Comp. Biochem. Physiol. Vol. 70A, pp. 309 to 313, 1981
Printed in Great Britain.

0300-9629/81/011309-05$02.00/0

PLASMA LEVELS OF AMINO ACIDS IN HUMANS, PIGS AND RATS AND THEIR CHANGES DURING LIVER TRANSPLANTATION IN THE PIG

R. MARTÍN DEL RIO, R. CAMPRODÓN, J. M. FABREGAT, J. M. RAMON and E. HERRERA

Departamento de Investigación, Centro Ramón y Cajal, Madrid; Unidad de Cirugía Experimental, Departamento de Cirugía, Ciudad Sanitaria "Francisco Franco", Barcelona and Cátedra de Fisiologia General, Facultad de Biologia, Universidad de Barcelona, Barcelona, Spain

(*Received* 16 *March* 1981)

Abstract—1. Plasma levels of individual amino acids in pigs are closer to those in humans than to those in rats.

2. During the liver transplant operation in the pig, in the unhepatic phase there is a rapid rise in plasma, alanine and ornithine levels and a decrease in glutamate, aspartate and arginine.

3. After liver transplant there is an increase in most amino acids which is most striking in alanine, lysine and ornithine while plasma levels of arginine remained undetectable.

4. These findings demonstrate the role of the liver as the main receptor of most amino acids released from muscle protein.

5. Although the method used is valid as an experimental model for liver transplant in humans, the preservation of the liver must be modified to avoid the intense proteolytic activity which contributes to the plasma amino acid changes observed after transplant.

INTRODUCTION

The liver is the main site of deamination of amino acids to ammonia and ketoacids which are later converted to carbohydrates, fatty acids or ketone bodies (Schepartz, 1973; Ruerman *et al.*, 1964). In the fasted state, there is an increased flux of amino acids from peripheral tissues toward the liver (Goldberg, 1980; Goldberg & Chang, 1978) where gluconeogenesis is enhanced (Schimmel & Knobil, 1970; Herrera *et al.*, 1969; Llobera & Herrera, 1980). It is well known that the kidney cortex may also participate in the synthesis of glucose from amino acids, especially in the fasted state (Owen *et al.*, 1969; Mehlman *et al.*, 1967). The *in vivo* comparative contribution of either organ to the changes in plasma amino acid levels that occur in the fasting state have not been established. In the present study, the liver transplant technique has been used in the pig to investigate the role of the liver in individual plasma amino acids levels in the fasting conditions. Since the basal plasma level of individual amino acids in the pig could substantially differ from that of other mammals, the study was initiated by securing such determinations in young humans and rats to make the proper comparisons.

MATERIALS AND METHODS

All subjects studied were fasted overnight before blood extraction. In young women volunteers (22–32 yr old), blood was collected from a cubital vein into heparinized syringes. Female Wistar rats (150–160 g) were sacrificed by decapitation and blood was collected from the neck into heparinized beakers. Basal blood samples were also collected from female Large White pigs (20–28 kg) under halophane anesthesia (Camprodón *et al.*, 1974) from the external jugular vein into a heparinized syringe.

Eight pigs received an orthotopic liver transplant under halophane anesthesia (Camprodón *et al.*, 1974; Camprodón *et al.*, 1973), according to a surgical technique already described (Camprodón *et al.*, 1974; Camprodón *et al.*, 1973; Camprodón *et al.*, 1975). After infusion through the portal vein of 2 l of cold (4°C) heparinized saline (5000 IU and heparin/l), the liver was preserved in ice. At different times blood samples were collected from the external jugular vein into heparinized tubes. All the blood samples were immediately centrifuged at 3000 rpm for 10 min at 4°C and plasma aliquots were kept frozen until processing. Plasma aliquots were deproteinized with 10% 5-sulphosalicylic acid in 0.1 N HCl and used for the evaluation of individual amino acids in a 121 MB Beckman amino acid autoanalyzer (Martín del Rio & Latorre Caballero, 1980). Norleucine was always used as internal standard. Statistical comparison between the groups was done with the Student *t*-test.

