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Biotin-Dependent Carboxylase Activities in Different CNS and Skin-Derived Cells, and their Sensitivity to Biotin-Depletion

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Abstract: The validity of various transformed and untransformed CNS and skin-derived cell cultures as a model for studying effects of biotin deficiency was tested. In biotin-sufficient conditions (0.1–10 μmol/L) all cell types showed considerable activities of the four biotin-dependent carboxylases. Notably, pyruvate carboxylase activity was also present in the different neuronal cells. One passage in low-biotin medium (6 –130 pmol/L) lowered mitochondrial carboxylase activities in all cell types, but to varying degrees. Sensitivity to biotin depletion was greatest in three neuronal cell types, Roc-1 oligodendroglia, and three keratinocyte cell types (carboxylase activities decreased to 2–11% of maximal); intermediate in primary astrocytes and C6 glioma (decreased to 12–28%), and least in SAOS2 sarcoma and skin fibroblasts (decreased to 32–85%). Transformed and untransformed cell lines of the same cell type showed similar sensitivity.

We conclude that cultures of different transformed CNS and keratinocyte cell types allow the study of effects of biotin deprivation. Carboxylase activities of neurons, oligodendroglia, and keratinocytes were much more sensitive to biotin depletion than fibroblasts. This may be an important factor in the pathogenesis of neurological and cutaneous abnormalities in congenital biotinidase deficiency where recycling of biotin is deficient.

Key words: Biotin deficiency, cultured CNS cells, NB2a neuroblastoma, GT1-7 neurons, primary cortical neurons, Roc-1 oligodendroglia, C6 glioma, primary rat brain cells, cultured skin-derived cells, skin fibroblasts, HaCat, follicular keratinocytes, interfollicular keratinocytes, carboxylase activities, biotinidase

Non-standard abbreviations: CNS, central nervous system; FCS, fetal calf serum; NBCS, newborn calf serum; PCC, pronionyl-CoA carboxylase; MCC, 3-methylcrotonyl-CoA carboxylase; PC, pyruvate carboxylase; ACC, acetyl-CoA carboxylase; GLDH, glutamate dehydrogenase; LDH, lactate dehydrogenase; PRBC, primary rat brain cells; PCN, primary cortical neurons.

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Introduction

Biotin, the cofactor of four mammalian carboxylases, is recycled from endogenous, proteolytically degraded carboxylases and released from its protein-bound forms in the diet by biotinidase [1]. Biotinidase deficiency (McKusick 253260), a rare inborn error of metabolism, is inherited as an autosomal recessive trait and leads to biotin deficiency, which in turn results in multiple carboxylase deficiency (MCD), i.e. decreased activities of the four biotin-dependent carboxylases, propionyl-CoA carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC), pyruvate carboxylase (PC), and acetyl-CoA carboxylase (ACC). These carboxylases play an essential role in important metabolic processes such as protein, carbohydrate and lipid metabolism. Thus MCD leads to severe metabolic derangement with typical organic aciduria and life-threatening illness.

Biotinidase deficiency is characterized clinically by early neurologic abnormalities such as seizures, ataxia and hypotonia, usually appearing between one and six months of age, often in the absence of organic aciduria and metabolic acidosis [2]. Later manifestations include cutaneous abnormalities such as skin rash and alopecia. Without treatment, irreversible brain damage including neurosensory hearing loss and optic atrophy may occur. Neurological abnormalities develop as a consequence of increasing biotin deficiency in the brain, and can be prevented by early administration of high doses of biotin. Their pathogenesis, however, is not yet fully understood. In particular it is not known why neurological symptoms usually appear before overt organic aciduria.

In order to investigate the pathophysiology of this disorder, models for the study of the effect of biotin deficiency on metabolic processes involved in the brain are required. Animals made biotin-deficient with dietary avidin are an inappropriate model, because in such an acquired deficiency the brain is more resistant to biotin-depletion [3] than in inherited biotinidase deficiency, in which carboxylase activities decrease earlier and more severely in the brain than in other organs [4]. We therefore investigated whether cultures of the three main cell types of the brain, astroglia, oligodendroglia, and neurons, could provide an alternative model. Since some earlier studies reported [5–7] that pyruvate carboxylase is not present in neuronal cells we first measured the specific activities of all biotin-dependent carboxylases in different types of CNS-cells grown in biotin-sufficient culture conditions. Their carboxylase activities were compared with those in different types of skin-derived cells. In addition, the sensitivity of each cell line to biotin depletion was studied by measuring carboxylase activities. The changes of activities in transformed and untransformed cells of the same cell type were compared in order to establish whether transformed cell lines might also provide adequate model systems.

