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Metabolism of amino acids by cultured rat Sertoli cells

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Abstract

Sertoli cells support spermatogenesis both spatially and energetically; for this reason, these cells have important adaptations. The energetic metabolism of Sertoli cells was adapted to provide lactate and pyruvate to developing germ cells, because these substrates are essential for spermatocytes and spermatids. In this study, we investigated whether Sertoli cells use alanine, leucine, valine, and glycine as energetic substrates and whether the simultaneous addition of other nutrients, such as glucose and glutamine, might affect the metabolism of these amino acids. Alanine, leucine, valine, and glutamine are almost totally oxidized to CO_2 by these cells. In contrast, glycine has been demonstrated to be a poor energetic substrate, being mainly incorporated into proteins, and their metabolism did not change in the presence of palmitic acid, glucose, and/or glutamine. The metabolism of the 3 other amino acids was modified by palmitic acid; besides, glucose changed alanine, leucine, and valine oxidation. Glutamine decreased the oxidation of alanine, leucine, and valine to CO_2. Conversely, both alanine and leucine decreased the oxidation of glutamine. Our present findings show that Sertoli cells can adapt its energy metabolism to the oxidative substrates available to fulfill their role in spermatogenic energetic supply.

1. Introduction

Sertoli cells are the somatic cells of the testis that are essential for testis formation and spermatogenesis. Sertoli cells facilitate the progression of germ cells in spermatozoa via direct contact and by controlling the environmental milieu within the seminiferous tubules [1-3]. It has been demonstrated that Sertoli cells can use glucose aerobically at a high rate and secrete lactate and pyruvate; lactate and pyruvate are essential for the maintenance of germ cells [4-6]. A small percentage of glucose is oxidized to CO_2 and even less is converted to other intermediates. Thus, it seems that glucose is not the major fuel for Sertoli cells.

Little is known about the oxidation of energy substrates other than glucose by Sertoli cells. Jutte et al [7] reported that Sertoli cells incubated with palmitic acid oxidized 66% of this substrate to CO_2, and they suggest that fatty acids seem to be a major energy substrate for Sertoli cells. In other experiments, Sertoli cells were incubated with valine, leucine, and isoleucine; these amino acids were selectively taken up by the cells, but their metabolic pathways were not investigated. Branched chain amino acid aminotransferase activity was found in Sertoli cells, and 15% to 30% of the 4-methyl-2-oxopentanoate formed by transamination of leucine was released, taken up by germ cells and converted to 2-hydroxy-4-methylvalerate [8,9]. However, this is not an energy-producing reaction and its physiological significance is unknown.

Furthermore, Grootegoed et al [10] have shown that besides glucose and fatty acids, the single oxidation of glutamine and leucine can yield much of the required energy by Sertoli cells. Nevertheless, the relative importance of the endogenous metabolism of these substrates on the bioenergetic balance of Sertoli cells, when more than one fuel is present in the culture medium, has not been investigated. The aim of the present work is to study the use of different amino acids as energy substrates by Sertoli cells and to determine if Sertoli cell metabolism can be influenced by the presence of other exogenous substrates. Thus, we studied the effect of alanine, glycine, leucine, valine, and glutamine on Sertoli cell metabolism. The substrates alanine, glycine, valine, and glutamine were chosen because of energy potential of the citric acid cycle and leucine for lipid formation. There are no

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Table 1
^{14}C-amino acid metabolism in Sertoli cells

	Glycine	Alanine	Valine	Leucine	Glutamine
CO_2	134 ± 17	3776 ± 226	3523 ± 270	3804 ± 605	3949 ± 466
Lipids	200 ± 21	582 ± 126	612 ± 76	298 ± 140	341 ± 43
Proteins	1314 ± 72	2923 ± 377	3698 ± 278	1159 ± 84	809 ± 51

The Sertoli cells were incubated for 1 hour in KRb with ^{14}C-amino acids (0.25μCi per 0.2 mmol/L). Results expressed as picomoles of amino acids oxidized to CO_2, converted to lipids, or incorporated to proteins per milligram protein per hour. Values are mean ± standard error. Representative data from 4 independent experiments.

reports about the use of alanine and glycine by Sertoli cells. Furthermore, glutamine is known to decrease branched chain amino acid oxidation and to produce lactate in the nervous system [11] and enterocytes [12]. Lactate is one of the most important products secreted by Sertoli cells for maintenance of germinative cells during spermatogenesis [4]; therefore, we studied the possibility that in Sertoli cells, glutamine can also be used to produce lactate. The study of biochemical interactions among essential energy substrates required to support the high metabolic demand of Sertoli cells may lead to a better understanding of the metabolic cross-talk between Sertoli and germ cells.

