The maximal activity of phosphate-dependent glutaminase and glutamine metabolism in late-pregnant and peak-lactating rats

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1. The maximal activity of phosphate-dependent glutaminase was increased in the small intestine, decreased in the liver and unchanged in the kidney of late-pregnant rats. This was accompanied by increases in the size of both the small intestine and the liver. 2. The maximal activity of phosphate-dependent glutaminase was increased in both the small intestine and liver but unchanged in the kidney of peak-lactating rats. 3. Enterocytes isolated from late-pregnant or peak-lactating rats exhibited an enhanced rate of utilization of glutamine and production of glutamate, alanine and ammonia. 4. Arteriovenous-difference measurements across the gut showed an increase in the net glutamine removed from the circulation in late-pregnant and peak-lactating rats, which was accompanied by enhanced rates of release of glutamate, alanine and ammonia. 5. Arteriovenous-difference measurements for glutamine showed that both renal uptake and skeletal-muscle release of glutamine were not markedly changed during late pregnancy or peak lactation; but pregnant rats showed a hepatic release of the amino acid. 6. It is concluded that, during late pregnancy and peak lactation, the adaptive changes in glutamine metabolism by the small intestine, kidneys and skeletal muscle of hindlimb are similar; however, the liver appears to release glutamine during late pregnancy, but to utilize glutamine during peak lactation.

INTRODUCTION

Although glutamine is not considered to be a dietary essential amino acid, it has several important roles in mammals (for reviews, see Kovacevic & McGivan, 1983; Ardawi & Newsholme, 1985b; Newsholme *et al.*, 1985); in small intestine, glutamine is known to be a major source of energy (Windmueller, 1984); in kidney, glutamine provides ammonia for acid/base balance (Pitts, 1973); in brain, it may function as a local store of glutamate, which is itself a neurotransmitter (Quastel, 1979); and its utilization and oxidation are important, if not essential, for rapidly dividing cells (Ardawi & Newsholme, 1985b; Newsholme *et al.*, 1985). In the rat, skeletal muscle is considered to be the major site of glutamine synthesis (Snell, 1980); however, the liver could synthesize or release glutamine, depending on the physiological condition of the animal (Lund, 1980).

Pregnancy and lactation are unique physiological conditions during which a number of metabolic changes are observed. In pregnancy, as the conceptus develops, metabolically active hormones of pregnancy are formed and released into the maternal circulation; at the same time the mother must respond to the ever-increasing demands of the conceptus growth, supplying all the materials needed for the synthesis and energy requirements through the placenta, which poses challenges to maternal fuel economy (for reviews, see Yen, 1973; Knopp *et al.*, 1981). In lactation, however, the synthesis of milk in lactating mammary glands requires the redirection of certain maternal fuels (e.g. glucose and fatty acids), which is accompanied by changes in the fuel-hormone relationship (for review, see Bauman & Elliot, 1983).

The regulation of glutamine metabolism could involve adaptive changes in the activity of key enzymes in the pathway of glutamine catabolism. One of these is phosphate-dependent glutaminase (EC 3.5.1.2), hereafter

referred to as 'glutaminase'. To date, there have been no reports on the adaptive changes of glutamine metabolism during late pregnancy or peak lactation. Therefore, the main object of the present work has been to provide information on whether adaptive changes occur during late pregnancy and peak lactation in the activity of glutaminase and glutamine metabolism by tissues known markedly to utilize (small intestine and kidneys) or synthesize (skeletal muscle) glutamine, or both (liver), respectively. The relevance of these changes to the overall regulation of glutamine metabolism is discussed.

MATERIALS AND METHODS

Animals

Female Wistar albino rats (250–300 g) were obtained from King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Virgin and 19-day-pregnant rats (late-pregnant rats) were used and the stage of pregnancy was determined as described previously (Leturque *et al.*, 1980). The average number of foetuses per pregnant rat was ten, and only rats bearing eight or more foetuses were included in the study. Lactating rats with between eight and ten pups were used after a lactation period of 15 days (peak-lactating rats). Non-lactating controls were rats whose pups had been weaned 3–4 weeks previously. Animals were maintained on a standard laboratory diet (Ardawi, 1986a) and water *ad libitum*, and were kept in a controlled environment (constant temperature 24 °C, and a light cycle of 12 h on, 12 h off).