RESULTS

In Table 1 the values are summarized of plasma levels of individual amino acids in female humans, pigs and rats after an overnight fast. The values in pigs are closer to those in humans than those in rats. However, the plasma levels of glutamine are significantly lower and those of alanine, aspartate, glycine, proline, ornithine, citrulline, valine and isoleucine are significantly higher in the pig as compared with the human. In the rat, the plasma levels of glutamate, ornithine, histidine and cystine are significantly reduced and those of glutamine, aspartate, glycine, threonine, serine, proline, lysine, arginine, citrulline, isoleucine, methionine, tyrosine, tryptophan and phenylalanine are significantly augmented when compared to their levels in humans.

The changes in amino acid levels in plasma during liver transplant in pigs are shown in Table 2, expressed as the percentage of values before the unhepatic phase. In the gluconeogenic amino acids group,

Table 1. Plasma levels of individual amino acids in young female humans, pigs and rats after overnight fast

	Human (5)	*P* (human vs pig)	*Pig* (8)	*P* (pig vs rat)	*Rat* (6–7)	*P* (rats vs humans)
Gluconeogenic amino acids						
Alanine	320 ± 25	<0.05	460 ± 38	NS	380 ± 16	NS
Glutamate	179 ± 7	NS	167 ± 14	<0.001	92 ± 4	<0.001
Glutamine	463 ± 24	<0.001	224 ± 33	<0.001	648 ± 17	<0.001
Aspartate	10 ± 1	<0.001	51 ± 3	<0.001	29 ± 1	<0.001
Glycine	233 ± 24	<0.01	669 ± 89	<0.05	408 ± 9	<0.001
Threonine	153 ± 15	NS	193 ± 42	<0.05	333 ± 19	<0.001
Serine	134 ± 9	NS	193 ± 29	<0.05	268 ± 5	<0.001
Imino acids						
Proline	126 ± 5	<0.001	280 ± 17	<0.001	146 ± 2	<0.01
Hydroxyproline			148 ± 8			
Basic amino acids						
Lysine	157 ± 11	NS	213 ± 33	<0.01	383 ± 38	<0.001
Arginine	81 ± 4	NS	119 ± 21	NS	154 ± 7	<0.001
Ornithine	67 ± 10	<0.05	167 ± 28	<0.001	29 ± 2	<0.01
Histidine	74 ± 3	NS	86 ± 12	NS	59 ± 3	<0.01
Citrulline	26 ± 2	<0.05	58 ± 8	NS	69 ± 3	<0.001
Branched chain amino acids						
Valine	201 ± 8	<0.05	324 ± 38	<0.01	191 ± 11	NS
Leucine	101 ± 6	<0.05	203 ± 28	<0.05	125 ± 11	NS
Isoleucine	53 ± 4	<0.01	152 ± 21	<0.01	81 ± 6	<0.01
Sulphur amino acids						
Cystine	113 ± 4				55 ± 3	<0.001
Methionine	23 ± 1				51 ± 3	<0.001
Taurine	99 ± 7	NS	90 ± 19	<0.05	162 ± 27	NS
Aromatic amino acids						
Tyrosine	51 ± 5	NS	85 ± 14	NS	74 ± 3	<0.01
Tryptophan	45 ± 14	NS	54 ± 7	<0.001	100 ± 7	<0.01
Phenylalanine	63 ± 3	NS	93 ± 14	NS	75 ± 3	<0.05

Values are expressed as means ± SEM and correspond to μmol/1. (*n* in parentheses). NS = not significant ($P > 0.05$).