2. Materials and methods

2.1. Materials

Hyamine hydroxide was purchased from the JT Baker Chemical Company, Phillisburg, NJ, and L-[U-^{14}C]leucine (315 mCi/mmol), L-[U-^{14}C]alanine (157 mCi/mmol), [U-^{14}C]glycine (57 mCi/mmol), L-[U-^{14}C]valine (266 mCi/mmol), and L-[U-^{14}C]glutamine (277 mCi/mmol) were from Amersham International, Berkinghamshire, UK. Opti Phase Tri safe 3 was from Wallac HisafeR Cocktail. All other chemicals were reagent grade.

2.2. Cell culture

Sertoli cells were prepared from Wistar rats aged 16 to 18 days, as previously described [13], according to the modification of Tung and Fritz [14]. Sertoli cells were plated (2.5×10^5 cells/cm^2) in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 HAM (DMEM/F12) supplemented with 1% fetal calf serum. Cells were cultured in a humidified 5% CO_2 atmosphere. After 24 and 48 hours, the cellular monolayer was washed and fresh medium was added. On the third day after plating, the medium was discarded and the attached Sertoli cells were harvested by centrifugation, after treatment with 0.125% trypsin/0.05% EDTA. The harvested cells were washed with DMEM/F12 supplemented with 5% fetal calf serum, and the concentration of protein was determined by the Lowry method [15]. Cell viability, as judged by trypan blue exclusions, was regularly higher than 90%.

2.3. Substrate oxidation and incorporation into lipids and proteins

Sertoli cells (approximately 6×10^6 cells, corresponding to 2.0 mg of protein), were incubated in 1 mL of Krebs-Ringer bicarbonate (KRb) with the specified concentrations of [^{14}C]-labeled substrates, in the absence or presence of other compounds, which might be used by Sertoli cells as energy-yielding substrates (as described in the legends of figures). Incubation was carried out in flasks after the content was gassed with a 95% O_2/5% CO_2 mixture for 1 minute and then sealed with rubber stopper. The suspension of Sertoli cells was incubated at 34°C for 1 hour in a Dubnoff metabolic shaker (60 cycles/min) according to Dunlop et al [16]. Incubation was stopped by adding

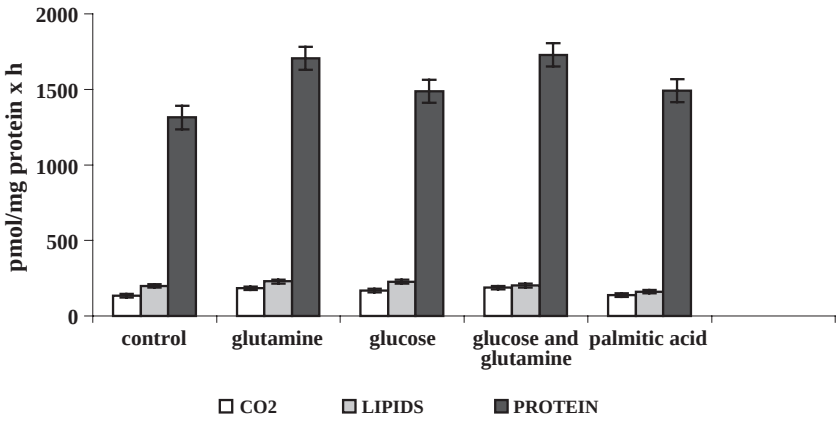

Fig. 1. Effect of the addition of palmitic acid, glucose, and/or glutamine on the metabolism of glycine. The Sertoli cells were incubated for 1 hour in KRb with [U-^{14}C]glycine (0.25μCi/0.2 mmol/L) in the presence or absence of palmitic acid (0.5 mmol/L), D-glucose (5 mmol/L), and/or glutamine (2.0 mmol/L). The oxidation to CO_2, the conversion to lipids, and the incorporation into protein were measured. Data are expressed as means ± SEM (4 different cell preparations).

