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Glucose and glutamine metabolism in rat thymocytes

Karl BRAND,*‡ John F. WILLIAMS† and Maurice J. WEIDEMANN†
**Institute for Physiological Chemistry, University of Erlangen-Nuremberg, D-8520 Erlangen, Federal Republic of Germany, and †Department of Biochemistry, Faculty of Science, The Australian National University, Canberra, A.C.T. 2600, Australia*

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1. The metabolism of glucose and glutamine in freshly prepared resting and concanavalin A-stimulated rat thymocytes was studied. Concanavalin A addition enhanced uptake of both glucose and glutamine and led to an increase in oxidative degradation of both substrates to CO_2. 2. With variously labelled [^{14}C]glucose, it was shown that the pathways of glucose dissimilation were equally stimulated by the mitogen. A disproportionately large percentage of the extra glucose taken up was converted into lactate, but concanavalin A also caused an increase in the oxidation of pyruvate as judged by the enhanced release of $^{14}CO_2$ from [2-^{14}C]-, [3,4-^{14}C]- and [6-^{14}C]-glucose. Addition of glutamine did not affect glucose metabolism. 3. The major end products of glutamine metabolism by resting and mitogen-stimulated rat thymocytes were glutamate, aspartate, CO_2 and NH_3. Virtually no lactate was formed from glutamine. Concanavalin A enhanced the formation of all end products except glutamate, indicating that more glutamine was metabolized beyond the stage of glutamate in the mitogen-activated cells. Addition of glucose caused a significant decrease in the rates of glutamine utilization and conversion into aspartate and CO_2 in the absence and in the presence of concanavalin A. 4. In the presence of glucose, almost all nitrogen of the metabolized glutamine was accounted for as NH_3 released via the glutaminase and/or glutamate dehydrogenase reactions. In the absence of glucose, part (18%) of the glutamine nitrogen was metabolized by the resting and to a larger extent (38%) by the mitogen-stimulated thymocytes via a transaminase or amidotransferase reaction.

Enhanced aerobic glycolysis is not unique to malignantly transformed cells, but has been observed also in mitogenically transformed lymphocytes (Roos & Loos, 1973; Culvenor & Weidemann, 1976; Hume *et al.*, 1978*a*; Hume & Weidemann, 1979). It has been suggested that stimulation of glycolysis by concanavalin A results from the co-ordinated activation of plasma-membrane glucose transport and phosphofructokinase in thymocytes (Yasmeen *et al.*, 1977; Kolbuch-Braddon & Weidemann, 1981). Glutamine utilization and oxidation is also stimulated by concanavalin A in lymphocytes isolated from mesenteric lymph nodes (Ardawi & Newsholme, 1983).

Mitogen-induced lymphocyte activation is a useful system for distinguishing metabolic differences between tumour-cell growth and controlled cell proliferation. In this paper we report a quantitative assessment of the metabolic fate of carbon from variously labelled [^{14}C]glucose in control and mitogenically stimulated immature rat thymocytes. In addition, the extent of glutamine metabolism and its metabolic fate have been investigated in these cells by measurement of the intermediates and products that accumulate during incubation of thymocytes with [U-^{14}C]-glutamine. Furthermore the effects of glucose on glutamine metabolism and of glutamine on glucose metabolism have been studied.

‡ To whom reprint requests should be addressed.

Materials and methods

Materials

Female outbred Wistar albino rats (7–9 weeks old) were used for all experiments. [U-^{14}C]-, [1-

^{14}C]-, [2-^{14}C]- and [6-^{14}C]-glucose and [U-^{14}C]-glutamine were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. [3,4-^{14}C]-Glucose was purchased from New England Nuclear, Boston, MA, U.S.A. Concanavalin A was from Sigma London Chemical Co., Poole, Dorset, U.K. L-Glutamine (A grade) was from Calbiochem–Behring, Div. Australian, Pty. Ltd. Kingsgrove, N.S.W., Australia, or E. Merck, Darmstadt, Germany. α-D-Glucose and 2-phenethylamine were from Merck. Enzymes, substrates and coenzymes used in metabolite assays were obtained from Boehringer, Mannheim, Germany. Dowex 50W-X8 (H^+ form, 100–200 mesh) and Dowex 1-X8 (acetate form, 100–200 mesh) were obtained from Bio-Rad Laboratories Pty. Ltd., Epping, N.S.W., Australia.