alanine is the only amino acid with a plasma concentration that increases during the unhepatic phase, as compared with basal values, while the levels of both glutamate and aspartate are significantly reduced and those of the other gluconeogenic amino acids are unchanged during this phase. Liver transplant produced a further increment in the plasma level of alanine which persisted until 90 min after transplant. Glutamate and aspartate recovered their basal levels after transplant and no other changes were observed in the remaining amino acids in this group. Proline levels did not change during the operation and hydroxyproline levels, after an initial decrease during the unhepatic phase, did not differ significantly from the basals. In the basic amino acids group, lysine did no change during the unhepatic phase (Table 2) but increased significantly from the first min after transplant and maintained a high level throughout the 90 min studied. Arginine almost disappeared from the blood at the onset of the unhepatic phase and its levels remained undetectable after transplant. Contrary to arginine, the levels of ornithine were immediately enhanced after hepatectomy and were further augmented after transplant. Neither citrulline nor histidine levels changed in plasma during the experiment. Among the sulphur amino acids, taurine increased progressively after hepatectomy and showed significantly high values at 30 min (Table 2). After transplant, taurine levels remained high, being significantly increased at 1, 5 and 15 min in comparison with basal values. In the aromatic amino acids group there was no change in tyrosine and phenylalanine during the unhepatic phase while they both increased after transplant, and phenylalanine values were significantly augmented at 15 min. On the contrary, tryptophan levels progressively decreased during the unhepatic phase and remained low until 30 min after transplant. Levels of the branched chain amino acids were unmodified during the unhepatic phase and rose after transplant to levels which were significant for leucine and isoleucine at 15 min and for valine at 90 min, in comparison with basal values.

DISCUSSION

Basal values of individual amino acid levels recorded in female humans and in rats after overnight fast were similar to those previously reported using a different methodology (Feling *et al.*, 1972; Young & Prenton, 1969; Palou *et al.*, 1977; Alemany *et al.*, 1978). Values in pigs differed from those recently reported by Montgomery *et al.* (1980) but in addition to using a different sex and strain of animal, they provided specifically treated diets (Montgomery *et al.*,

Table 2. Percentual changes of plasma levels of individual amino acids during the liver transplant in the pig

	Unhepatic phase				Post-transplant					
Minutes	1	5	15	30	1	5	15	30	60	90
Gluconeogenic amino acids										
Alanine	**133 ± 9	*131 ± 12	**147 ± 14	*159 ± 19	**152 ± 14	166 ± 30	***251 ± 46	**208 ± 40	**208 ± 48	*254 ± 69
Glutamate	*77 ± 9	**67 ± 8	**59 ± 3	***62 ± 3	95 ± 11	85 ± 19	108 ± 8	103 ± 9	118 ± 8	101 ± 11
Glutamine	84 ± 16	97 ± 6	119 ± 14	109 ± 14	126 ± 34	91 ± 13	116 ± 5	84 ± 12	97 ± 3	89 ± 14
Aspartate	83 ± 10	*71 ± 13	*73 ± 14	91 ± 11	94 ± 23	89 ± 7				
Glycine	112 ± 6	82 ± 15	108 ± 9	107 ± 9	112 ± 10	113 ± 17	101 ± 14	104 ± 18	115 ± 18	109 ± 4
Threonine	113 ± 6	99 ± 9	109 ± 12	108 ± 10	108 ± 9	112 ± 14	134 ± 16	126 ± 23	119 ± 14	110 ± 9
Serine	109 ± 10	94 ± 10	101 ± 12	99 ± 15	97 ± 19	107 ± 11	123 ± 15	116 ± 10	108 ± 9	109 ± 6
Imino acids										
Proline	97 ± 7	90 ± 9	99 ± 11	102 ± 9	97 ± 10	103 ± 14	97 ± 9	100 ± 17	126 ± 18	
Hydroxyproline	*81 ± 6	87 ± 8	114 ± 3	97 ± 5	108 ± 6	94 ± 4	110 ± 8			
Basic amino acids										
Lysine	109 ± 8	113 ± 19	119 ± 14	107 ± 9	***140 ± 7	**148 ± 10	*172 ± 19	**153 ± 9	**142 ± 3	**179 ± 6
Arginine	***20 ± 6	***8 ± 1	***35 ± 3	***32 ± 11	ND	ND	ND	ND	ND	ND
Ornithine	*173 ± 30	**182 ± 23	*160 ± 20	*171 ± 26	**194 ± 25	*180 ± 15	*299 ± 70	**361 ± 81	**353 ± 52	*413 ± 110
Citrulline	84 ± 9	87 ± 10	95 ± 9	92 ± 8	103 ± 17	88 ± 17	115 ± 15	118 ± 9	121 ± 31	
Histidine	98 ± 12	97 ± 8	98 ± 15	109 ± 15	92 ± 10	72 ± 21	101 ± 18	99 ± 22	119 ± 18	113 ± 9
Sulphur amino acids										
Taurine	114 ± 22	116 ± 20	155 ± 28	*163 ± 23	*174 ± 29	*196 ± 45	*170 ± 22			
Aromatic amino acids										
Tyrosine	102 ± 10	80 ± 15	85 ± 15	99 ± 12	96 ± 20	94 ± 15	118 ± 21	161 ± 50	147 ± 31	135 ± 22
Phenylalanine	105 ± 8	91 ± 15	92 ± 8	103 ± 10	104 ± 13	89 ± 21	*146 ± 14	146 ± 26	135 ± 89	151 ± 36
Tryptophan	85 ± 11	89 ± 9	76 ± 10	*69 ± 10	*65 ± 14	*63 ± 10	*52 ± 12	*53 ± 17	79 ± 17	98 ± 6
Branched chain amino acids										
Valine	106 ± 5	97 ± 4	98 ± 7	95 ± 5	94 ± 5	93 ± 9	115 ± 6	113 ± 7	114 ± 12	**128 ± 7
Leucine	98 ± 15	105 ± 7	104 ± 6	106 ± 4	116 ± 12	110 ± 13	**145 ± 12	**157 ± 9	**133 ± 11	**166 ± 10
Isoleucine	92 ± 16	99 ± 6	92 ± 7	92 ± 10	95 ± 12	93 ± 15	*126 ± 10	**161 ± 16	*110 ± 3	*123± 5