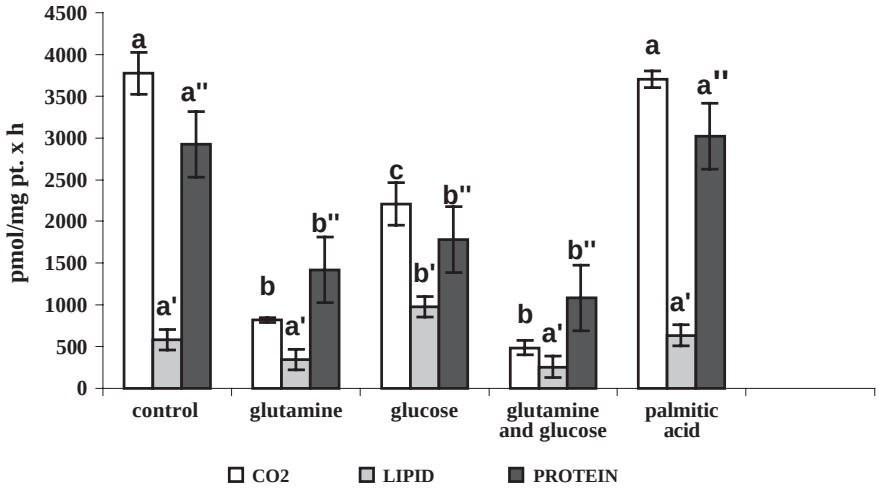

Fig. 2. Effect of the addition of palmitic acid, glucose, and/or glutamine on the metabolism of alanine. The Sertoli cells were incubated for 1 hour in KRb with L-[U-^{14}C]alanine (0.25μCi/0.2 mmol/L) in the presence or absence of palmitic acid (0.5 mmol/L), D-glucose (5 mmol/L), and/or glutamine (2.0 mmol/L). The oxidation to CO_2, the conversion to lipids, and the incorporation into protein were measured. Values are expressed as means ± SEM (four different cell preparations). Different letters indicate statistically significant differences. a different from c, $P < .05$; a different from b, $P < .01$; a′ different from b′, $P < .01$; a″ different from b″, $P < .01$.

0.25 mL of 50% trichloroacetic acid (TCA) through the rubber stopper. Hyamine hydroxide (1 mol/L, 0.25 mL) was then injected into the central wells. The flasks were shaken for an additional 30 minutes at 34°C to trap CO_2. Subsequently, the content of the central well was transferred to vials and assayed for CO_2 radioactivity in a liquid-scintillation counter. The contents of the flask were homogenized and transferred to tubes. After centrifugation, the precipitate was washed 3 times with 10% TCA, and lipids were extracted with chloroform-methanol (2:1). The chloroform-methanol phase was evaporated in vials and radioactivity was measured. The lipid-free protein pellet was dissolved in concentrated formic acid and radioactivity was measured. All results were expressed considering the initial specific activity of the incubation medium. Assays were linear with respect to time of incubation.

2.4. Statistical analysis

Differences among groups were evaluated with analysis of variance, followed by the Duncan multiple range test,