Methods

Rat thymus lymphocytes were prepared as described by Culvenor & Weidemann (1976); viability was greater than 90% as judged by Trypan Blue exclusion. Incubations were performed at 37°C in 20ml plastic scintillation vials. Freshly prepared thymocytes (at a density of 1×10^8–5×10^8 cells) were incubated in a total volume of 5ml of incubation medium, which consisted of phosphate-buffered saline, pH7.4 (Culvenor & Weidemann, 1976). Vials were gassed with 100% O_2 for 20s, then tightly stoppered with rubber caps and shaken continuously (120 oscillations/min). Incubations were initiated by the addition of [^{14}C]-glucose (4mM) or [^{14}C]glutamine (4mM) as substrate and terminated after 3h by the addition of 1ml of 1M-$HClO_4$. Shaking was continued for an additional 30min to trap all the $^{14}CO_2$ evolved in 0.5ml of phenethylamine. The specific radioactivity of the various [^{14}C]glucoses varied from 40×10^9 to 110×10^9d.p.m./mol, except for [3,4-^{14}C]glucose, of specific radioactivity in the range 15×10^9–20×10^9d.p.m./mol. The specific radioactivity of [U-^{14}C]glutamine was in the range of 10×10^9–20×10^9d.p.m./mol. In pilot studies it was established that substrate utilization and $^{14}CO_2$ release were linear with time for up to 4h. Samples in which 1M-$HClO_4$ was injected before the addition of radioactive substrate were used as blanks (0min of incubation). The $HClO_4$-insoluble cell pellet was washed twice with 0.1M-$HClO_4$. The combined supernatant fractions were neutralized with 1M-KOH and adjusted with buffer to a final volume of 10ml. Phenethylamine, which was placed in an Eppendorf cup during the incubation, was transferred quantitatively to scintillation vials and the radioactivity determined. The minimal amount of $^{14}CO_2$ radioactivity in blanks stopped at zero time was subtracted from the experimental assays.

Metabolite assays

Metabolites in neutralized cell extracts were determined spectrophotometrically by using enzymic assays: glucose by the coupled hexokinase/glucose-6-phosphate dehydrogenase method described by Bergmeyer *et al.* (1974*a*); glutamine and NH_3 by the method of Windmueller & Spaeth (1974); glutamate by the method of Bernt & Bergmeyer (1974); lactate by the method of Gawehn & Bergmeyer (1974); aspartate by the method of Bergmeyer *et al.* (1974*b*); and pyruvate by the method of Czok & Lamprecht (1974). The chromatographic fractionation of extracts was performed essentially as described by Quadflieg & Brand (1978), with tandem ion-exchange columns. The amino acid fraction was separated by eluting the Dowex 50W (H^+ form) with 2M-NH_3. The eluate was evaporated to dryness, the amino acid residue was dissolved in 1ml of distilled water and a 100μl sample taken for amino acid analysis with a Biotronic amino acid analyser model LC 6000.

Results and discussion

Quantitative aspects of [^{14}C]glucose metabolism

Hume *et al.*, (1978*a*) reported that concanavalin A addition doubled [U-^{14}C]glucose uptake by rat thymocytes and caused a specific increase in pyruvate oxidation and a disproportionately large conversion of glucose into lactate. Acetoacetate addition strongly suppressed pyruvate oxidation in the presence of [U-^{14}C]glucose, but did not prevent concanavalin A from stimulating this process. In an extension of this work, using variously ^{14}C-labelled glucose, we have studied the effects of concanavalin A and glutamine on the individual reactions of aerobic glucose degradation. Concanavalin A significantly increased glucose utilization, lactate production and $^{14}CO_2$ release from [U-^{14}C]glucose, confirming the results of Hume *et al.* (1978*a*). Glutamine (4mM) did not affect these reactions when compared with the control incubations (no additions), although thymocytes utilized considerable amounts of glutamine in the presence of 4mM-glucose (Table 1). Concanavalin A caused an almost equivalent increase of $^{14}CO_2$ liberation from [^{14}C]glucose labelled in carbon atoms 1, 2, 3 and 4 and 6 (Table 2). The enhanced $^{14}CO_2$ released from [3,4-^{14}C]glucose in the presence of the mitogen demonstrates directly that pyruvate oxidation is stimulated to the extent suggested by Hume *et al.* (1978*a*,*b*). The fact that the ratio of $^{14}CO_2$ released from [1-^{14}C]glucose to that from [6-^{14}C]glucose is both small and similar in the absence (1.5) and presence (1.3) of concanavalin A suggests that glucose metabolism via the oxidative segment of the pentose pathway is not

