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Placental formation of lactate from transferred L-alanine and its impairment by aminooxyacetate in the late-pregnant rat

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After 20 min infusion of L-[U-^{14}C]alanine through the left uterine artery in 21-day-pregnant rats, the radioactivity in the plasma of fetuses from the left uterine horn was much higher than in their mothers and was composed of approximately equal parts of [^{14}C]alanine and [^{14}C]lactate, with a minor percentage of [^{14}C]glucose. Radioactivity in fetal plasma was much lower when the mothers were infused with α-amino[^{14}C]isobutyric acid. The simultaneous infusion of aminooxyacetate decreased materno-fetal transfer of radioactivity from [^{14}C]alanine but not from α-amino[^{14}C]isobutyric acid, and this effect corresponded to a complete disappearance of the [^{14}C]lactate in fetal plasma without affecting [^{14}C]alanine levels or alanine concentration in the fetuses. Placenta slices in vitro metabolized L-[U-^{14}C]alanine into [^{14}C]lactate and $^{14}CO_2$ at the rate of 7 nmol/g per min, and this process was inhibited by the presence of 1 mM aminooxyacetate in the medium. Placental uptake of α-amino[^{14}C]isobutyric acid was half that of [U-^{14}C]alanine, and aminooxyacetate did not affect this parameter with either of the labelled compounds. Results indicate that the lower transfer to the rat fetus of the ^{14}C atoms from α-amino[^{14}C]isobutyric acid as compared to that from [^{14}C]alanine is due not only to the diminished placental carrier system of the former but also to its non-metabolyzable condition. It is proposed that the capacity of the placenta to metabolyze L-alanine to lactate and the subsequent release of lactate to the fetus constitute important factors for the fetal metabolic economy.

Introduction

Amino acid concentrations in fetal plasma are higher than in maternal plasma [1–4]. Amino acids are required by the fetus not only as primary substrates for protein biosynthesis [5] but also as metabolic fuels [6,7]. The mechanism by which amino acids are transferred across the placenta to the fetal circulation is not yet completely understood. An active amino acid placental uptake from maternal circulation, followed by passive release down a concentration gradient into fetal circulation [8], or a protein catabolic/anabolic cycle in the placenta [9] are among the proposed transfer mechanisms. Using a sheep preparation, Battaglia and his group showed that lactate acquired by the fetus from umbilical circulation is produced by the placenta [10] and, using the in vitro human placenta, they also found that placental lactate production exceeded the amount of glucose utilized

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[11], suggesting that amino acid metabolism is a likely source for production of the remaining lactate. In the present study we tested this possibility by selectively infusing either labelled L-alanine or α-aminoisobutyric acid through one uterine horn in the pregnant rat, following our recently reported technique [12]. The work was extended to determine the effect of aminooxyacetate which is a potent aminotransferase inhibitor, and the results were corroborated by using the 'in vitro' incubation of placental slices. Results indicate that rat placenta produces significant amounts of lactate from maternal alanine, and that this lactate is released to the fetal circulation.