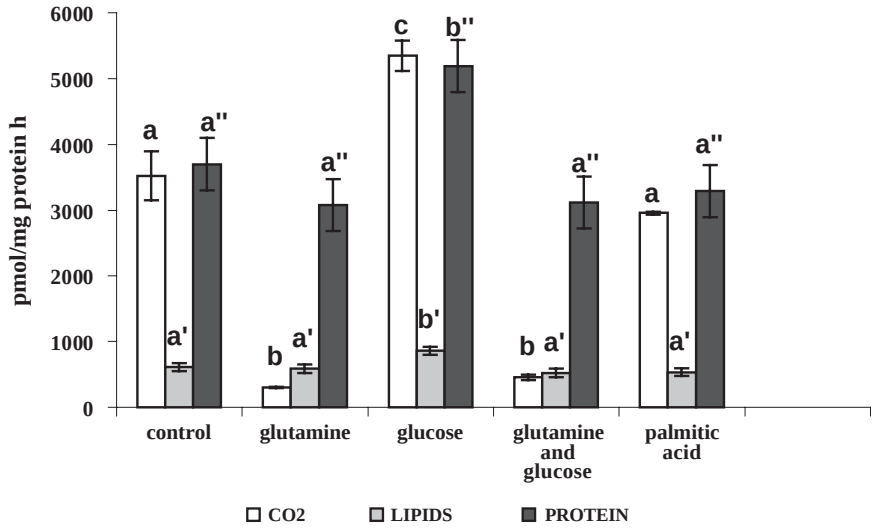

Fig. 3. Effect of the addition of palmitic acid, glucose, and/or glutamine on the metabolism of valine. The Sertoli cells were incubated for 1 hour in KRb with L-[U-^{14}C]valine (0.25μCi per 0.2 mmol/L) in the presence or absence of palmitic acid (0.5 mmol/L), D-glucose (5 mmol/L), and/or glutamine (2.0 mmol/L). The oxidation to CO_2, the conversion to lipids, and the incorporation into protein were measured. Values are expressed as means ± SEM (4 different cell preparations). Different letters indicate statistically significant differences. a different from c, $P < .05$; a different from b, $P < .01$; a′ different from b′, $P < .05$; a″ different from b″, $P < .05$.

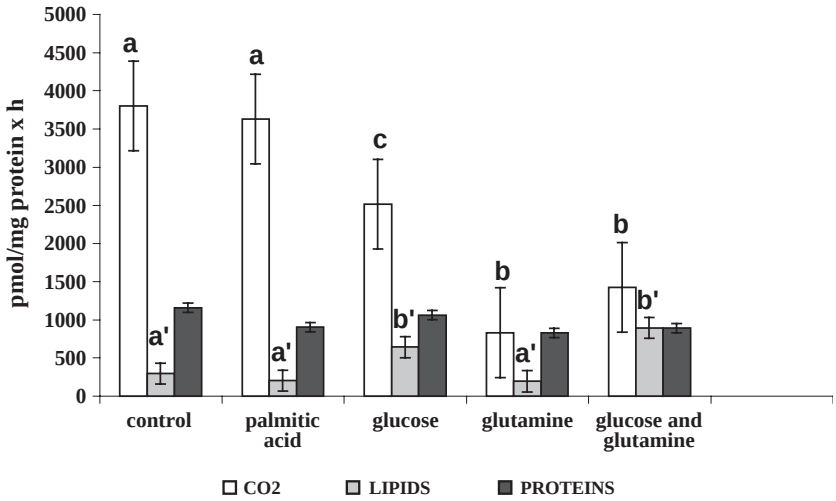

Fig. 4. Effect of the addition of palmitic acid, glucose and/or glutamine on the metabolism of leucine. The Sertoli cells were incubated for 1 hour in KRb with L-[U-^{14}C]leucine (0.25 μCi/0.2 mmol/L) in the presence or absence of palmitic acid (0.5 mmol/L), glucose (5 mmol/L) and/or glutamine (2.0 mmol/L). The oxidation to CO_2, the conversion to lipids and the incorporation into proteins were measured. Values are expressed as means ± SEM (four different cell preparations). Different letters indicate statistically significant differences. a different from c $P < .05$, a different from b $P < .01$, a′ different from b′ $P < .01$.

as appropriate. A probability level of $P < .05$ was considered significant.

3. Results

Table 1 shows basal amino acid metabolism by Sertoli cells. [U-^{14}C]glycine showed the lowest CO_2 production. In contrast, the incorporation of leucine and glutamine into protein was lower than that of the other amino acids. Alanine and valine were mainly converted to lipids.

