprecipitation was determined. The specific activity of the enzyme (munits/mg of protein) on days 3 and 7, respectively, was in glioma cells, 106 and 132, and in neuroblastoma cells, 138 and 198.

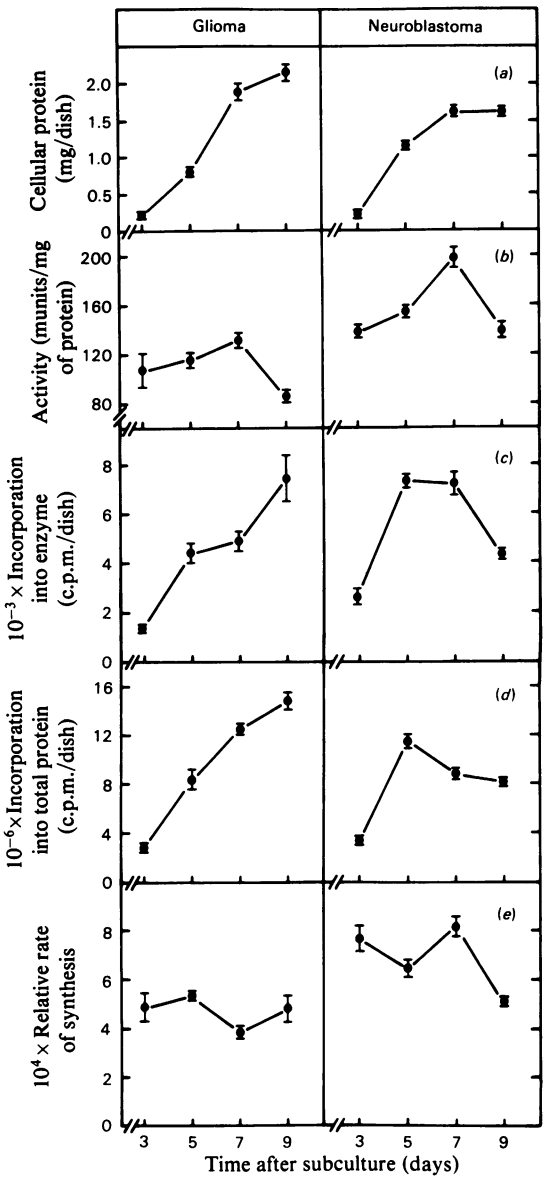

Fig. 3. *Cellular protein (a), 3-oxoacid CoA-transferase specific activity (b), incorporation of radioactivity into 3-oxoacid CoA-transferase (c) and into total protein (d), and relative rate of synthesis of 3-oxoacid CoA-transferase (e) on days 3, 5, 7 and 9 of growth after subculture of glioma and neuroblastoma cells*

Enzyme activity, protein, and incorporation of radioactivity into the enzyme and total protein were measured as described in the Materials and methods section. The results are means ± S.E.M. for four to six dishes.

To evaluate the role of enzyme degradation in the regulation of 3-oxoacid CoA-transferase activity, glioma and neuroblastoma cells on day 4 after subculture were pulse-labelled for 24 h with L-[^{35}S]-methionine. Subsequently, they were exposed to 10 mM-methionine (unlabelled) during a 6 h chase period (to minimize reutilization of radioisotope) and studied at specified times thereafter so that the decay of radioactivity in 3-oxoacid CoA-transferase could be compared with the decay of radioactivity in total cellular protein. It is possible that rates of degradation were underestimated, owing to reutilization of radioisotope. The apparent half-life for total cellular protein in both cell lines was about 28 h. The validity of this observation in glioma cells was confirmed in another experiment (results not shown). The corresponding value for 3-oxoacid CoA-transferase was 46 h in glioma cells and 50 h in neuroblastoma cells (Fig. 4). Therefore, the rate of degradation of 3-oxoacid CoA-trans-