Materials and Methods

CNS cells: The CNS cells originate from the brain of mice (GT1-7, NB2a) or rats (C6, Roc-1, primary brain cells, primary neurons). Primary rat brain cell (PRBC) cultures were established from newborn rat brain suspensions as described earlier [8]. This type of culture is known to contain mainly astrocytes. Rat C6 glioma cells are a well defined transformed cell line with similarities to astrocytes and were obtained from the American Type Culture Collection. Roc-1 oligodendroglia cells are a transformed hybrid cell line between C6 glioma cells and rat oligodendrocytes with mainly oligodendroglial characteristics (Dr. A. McMorris, Wistar Institute, personal communication). NB2a neuroblastoma cells are a well-defined transformed cell line from mouse brain. These cells are acetylcholine esterase-positive cells with characteristics of immature neurons. GT1-7 neurons are differentiated hypothalamic mouse cells that secrete gonadotropin-releasing hormone [9], kindly provided by Prof. Pamela L. Mellon (San Diego, USA). Primary cortical neurons (PCN) were established from fetal (16th day of gestation) rat brain as described earlier [10, 11].

Skin-derived cells: Human skin fibroblasts were grown from skin biopsies of healthy individuals. SAOS2 sarcoma cells are a well-defined transformed human cell line obtained from the American Type Tissue Collection. Human outer root sheath cell (ORS; follicular keratinocytes) cultures were set up from hair roots, and human interfollicular keratinocytes (SHK1) from skin of mammary gland obtained from healthy individuals after breast size reduction. Primary cultures were established in the presence of a skin fibroblast feeder layer as described earlier [12, 13]. Further cultures of ORS and SHK1 cells were performed in the absence of fibroblasts. HaCat cells are a well-defined transformed keratinocyte cell line. All human biopsies were obtained with informed consent of the donors.

Culture procedures and media: Basal medium was either Dulbecco's modified Eagle medium (DMEM, Gibco BRL) or Earl's minimal essential medium (MEM, Gibco BRL) (except for ORS, SHK1 and PCN cells; see below) supplemented with antibiotics (200 000 U penicillin/L (PRBC), 50 000 U penicillin and 500 mg/L streptomycin (PN), or 10 mL Antibiotic-Antimycotic/L from Gibco BPL (others), variable amounts of glucose and glutamine,

Table I: Biotin concentration in different commercially available sera for culture media

	Biotin, nmol/L		
	n	range	geometric mean
Fetal calf serum (FCS)	59	6.2 –1325	83.8
Newborn calf serum (NBCS)	10	0.74–3.11	1.62
Horse serum (HS)	6	0.94–1.96	1.30

n = number of different serum preparations.

and 10% (v/v) heat-inactivated animal serum. Since DMEM and MEM are biotin-free, the final biotin concentration of the medium depends on the serum supplement (Table I). The biotin concentration of fetal calf serum (FCS; AMIMED®) is about 100 times higher than that of newborn calf serum (NBCS; Gibco BRL or BIOSPA GmbH, Germany) or horse serum (HS; Socochim SA). The biotin concentration of each medium was determined prior to use because the biotin concentrations of the sera show considerable batch-to-batch variation (Table I) and even basal media may be contaminated with biotin.

The C6, Roc-1, NB2a, and HaCat cells were routinely grown in DMEM-FCS medium supplemented with 4 mmol/L glutamine and a final glucose concentration of 3 g/L. Skin fibroblasts and SAOS2 cells were cultured in MEM-FCS medium with 2 mmol/L glutamine and a final glucose concentration of 1 g/L. The biotin concentration of both FCS-based media was 65 nmol/L originating from the biotin in the FCS. For biotin-depletion, these cell lines were subcultured in a low-biotin medium prepared by replacing FCS with NBCS resulting in a final biotin concentration of 0.1 nmol/L.

PRBC were grown for the first 10 days in DMEM-FCS medium supplemented with 4 mmol/L glutamine and a final glucose concentration of 4.4 g/L. For biotin-depletion, the cell layers were rinsed with phosphate-buffered saline (PBS) and the medium was changed, without subcultivation of the cells, to low-biotin medium (FCS replaced by NBCS).

GT1-7 neurons were grown first in DMEM supplemented with 5% FCS and 5% HS as described [14]. For biotin-depletion, the cells were subcultured in a low-biotin medium prepared by replacing FCS with HS resulting in a final biotin concentration of 0.11 nmol/L.

Cultures of primary neurons were established in DMEM-FCS medium at a density of 10.5×10^6 cells/100 mm Petri-dish as described [10]. After three hours the medium was replaced by a serum- and biotin-free medium with DMEM supplemented as previously described [10]. The final biotin concentration of this medium was 0.13 nmol/L. The medium was changed on the 3rd and 5th day after seeding and cells were harvested for carboxylase assay after 7 days in low-biotin medium.

ORS and SHK1 keratinocytes were grown in serum-free keratinocyte growth medium (KGM, PromoCell® bioscience alive, Heidelberg, Germany) supplemented with 10 nmol/L biotin. For biotin-depletion, the cells were subcultured in a low-biotin KGM with a biotin concentration of 0.13 nmol/L.

To obtain maximal activities of the biotin-dependent carboxylases, parallel cultures of each cell line were made biotin-sufficient by growing them in the corresponding low-biotin medium supplemented with 1 or 10 µmol/L biotin.