Materials and Methods

Studies in vivo

Fed pregnant Wistar rats at day 21 of gestation (351 ± 14 g) were anesthetized with an intravenous injection of sodium pentobarbital (33 mg/kg body weight). Animals were subjected to the surgical procedure already described [12], and a PE-10 cannula (Intramedic, U.S.A.) was introduced counter-current into the left external iliac artery to the level of the left uterine artery. The superior vesical artery, the superior gluteal artery and the hypogastric trunk were clamped. In this way, the infusion medium containing a ^{14}C-labelled tracer given through the cannula becomes diluted with maternal blood reaching the left uterine artery, and the left uterine horn receives the tracer directly before it becomes diluted in the mother's general circulation. After a preinfusion of the rats with plain saline (0.9% NaCl) for 20 min, the same medium was injected for another 20 min at 12.5 μl/min containing 10 μCi of either L-[U-^{14}C]alanine or [1-^{14}C]aminoisobutyric acid (from Amersham International, Amersham, U.K.; specific activities, 10 mCi/mmol and 58 mCi/mmol, respectively). When aminooxyacetate effects were studied, this compound (from Sigma, U.S.A.) was dissolved in the medium at a concentration of 80 μg/μl and given during both the 20 min of preinfusion and the 20 min of actual infusion together with the tracer. After infusion, maternal blood was collected from the abdominal aorta into heparinized syringes and fetuses and placentas from the left uterine horn were immediately excised. Placentas were rinsed in saline, cut in two parts and after blotting on Whatman filter paper they were placed in 1 ml of 30% KOH and digested for 20 min in boiling water bath. An aliquot of the digests was placed in scintillation vials, decolored with H_2O_2, and neutralized with 1 M HCl for counting total radioactivity in a xylene/Triton X-100/PPO/POPOP based scintillation cocktail. Fetuses were decapitated, and blood was collected and pooled into heparinized receptacles. After plasma separation, aliquots were used for total radioactivity counting in the same scintillation liquid as above. Other plasma aliquots were deproteinized with 10% sulfosalycilic acid in 0.1 M HCl, and protein free supernatants were used for alanine determination in a Beckman 121 MB autoanalyzer. The remaining plasma was deproteinized with 10% $HClO_4$ and neutralized with saturated $KHCO_3$. The neutralized protein-free supernatants were used for glucose [13] and lactate [14] measurements. Plasma [^{14}C]alanine, [^{14}C]-lactate and [^{14}C]glucose were separated [15] by passing 100 μl of the protein-free supernatants over microcolumns (4 mm i.d.) containing cationic (AG50 W-X8, 200–400 mesh, hydrogen form, from Bio-Rad Laboratories, Richmond, CA) and anionic resins (AG1-X8, 200–400 mesh, chloride form; Bio-Rad Laboratories). Columns were eluted sequentially with 5 ml deionized distilled water, 5 ml of 0.5 M formic acid and 5 ml of 2 M ammonia. Recoveries in the corresponding eluates for the tracers added to non-radioactive plasma before precipitation were as follows: L-[U-^{14}C]alanine, 91 ± 2%; α-amino[^{14}C]isobutyric acid, 93 ± 3%; L-[U-^{14}C]lactate, 88 ± 3%; and D-[U-^{14}C]glucose, 97 ± 2%, in their respective fractions. Contamination of the other tracers in each of the eluates was always less than 0.4%. Results of radioactivity were always corrected by considering $1 \cdot 10^6$ dpm as the total infused radioactivity per rat. Statistical comparison between groups was carried out using the Student's *t*-test.

Studies in vitro with placental slices

Other fed 21-day-pregnant rats were killed by decapitation, and placentas were immediately excised and placed into cold Krebs-Ringer Bicarbonate solution containing 5 mM glucose. Incubation of placental tissue was performed accord-

ing to Smith et al. [16] with some modifications. Placentas were cut in 1 mm thick slices, discarding a 2-mm section from the edge. Slices were rinsed with Krebs-Ringer bicarbonate/5 mM glucose, weighed, and blotted on Whatman No. 1 filter paper. Slices were preincubated with shaking (100 cycles/min) in Krebs-Ringer bicarbonate buffer containing 10 mM glucose for 2 h at 37°C in an atmosphere of O_2/CO_2 (95 : 5). After this period, slices with jagged edges were discarded and a second incubation was initiated. For this, six placental slices (66–74 mg) per vial were placed into 1 ml Krebs-Ringer bicarbonate/5 mM glucose containing either 0.5 μCi of L-[U-^{14}C]alanine and 0.4 mM L-alanine or 0.5 μCi of α-amino[^{14}C]isobutyric acid, and 0.4 mM α-aminoisobutyric acid, in the absence or the presence of 1 mM aminooxyacetic acid. Incubation was performed as described above for 50 min. Six vials were run for each experimental condition, and while three of the vials were employed to measure $^{14}CO_2$ production by trapping it into hyamine hydroxyde, as previously described [17], the other three were used for radioactivity measurement in media and tissues. For the latter procedure, the tissues were rinsed at the end of the incubation with plain Krebs-Ringer bicarbonate buffer, blotted on Whatman No. 1 paper, and digested in 30% KOH for radioactivity counting as for the in vivo studies. Aliquots of 100 μl of media were used for total radioactivity measurement and other 100-μl aliquots were processed for ^{14}C separation as indicated above for plasma in the in vivo studies.