Chemicals and enzymes

All chemicals and enzymes were obtained from the same sources as described previously (Ardawi & Newsholme, 1985a; Ardawi, 1986a).

Table 1. Maximal activity of phosphate-dependent glutaminase in the small intestine of late-pregnant and peak-lactating rats

Intestinal homogenates were isolated and extracted from virgin, 19-day-pregnant, 15-day-lactating and non-lactating rats, and glutaminase activities were determined as described in the Materials and methods section. Values are presented as means $\pm$ S.D., where n is the number of rats used. Values significantly different from virgin or non-lactating controls are shown: * $P < 0.05$; ** $P < 0.001$. Percentage changes in intestinal glutaminase activity of pregnant or lactating rats as compared with the corresponding controls are given in parentheses.

	Small intestine wt. (g)	Glutaminase activity					
		Intestinal-mucosal scrapings			Whole small intestine		
		n	($\mu\text{mol}/\text{min}$ per g wet wt.)	(nmol/min per mg of protein)	n	($\mu\text{mol}/\text{min}$ per g wet wt.)	(nmol/min per mg of protein)
Virgin	4.81 ± 0.47	12	12.61 ± 2.95	117.73 ± 25.97	18	6.84 ± 1.63	33.43 ± 7.45
Pregnant	$7.66 \pm 1.65^{**}$	17	20.92 ± 5.94 (66%)**	$208.18 \pm 35.94^{**}$	19	9.22 ± 1.87 (35%)**	42.87 ± 8.61 (28%)**
Non-lactating	5.02 ± 0.48	6	13.22 ± 2.78	120.50 ± 15.60	6	6.90 ± 1.56	35.10 ± 6.66
Lactating	$6.51 \pm 1.82^{**}$	12	17.58 ± 3.51 (33%)**	$164.86 \pm 32.64^{**}$	12	8.52 ± 2.24 (23%)*	80.12 ± 21.26 (128%)**

Preparation of homogenates and assay of glutaminase activity

Animals were killed by cervical dislocation at day 19 of pregnancy or day 15 of lactation. The small intestine (from the duodenum to the caecum) was rapidly removed, washed by forcing ice-cold 0.9% NaCl through the lumen, and was cut longitudinally. Intestinal mucosa was separated from the underlying muscle by scraping with a microscope slide, weighed and homogenized in 5 vol. of extraction medium (Ardawi & Newsholme, 1982) by using a Polytron homogenizer (PCU-2, at position 3) for 10–20 s at 0 °C. In some experiments, the whole of the small intestine (mucosa plus underlying muscle layers) was extracted as described above. The liver and kidneys were also quickly removed, freed of connective tissue, rinsed in ice-cold saline (0.9% NaCl), blotted dry, and weighed. Liver and kidneys were homogenized in 10 vol. of extraction medium (Ardawi & Newsholme, 1984) by using a Polytron homogenizer (PCU-2, at position 4) for 10 s at 0 °C. Glutaminase activity in intestinal or renal homogenates was determined by the method of Curthoys & Lowry (1973). The hepatic enzyme was assayed by the method described by Watford *et al.* (1984). Tissue preparations were treated immediately before assay with 0.05% (v/v) Triton X-100. Preliminary experiments established that extraction and assay procedures were such as to produce maximum enzyme activities (see Crabtree *et al.*, 1979).

Preparation and incubation of enterocytes

Enterocytes were prepared as described by Watford *et al.* (1979). Incubations of cells were performed at 37 °C and terminated as described previously (Ardawi & Newsholme, 1985a).

Arteriovenous-difference measurements

Rats were anaesthetized with diethyl ether, and blood was withdrawn from venous vessels. Samples were taken into heparinized syringes from hepatic-portal, hepatic, renal and femoral veins and from the abdominal aorta. Samples (0.5–1.0 ml) were quickly added to 1.0 ml of ice-cold HClO_4 (10%, v/v) and used for determination of metabolites after deproteinization and neutralization. Arteriovenous differences were calculated for gut

(aorta–portal vein), kidney (aorta–renal vein), hind-limb (aorta–femoral vein) and liver [$\frac{2}{3}$ portal vein + $\frac{1}{4}$ aorta)–hepatic vein].

Determination of metabolites

Glutamine and other metabolites in neutralized extracts were determined by the same methods as described previously (Ardawi & Newsholme, 1983). Protein was determined by the procedure of Lowry *et al.* (1951).