Since it is known that specific activities of the biotin-dependent carboxylases may be two- to three-fold higher in confluent or postconfluent cells than in nonconfluent cultures, all cell lines (except PCN cells which do not proliferate) were grown to confluence as judged by microscopic examination. As shown in Tables II and III, the need

Table II: Biotin-dependent carboxylase (PCC, MCC, PC, ACC) and biotinidase activities (pmol/min/mg protein), as well as control enzyme activities (GLDH, LDH; nmol/min/mg protein) in different CNS cells grown in biotin-supplemented and biotin-depleted medium for one passage. Values are the mean of duplicate determinations in a representative experiment

	Biotin in medium,	Culture time,	Enzymes						
Cell line	M[1]	days	PCC	MCC	PC	ACC	GLDH	LDH	Biotinidase
Primary rat brain cells	10^{-5}	10	5528	1575	5739	1087	37.5	660	23.8
(mainly astrocytes)	10^{-10}	10	1570	316	1176	724	48.9	670	28.1
C6 glioma	10^{-5}	5	3852	1550	5964	1172	47.4	3503	50.4
	10^{-10}	5	692	180	734	805	44.6	3122	48.1
Roc-1 oligodendroglia	10^{-5}	5	4731	2317	5670	1392	133.2	5161	37.7
	10^{-10}	5	444	97.9	252	602	151.2	4794	31.6
NB2a neuroblastoma	10^{-5}	4.5	1800	908	4904	2526	12.8	2532	3.8
	10-10	4.5	96.7	46.3	114	270	13.1	2538	4.1
GT1-7 neurons	0.6×10^{-6}	21	3561	1242	6839	3550	20.7	1504	23.4
	1.1×10^{-10}	21	122	54.5	129	1580	20.8	1265	–
Primary cortical neurons	4×10^{-6}	8	1855	852	3334	1644	20.4	568	8.9
	1.3×10^{-10}	8	132	97.0	118	701	16.8	622	–

[1] For detail of the composition of the media, see Materials and Methods.

Table III: Biotin-dependent carboxylase (PCC, MCC, PC, ACC) and biotinidase activities (pmol/min/mg protein), as well as control enzyme activities (GLDH, LDH; nmol/min/mg protein) in different skin-derived cells grown in biotin-supplemented and biotin-depleted medium for one passage. Values are the mean of duplicate determinations in a representative experiment

Cell line	Biotin in medium, M[1]	Culture time, days	Enzymes PCC	MCC	PC	ACC	GLDH	LDH	Biotinidase
Skin fibroblasts	10^{-5}	10	561	430	773	634	23.4	3463	21.0
	10^{-10}	10	481	285	578	632	25.8	3703	–
SAOS2 sarcoma	10^{-5}	10	663	307	2878	540	18.9	2302	21.1
	10^{-10}	10	379	117	1679	481	23.1	2669	20.5
ORS cells	10^{-6}	10	582	230	3808	–	19.1	–	18.8
	0.6×10^{-10}	10	61.3	11.5	383	–	17.5	–	14.5
SHK1 keratinocytes	10^{-5}	9	919	414	4652	480	28.4	679	17.3
	10^{-10}	9	92.0	6.7	454	410	25.6	933	17.2
HaCat keratinocytes	10^{-5}	7	563	785	1595	1052	64.3	2357	–
	10^{-10}	7	50.7	43.7	44.7	430	70.1	2106	17.3

[1] For details concerning the composition of the media see Materials and Methods.