Figs. 1-5 show the effect of addition of other possible energy substrates upon the metabolism of these amino acids. Glucose, palmitic acid, and glutamine had no effect on the metabolism of glycine (Fig. 1). Neither did palmitic acid affect the metabolism of any amino acid tested (Figs. 2-5). Both glucose and glutamine (separately or together) decreased CO_2 production and protein synthesis from [U-^{14}C] alanine; however, glucose increased lipid synthesis (Fig. 2).

Fig. 3 shows that glucose increased the oxidation of valine to CO_2. Conversely, glutamine, with or without glucose, diminished this CO_2 production. Glucose also increased lipid and protein synthesis.

With respect to [U-^{14}C]leucine metabolism, glucose and glutamine decreased CO_2 production; however, glucose, but not glutamine, increased the metabolism of leucine to lipids (Fig. 4).

Because it was shown that glutamine decreases CO_2 production from alanine, valine, and leucine, we determined the effect of these amino acids on the metabolism of [U-^{14}C]glutamine. Fig. 5A shows that alanine and leucine decreased the oxidation of glutamine to CO_2, but did not alter either lipid or protein synthesis. Glucose, palmitic acid, and glycine did not affect glutamine metabolism (Fig. 5B). To investigate if glutamine can be converted to lactate, we plated Sertoli cell and cultured it for 24 hours at 32°C with [U-^{14}C]glutamine in the absence of glucose. Incubation medium after elution from Dowex 1-X8 ion-exchange column chromatography was analyzed by thin layer chromatography, and radioactivity was found in the region corresponding to authentic lactic acid (data not shown).

4. Discussion

Our results indicate that glucose is not the major fuel for Sertoli cells. These cells may use other energy substrates when presented alone to the cells, but there are no studies about how these cells satisfy their energy requirements by a mixture of substrates.

The primary aim of the present investigation was to test whether one or other amino acid is the preferred substrate for the bioenergetic balance of the Sertoli cell. Glutamine is reported to be the main energy substrate for proliferating cells [17]. In many tissues, it is mainly oxidized to CO_2, but it also enters the gluconeogenic pathway in liver and kidney. Moreover, in cultured astrocytes, 33% of the glutamate entering Krebs cycle as α-oxoglutarate is converted to lactate [18]. It was reported that cultured Sertoli cells can also use glutamine as an energy substrate [8]. The present paper shows that alanine, leucine, and valine oxidation may also be very important for Sertoli cells. In contrast, glycine does not seem to be an adequate energy substrate, but it is more effectively incorporated into proteins and partially converted to lipids.

In vertebrates, the cleavage of glycine has been demonstrated to be the main pathway by which glycine and serine are catabolized [19]. In the central nervous system (CNS), glycine is tightly linked to intermediary metabolism and can be easily obtained from serine through the withdrawal of one carbon atom by serine hydroxymethyl transferase [20]. This route is also the main pathway for

