Biochem. J. (1990) 265, 911–914 (Printed in Great Britain)

Intermediary metabolism in the Swarm rat chondrosarcoma chondrocyte

Catherine A. SPENCER, T. Norman PALMER and Roger M. MASON*

Department of Biochemistry, Charing Cross and Westminster Medical School, University of London, Fulham Palace Road, London W6 8RF, U.K.

The rat chondrosarcoma chondrocyte has the dual capacity to metabolize glucose (mainly via glycolysis) and glutamine (via an oxidative pathway). Glutamine metabolism, unlike that of glucose, is unable to sustain intracellular ATP concentrations. Glutamine consumption by the chondrosarcoma chondrocyte, however, is significantly in excess of its utilization as an amide-group donor in hexosamine synthesis, implying a novel and major role in cell metabolism.

INTRODUCTION

Chondrocytes metabolize glucose primarily through glycolysis [1], a finding consistent with the low O_2 consumption of cartilage in comparison with other tissues [2]. However, chondrocytes contain mitochondria [3] and possess active mitochondrial oxidative enzymes [4]. Thus aerobic processes may also occur.

The Swarm rat chondrosarcoma contains well-differentiated chondrocytes and has been used widely to study proteoglycan synthesis [5,6]. Primary confluent cultures of the chondrocytes can be maintained in medium containing glucose [5] and acidify the medium overnight, suggesting that they produce large amounts of lactate (R. M. Mason, unpublished work). However, they also contain mitochondria [7,8]. We have used explants of the tumour to investigate its metabolism of potential energy substrates and the dependency of proteoglycan synthesis on this metabolism.

MATERIALS AND METHODS

Tumours were removed (between 10:00 and 11:00 h) from animals 5 weeks after passage and experiments were started immediately thereafter. A suspension of sieved chondrosarcoma tissue in Hanks Balanced Salts solution was prepared [9]. After being washed in Hanks Balanced Salts solution the tissue suspension was washed three times in Krebs–Ringer bicarbonate buffer, pH 7.4, equilibrated with O_2/CO_2 (19:1), and maintained at 37 °C in Krebs–Ringer bicarbonate buffer (1:3, w/v).

Freshly prepared chondrosarcoma suspension (approx. 100 mg dry wt.) was incubated in 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 10 munits of insulin/ml, 1.2 mM-$MgSO_4$ and substrates as appropriate in the peripheral compartment of sealed 25 ml glass flasks fitted with a central well and equilibrated in O_2/CO_2 (19:1). Flasks were shaken continuously (120 strokes/min) and incubated at 37 °C. After termination of the incubation by the addition of 0.2 ml of 60% (w/v) $HClO_4$, $^{14}CO_2$ production was determined by entrapment in Hyamine 10-X hydrochloride solution over 1 h at 37 °C [10], or at 0–4 °C when pyruvate, glutamine, glutamate or NH_3 was to be assayed. The KOH-neutralized $HClO_4$ tissue extracts were assayed by published methods for alanine [11], ammonia [12], aspartate and asparagine [13], glutamate [14], lactate [15], malate [16], pyruvate [17] and glycogen [10].

For adenine nucleotide determinations, incubations were carried out in 2 ml plastic Eppendorf tubes and terminated by freezing the tissue in liquid N_2 after a 30 s spin at 10000 *g* and removal of the supernatant. The frozen tissue was extracted with 6% (w/v) $HClO_4$ in a microhomogenizer system (Biomedix, Pinner, Middx., U.K.) [18]. After neutralization, nucleotides were measured spectrophotometrically (254 nm) after separation on a Whatman Partisphere-SAX anion-exchange h.p.l.c. column by using a 50 min gradient of KH_2PO_4 from 0.1 M to 0.6 M. Quantitative determination was achieved by calibration with standards.

To measure [^{35}S]glycosaminoglycan synthesis, 20 μCi of [^{35}S]sulphate/ml was included in the incubation. The tissue/$KClO_4$ pellets were extracted with 1 ml of 0.5 M-NaOH for 18 h at room temperature to release [^{35}S]glycosaminoglycans. After neutralization, one-tenth of this extract and one-tenth of the neutralized $HClO_4$ extract were dialysed at 4 °C against running tap water (72 h) to remove unincorporated [^{35}S]sulphate, and the macromolecular ^{35}S radioactivity was measured.