Values are exposed as means ± SEM of 8 animals. They correspond to the percentage of the basal values found before the unhepatic phase (shown in the Table 1). Statistical comparison with the basals (100%) is shown by asterisks: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, No asterisks = not significant ($P > 0.05$). ND = not detectable.

1980; Montgomery *et al.*, 1978) that may affect the circulating amino acid patterns. The levels of individual plasma amino acids observed in our study in humans and pigs were very similar and differed substantially from those of the rat which were usually higher. These findings suggest that the pig is a better experimental model than the rat for the investigation of amino acid metabolism in humans. Although any comparisons between these species must be made with caution due to their obvious differences, results indicate that, in the rat, muscle protein breakdown is faster compared with utilization of amino acids by the splacnic bed organs, producing higher amino acid levels in plasma, in comparison with these activities in humans or pigs. This amino acid balance is seen most clearly during orthotopic liver transplant in the pig. The marked alterations of plasma amino acid levels during liver transplant in the overnight fasted pig is probably due to the equilibrium established between their incorporation to circulation, coming from muscle protein breakdown, and their removal or excretion, as it has been described in human injury (Williamson, 1980). During this unhepatic phase, alanine is the only gluconeogenic amino acid with an augmented plasma level. This finding coincides well with the recently proposed role of muscle alanine as the main gluconeogenic substrate for the liver (Snell, 1980). Although muscle proteolysis is augmented in the fasted state (Goldberg & Chang, 1978), it has been suggested that other amino acids (mainly the branched chain ones) (Snell, 1980) are converted to alanine and released to the circulation for its conversion to glucose by the liver. Thus the specific increase in the plasma level of alanine during the unhepatic phase is in agreement with hypothesis. The reduction of plasma levels of other gluconeogenic amino acids during this phase, such as glutamate and aspartate, may be explained by their important role in the transfer of α-keto acid compounds throughout the mitochondria membrane (Williamson, 1976) and their use as energetic substrates (Feling, 1973). It may be possible that their decrease in the unhepatic phase in plasma corresponds to their augmented utilization by peripheral tissues to compensate for the reduction in available glucose which is usually derived from the liver so that its level must be reduced, as has been shown in the rat during hepatectomy (Carmaniu & Herrera, 1980).