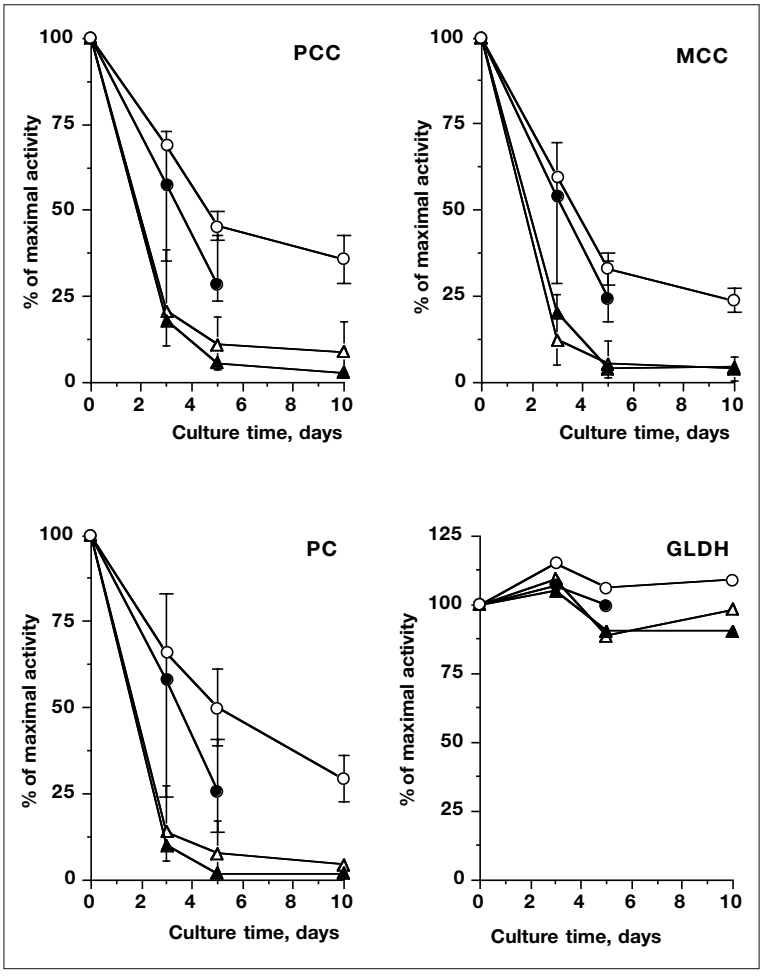

Figure 1: Decrease of the activities of the mitochondrial biotin-dependent carboxylases PCC, MCC, and PC in different CNS cells after 3, 5 and 10 days growth in low-biotin medium. The activity of GLDH (biotin-independent mitochondrial enzyme) is given as control. The cell lines were: PRBC (○), C6 glioma (●), Roc-1 oligodendroglia (△), NB2a neuroblastoma (▲). The activities in low-biotin medium are expressed as percent of activities of parallel cultures in medium supplemented with 1 µmol/L biotin. In C6 and Roc-1 cells the activities are mean and the range (vertical lines) of activities obtained in 3 separate experiments and in PRBC from 2 experiments. In NB2a cells the results of a single experiment are given.

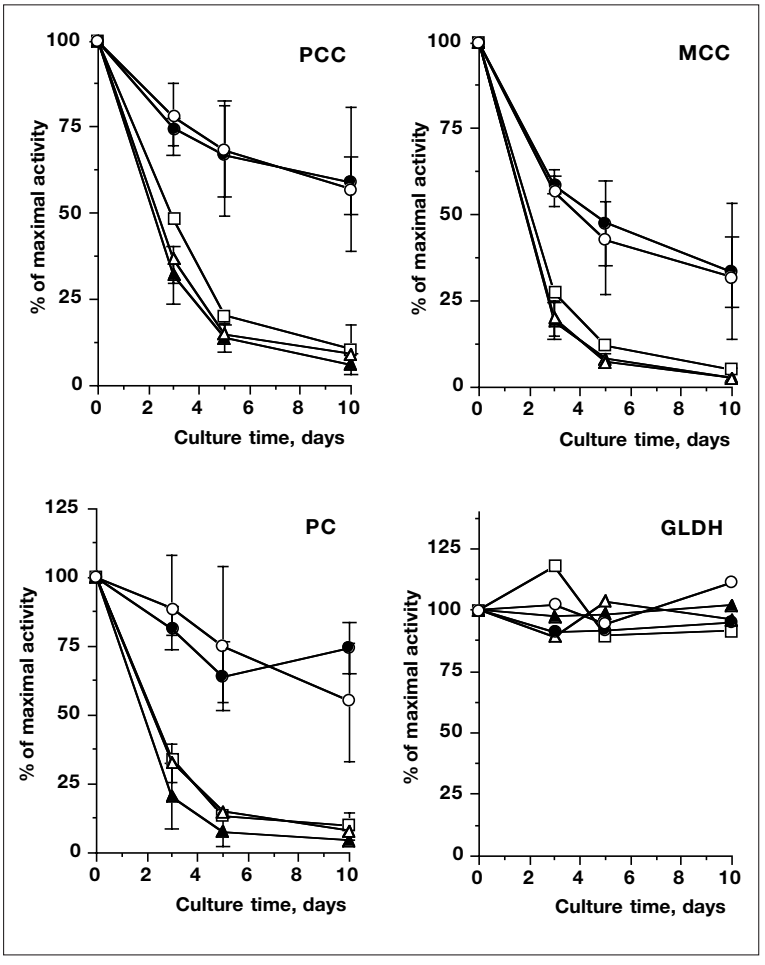

Figure 2: Decrease of the activities of the mitochondrial biotin-dependent carboxylases PCC, MCC, and PC in different skin-derived cells after 3, 5 and 10 days growth in low-biotin medium. The activity of GLDH (biotin-independent mitochondrial enzyme) is given as control. The cell lines were: skin fibroblasts (○), SAOS2 sarcoma cells (●), SHK1 keratinocytes (△), HaCat keratinocytes (▲), ORS cells (□). The activities in low-biotin medium are expressed as percent of activities of parallel cultures in medium supplemented with 1 µmol/L biotin. In skin fibroblasts the activities are mean and the range (vertical lines) of activities obtained in 4 separate experiments, in SHK1 keratinocytes from 3 experiments, in SAOS 2, and HaCat cells from 2 experiments. In ORS cells the results of a single experiment are given.

to use confluent cultures resulted in widely variable culture time (4.5–21 days) reflecting the different growth rate of the various cell types. For the study of the decrease of carboxylase activities in low-biotin media (Fig. 1 and 2) the level of seeding of the cultures was chosen individually for each cell line so that the cells reached confluence between the 5th and the 10th day. The exception was PRBC, which were not subcultured but made biotin-depleted by simply changing the medium (see above).