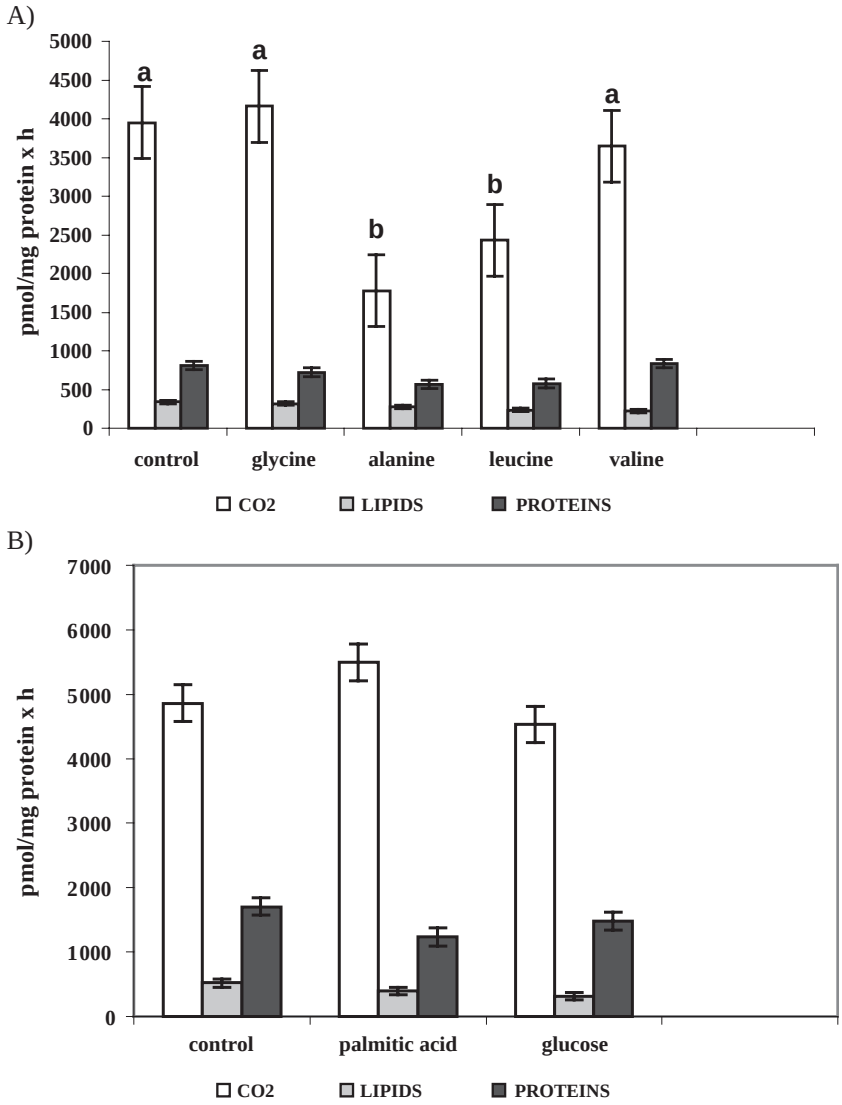

Fig. 5. A and B, Effect of the addition of glycine, alanine, leucine, valine, glucose, and palmitic acid on the metabolism of glutamine. The Sertoli cells were incubated for 1 hour in KRb with L-[U-^{14}C]glutamine (0.25μCi per 2.0 mmol/L). A, In the presence or absence of glycine (1.0 mmol/L), alanine (1.0 mmol/L), leucine (1.0 mmol/L), and valine (1.0 mmol/L). B, In the presence or absence of palmitic acid (0.5 mmol/L) and glucose (5 mmol/L). The oxidation to CO_2, the conversion to lipids, and the incorporation into protein were measured. Values are expressed as means ± SEM (4 different cell preparations). Different letters indicate statistically significant differences (a different from b, $P < .05$).

glycine oxidation in different regions of the rat CNS, where this amino acid can also be converted to serine and incorporated into phospholipids [21]. We have previously observed that [1-^{14}C]glycine is converted to serine in Sertoli cells, and then incorporated into phospholipids (data not shown). However, the oxidative metabolism of glycine has not previously been investigated in Sertoli cells.

In this work, we observed that Sertoli cells has insignificant capacity for oxidized glycine to CO_2; however, creatine that is crucial for providing energy to spermatogenic cells is synthesized in the Sertoli cells from glycine and arginine [22]. Moreover, in Sertoli cells, glycine is used for glutathione (GSH) synthesis, which in turn is released by these cells and may provide GSH as a source for cysteine, as well as reducing power for the spermatogenic cells. In addition, the biosynthetic rate of protamine production is very high during the spermiogenic process, and large amounts of cysteine are required for these cells [23]. The present study shows that the glycine cleavage system in Sertoli cells seems to have other function than that in the CNS, because besides it is to be converted to serine and incorporated into phospholipids, glycine was poorly oxidized to CO_2, and it is likely that the enzymatic activities of the glycine oxidative pathway are not significant in Sertoli cells.