The enhancement in taurine levels during the unhepatic phase also deserves comment. Although this amino acid is not a component of tissue proteins (Huxtable, 1976), in the fasting state it must come from the heart and/or skeletal muscle which contain the highest amounts in the body (Awapara, 1976). Thus its rise in plasma during the unhepatic phase may represent an augmented release into circulation of this amino acid from those tissues or a reduced removal due to the absence of the liver. Further work should clarify these possibilities. The changes in the plasma levels of basic amino acids directly related to the urea cycle are also striking during the unhepatic phase. The disappearance of arginine from circulation is in opposition to the increase in ornithine and suggests that the changes in the former are not due to an augmented catabolism but to its impaired synthesis throughout the urea cycle. The augmented levels of ornithine in plasma may also be influenced by its formation from arginine in the kidney (Soberon & Palacios, 1976) but this contribution should be minor.

The amino acid changes after liver transplant do not reflect their restored uptake from circulation. On the contrary, liver transplant produces an increment in the plasma levels of most amino acids compared not only to basal levels but to levels during the unhepatic phase. This finding indicates that the new transplanted liver releases amino acids into circulation. This effect is probably the result of the proteolytic processes that may occur during the preservation time which give rise to an accumulation of free amino acids that are released into the plasma as soon as the circulation is restored through the transplanted liver. The difficulties of the transplanted liver in recuperating its normal function is also suggested by the maintenance of very low levels of arginine in plasma up to the 90 min studied. Although all the transplanted pigs in this investigation lived for over two months following surgery, it is evident that the preservation procedure used requires specific changes to obtain a more rapid restoration of all hepatic functions. The method employed is valid, however, as an experimental model for liver transplant in humans and for investigations of the role of the liver in certain metabolic parameters.

Acknowledgements—The authors wish to thank Caroline S. Delgado for her editorial help. Part of this study was performed with a grant from the Descuento Complementario de la Industria Farmacéutica to Dr R. Camprodón.

REFERENCES

Alemany M., Palou A., Codina J. & Herrera E. (1978) Changes in plasma amino acids levels and *in vivo* gluconeogenesis from alanine in rats chronically treated with sulfonylureas. *Diabetes & Metab.* **4**, 181–186.

Awapara, J. (1976) The metabolism of taurine in the animal. In *Taurine* (Edited by Huxtable R. & Barbean A.), pp. 1–19. Raven Press, New York.

Camprodon R., Guerrero J. A., Salva J. A., Jornet J., Piñeiro H., Segura J., Salva R., Esquerda J. E. & Ribas D. (1975) Aspectos de la preservación del hígado en relación al transplante hepático. *Rev. Quirurg. Esp.* **2**, 53–59.

Camprodon R., Jornet J., Salva J. A., Guerrero J. A., Piñero H., Mendoza C. G., Miralles J. A. & Kanarek B. (1973) Transplante hepático ortotópico en el cerdo. *Revta. esp. Enferm. Apar. dig. Nutr.* XL, 115–126.

Camprodon R., Jornet T., Salva J. A., Guerrero J. A., Piñero H., & Mendoza C. G. (1974) Problemas anestésicos del transplante hepático ortotópico en el cerdo. *Revta esp. Anest.* **21**, 68–74.

Carmaniu S. & Herrera E. (1980) Extra-hepatic utilization of ^{14}C-glucose and ^{14}C-glycerol in the eviscerated rat. *Diabetes & Metab.* **6**, 239–244.

Feling Ph., Kim J., Lynch V. & Hendler R. (1972) Amino acid metabolism during starvation in human pregnancy. *J. clin. Invest.* **51**, 1195–1202.

Feling P., Wharen J., Karl I., Cerasi E., Luft R. & Kipnis D. M. (1973) Glutamine and glutamate metabolism in normal and diabetic subjects. *Diabetes* **22**, 573–576.