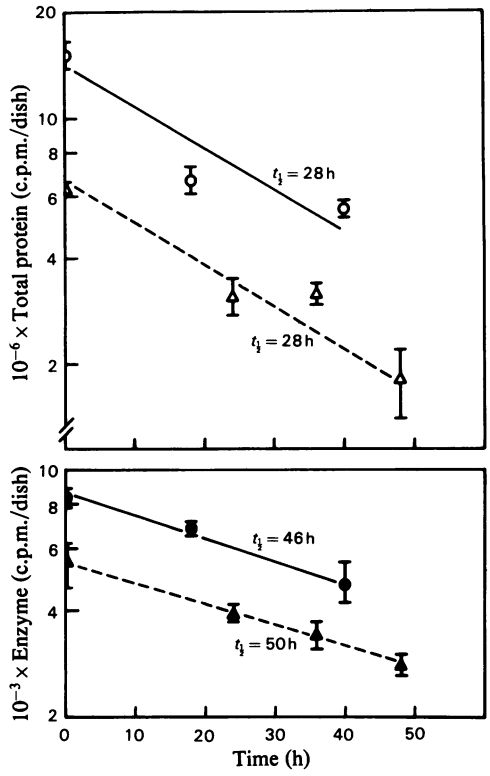

Fig. 4. *Rates of degradation of 3-oxoacid CoA-transferase and total cellular protein in glioma (●, ○) and neuroblastoma (▲, △) cells*

Cells were labelled for 24h with L-[^{35}S]methionine, incubated in complete medium containing 10mM unlabelled methionine for 6h, and studied at specified times thereafter. The results are means ±S.E.M. for four or five dishes.

ferase is relatively low and similar in both cell lines.

The time required for a protein to achieve a new steady state is directly proportional to its half-life, as Berlin & Schimke (1965) elegantly demonstrated. The mean relative rate of synthesis of 3-oxoacid CoA-transferase in neuroblastoma cells during days 3–7 was 7.43×10^{-4}, whereas the rate constant of degradation of the enzyme was only 56% of the rate constant of degradation of total cellular protein. Under steady-state conditions, the concentration of an enzyme is equal to its rate of synthesis divided by its rate constant of degradation. Therefore, the steady-state concentration of 3-oxoacid CoA-trasferase in neuroblastoma cells is 0.133% of total protein. Since purified 3-oxoacid CoA-transferase has a specific activity of 150 units/mg of protein, the expected steady-state specific activity is 200munits/mg of protein. This steady-state concentration is not achieved until day 7, when the specific activity of the enzyme was 198 ± 8munits/mg of protein. Hence, the increase in enzyme specific activity from day 3 to day 7, during which time the relative rate of synthesis does not increase, represents the approach to steady-state conditions for the enzyme. Conversely, the 30% decrease in 3-oxoacid CoA-transferase specific activity between days 0 and 3 in neuroblastoma cells is presumably due to the more rapid approach of other cellular proteins to steady state, which results in a decline in the ratio of 3-oxoacid CoA-transferase, as well as other slowly degraded proteins, to total cellular protein. These conclusions are not affected by the delay in the approach of all proteins to steady state caused by the proliferation of cells during the first 7 days after subculture. Analysis of the data obtained in studies of glioma cells yields similar conclusions.

The regulation of 3-oxoacid CoA-transferase and the other two enzymes of ketone-body metabolism, D-3-hydroxybutyrate dehydrogenase and acetoacetyl-CoA thiolase, in developing rat brain has been the subject of numerous reports. The activities of these enzymes rise severalfold during the suckling period, but gradually decline by 50–70% after weaning (Page *et al.*, 1971; Tildon *et al.*, 1971). Thus their postnatal development is apparently associated with the hyperketonaemia induced by the high fat content of rat milk. Furthermore, experimentally induced hyperketonaemia can cause premature increases in the activities of ketone-body-metabolizing enzymes. Brain 3-oxoacid CoA-transferase activity was increased 2-fold in 20-day-old foetuses of dams fed on a high-fat diet during pregnancy (Dierks-Ventling, 1971). Similarly, 1-day-old pups of dams fed on a high-fat diet during pregnancy exhibited a 2-fold increase in brain D-3-hydroxybutyrate dehydrogenase activity (Sherman & Wilson, 1978). Activities of this enzyme were elevated nearly 3-fold in 1-day-old pups of rats which had been starved during the last 5 days of gestation (Thaler, 1972). In contrast, weaning of rats to a high-fat diet for 3 weeks resulted in only a 13% higher activity of brain D-3-hydroxybutyrate dehydrogenase compared with age-matched rats weaned on laboratory chow (Pull & McIlwain, 1971). In adult rats, hyperketonaemia induced either by feeding on a high-fat diet (Dierks-Ventling & Cone, 1971) or by starvation (Williamson *et al.*, 1971; Bassler *et al.*, 1973) had no effect on the activities of acetoacetyl-CoA thiolase or 3-oxoacid CoA-transferase, respectively, in brain. Hence, adaptive changes in the activities of ketone-body-metabolizing enzymes in response to hyperketonaemia are apparently restricted to an early period of development of rat brain.