RESULTS AND DISCUSSION

Metabolism of glucose and glutamine by the Swarm chondrosarcoma

Glucose is converted rapidly into lactate, pyruvate, alanine and CO_2 by Swarm chondrosarcoma tissue *in vitro* (Table 1). Lactate production was linear over the 2 h incubation period (linear regression: $r = 0.939$, $P < 0.001$), and together with pyruvate and alanine (the other major C_3 metabolites of glucose) accounted for about 80% of the glucose carbon skeleton utilized.

The pathway of CO_2 generation from glucose was investigated by using glucose labelled at either C-1 or C-6. Only $^{14}CO_2$ derived from [1-^{14}C]glucose (6.3 ± 0.2 μmol/h per g dry wt.) was produced, indicating that decarboxylation is exclusively via the pentose phosphate

* To whom correspondence should be addressed.

Table 1. Glucose and glutamine metabolism in Swarm rat chondrosarcoma tissue *in vitro*

Suspensions of chondrosarcoma tissue were incubated with substrate [5 mM-glucose; 10 mM-glutamine (Expt. 1) or 2.5 mM-glutamine (Expt. 2)] at 37 °C for 2 h. $^{14}CO_2$ was trapped and neutralized $HClO_4$ extracts were assayed for metabolites as indicated in the Materials and methods section. Expt. 1 data are from one experiment and are given as means ± S.E.M. ($n > 4$). Four other experiments gave similar results. Expt. 2 data are from another separate experiment ($n = 6$). Significant differences between metabolite concentrations in the presence of a single substrate and with both substrates present (calculated by Student's t method) are denoted as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Abbreviations: N.A., not applicable; N.D., not determined. To facilitate measurement of glutamine disappearance, the initial glutamine concentration was lowered to 2.5 mM. Glutamate production in the presence of glutamine was independent of glutamine concentration over the range 2.5–10 mM and therefore the deduced reaction stoichiometry can be extrapolated to apply at 10 mM-glutamine.

	Rate of utilization of substrate or production of metabolite (μmol/h per g dry wt.)			
Substrate ...	None	Glucose	Glutamine	Glucose + glutamine
Expt. 1 (production)				
Lactate	74.9±3.0	582.5±21.5	78.2±2.1***	617.9±19.4
Pyruvate	0.0	11.1±0.7*	3.4±0.1***	15.9±1.8
Alanine	12.7±0.7	24.3±1.5***	47.3±6.5**	120.7±17.0
Glutamate	22.1±2.4	N.D.	59.4±8.5	73.5±7.9
NH_3	1.2±0.7	N.D.	59.3±0.4	52.4±2.8
Aspartate	0.7±0.4	N.D.	24.1±1.9***	3.4±1.2
Asparagine	0.0	N.D.	38.0±0.7	45.9±8.9
CO_2 (glutamine)	N.A.	N.A.	140.0±9.0	92.8±23.8
(glucose)	N.A.	6.3±0.4	N.A.	6.3±0.2
Expt. 2 (utilization)				
Glucose	N.A.	−233.2±5.6*	N.A.	−272.1±15.6
Lactate	63.4±3.9	414.0±19.3*	N.D.	509.1±30.1
Glutamine	1.0±1.1	N.A.	−80.0±16.7	−80.4±10.7
Glutamate	20.2±1.7	N.D.	64.3±6.7	±47.2±4.9

pathway and that there is no capacity for mitochondrial pyruvate oxidation via the pyruvate dehydrogenase complex. Thus from the data in Table 1 approx. 40 molecules of glucose are metabolized through glycolysis to lactate for every 1 molecule of glucose entering the pentose phosphate pathway. Concentrations of glycogen and rates of synthesis (measured as ^{14}C incorporation from [U-^{14}C]glucose into glycogen) were negligible in these chondrocytes (results not shown).