Cells were harvested either by scraping with a rubber-policeman (ORS, SHK1, PCN) or by trypsinization (all other cell lines). Cells were sedimented by centrifugation (200 × g), washed twice with PBS, and cell pellets were stored at –75°C until assayed for enzyme activities.

Other methods: Biotin concentrations in sera and complete media were determined by a microbiological assay using *Lactobacillus plantarum* ATCC 8014 [15] before addition of antibiotics.

Biotinidase activity was determined in cell extracts by an HPLC method using the natural substrate biocytin [16]. Cell extracts were prepared by suspending cell pellets in ice-cold buffer (5 mmol/L K-phosphate buffer pH 6.0, 0.25 mmol/L dithiothreitol and 0.1% Triton X-100) and passing the cell suspension 12–16 times through a 0.6 × 23 mm needle over 20 minutes. After centrifugation (5 min, 14 000 rpm, Eppendorf centrifuge) the supernatant was used immediately for the assay.

The activities of the biotin-dependent mitochondrial carboxylases (PCC, MCC, PC) were assayed in crude cell homogenates by measuring the incorporation of ^{14}C-bicarbonate into acid-nonvolatile products as described [17, 18]. Cytosolic ACC-activity was assayed by measuring the citrate-activated incorporation of ^{14}C-bicarbonate into malonyl-CoA as described [4, 16]. Activities of glutamate dehydrogenase (GLDH) and lactate dehydrogenase (LDH) were determined as mitochondrial and cytosolic biotin-independent control enzymes, respectively, by NADH-linked assay [19].

Protein concentration in homogenates was determined after trichloroacetic acid precipitation by a modification of the Lowry method [17].

Results

Specific activities of carboxylases in biotin-supplemented and biotin-depleted cells: Enzyme activities in cells grown for one subculture or for the noted culture time (primary cultures) in the corresponding low-biotin media with and without biotin supplementation are shown in Tables II and III. All cell types showed considerable levels of activities of all four biotin-dependent carboxylases in the presence of biotin. Importantly, PC-activity was present in all three neuronal cell types and specific activities were comparable to those in the glial cell types.

Carboxylase activities in CNS-cells were generally higher than in the skin-derived cells. However, specific activities may vary even in the same cell line depending on the stage of confluence, the composition of the medium, and the frequency of medium changes. No difference in activity was observed when the biotin concentration of the culture medium was varied between 0.01 and 10 μmol/L (results not shown).

In low-biotin medium, the activities of the biotin-dependent carboxylases were lower than in biotin-supplemented medium in all cell types, but to a variable degree. Activity of the cytosolic ACC was less sensitive to biotin-depletion of the medium than the activities of the mitochondrial carboxylases in all cell types. Among the CNS-cells all neuronal cell types as well as Roc-1 oligodendroglia were extremely sensitive to biotin-depletion of the culture medium. The mitochondrial carboxylase activities decreased to l2–11% of activities in biotin-supplemented medium. PRBC and C6 glioma cells were less sensitive, retaining higher activities (12–28% of maximal activities) in the low-biotin medium. Among the skin-derived cells, the three keratinocyte cell types were highly sensitive to biotin depletion. The mitochondrial carboxylase activities decreased to 10% or less whereas skin fibroblasts and SAOS 2 sarcoma cells were the most resistant cell types (remaining activity 32–85%). As expected, biotin deprivation had no effect on the activities of the biotin-independent control enzymes.

Biotinidase activity was present in all types of cells, indicating the ability to recycle biotin. However, the activity in two neuronal cell types, i.e. NB2a neuroblastoma cells and primary cortical neurons, was considerably lower than in the glial cells (Table II). As expected, the activity of biotinidase was not dependent on biotin.

Decrease of carboxylase activities during biotin deprivation: To compare the sensitivity of the different cell types to biotin depletion more precisely, the time course of the decrease of mitochondrial carboxylase activities was followed in selected cell lines or strains. Activities measured in cells grown in the corresponding low-biotin media for 3, 5 or 10 days, were expressed as percent of activities measured in parallel cultures supplemented with 1 μmol/L biotin. The results are shown in Figures 1 and 2. Overall comparison of the activities of the three mitochondrial carboxylases revealed no marked differences in their sensitivity to biotin depletion. However, in some cells MCC activity decreased more rapidly than PCC and PC activities.