Because alanine, leucine, and valine are preferentially oxidized to CO_2 in Sertoli cells, we investigated whether the presence of glucose could change the catabolism of these amino acids. Sertoli cells do not use glucose as a main energy substrate; as was cited above, Robinson and Fritz [5] showed that only a small fraction of the pyruvate produced

from glucose is used by Sertoli cells via the Krebs cycle. Instead, the pyruvate is converted to lactate and both are secreted by these cells [4,9]. However, glucose modified the metabolism of alanine, leucine, and valine. The oxidation of valine to CO_2, its incorporation into proteins, and its conversion to lipids were increased by glucose, whereas the synthesis of lipids from alanine and leucine was increased, and oxidation to CO_2 was decreased by glucose. Incorporation of alanine into proteins also decreased in the presence of glucose. It is possible that low amounts of acetyl-CoA arising from glucose modify alanine and leucine oxidation to CO_2 by competing with the acetyl-CoA produced by these amino acids. In the case of valine oxidation, the acetyl-CoA reacts with oxaloacetate, which can be produced from valine, to form citrate, which could stimulate the Krebs cycle.

The stimulation of the conversion of valine to lipids by glucose may be the result of stimulation of the pentosis cycle, which has been shown to be an important destination for glucose in Sertoli cells [5]. The oxidation of valine and leucine is dependent upon α-ketodehydrogenase, which is regulated by covalent modification. Glucose, however, has different effects upon the oxidation of valine and leucine to CO_2; thus, it is possible that different α-ketodehydrogenases are present in Sertoli cells, as described in bovine liver [24].

In the CNS of 14-day-old rats, glucose (5-10 mmol/L) stimulates the incorporation of leucine and glycine into proteins [25]. The decrease in the incorporation of L-[U-^{14}C]alanine and the increase in the incorporation of L-[U-^{14}C]valine into proteins in the Sertoli cells, however, are difficult to explain.

It was previously demonstrated that the oxidation of fatty acids is a major energy source for cultured Sertoli cells [7,10]. Nevertheless, it was observed that addition of palmitic acid did not modify the metabolism of any of the amino acids tested.

We postulated that alanine, valine, leucine, or glutamine play an important role as substrates for Sertoli cell energy metabolism. We investigated a possible preference of these cells for one of them. Glutamine inhibited the oxidation of leucine, valine, and alanine, without modifying the conversion of these amino acids to lipids. Glutamine also decreased the incorporation of alanine into proteins. The decreased oxidation of alanine, valine, and leucine may be related to the oxidation of glutamine, which can compete in the transamination reactions, decreasing the formation of ketoacids from these amino acids.

To understand the role of glutamine in the metabolism of alanine, valine, and leucine, we studied the action of these amino acids on glutamine metabolism. In addition, we also studied the effect of glucose and palmitic acid upon this metabolism. Glucose, palmitic acid, and valine did not modify the metabolism of glutamine. However, alanine and leucine decreased glutamine oxidation, possibly because of the transamination reaction previously cited. Pyruvate is formed from alanine via a transaminase reaction and can inhibit the oxidation of glutamine because glutamine is an important source of lactate via transformation of malate to pyruvate by malic enzyme.

The most important finding of the present work is the observation that glycine is not an adequate energy-yielding substrate for Sertoli cells, but it seems probable that glycine may have some physiological significance in protein and phospholipid synthesis in addition to the precursor for creatine and glutathione synthesis. Moreover, it is important to note the 3- to 4-fold decrease in leucine, valine, and alanine oxidation in the presence of glutamine, and the well-known stimulatory effect of exogenous glucose upon lipid synthesis, because glucose replaces the vital citric acid cycle intermediates.

Comparison of data obtained using the various amino acids tested here and the influence exerted by the presence of other energy substrates is crucial for a better understanding of the most important pathways of energy metabolism in the Sertoli cells, as well as the relationship of this metabolism with the development of germinal cells, such as in lactate formation from glutamine, which is essential for the maintenance of germ cells.