Glutamine metabolism by the chondrosarcoma produces alanine, glutamate, NH_3, aspartate, asparagine and CO_2 (Table 1), in close agreement with the metabolites of glutamine produced by rat enterocytes [19], rat mesenteric lymphocytes [20] and rat colonocytes [21]. Hanson & Parsons [22] have proposed a pathway to account for the pattern of metabolites produced in rat intestinal mucosa: glutamine → glutamate + NH_3 → 2-oxoglutarate → (citric acid cycle) → malate → pyruvate + CO_2 → alanine. Rates of metabolite production in the chondrosarcoma with glutamine as sole exogenous substrate were linear over 2 h, e.g. alanine and $^{14}CO_2$ from [U-^{14}C]glutamine (linear regression: $r = 0.981$, $P < 0.001$, and $r = 0.933$, $P < 0.001$ respectively). The fates of the amido nitrogen of glutamine [namely conversion into NH_3, asparagine and hexosamine (see below)] and of the amino nitrogen (namely conversion into alanine and aspartate) almost completely account for the rate of glutamine metabolism. The present data do not allow us to comment on the fate of the carbon skeleton of glutamine. Endogenous and substrate-dependent rates of O_2 consumption by chondrosarcoma tissue suspensions varied considerably among preparations and also within each preparation. Despite this, the addition of glutamine (10 mM) to the tissue consistently caused an increase in O_2 consumption over the endogenous rate [mean increase 16±1% over the endogenous rate ($n = 3$ experiments; $P < 0.001$)].

Glutamine does not activate or inhibit lactate and CO_2 production from glucose, and the increased rate of pyruvate synthesis in the presence of both substrates is no more than additive. However, alanine production is clearly potentiated in the presence of glutamine plus glucose (Table 1). At the same time the rate of aspartate production from glutamine is decreased ($P < 0.001$), but no other metabolites are significantly affected. Hence there is no clear-cut pattern of activation or inhibition of metabolism by either glucose of glutamine. In contrast, in the lymphocyte the metabolism of glucose and glutamine are mutually stimulated [20].

The rates of metabolism of both glucose and glutamine by chondrosarcoma are comparable with those in some other rapidly dividing cell types (Table 2). In contrast with these isolated cells, chondrosarcoma tissue is composed largely of extracellular matrix with approx. 30% of its dry weight accounted for by collagen and chondroitin sulphate alone [9]. Therefore the rates of metabolism per g dry wt. of isolated chondrocytes (2×10^8 cells per g dry wt. of tissue) would be significantly greater than shown. Thus glutamine utilization by the chondrosarcoma is equivalent to over 30% of the rate of glucose utilization and, allowing for the contribution of extracellular matrix to dry weight, glutaminolysis in the chondrosarcoma chondrocyte is at least equivalent to that in the lymphocyte.

Table 2. Comparison of rates of glucose and glutamine utilization in different cell types

Data for enterocytes, lymphocytes and colonocytes were taken from refs. [19], [20] and [21] respectively. Substrate concentrations were: *, 2 mM; **, 5 mM, ***, 10 mM.

Cell type	Substrate...	Rate of utilization (μmol/h per g dry wt.) Glucose	Glutamine
Enterocyte		720***	660**
Lymphocyte		37**	160*
Colonocyte		391***	330**
Chondrosarcoma chondrocyte		233**	80***

Two properties of chondrosarcoma chondrocytes may be important in the context of glutamine utilization. Firstly, they are tumour cells, and high rates of glycolysis and of glutamine utilization are characteristic of rapidly dividing cells [23]. Secondly, they are chondrocytes and have a mandatory requirement for glutamine in the synthesis of glycosaminoglycans, major extracellular matrix components of cartilage and chondrosarcoma [24].

Adenine nucleotide concentrations in chondrosarcoma tissue

An energy substrate must have the capacity to maintain intracellular ATP concentrations, at least under basal conditions. Thus we investigated whether glutamine has this capacity in the chondrosarcoma. Adenine nucleotides were measured by h.p.l.c. The concentrations (Table 3) are approximately one-tenth those in liver [25], but the actual intracellular concentrations are presumably substantially higher, given that the extracellular matrix accounts for at least 30% of the total chondrosarcoma on a weight basis.

In the presence of both glucose and glutamine, the concentrations of ATP and total nucleotides were maintained over a 2 h incubation period (Table 3). The [ATP]/[AMP] ratio decreased only marginally during this time. In the absence of glucose, the ATP and total nucleotide concentrations decreased significantly over 2 h, as did the [ATP]/[AMP] ratio, irrespective of whether glutamine was present. Thus the utilization of glutamine alone does not provide sufficient ATP to sustain the energy status of the chondrosarcoma. This might imply that the major role of glutamine utilization in the chondrosarcoma chondrocytes is in the generation of UDP-*N*-acetylhexosamines for glycosaminoglycan synthesis, although this explanation fails to account for the release of NH_3 and the increase in O_2 consumption produced by glutamine. Therefore we investigated whether the high level of utilization of glutamine corresponds to a high rate of glycosaminoglycan synthesis by the tumour.