The rate of the decrease of carboxylase activities revealed differences between the various cell types. In NB2a neuroblastoma, Roc-1 oligodendroglia, and the keratinocytes SHK1, ORS, and HaCat, the activities decreased rapidly to below 50% after three days and below 20% after five days of biotin deprivation. Out of the skin-derived cells, the mesenchymal skin fibroblasts and SAOS2 sarcoma cells were again clearly more resistant to biotin depletion than all of the keratinocytes, which are of ectodermal origin. They retained 32% to 74% of carboxylase activities after 10 days of biotin deprivation. From the CNS-cell types, PRBC (33–50% after 5 days), and to a lesser extent C6 glioma cells (24–28% after 5 days), were more resistant than NB2a and Roc-1 cells. As expected, there was no decrease in the activity of biotin-independent control enzyme GLDH.

Comparison of transformed cell lines and untransformed cell strains of the same cell type showed similar sensitivities to biotin deprivation. The rates of decrease of the carboxylase activities in skin fibroblasts and SAOS2 sarcoma as well as in SHK1 and HaCat keratinocytes were nearly identical. The difference between C6 glioma cells and PRBC (mainly astrocytes) may result from the differences in the culture procedure and rates of proliferation. PRBC were not subcultured and are slowly proliferating cells, whereas C6 cells proliferate rapidly.

Reactivation of carboxylase activities: The influence of biotin deficiency on *de novo* synthesis of apocarboxylases was investigated by studying the short-term reactivation of apoPCC by biotin. Cells were grown to confluence in low-biotin medium to induce PCC deficiency. Biotin was added to the medium (10 μmol/L) and after 30 minutes incubation at 37°C, the medium was removed, cell layers were washed with PBS, harvested and assayed for PCC activity. Although it is very unlikely that a considerable amount of apoPCC can be synthesized by fibroblasts during the short incubation period of 30 minutes, parallel cultures were incubated with added biotin in the presence of cycloheximide at a concentration (20 μg/mL medium) that is known to inhibit protein synthesis. As shown in Figure 3, this short-term incubation with biotin resulted in reactivation of PCC from 63–3% to 80–117% of maximal activity in the different cell types. This indicates that biotin depletion did not affect the synthesis of apoPCC. As expected this reactivation was not affected by cycloheximide.

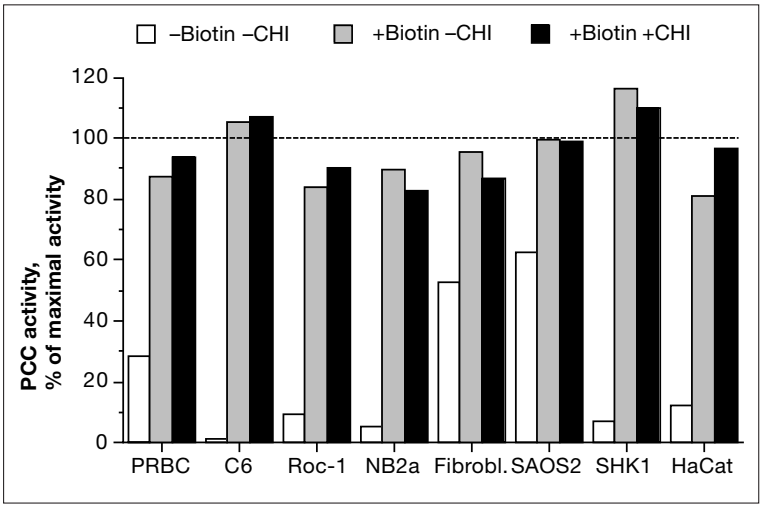

Figure 3: Short-term reactivation of deficient PCC activity with biotin in the presence and absence of cycloheximide (CHI). Cells were subcultured in low-biotin medium and grown to confluence before reactivation. The white bars show the basal activities in low-biotin medium without added biotin, the grey bars after 30 minutes incubation at 37°C with biotin (10 µmol/L) in the absence of CHI, and the black bars after 30 minutes incubation with biotin in the presence of CHI (20 µg/mL). Activities are expressed as percent of those obtained in parallel cultures grown in low-biotin medium supplemented with biotin (10 µmol/L).

Discussion

This study clearly demonstrates the presence of all four biotin-dependent carboxylases in different types of CNS cells and skin-derived cells, both transformed and untransformed, when grown in biotin-sufficient culture medium. This is in contrast to earlier enzymatic [7] and immunocytochemical [5, 6] studies that described PC as a glia-specific enzyme and not present in neurons. In fact the specific activity of PC in 3 different neuronal cell lines is even higher than that of the other three biotin-dependent carboxylases. The presence of PC activity cannot be explained due to cellular transformation since PC was active even in primary cortical neurons. The absence of PC activity in primary neurons in the study of Yu *et al* [7] can be explained by biotin deficiency since the cells were grown in a medium with 5% horse serum as the only source of biotin. This results in a final biotin concentration of about 0.05 nmol/L (see Table I). We have clearly demonstrated in the present study that activities of all mitochondrial carboxylases were severely reduced in the three different neuronal cell types grown with 0.1 nmol/L biotin. The reported absence of immunohistochemical staining for PC in cultured neurons [5, 6] remains to be explained.