Results

After infusion of L-[U-^{14}C]alanine for 20 min through the left uterine artery in 21-day-pregnant rats, radioactivity was much higher in the plasma of fetuses from the left uterine horn than in their mothers (Table I). Total radioactivity per gram in placentas from the left uterine horn was higher than the amount present per milliliter in either maternal or fetal plasma (Table I). Simultaneous infusion with aminooxyacetate (0.97 mg/min) and L-[U-^{14}C]alanine did not affect total radioactivity in either the maternal plasma or the placenta, but did significantly reduce radioactivity in fetal plasma, thus enhancing the placental/fetal plasma radioactivity ratio (Table I). (The amount of radioactivity coming from infused [U-^{14}C]alanine that appeared in placental proteins was estimated. Proteins were precipitated three times with trichloroacetic acid (2.5%, final concentration), digested with 15% KOH for 1 h at 100°C and treated with hydrogen peroxyde for counting. The percentage of placental total radioactivity which corresponded to ^{14}C-proteins was 7.6 ± 0.6 in animals infused with the tracer ($n = 5$) and 7.8 ± 0.9 ($n = 3$) in those receiving the tracer and aminooxyacetate simultaneously, the difference between the two groups being non-significant.) When α-amino[1-^{14}C]isobutyric acid was the infused tracer, maternal was higher than fetal plasma radioactivity, and the fetal level was lower than with L-[U-^{14}C]alanine (Table I). Placental radioactivity was similar following infusions with α-amino[1-^{14}C]isobutyric acid and L-[U^{14}C]alanine, but placenta/fetus plasma radioactivity ratio was greatly enhanced with the former tracer (Table I). In contrast with L-[U-^{14}C]alanine, aminooxyacetate did not modify any recorded radioactivity values after infusion with α-amino[1-^{14}C]isobutyric acid (Table I).

Distribution of radioactivity in plasma components was estimated after the infusion of tracers through the left uterine artery in the mothers. Values corresponding to the experiments with L-[U-^{14}C]alanine are summarized in Table II, but radioactivity levels following infusion with α-amino[1-^{14}C]isobutyric acid are not shown, because over 98% of total maternal and fetal plasma radioactivity always corresponded to the administered non-metabolizable tracer. In animals infused with L-[U-^{14}C]alanine, 34 and 25% of the mother's plasma radioactivity corresponded to [^{14}C]lactate and [^{14}C]glucose, respectively, whereas the remaining 41% corresponded to [^{14}C]alanine (Table II). Radioactivity distribution in fetal plasma was 50% for [^{14}C]alanine, 48% for [^{14}C]lactate, and 2% for [^{14}C]glucose (Table II). Comparison of absolute radioactivity values in fetal and maternal plasma indicates that both [^{14}C]alanine and [^{14}C]lactate were significantly higher and [^{14}C]glucose significantly lower in fetuses (Table II). Plasma concentrations of both alanine and lactate as well as their respective specific activities were significantly higher in fetuses than in their mothers, whereas

TABLE I

EFFECT OF AMINOOXYACETIC ACID (AOA) ON PLASMA AND PLACENTA RADIOACTIVITY 20 MIN AFTER INFUSION THROUGH THE LEFT UTERINE ARTERY WITH EITHER L-[U-^{14}C]ALANINE OR α-AMINO[1-^{14}C]ISOBUTYRIC ACID IN THE 21-DAY-PREGNANT RAT

The tracers were infused simultaneously with or without aminooxyacetic acid (0.97 ng/min). Both placentas and fetuses correspond to those from the left uterine horns. Values are expressed as mean ± S.E., with the number of animals per group given in parentheses. *P* corresponds to the statistical comparisons versus without AOA. [b,c] Statistical comparisons versus maternal plasma; [e,f] statistical comparisons versus L[U-^{14}C]-alanine; [b,c] $P < 0.01$; [c,f] $P < 0.001$.

	Maternal plasma (cpm/ml)	Fetal plasma (cpm/ml)	Placenta (cpm/g)	Placenta/fetal plasma ratio
L-[U-^{14}C]Alanine				
without AOA (6)	2323 ± 229	12223 ± 2097 [c]	21869 ± 3715	1.92 ± 0.36
with AOA (5)	2466 ± 220	6650 ± 984 [b]	30347 ± 4120	4.60 ± 0.40
P	n.s.	< 0.05	n.s.	< 0.01
α-Amino[1-^{14}C]isobutyric acid				
without AOA (4)	8482 ± 241 [f]	2675 ± 253 [c,f]	34564 ± 4041	14.04 ± 1.23 [f]
with AOA (4)	8550 ± 293 [f]	2705 ± 97 [c,e]	27743 ± 5710	11.97 ± 1.35 [f]
P	n.s.	n.s.	n.s.	n.s.

glucose levels were slightly lower in the former. Infusion of aminooxyacetic acid to mothers receiving L-[U-^{14}C]alanine appeared to reduce significantly the capacity to metabolize the administered tracer, as indicated by increased plasma [^{14}C]alanine values and the sharp decrease in both