Table 1. *Effects of glutamine and concanavalin A on glucose metabolism of rat thymocytes*
Mean values ± S.E.M. are given as μmol/60min per 10^{10} viable cells, with the numbers of experiments in parentheses. Glucose concentration in all incubations was 4mM. For details, see the Materials and methods section. Significant differences from controls (no addition) are shown by $^{a}P<0.001$ and $^{b}P<0.05$.

Additions	Glucose utilized	$^{14}CO_2$ liberated from [U-^{14}C]glucose	Lactate formed	Pyruvate formed	Glutamine utilized	Glutamate formed
None	24.5±1.2 (21)	28.2±2.6 (5)	19.4±1.5 (19)	1.2±0.1 (19)	–	–
Glutamine (4mM)	22.4±1.2 (19)	23.4±2.6 (5)	19.0±1.1 (20)	0.9±0.1 (21)	21.5±2.7 (10)	17.8±1.9 (10)
Concanavalin A (100 μg/incubation)	45.4[a]±1.3 (22)	41.6[b]±3.6 (5)	52.0[a]±2.5 (21)	2.3±0.3 (21)	–	–

Table 2. *Effects of glutamine and concanavalin A on $^{14}CO_2$ production from various ^{14}C-labelled glucoses by rat thymocytes*
Mean values ± S.E.M. for five experiments are given as μmol/60min per 10^{10} cells. Glucose concentration in all incubations was 4mM. Significant difference from controls (no addition) is shown by $^{a}P<0.05$.

	$^{14}CO_2$ released from glucose labelled in position:						
Additions	C-1	C-2	C-3,4	C-6	U	C-1 / C-6	F-type pentose cycle (%)
None	3.5±0.4	5.1±0.7	14.7±2.7	2.4±0.4	28.2±2.6	1.5	1.7
Glutamine (4mM)	3.0±0.3	4.0±0.7	14.1±3.0	1.6[a]±0.3	23.4±2.6	1.9	2.3
Concanavalin A (100μg)	4.7±0.5	7.0±1.0	21.0[a]±2.6	3.6±0.5	41.6±3.6	1.3	0.9

enhanced relative to tricarboxylic acid-cycle oxidations in mitogen-activated rat thymocytes. This conclusion is confirmed by the very low measured values (Katz & Wood, 1963) of the F-type (Longenecker & Williams, 1980) pentose cycle (less than 2.5%; Table 2) in thymocytes irrespective of the presence of concanavalin A or glutamine. Glutamine, on the other hand, decreased glucose oxidation via the tricarboxylic acid cycle, as judged from the diminished $^{14}CO_2$ release from [6-^{14}C]glucose, resulting in a slight increase in the ratio of $^{14}CO_2$ release from [1-^{14}C]glucose to that from [6-^{14}C]glucose, from 1.5 to 1.9. From the results reported in Tables 1 and 2 it appears that concanavalin A caused an increase in glucose metabolism in rat thymocytes by an approximately equal enhancement of all reactions of glucose dissimilation (except the F-pentose cycle), whereas glutamine slightly decreased glucose oxidation via the citric acid cycle.