This study emphasizes the importance of the measurement of biotin concentration in culture media supplemented with different sera, particularly when cultured cells are used for investigation of biotin metabolism. The addition of 10% FCS supplies sufficient biotin (10 nmol/L) to achieve maximal activities of the biotin-dependent enzymes. In contrast, addition of 10% NBCS or HS results in a very low biotin concentration of 0.07–0.31 nmol/L in the medium. This corresponds to about one tenth of the physiological plasma concentration of biotin in man and many animals [16], and results in cellular biotin deficiency and in decreased activities of the biotin-dependent carboxylases.

In this study we took advantage of the low biotin content of NBCS and HS to prepare low-biotin media for those cells that require serum for growth. An earlier study showed that biotin depletion resulting in a decrease of PRBC carboxylase activities to 15%, can also be achieved using a FCS-containing medium supplemented with avidin to bind biotin for inactivation [20]. However, the use of excess avidin in the medium may contaminate the cells with free avidin and may interfere in experiments related to transport and metabolism of biotin and its derivatives, most of which are also bound by avidin [21].

Severe biotin deficiency, as indicated by low activities of mitochondrial carboxylases, could be easily produced within a few days in neuronal cells, Roc-1 oligodendroglia, and in keratinocytes, but less well in C6 glioma and PRBC cultures, whereas SAOS2 sarcoma cells and skin fibroblasts were more resistant and became only moderately biotin-deficient in our culture system. These effects on carboxylase activities were achieved after just a single passage in either serum- and biotin-free media or in media supplemented with low-biotin sera. The higher sensitivity of mitochondrial carboxylase activities to biotin-depletion as compared with the cytosolic ACC activity is in accordance with earlier studies in skin fibroblasts and in keratinocytes [22–24]. The decrease in carboxylase activities must be related to biotin deficiency rather than to decreased viability of cells, since the activities of the biotin-independent control enzymes were not affected. Since biotinidase activity was present in all cell lines, the extreme sensitivity of some of the cell lines to biotin depletion cannot be explained by an inability to recycle biotin. The short-term reactivation studies showed that biotin deprivation affects neither the synthesis nor the degradation

rate of apocarboxylases in these cell types. Therefore the study of effects on holocarboxylase formation in these cell lines is feasible. Finally, the carboxylase activities in transformed and untransformed variants of the same cell type showed a similar sensitivity to biotin depletion.

In conclusion, cultured cells, particularly different CNS- and keratinocyte cell lines, provide a good model to study the effect of biotin deficiency on general metabolic processes. The results showed that transformed cell lines can replace more complicated primary cell cultures, thus allowing rapid and easy culture techniques. The rapid decrease of carboxylase activities in cultured neuronal and oligodendroglial cells due to biotin depletion observed in this study may play a role in the pathogenesis of the development of the early and progressive manifestation of neurological abnormalities in biotinidase deficiency where biotin recycling is not possible. Indeed, in a patient with lethal outcome of biotinidase deficiency, PCC activity was severely decreased in the brain (3% of mean control value) but moderately decreased in liver (29%) and kidney (42%) [4]. In contrast, in acquired biotin deficiency, the carboxylase activities are better conserved in the brain and in other tissues [3]. Our findings suggest that the conservation of carboxylase activities in acquired biotin deficiency is not due to a slow turnover of carboxylases within brain cells. The lack of this conservation of carboxylase activities in biotinidase deficiency point to a key role of biotinidase in the supply of biotin in the brain *in vivo*. The clinical manifestation of skin lesions in both acquired biotin deficiency and biotin deficiency caused by biotinidase deficiency is well explained by the marked sensitivity of carboxylase activities in keratinocytes to biotin depletion.