TABLE II

EFFECT OF AMINOOXYACETIC ACID (AOA) ON PLASMA RADIOACTIVITY DISTRIBUTION 20 MIN AFTER INFUSION THROUGH THE LEFT UTERINE ARTERY WITH L-[U-^{14}C]ALANINE IN THE 21-DAY-PREGNANT RAT

The tracer was infused simultaneously with or without aminooxyacetic acid (0.97 ng/min). Fetal plasma correspond to that of fetuses from the left uterine horns. Values are expressed as mean ± S.E. of 5 animals per group. *P* corresponds to the statistical comparisons versus without AOA. [a,b,c] Statistical comparisons versus maternal plasma; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

	Without AOA ($n = 6$)	*P*	With AOA ($n = 5$)
Maternal plasma			
[^{14}C]Alanine (cpm/ml)	1021 ± 144	< 0.001	2493 ± 230
Alanine (μM)	460 ± 8	< 0.001	680 ± 21
[^{14}C]Alanine spec. act. (cpm/μmol)	2152 ± 298	< 0.05	3692 ± 359
[^{14}C]Lactate (cpm/ml)	861 ± 43	< 0.001	67 ± 26
Lactate (mM)	4.1 ± 0.4	n.s.	3.8 ± 0.4
[^{14}C]Lactate spec. act. (cpm/μmol)	228 ± 21	< 0.001	20 ± 4
[^{14}C]Glucose (cpm/ml)	627 ± 34	< 0.001	24 ± 16
Glucose (mM)	5.3 ± 0.6	n.s.	5.8 ± 0.6
[^{14}C]Glucose spec. act. (cpm/μmol)	97 ± 17	< 0.001	4.2 ± 3.1
Fetal plasma			
[^{14}C]Alanine (cpm/ml)	6697 ± 1136 [c]	n.s.	6881 ± 609 [c]
Alanine (μM)	1185 ± 105 [c]	n.s.	1216 ± 66 [c]
[^{14}C]Alanine spec. act. (cpm/μmol)	5651 ± 1131 [a]	n.s.	5670 ± 1207
[^{14}C]Lactate (cpm/ml)	6535 ± 1171 [c]	< 0.001	160 ± 120
Lactate (mM)	15 ± 1 [c]	n.s.	15 ± 2 [c]
[^{14}C]Lactate spec. act. (cpm/μmol)	469 ± 75 [b]	< 0.01	12 ± 8
[^{14}C]glucose (cpm/ml)	286 ± 40 [c]	< 0.001	1 ± 1
Glucose (mM)	4.8 ± 0.4	n.s.	4.9 ± 0.3
[^{14}C]Glucose spec. Act. (cpm/μmol)	51 ± 7 [b]	< 0.001	0.2 ± 0.2

TABLE III

EFFECT OF AMINOOXYACETIC ACID (AOA) ON THE IN VITRO UTILIZATION OF EITHER L[U-^{14}C]ALANINE OR α-AMINO[1-^{14}C]ISOBUTYRIC ACID BY PLACENTAL SLICES

Placental slices (66–74 mg/vial) were incubated for 50 min in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5 mM glucose and the indicated components. Values correspond to the means of triplicates from a single experiment.

	L-[U-^{14}C]alanine (0.4 mM)		α-Amino[1-^{14}C]isobutyric acid (0.4 mM)	
	Basal	AOA (1 mM)	Basal	AOA (1 mM)
Total radioactivity in tissue (cpm)	64753	69386	36225	36260
Total radioactivity in medium (cpm)	435000	421080	467720	465000
^{14}C-amino acid in medium (cpm)	398108	420858	460404	460247
[^{14}C]Lactate in medium (cpm)	31255	819	136	7
$^{14}CO_2$ (cpm)	4977	79	157	63
Estimated ^{14}C-amino acid utilization for lactate and CO_2 production (nmol/g per min)	7.12	0.20	0.06	0.01

[^{14}C]lactate and [^{14}C]glucose (Table II). Treatment with aminooxyacetic acid also produced an increase in maternal plasma alanine concentration and [^{14}C]alanine specific activity, without affecting either lactate or glucose concentrations and producing a marked reduction in their respective ^{14}C specific activities (Table II). Maternal aminooxyacetic acid treatment did not affect fetal plasma values of [^{14}C]alanine, alanine concentration and [^{14}C]alanine specific activity, but greatly reduced fetal plasma [^{14}C]lactate and [^{14}C]glucose values and their respective specific activities, without modifying either lactate or glucose concentrations (Table II).