It is of interest to compare our results, obtained with immature rat thymocytes, with those reported by Ardawi & Newsholme (1983), who used mature T-lymphocytes from rat mesenteric lymph nodes. The rates of glucose utilization in their experiments were slightly higher than those reported here, but, in contrast with our findings, they observed an increase in glucose utilization from 37.1 to 60.8 μmol/h per g dry wt. in the presence of 2mM-glutamine. In the present experiments 40 and 42% of the glucose taken up by unstimulated thymocytes was recovered in lactate in the presence and the absence of 4mM-glutamine respectively, whereas Ardawi & Newsholme (1983) recovered 55 and 79% respectively. Concanavalin A in the incubations reported here increased the conversion of glucose into lactate in immature thymocytes to 57%.

Quantitative aspects of [U-^{14}C]glutamine metabolism

The rate of glutamine utilization by incubated rat thymocytes was 0.59 μmol/min per 10^{10} cells, which is approximately one-fifth of that measured in mature lymphocytes (Ardawi & Newsholme, 1983). The major end products were glutamate (61%), aspartate (25%) and $^{14}CO_2$ (21%), yielding a complete carbon balance (Table 3). Approx. 50% of the glutamine metabolized beyond the stage of glutamate could be recovered as aspartate, and the remainder as $^{14}CO_2$, indicating that glutamine in these cells significantly contributed to respiration. The same recovery of glutamine in the products glutamate (61%) and aspartate (27%) was reported by Ardawi & Newsholme (1983), although they did not measure CO_2. They found insignificant lactate formation when glutamine was the sole substrate.

When both glucose and glutamine were present in the incubation medium, the rate of glutamine utilization by the immature thymocytes decreased significantly, and more glutamate (72%) but less aspartate (13%) was produced. In contrast with our results, Ardawi & Newsholme (1983) observed an increase of almost 1.4-fold in the rate of glutamine utilization by mature lymphocytes incubated with 5mM-glucose, and nearly all of the glutamine utilized was recovered as glutamate (63%) and aspartate (31%). Only 26.7% of the glucose utilized

Table 3. *Effects of glucose and concanavalin A on the metabolism of [U-^{14}C]glutamine by isolated rat thymocytes*

Thymocytes were incubated (1×10^8–4×10^8 cells) for 180 min in 5 ml of incubation medium containing 4 mM-glutamine. For details see the Materials and methods section. Rates of utilization (indicated by a minus sign) or production are presented as means ± S.E.M. and are expressed as μmol/60 min per 10^{10} cells. Significant difference from controls (no additions) is indicated by $^aP < 0.001$ and $^bP < 0.05$. Significant difference from incubation with 100 μg of concanavalin A only is indicated by $^cP < 0.001$, or from that with 4 mM-glucose by $^dP < 0.001$.

Additions to incubations	*n*	Glutamine utilized	Glutamate formed	[^{14}C]Glutamine in aspartate	[^{14}C]Glutamine in CO_2	[^{14}C]Glutamine in protein	Glutamine in products (%)	Ammonia formed	Glucose utilized	Pyruvate formed	Lactate formed	Glucose in trioses (%)
None	11	−35.1 ± 1.6	21.3 ± 2.4	8.7 ± 0.9	7.3 ± 0.5	0.3 ± 0.03	108	40.0 ± 1.1			1.0 ± 0.2	
Glucose (4 mM)	9	−22.7[a] ± 2.1	16.3 ± 1.3	3.0[a] ± 0.3	3.0[a] ± 0.4	1.1[a] ± 0.1	103	28.3[a] ± 1.8	−24.3 ± 0.5	1.0 ± 0.2	12.0 ± 0.7	26.7
Concanavalin A (100 μg/incubation)	11	−45.3[a] ± 0.8	21.7 ± 2.2	15.0[a] ± 0.9	9.3[b] ± 1.0	0.4[b] ± 0.1	102	42.7 ± 2.7			0.3 ± 0.1	
Glucose (4 mM) + concanavalin A	10	−20.4[a,c] ± 1.3	13.8[b] ± 1.2	2.0[a,c] ± 0.1	5.0[a,c] ± 0.2	1.4[a,c] ± 0.1	109	21.7[a,c] ± 0.5	−43.3[d] ± 2.1	2.3[d] ± 0.1	39.0[d] ± 0.1	47.7

by the immature thymocytes was converted into lactate and pyruvate in the presence of 4 mM-glutamine.