Glycosaminoglycan synthesis by chondrosarcoma chondrocytes

With glucose as the sole exogenous substrate, the basal rate of glycosaminoglycan synthesis by chondrosarcoma chondrocytes was increased (Table 4). Glutamine stimulated glycosaminoglycan synthesis to a small extent, but in the presence of both substrates the rate of synthesis was potentiated. This is presumably due to the generation of ATP from glucose accompanied by the provision of precursors from glutamine. Glutamine could be replaced by glucosamine, which is an alternative precursor of UDP-*N*-acetylhexosamines in glycosaminoglycan synthesis but is not an energy substrate. This demonstrates that, although the cells have the capacity for glutamine utilization, there is no mandatory requirement for it in glycosaminoglycan synthesis, other than as an amide donor in hexosamine synthesis.

The glycosaminoglycans synthesized by the chondrosarcoma are chondroitin 4-sulphate and a small amount of chondroitin 6-sulphate [26], each composed of the repeating disaccharide unit D-glucuronic acid-*N*-acetyl-D-galactosamine *O*-sulphate. Thus for each mol of sulphate incorporated into these glycosaminoglycans 1 mol of glutamine would be utilized in generating the hexosamine moiety. The rate of glycosaminoglycan synthesis (Table 4) in the presence of glucose plus glutamine (3.5 μmol of [^{35}S]sulphate incorporated/h per g dry wt.)

Table 3. Concentrations of adenine nucleotides in incubated chondrosarcoma chondrocytes

Suspensions of chondrosarcoma tissue were incubated for up to 2 h at 37 °C with substrate (5 mM-glucose; 10 mM-glutamine). Nucleotides were measured in $HClO_4$ extracts of the tissue. Means ± S.E.M. are given ($n = 9$ for 0 min values; $n = 3$ or 4 for 60 min and 120 min values). Significant differences, calculated by Student's t test, from control (0 min) concentrations of nucleotides are indicated: *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

Substrate	Incubation time (min)	Nucleotide concentration (μmol per g dry wt.) ATP	ADP	AMP	Total adenine nucleotides	[ATP]/[AMP] ratio
	0	3.2±0.2	1.7±0.1	0.4±0.1	5.3	8.4
Glucose + glutamine	60	3.4±0.3	1.1±0.2*	0.4±0.1	4.9	8.6
	120	3.4±0.3	1.2±0.1	0.5±0.1	5.1	7.0
Glutamine	60	2.2±0.3*	1.5±0.2	0.8±0.3	4.4	2.9
	120	1.5±0.3***	1.3±0.2	1.0±0.1***	3.8	1.5
None	120	1.5±0.2***	1.0±0.1*	0.9±0.2**	3.4	1.7

Table 4. Incorporation of [^{35}S]sulphate into glycosaminoglycans by Swarm rat chondrosarcoma tissue

Chondrosarcoma tissue was incubated with substrate(s) and 20 μCi of [^{35}S]sulphate/ml, and [^{35}S]glycosaminoglycans were measured. Data are presented as means ± S.E.M. and are for a single experiment ($n = 6$). Similar data were obtained from four other preparations of the tissue. Significant differences from the control (without substrate) were calculated by Student's t method: $*P < 0.005$; $**P < 0.001$.

Substrate	[^{35}S]Sulphate incorporated (μmol/h per g dry wt.)
None	0.47 ± 0.02
Glucose (5 mM)	1.24 ± 0.09**
Glutamine (10 mM)	0.69 ± 0.07*
Glucosamine (2 mM)	0.93 ± 0.16**
Glucose (5 mM) + glutamine (10 mM)	3.50 ± 0.49**
Glucose (5 mM) + glucosamine (2 mM)	2.43 ± 0.04**

is much lower than the rate of glutamine disappearance (Table 1). This implies that glutamine has an additional role in these cells apart from providing hexosamine precursors for glycosaminoglycan synthesis.