Goldberg A. L. (1980) The regulation of protein turnover by endocrine and nutritional factors. *Plasticity of muscle* (Edited by Pette E.), pp. 469–492. de Gruyter, New York.

GOLDBERG A. L. & CHANG T. W. (1978) Regulation and significance of amino acid metabolism in skeletal muscle. *Fedn. Proc.* **37,** 2301–2307.

HERRERA E., KNOPP R. H. & FREINKEL N. (1969) Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis, and nitrogen metabolism during late gestation in the fed and fasted rat. *J. clin. Invest.* **48,** 2260–2272.

HUXTABLE R. (1976) Metabolism and function of taurine in the heart. In *Taurine*, (Edited by HUXTABLE R. & BARBEAU A.), pp. 99–119. Raven Press, New York.

LLOBERA M. & HERRERA E. (1980) Effects of starvation on *in vivo* gluconeogenesis in hypo- and hyperthyroid rats. *Endocrinology* **106,** 1628–1633.

MARTIN DEL RIO R. & LATORRE CABALLERO A. (1980) Presence of γ-aminobutyric acid in rat ovary. *J. Neurochem.* **34,** 1584–1587.

MEHLMAN M. A., WALTER P. & LARDY H. A. (1967) Path of carbon in gluconeogenesis and lipogenesis. VII. The synthesis of precursors for gluconeogenesis from pyruvate and bicarbonate by rat kidney mitochondria. *J. biol. chem.* **242,** 4594–4602.

MONTGOMERY G. W., FLUX D. S. & CARR J. R. (1978) Feeding patterns in pigs: The effects of amino acid deficiency. *Physiology and Behav.* **20,** 693–698.

MONTGOMERY G. W., FLUX D. S. & GREENWAY R. M. (1980) Tryptophan deficiency in pigs: changes in food intake and plasma levels of glucose, amino acids, insulin and growth hormone. *Horm. Metab. Res.* **12,** 304–309.

OWEN O. E., FELIG P., MORGAN A. P., WAHREN J. & CAHILL JR. G. F. (1969) Liver and kidney metabolism during prolonged starvation *J. clin. Invest.* **48,** 574–583.

PALOU A., AROLA LL. & ALEMANY M. (1977) Plasma amino acid concentrations in pregnant rats and in 21-day foetuses. *Biochem. J.* **166,** 49–55.

RUERMAN M. B., MACDONALD M., NEUFELDT N., PARK B. & BERGER M. (1964) On the regulation of blood alanine. In *Regulation of Hepatic Metabolism* (Edited by Lundquist, F. & Tygstrup, N.) pp. 209–218, Scandinavian University Books, Copenhagen.

SCHEPARTZ B. (1973) *Regulation of Amino Acid Metabolism in Mammals.* Saunders, Philadelphia.

SCHIMMEL R. J. & KNOBIL E. (1970) Insulin, free fatty acids, and stimulation of hepatic gluconeogenesis during fasting. *Am. J. Physiol.* **218,** 1540–1547.

SNELL K. (1980) Muscle alanine synthesis and hepatic gluconeogenesis. *Biochem. Soc. Trans.* **8,** 205–213.

SOBERON G. & PALACIOS R. (1976) Arginase. In *The Urea Cycle* (Edited by GRISOLIA S., BAGUENA S. & MAYOR F.), pp. 221–235. Wiley, New York.

WILLIAMSON J. R. (1976) Role of anion transport in the regulation of metabolism. In *Gluconeogenesis: Its Regulation in Mammalian Species* (Edited by HANSON R. W. & MEHLMAN M. A.), pp. 165–220. Wiley, London.

WILLIAMSON D. H. (1980) Muscle protein degradation and amino acid metabolism in human injury. *Biochem. Soc. Trans.* **8,** 497–499.

YOUNG M. & PRENTON M. A. (1969) Maternal and fetal plasma amino acid concentrations during gestation and in retarded fetal growth. *J. Obstet. Gynaec. Br. Commonw.* **76,** 333–344.