To determine the direct role of the placenta in the observed changes after in vivo infusions, placenta slices from 21-day-pregnant rats were incubated with either L-[U-^{14}C]alanine (0.4 mM) or α-amino[1-^{14}C]isobutyric acid (0.4 mM) in the presence or the absence of 1 mM aminooxyacetate. As shown in Table III, [^{14}C]alanine was efficiently incorporated by placental slices as radioactivity in tissue, representing 13% of total radioactivity in the medium. A significant level of radioactivity also appeared both in lactate and CO_2 (Table III), indicating an efficient transamination of [^{14}C]-alanine by the placenta preparation. Using these parameters, the estimated alanine metabolization by placenta slices was 7.12 nmol/g per min (Table III). The presence of aminooxyacetic acid in the incubation medium did not modify the incorporation of [^{14}C]alanine into the tissue, but almost completely inhibited its metabolization to lactate and CO_2. The estimated alanine metabolization value in the presence of aminooxyacetic acid was almost negligible (0.2 nmol/g per min) (Table III). Placenta slices also incorporated α-amino-[^{14}C]isobutyric acid, although to a lesser extent than [^{14}C]alanine. As expected, radioactivity appeared in lactate and CO_2 was negligible after incubation with α-amino[^{14}C]isobutyric acid (Table III). As with [^{14}C]alanine, aminooxyacetic acid did not modify incorporation of radioactivity from α-amino[^{14}C]isobutyric acid into the tissue (Table III).

Discussion

The present results show that [^{14}C]alanine transfer through the placenta is more efficient than that of α-amino[^{14}C]isobutyric acid and that an important fraction of the former reaches the fetus in the form of [^{14}C]lactate, this process being completely blocked by aminooxyacetate, a known potent inhibitor of aminotransferase activities. This higher capacity of the placenta to handle L-alanine than α-aminoisobutyric acid was also observed in our in vitro preparations and is in agreement with previous studies in guinea pig placenta [18]. The difference between placental uptake of L-alanine and α-aminoisobutyric acid is probably due to the different carrier systems involved [19,20]. Half of

the maternal alanine reaching fetal circulation appeared as lactate, and this conversion process seems to occur mainly in the placenta itself, as indicated by the fact that when incubated in vitro, placenta slices also produced a notable amount of [^{14}C]lactate from L-[^{14}C]alanine, in accordance with the presence of alanine aminotransferase activity in rat placenta [21,22]. To our knowledge this is the first description of lactate production from alanine by the placenta, and it could explain the reported lack of correlation between glucose utilization and lactate production in human placenta preparations [11,23,24], demonstrating that placental amino acid metabolism is an important source of lactate production. The process seems to be linked to transfer of L-alanine (and presumably other amino acids) to the fetus for two reasons: (i) transfer of the non-metabolizable amino acid, α-aminoisobutyric acid, to the fetus is much less efficient than the transfer of alanine; and (ii) in the presence of aminooxyacetic acid, there is a dramatic drop in the ability of the placenta to convert L-alanine into either lactate or CO_2, in agreement with its known inhibitory action on aminotransferases activity, and a concomitant abolishment of the [^{14}C]lactate present in fetal plasma after maternal infusion with [^{14}C]alanine. Aminooxyacetic acid did not modify the in vitro placental uptake of these compounds or the amount of label appearing as either [^{14}C]alanine or α-amino[^{14}C]isobutyric acid in the fetal plasma after their respective infusion into the maternal uterine artery, suggesting that placental carrier systems for L-alanine are independent of its metabolization.

The present results, showing the capacity of the placenta to metabolyze L-alanine to lactate and its subsequent release to the fetus, may be considered together with other reported findings in sheep [10,25] as showing that glucose taken up by the placenta from the maternal side is partially converted to lactate and subsequently transferred to the fetus. These results demonstrate the role of the placenta in modifying the molecular quality of substrates reaching the fetus. We have previously shown the high rate of lactate production by the fetoplacental unit of the rat in vivo [26]. The finding that a considerable amount of L-alanine and glucose carbons reach the fetus as lactate, together with its high levels in fetal plasma and the known capacity of the fetal tissues to use it as an important oxydative fuel source [27,28], suggest that placental production of lactate from maternal substrates constitutes an important factor for fetal metabolic economy and development.

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