Expression of results

Glutaminase activity is expressed as μmol of glutamate formed/min per g wet wt. of tissue and as nmol of glutamate formed/min per mg of protein at 37 °C. Changes in concentrations of substrate or metabolites during the incubation of isolated enterocytes were determined from the net change between zero time and 20 min incubation. Rates of substrate utilization or metabolite production are expressed as $\mu\text{mol}/\text{min}$ per g dry wt. of cells. The results are expressed as means $\pm$ S.D., and comparisons between sets of data were made by Student's t test.

RESULTS

Tables 1 and 2 summarize the changes in weight of the small intestine, liver and kidney obtained from late-pregnant or peak-lactating rats. Both the small intestine and liver showed an increase in size, whereas the size of the kidney was not markedly changed.

Intestinal glutaminase activity

The maximal activity of glutaminase in intestinal mucosa of female rats (measured in scrapings or the whole of the small intestine) is higher than those found for male rats [being 63.17 ± 5.75 ($n = 14$) or 7.12 ± 2.03 ($n = 8$) nmol/min per mg of protein measured in scrapings or whole of the small intestine of male rats respectively; see also Budohoski *et al.* (1982), Watford *et al.* (1984) and Ardawi (1986b)], and is presented in Table 1. A marked increase in the activity of glutaminase was evident in both late pregnancy and peak lactation, whether measured in intestinal scrapings or in the whole

Hepatic and renal homogenates were prepared and glutaminase activities were determined as described in the Materials and methods section. Values are presented as means $\pm$ S.D., where n is the number of rats used. Values significantly different from virgin or non-lactating controls are shown: * $P < 0.05$; ** $P < 0.001$. Percentage changes in hepatic or renal glutaminase activities of pregnant and lactating rats as compared with the corresponding controls are given in parentheses.

	Hepatic glutaminase activity			Kidney wt. (% of body wt.)	Renal glutaminase activity			
	Liver wt. (% of body wt.)	<i>n</i>	(μ mol/min per g wet wt.)		(nmol/min per mg of protein)	(μ mol/min per g wet wt.)	(nmol/min per mg of protein)	
Virgin	3.79 $\pm$ 0.37	16	5.20 $\pm$ 0.85	31.18 $\pm$ 4.89	0.34 $\pm$ 0.02	19	26.16 $\pm$ 4.72	244.12 $\pm$ 40.37
Pregnant	4.52 $\pm$ 0.24**	12	3.92 $\pm$ 0.56 (25%)*	22.86 $\pm$ 3.52 (27%)**	0.27 $\pm$ 0.01	12	24.88 $\pm$ 3.14 (5%)	228.36 $\pm$ 33.60 (7%)
Non-lactating	3.91 $\pm$ 0.46	10	5.12 $\pm$ 0.90	30.56 $\pm$ 5.10	0.33 $\pm$ 0.04	6	25.90 $\pm$ 3.80	250.70 $\pm$ 36.10
Lactating	4.23 $\pm$ 0.29*	10	5.89 $\pm$ 0.43 (15%)*	36.09 $\pm$ 2.92 (18%)*	0.31 $\pm$ 0.02	9	24.55 $\pm$ 2.56 (5%)	227.29 $\pm$ 24.48 (9%)

Hepatic and renal glutaminase activity

The maximal activity of renal glutaminase was unchanged during late pregnancy or peak lactation (Table 2).

The rate of glutamine utilization by incubated rat enterocytes was 12.26 or 11.93 $\mu\text{mol}/\text{min}$ per g dry wt. when measured in cells isolated from virgin or non-lactating control rats respectively. Glutamate, alanine and ammonia were the major nitrogenous end-products (Table 3). Late pregnancy or peak lactation, however, caused an increase in the rates of glutamine utilization and of glutamate, alanine and ammonia production (Table 3). These findings are consistent with the increased metabolic capacity of the small intestine of late-pregnant or peak-lactating rats, as indicated by higher glutaminase activities during such conditions (Table 1), and suggest a different form of regulation of intestinal glutamine metabolism.