Conclusions

The results indicate that the rat chondrosarcoma chondrocyte is extremely active in anaerobic metabolism, even under aerobic conditions. Glycolysis provides for the energy requirements of the cell and biosynthetic processes such as proteoglycan synthesis. Decreased glycolytic activity might therefore be expected to lead to depletion of cartilage-matrix proteoglycans. Metabolism of glucose via the pentose phosphate pathways operates at a low rate compared with flux through the glycolytic pathway.

The chondrosarcoma shows evidence of significant glutamine consumption at a rate at least 20-fold in excess of the level required for synthesis of hexosamine precursors for glycosaminoglycans. However, glutamine is not an energy substrate for these cells, and the significance of its high rate of utilization in the chondrosarcoma chondrocytes is presently unknown. It is noteworthy that we have so far been unable to detect citric acid-cycle intermediates coming from [U-^{14}C]glutamine (C. A. Spencer, unpublished work), and so glutamine metabolism in these cells may follow a pathway different from that proposed by Hanson & Parsons [22].

We are grateful to CIBA–GEIGY Pharmaceuticals, Horsham, Sussex, U.K., and to the Central Research Fund of the University of London for financial support.

Received 20 July 1989/17 November 1989; accepted 30 November 1989

REFERENCES

1. Stockwell, R. A. (1979). Biology of Cartilage Cells, pp. 81–85, Cambridge University Press, Cambridge
2. Oegama, T. R., Jr. & Thompson, R. C., Jr. (1986) in Articular Cartilage Biochemistry (Kuettner, K. E., Schleyerbach, R. & Hascall, V. C., eds.), pp. 257–271, Raven Press, New York
3. Yamamoto, T. & Gay, C. V. (1988) J. Histochem. Cytochem. **36**, 1161–1166
4. Sampson, H. W. & Cannon, M. S. (1986) Histochem. J. **18**, 233–238
5. Kimura, J. H., Hardingham, T. E., Hascall, V. C. & Solursh, M. (1979) J. Biol. Chem. **254**, 2600–2609
6. Thonar, E. J.-M. A., Lohmander, L. S., Kimura, J. H., Fellini, S. A., Yanagishita, M. & Hascall, V. C. (1983) J. Biol. Chem. **258**, 11564–11570
7. Hascall, G. K. (1980) Anat. Rec. **198**, 135–146
8. Lancaster, C. A., Fryer, P. R., Griffiths, S. & Mason, R. M. (1989) J. Cell Sci. **92**, 271–280
9. Mason, R. M. & Bansal, M. K. (1987) Connect. Tissue Res. **16**, 177–185
10. Palmer, T. N., Caldecourt, M. A. & Sugden, M. C. (1983) Biochem. J. **216**, 63–70
11. Williamson, D. H. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1679–1682, Academic Press, London
12. Kun, E. & Kearney, E. B. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1802–1805, Academic Press, London
13. Bergmeyer, H. U., Bernt, E., Möllering, H. & Pfleiderer, G. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1696–1700, Academic Press, London
14. Bernt, E. & Bergmeyer, H. U. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1704–1708, Academic Press, London
15. Gutmann, I. & Wahlefeld, A. W. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1464–1468, Academic Press, London
16. Gutmann, I. & Wahlefeld, A. W. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1585–1589, Academic Press, London
17. Czok, R. & Lamprecht, W. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 1446–1451, Academic Press, London
18. Hearse, D. J. (1984) Cardiovasc. Res. **18**, 384–390
19. Watford, M., Lund, P. & Krebs, H. A. (1979) Biochem. J. **178**, 589–596
20. Ardawi, M. S. M. & Newsholme, E. A. (1983) Biochem. J. **212**, 835–842
21. Ardawi, M. S. M. & Newsholme, E. A. (1985) Biochem. J. **231**, 713–719
22. Hanson, P. J. & Parsons, D. S. (1980) Biochem. Soc. Trans. **8**, 506–509
23. Newsholme, E. A., Crabtree, B. & Ardawi, M. S. M. (1985) Biosci. Rep. **5**, 393–400
24. Bollet, A. J. & Shuster, A. (1960) J. Clin. Invest. **39**, 1114–1118
25. Williamson, D. H. & Brosnan, J. T. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 2266–2302, Academic Press, London
26. Mason, R. M., Kimura, J. H. & Hascall, V. C. (1982) J. Biol. Chem. **257**, 2236–2245