Acknowledgments

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References

[1] Jégou B, Sharpe RM. Paracrine mechanisms in testicular control. In: de Krester D, editor. Molecular biology of the male reproductive system. San Diego (Calif): Academic Press; 1993. p. 271-310.
[2] Jégou B. The Sertoli-germ cell communication network in mammals. Int Rev Cytol 1993;147:25-96.
[3] Griswold MD. The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol 1998;9:411-6.
[4] Jutte NH, Jansen R, Grootegoed JA, Rommers FF, Van Der Molen HG. FSH stimulation of the production of pyruvate and lactate by rat Sertoli cells may be involved in hormonal regulation of spermatogenesis. J Reprod Fertil 1983;68:219-26.
[5] Robinson R, Fritz IB. Metabolism of glucose by Sertoli cells in culture. Biol Reprod 1981;24:1032-41.
[6] Grootegoed JA, Jansen R, Van Der Molen HJ. The role of glucose, pyruvate and lactate in ATP production by rats spermatocytes and spermatids. Biochim Biophys Acta 1984;767:248-56.
[7] Jutte NH, Eikvar L, Levy FO, Hansson V. Metabolism of palmitate in cultured rat Sertoli cells. J Reprod Fertil 1985;73:497-503.
[8] Grootegoed JA, Jutte NH, Jansen R, Van Der Molen HJ. Hormonal activation of the supporting role of Sertoli cells in spermatogenesis. Horm Cell Regul 1983;7:299-316.
[9] Grootegoed JA, Jansen R, Van Der Molen HJ. Intercellular pathway of leucine catabolism in rat spermatogenic epithelium. Biochem J 1985;226:889-92.
[10] Grootegoed JA, Oonnk RB, Jansen R, Van Der Molen HJ. Metabolism of radiolabelled energy-yielding substrates by rat Sertoli cells. J Reprod Fertil 1986;77:109-18.
[11] Sonnewald U, Westergaard N, Schouboe A. Glutamate transport and metabolism in astrocytes. Glia 1997;21:56-63.

[12] Watford M. Glutamine metabolism in rat small intestine: synthesis of three-carbon products in isolated enterocytes. Biochim Biophys Acta 1994;1200:73-8.
[13] Guma FCR, Bernard EA. Effect of retinol on glycoprotein synthesis by Sertoli cells in culture: dolichyl phosphomannose synthase activation. Int J Androl 1994;17:50-5.
[14] Tung PS, Fritz IB. Extracellular matrix rat Sertoli cell histotype expression in vitro. Biol Reprod 1984;30:213-27.
[15] Lowry OH, Rosebrough AL, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
[16] Dunlop DS, Van Elden W, Lajtha A. Measurements of rates of protein synthesis in rat brain slices. J Neurochem 1974;22:821-39.
[17] Zielke HR, Ozand PT, Tildon JT, Seudalian DA, Cornblath M. Reciprocal regulation of glucose and glutamine utilization by cultured human diploid fibroblast. J Cell Physiol 1978;95:41-8.
[18] Kaneko T, Iuchi Y, Kobayashi T, Fujii T, Saito H, Kurachi H, et al. The expression of glutathione reductase in the male reproductive system of rats supports the enzymatic basis of glutathione function in spermatogenesis. Eur J Biochem 2002;269:1570-8.
[19] Yoshida T, Kikuchi G. Comparative study on major pathways of glycine and serine catabolism in vertebrate livers. Biochem J 1972;72:1503-16.
[20] Daly EC, Nadi NS, Aprison MH. Regional distribution and properties of glycine cleavage system within the central nervous system of the rat: evidence for endogenous inhibitor during in vitro assay. J Neurochem 1976;36:179-85.
[21] Fagundes IS, Rotta LN, Schweigert ID, Valle SC, Oliveira KR, Krüger AH, et al. Glycine, serine and leucine metabolism in different regions of rat central nervous system. Neurochem Res 2001;26:245-9.
[22] Moore NP, Gray TJB, Timbrell JA. Creatine metabolism in the seminiferous epithelium of rats. I. Creatine synthesis by isolated and cultured cells. J Reprod Fertil 1998;112:325-30.
[23] Westergaard N, Sonnewald U, Schousboe A. Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. Dev Neurosci 1995;17:203-11.
[24] Connelly JL, Danner DJ, Bowden JA. Branched chain alpha-keto acid metabolism. I. Isolation, purification, and partial characterization of bovine liver alpha-ketoisocaproic and alpha-keto-beta-methyl-valeric acid dehydrogenase. J Biol Chem 1968;243:1198-203.
[25] Oliveira KR, Rotta LN, Valle SC, Pilger DA, Nogueira CW, Feoli AM, et al. Ontogenic study of the effects of energetic nutrients on amino acids metabolism of rat cerebral cortex. Neurochem Res 2002;27:513-8.