Arterial glutamine concentration was not markedly changed in late pregnancy or peak lactation (Table 4). Arteriovenous-difference measurements of glutamine and its major nitrogenous end-products across the gut of virgin, late-pregnant, non-lactating and peak-lactating rats are presented in Table 4. Virgin and non-lactating rats exhibited a net glutamine removal from the circulation, which was accompanied by the production of glutamate, alanine and ammonia. These results are consistent with other work (Felig *et al.*, 1973; Schrock & Goldstein, 1981; Brosnan *et al.*, 1983; Watford *et al.*, 1984; Ardawi, 1986b). Net glutamine removal from the circulation was increased by approx. 40 and 19% in late pregnancy and peak lactation respectively, and was accompanied by enhanced rates of release of glutamate, alanine and ammonia (Table 4).

Arteriovenous-difference measurements for glutamine across the liver, kidney and hindlimb in late-pregnant and peak-lactating rats are presented in Table 5. Both renal uptake and skeletal-muscle release of glutamine were not markedly changed during late pregnancy or peak lactation; however, pregnant rats showed a hepatic release of glutamine, rather than its removal.

Table 3. Glutamine metabolism by incubated enterocytes isolated from late-pregnant or peak-lactating rats

Enterocytes were isolated from virgin, 19-day-pregnant, non-lactating and 15-day-lactating rats and incubated in the presence of 5 mM-glutamine as described in the Materials and methods section. Rates are given as means $\pm$ S.D., for five separate experiments. A negative sign indicates utilization. Significant differences in rates of utilization of glutamine or production of glutamate, alanine and ammonia from cells of virgin or non-lactating rats are shown: * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$.

	Rate of utilization or production (μ mol/min per g dry wt.)			
	Glutamine	Glutamate	Alanine	Ammonia
Virgin	-12.26 ± 1.97	3.41 ± 0.34	2.44 ± 0.38	9.92 ± 0.90
Pregnant	$-16.06 \pm 1.70^{**}$	$4.16 \pm 0.45^{*}$	$3.20 \pm 0.30^{**}$	$13.34 \pm 2.14^{*}$
Non-lactating	-11.93 ± 1.31	3.46 ± 0.71	2.62 ± 0.49	9.70 ± 2.80
Lactating	$-17.12 \pm 1.45^{***}$	$4.26 \pm 0.48^{*}$	$3.14 \pm 0.29^{*}$	$13.87 \pm 1.93^{**}$

Table 4. Arteriovenous-difference measurements for glutamine, glutamate, alanine and ammonia across the portal-drained viscera of late-pregnant and peak-lactating rats

Measurements were made as described in the Materials and methods section, and results are presented as means $\pm$ S.D. for five animals. Values are expressed as μ mol of metabolite/ml of whole blood; a negative sign indicates release. Significant differences from virgine or non-lactating controls are shown: * $P < 0.05$; ** $P < 0.005$.

	Arterial glutamine concn.	Arteriovenous difference			
		Glutamine	Glutamate	Alanine	Ammonia
Virgin	0.67 ± 0.09	0.101 ± 0.02	-0.042 ± 0.01	-0.113 ± 0.04	-0.278 ± 0.02
Pregnant	0.59 ± 0.06	$0.141 \pm 0.03^{**}$	$-0.068 \pm 0.01^{**}$	$-0.175 \pm 0.01^{**}$	$-0.322 \pm 0.03^{*}$
Non-lactating	0.64 ± 0.08	0.108 ± 0.03	-0.048 ± 0.01	-0.120 ± 0.06	-0.288 ± 0.05
Lactating	0.62 ± 0.07	$0.129 \pm 0.01^{*}$	$-0.056 \pm 0.01^{*}$	$-0.143 \pm 0.01^{*}$	$-0.298 \pm 0.02^{*}$

Table 5. Arteriovenous-difference measurements for glutamine across the gut, liver, kidney and hindlimb of late-pregnant and peak-lactating rats

Measurements were made as described in the Materials and methods section, and results are presented as means $\pm$ S.D. for five animals. Values are expressed as nM-glutamine in whole blood, and negative signs indicate release.