Concanavalin A caused a significant stimulation of glutamine utilization and a change in the pattern of product formation. Relatively less glutamate (49%), but more aspartate (33%), was produced in comparison with control incubations. The absolute, but not the relative (21%), amount of $^{14}CO_2$ released from [U-^{14}C]glutamine was significantly increased in the mitogen-activated thymocytes, suggesting that glutamine is a suitable energy substrate for proliferating thymocytes. All of the increased glutamine utilized was accounted for as glutamate, aspartate and CO_2. It is noteworthy that concanavalin A had a similar effect on glutamine metabolism in mature lymphocytes. Ardawi & Newsholme (1983) observed a 1.5-fold increase in glutamine utilization, but without a corresponding increase in glutamate production, indicating that, consistent with our finding, more glutamine was metabolized via the citric acid cycle in mitogen-stimulated lymphocytes. Addition of glucose to incubations containing glutamine and concanavalin A not only abolished the mitogen-induced increment of glutamine utilization by rat thymocytes but also decreased this rate below that observed in the controls. The rates of substrate utilization and product formation were similar to those found when glucose and glutamine but no mitogen were present in the incubations (Table 3). From these results it can be concluded that glucose rather than glutamine is the preferred fuel of respiration for immature rat thymocytes. This conclusion is supported by the following findings: (i) glucose utilization can be stimulated by concanavalin A (Table 1) even in the presence of glutamine (Table 3); (ii) addition of glucose causes a decrease in the extent of glutamine metabolism (Table 3), whereas addition of glutamine does not decrease glucose metabolism by rat thymocytes (Table 1). Approx. 48% of the increased glucose utilized in the mitogen-activated cells could be accounted for as lactate.

The marked differences between our results, obtained with immature rat thymocytes, and those of Ardawi & Newsholme (1983), who used mature lymphocytes from mesenteric lymph nodes, mainly concern the mutual effects of glucose and glutamine on their respective metabolic fates. In the immature cells, although glutamine had no significant effect on glucose metabolism, glucose markedly decreased the rates of glutamine utilization and product formation, even in the presence of concanavalin A. In mature lymphocytes, however, glutamine stimulated the rate of glucose utilization and glucose increased, rather than decreased, glutamine metabolism: all of the increased glucose

utilization could be accounted for as lactate, and all of the increased glutamine utilization could be accounted for as glutamate and aspartate (Ardawi & Newsholme, 1983). Thus the oxidative degradation of neither substrate was increased. In the immature thymocytes, however, the presence of 4mM-glucose not only decreased the rate of glutamine utilization but caused an even more pronounced decrease in the conversion of glutamine into aspartate. Incorporation of [U-^{14}C]-glutamine into protein was slightly enhanced in incubations with glucose present (Table 3). It is particularly striking that in the absence of glucose virtually no lactate was detected in the incubations, indicating that, in contrast with mature lymphocytes, no pathway was active in converting glutamine carbon atoms into lactate. As reported previously with sliced rat spleen (Suter & Weidemann, 1976), glucose also affected the rate of ammonia formation from glutamine during the incubation. From the measured rates of glutamine utilization and glutamate formation it is possible to calculate the amount of ammonia that could theoretically be liberated by the action of glutaminase and glutamate dehydrogenase during the incubation. Without additions this calculation yields 48.9, with glucose present, 29.1, with concanavalin A present, 68.9, and with both glucose and concanavalin A, 27.0 (μmol/h per 10^{10} cells). Comparing these calculated values of ammonia formation with those actually measured (Table 3), it appears that in the presence of glucose virtually all of the glutamine nitrogen not accounted for as glutamate is converted into ammonia. In the controls (8.9μmol), and especially in the incubation with concanavalin A present (26.2μmol), a substantial amount of glutamine nitrogen was not released as ammonia, but was released via a transaminase or amidotransferase reaction (e.g. for the synthesis of purine and pyrimidine nucleotides). Further studies on glucose and glutamine metabolism in mitogen-stimulated proliferating cultured thymocytes are required to elucidate the contribution of glutamine to the respiratory fuel and energy supply in proliferating cells.

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