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References

1. Wolf, B. (1995) Disorders of biotin metabolism. In: The metabolic and molecular bases of inherited disease (Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., eds.) pp. 3151–3177, McGraw-Hill, New York.
2. Baumgartner, R., Suormala, T. (1995) Biotin-responsive multiple carboxylase deficiency. In: Inborn Metabolic Diseases. Diagnosis and treatment (Fernandes, J., Saudubray, J.-M., Van den Berghe, G., eds.), pp. 239–245, Springer-Verlag, Berlin, Heidelberg.
3. Bhagavan, H.N., Coursin, D.B. (1970) Depletion of biotin from brain and liver in biotin deficiency. J. Neurochem. 17, 289–290.
4. Baumgartner, E.R., Suormala, T.M., Wick, H., Probst, A., Blauenstein, U., Bachmann, C., Vest, M. (1989) Biotinidase deficiency: A cause of subacute necrotizing encephalomyelopathy (Leigh Syndrome). Report of a case with lethal outcome. Pediat. Res. 26, 260–266.
5. Cesar, M., Hamprecht, B. (1995) Immunocytochemical examination of neural rat and mouse primary cultures using monoclonal antibodies raised against pyruvate carboxylase. J. Neurochem. 64, 2312–2318.
6. Shank, R.P., Bennett, G., Freytag, S.O., Campbell, G.L. (1985) Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. Brain Res. 329, 364–367.
7. Yu, A.C.H., Drejer, J., Hertz, L., Schousboe, A. (1983) Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. J. Neurochem. 41, 1484–1487.
8. Wiesmann, U.N., Hofmann, K., Burkhart, T., Herschkowitz, N. (1975) Dissociated cultures of newborn mouse brain I. Metabolism of sulfated lipids and mucopolysaccharides. Neurobiology 5, 305–315.
9. Mellon, P.L., Windle, J.J., Goldsmith, P.C., Padula, C.A., Roberts, J.L., Weiner, R.I. (1990) Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. Neuron. 5, 1–10.
10. Brewer, G.J., Cotman, C.W. (1989) Survival and growth of hippocampal neurons in different medium at low density: advantages of a sandwich culture technique or low oxygen. Brain Res. 494, 65–74.
11. Gomeza, J., Casado, M., Gimenez, C., Aragón, C. (1991) Inhibition of high affinity GABA uptake in primary astrocyte cultures by phorbol esters and phospholipase C. Biochem. J. 275, 435–439.
12. Limat, A., Noser, F.K. (1986) Serial cultivation of single keratinocytes from the outer root sheath of human scalp hair follicles. J. Invest. Dermatol. 87, 485–488.
13. Limat, A., Hunziker, T., Boillat, C., Bayreuther, K., Noser, F. (1989) Post-mitotic human dermal fibroblasts efficiently support the growth of human follicular keratinocytes. J. Invest. Dermatol. 92, 758–762.
14. Bosma, M.M. (1993) Ion channel properties and episodic activity in isolated immortalized gonadotropin-releasing hormone (GnRH) neurons. J. Memb. Biol. 136, 85–96.
15. Bernoulli, C. (1999) Entwicklung einer sensitiven Methode zur Biotinbestimmung in Plasma und Liquor: Ermittlung von Referenzwerten in der Pädiatrie, Erstellen des Biotinstatus bei Biotinidasemangel-Patienten und Bestimmen der Proteinbindung in Plasma. Inauguraldissertation, Philosophisch-Naturwissenschaftliche Fakultät der Universität, Basel.
16. Suormala, T.M., Baumgartner, E.R., Wick, H., Scheibenreiter, S., Schweitzer, S. (1990) Comparison of patients with complete and partial biotinidase deficiency: biochemical studies. J. Inher. Metab. Dis. 13, 76–92.

17. Suormala, T., Wick, H., Bonjour, J.-P., Baumgartner, E.R. (1985) Rapid differential diagnosis of carboxylase deficiencies and evaluation for biotin-responsiveness in a single blood sample. Clin. Chim. Acta 145, 151–162.
18. Suormala, T., Ramaekers V.T.H., Schweitzer, S., Fowler, B., Laub, M.C., Schwermer, C., Bachman, J., Baumgartner, E.R. (1995) Biotinidase Km-variants: detection and detailed biochemical investigations. J. Inher. Metab. Dis. 18, 689–700.
19. Leighton, F., Poole, B., Beaufay, H. (1968) The large-scale separation of peroxisomes, mitochondria and lysosomes from the liver of rats injected with Triton WR-1339. J. Cell. Biol. 37, 482–513.
20. Rodriguez-Pombo, P., Sweetman, L., Ugarte, M. (1992) Primary cultures of astrocytes from rat as a model for biotin deficiency in nervous tissue. Mol. Chem. Neuropath. 16, 33–44.
21. Baur, B., Suormala, T., Bernoulli, C., Baumgartner, E.R. (1998) Biotin determination by three different methods: specificity and application to urine and plasma ultrafiltrates of patients with and without disorders in biotin metabolism. Internat. J. Vit. Nutr. Res. 68, 300–308.
22. Limat, A., Suormala, T., Hunziker, T., Waelti, E.R., Braathen, L.R., Baumgartner, R. (1996) Proliferation and differentiation of cultured human follicular keratinocytes are not influenced by biotin. Arch. Dermatol. Res. 288, 31–38.
23. Packman, S., Caswell, N., Gonzalez-Rios, M.C., Kadlecek, T., Cann, H., Rassin, D., McKay, C. (1984) Acetyl CoA carboxylase in cultured fibroblasts: Differential biotin dependence in the two types of biotin-responsive multiple carboxylase deficiency. Am. J. Hum. Genet. 36, 80–92.
24. Suormala, T., Fowler, B., Duran, M., Burtscher, A., Fuchshuber, A., Tratzmüller, R., Lenze, M.J., Raab, K., Baur, B., Wick, H., Baumgartner, R. (1997) Five patients with a biotin-responsive defect in holocarboxylase formation: evaluation of responsiveness to biotin therapy *in vivo* and comparative studies *in vitro.* Pediatr. Res. 41, 666–673.

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