Tissues	State of animals . . .	Arteriovenous difference (nM)			
		Virgin	Pregnant	Non-lactating	Lactating
Gut		101 ± 22	141 ± 30	123 ± 15	124 ± 10
Liver		31 ± 11	-14 ± 5	23 ± 10	25 ± 12
Kidney		17 ± 5	18 ± 6	16 ± 5	19 ± 7
Hindlimb		-76 ± 19	-90 ± 17	-75 ± 13	-74 ± 10

DISCUSSION

Glutamine metabolism in late pregnancy

Pregnancy is a unique metabolic state in the fuel-hormone relationship in which the mother has to provide fuels not only for her own energy supply, but also for the growth and metabolic needs of the conceptus. As pregnancy advances, the increasing demands of the growing foetus result in certain adaptive responses which ensure the continuous supply of fuels for both maternal and foetal energy metabolism (for reviews, see Yen, 1973; Knopp *et al.*, 1981). The present work demonstrates that late pregnancy is characterized

by an enhanced rate of glutamine utilization by the small intestine both *in vitro* and *in vivo*. This increase in glutamine utilization by the small intestine could be attributed to three factors; first, the sparing of glucose (owing to the decrease in glucose concentration during late pregnancy, as compared with virgin rats, by approx. 22%; $P < 0.05$; results not shown) to be used by other maternal tissues (e.g. brain) and the foetus; the latter has a continuous demand for glucose (see Shambaugh *et al.*, 1977a,b). Thus the role of glutamine as a metabolic fuel is likely to be more important in the small intestine than that of glucose in late pregnancy, and therefore glutamine could partly replace glucose. Second, the

increased rate of intestinal glutamine utilization was accompanied by higher rates of release of alanine during late pregnancy, which could be an adaptive mechanism that ensures a continuous supply of alanine for glucose production via hepatic gluconeogenesis. Third, in pregnant rats there is a marked tendency towards diminished skeletal-muscle amino acid catabolism, which serves to prevent nitrogen losses during the late phase of pregnancy (see Knopp *et al.*, 1981). Consequently, the increased release of alanine via enhanced intestinal glutamine metabolism prevents any increase in alanine release by maternal skeletal muscle.

Glutamine is known to be mainly synthesized and released by skeletal muscle (see Snell, 1980). In the present work, there was no significant increase in the release of glutamine by skeletal muscle during late pregnancy, as indicated by arteriovenous-difference measurements across the hindlimb. However, late pregnancy was characterized by hepatic glutamine release rather than an uptake, which was accompanied by diminished capacity to utilize glutamine (as indicated by lowered hepatic glutaminase activity) (Table 5). The liver is known to function as either a glutamine-synthesizing or -utilizing tissue, depending on the physiological state of the rat. In normal fed (Lund & Watford, 1976), starved (Aikawa *et al.*, 1973), or high-carbohydrate-fed (Remesy *et al.*, 1978) rats there appears to be glutamine output, but livers of rats fed on a high-protein diet remove glutamine (Yamamoto *et al.*, 1974). Therefore, in late pregnancy it is possible that the contribution of the liver to circulating glutamine provides an extra source of this amino acid, which is known to be removed at a considerable rate by the developing foetus (Ishikawa, 1976).

Glutamine metabolism in peak lactation

The present work demonstrates that peak lactation is characterized by enhanced rates of intestinal glutamine metabolism. There are two lines of evidence to support this. First, the potential of glutamine utilization (glutaminase activity) per cell was increased and accompanied by higher rates of glutamine catabolism by enterocytes isolated from peak-lactating rats. Second, a marked increase in net glutamine removal by the small intestine was associated with high rates of release of glutamate, alanine and ammonia.

Lactation in rats and other animals is usually associated with the mobilization of adipose-tissue lipid, owing to the demand for nutrients for milk production, and glucose is considered to be the major substrate for the synthesis of lactose and fatty acids by the mammary gland of non-ruminants (Bauman & Elliot, 1983; Jones *et al.*, 1984). Lactating mammary glands removed large amounts of amino acids from the arterial plasma for milk protein synthesis, and glutamine appears to be used as a metabolic fuel by this tissue (see Lund, 1980). In the present work, the increased rate of removal of glutamine by the small intestine of peak-lactating rats could have been due to two factors: first, the sparing of glucose by glutamine, and hence directing more glucose to the mammary gland for milk synthesis; this is supported by the fact that blood glucose was lowered by approx. 25% ($P < 0.05$ compared with non-lactating rats; M. S. M. Ardawi, unpublished work) during peak lactation and accompanied by a decrease in insulin (23%, $P < 0.05$; M. S. M. Ardawi, unpublished work); second, the pro-

vision of alanine to be used for the synthesis of glucose via hepatic gluconeogenesis.

Therefore, although the metabolic and hormonal status during late pregnancy and peak lactation is different, the adaptive changes in glutamine metabolism by the small intestine seem to be similar. However, the liver appears to release glutamine during late pregnancy, but to utilize glutamine during peak lactation.

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