By including ketone bodies in the culture

Table 1. *Effects of acetoacetate and* DL-*3-hydroxybutyrate on cellular protein, specific activity and relative rate of synthesis of 3-oxoacid CoA-transferase in neuroblastoma cells*

The cells were grown in modified minimal essential medium, supplemented with either acetoacetate or DL-3-hydroxy-butyrate as indicated. Each treatment was continued for four consecutive subcultures. On days 3 and 5 of the fourth subculture, the cells were labelled with L-[^{35}S]methionine for 2h and determinations were performed as described in the Materials and methods section. The relative rate of 3-oxoacid CoA-transferase synthesis is expressed as the ratio of radioactivity (c.p.m.) in the enzyme to that in total cellular protein. The results are the means ± S.E.M. for five dishes. Abbreviation: n.s., not significant.

Time in culture (days)	Addition	Cellular protein (μg of protein/dish)	Specific activity (munits/mg of protein)	Incorporation of [^{35}S]methionine into		Relative rate of synthesis of the enzyme ($\times 10^4$)
				Enzyme (c.p.m.)	Total protein (c.p.m. $\times 10^{-6}$)	
3	None	237 ± 8	139 ± 5	2598 ± 265	3.40 ± 0.27	7.65 ± 0.52
	0.5 mM-Acetoacetate	319 ± 11	184 ± 9	4012 ± 157	4.25 ± 0.25	9.48 ± 0.37
		($P < 0.001$)	($P < 0.005$)	($P < 0.005$)	(n.s.)	($P < 0.025$)
	2.5 mM-DL-3-Hydroxybutyrate	153 ± 11	174 ± 16	1810 ± 104	2.08 ± 0.04	8.75 ± 0.58
		($P < 0.001$)	(n.s.)	($P < 0.05$)	($P < 0.005$)	(n.s.)
5	None	1160 ± 27	155 ± 5	7346 ± 278	11.49 ± 0.55	6.44 ± 0.35
	0.5 mM-Acetoacetate	1280 ± 50	162 ± 7	5297 ± 296	9.25 ± 0.28	5.77 ± 0.45
		(n.s.)	(n.s.)	($P < 0.005$)	($P < 0.01$)	(n.s.)
	2.5 mM-DL-3-Hydroxybutyrate	1160 ± 19	155 ± 4	7020 ± 122	10.59 ± 0.36	6.81 ± 0.13
		(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)

medium, it was possible to examine their direct effect on 3-oxoacid CoA-transferase in these two cell lines. Acetoacetate (0.5 mM) increased 3-oxoacid CoA-transferase specific activity in neuroblastoma cells on day 4 (Patel *et al.*, 1981), but DL-3-hydroxybutyrate (2.5 mM) had no effect. Neither acetoacetate nor DL-3-hydroxybutyrate affected the specific activity of 3-oxoacid CoA-transferase in glioma cells (Patel *et al.*, 1981). In the present study, acetoacetate (0.5 mM) significantly enhanced 3-oxoacid CoA-transferase specific activity on day 3 (Table 1). This increase was associated with a 23% stimulation ($P < 0.025$) of the relative rate of synthesis of the enzyme on day 3. This value, 9.48×10^{-4}, was higher than that observed at any time in untreated cells (Fig. 3*e*). An effect of acetoacetate on neuroblastoma cells on day 5 was not observed. DL-3-Hydroxybutyrate (2.5 mM) affected neither the specific activity nor the relative rate of synthesis of 3-oxoacid CoA-transferase, although it inhibited cellular growth. Whether this effect was due to the presence of the L-isomer is unknown.

The increase in the relative rate of synthesis of 3-oxoacid CoA-transferase caused by acetoacetate in culture suggests that substrate-mediated induction of 3-oxoacid CoA-transferase synthesis may be an important regulatory mechanism *in vivo*. Observation of the acetoacetate effect only in the early growth phase of neuroblastoma cells in culture may be relevant to the existence of a significant adaptive response of the ketone-body-metabolizing enzymes to ketosis only in prenatal rat brain (Dierks-Ventling, 1971; Thaler, 1972; Sherman & Wilson, 1978). It has been established previously that acetoacetate serves not only as an energy source for the developing brain but also as a precursor for brain lipogenesis (Robinson & Williamson, 1980). The effect of acetoacetate on 3-oxoacid CoA-transferase synthesis in neuroblastoma cells shows that acetoacetate can also serve as a metabolic signal capable of specifically causing an increase in the relative rate of synthesis of the enzyme responsible for the first committed step of its degradation. Further studies are required to elucidate the mechanism of action of acetoacetate on the synthesis of this enzyme.

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