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Glutamate Metabolism and Placental Transfer in Pregnancy

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For more than 50 years it has been recognized that amino acid levels in the fetus generally exceed those in the mother (5). The concentration of most amino acids in fetal plasma is approximately twice that of the mother (3), implying active transport by the placenta. Although this characteristic connotes certain obvious advantages with respect to facilitating fetal growth and development, it also carries a potential for adverse effects if the transplacental gradient is maintained in the face of elevated maternal levels of those amino acids known to be toxic in high concentration. Phenylalanine is a classic example of this phenomenon, for infants born to women with hyperphenylalanemia, though themselves genetically normal, frequently exhibit growth retardation and permanent brain damage as a consequence of their prolonged intrauterine exposure to high phenylalanine levels (2).

Glutamate represents an amino acid of special concern because of the demonstration of a neurotoxic effect of high doses in certain species. Studies of fetal toxicity of maternally administered glutamate have yielded conflicting results. Whereas Murakami and Inouye (6) reported brain lesions in the mouse fetus following maternal glutamate administration, no such effects were found by Lucas and Newhouse (4) in the mouse or by Newman et al. (7) in the monkey.

The question of fetal toxicity of maternally administered glutamate hinges in large part on the matter of placental transfer. Although the indirect study mentioned above has suggested that glutamate crosses the placenta, other more direct investigations indicate that it does not. Dierks-Ventling and associates (1) injected glutamate (1 g/kg) into the tail vein of pregnant rats and found no increase in fetal glutamate levels despite a 35-fold increase in the maternal concentration. In an *in vitro* study, Schneider and Dancis (9) measured uptake and release of 10 amino acids by human placental slices; acidic amino acids (glutamate and aspartate) and serine were taken up rapidly but released very poorly, consistent with poor to absent placental transfer, whereas the efflux system for other amino acids was much more efficient.

We have conducted studies of the bidirectional transfer of glutamate across the primate placenta, described in detail elsewhere (12). For comparative purposes, we

have also investigated maternal-fetal transmission of aspartate (8), the other dicarboxylic amino acid normally present in plasma.

METHODS

Rhesus monkeys (*Macaca mulatta*) were studied during the last third of pregnancy. This species was utilized because it has a hemochorial placenta virtually identical morphologically, and in most instances functionally, with that of the human. The rhesus placenta is usually bipartite with each lobe connected by interplacental vessels that can be identified by transillumination of the exposed uterus, exposed by incising the myometrium, and catheterized with a T-tube. This preparation permits access to the fetal circulation under physiologic conditions; i.e., with the fetus *in utero* and the amniotic sac intact. Amniotic fluid samples can be obtained by intermittent transuterine puncture.

The animals were prepared by inserting venous catheters into the inferior vena cava (via a saphenous vein) and into an arm vein. Following tranquilization with phencyclidine hydrochloride and induction of inhalation anesthesia, the uterus was exposed through a midline celiotomy incision and an interplacental vein catheterized with a silastic T-tube.

Maternal-fetal glutamate transfer was examined in five animals in which monosodium L-glutamate (MSG) was infused into an antecubital vein over 1 hr and sequential samples were obtained from the maternal vena cava and the interplacental vessel at intervals during the infusion and for 3 hr thereafter. The dosage of glutamate administered ranged from 0.16 to 0.4 g/kg maternal weight, and each infusion included added 3-4-^{14}C-L-glutamate in amounts of 50 to 75 μCi.

Fetal-maternal glutamate transfer was examined in three animals. In one, 5 g/kg fetal weight (with 60 μc ^{14}C-glutamate) was infused into an interplacental artery over 1 hr and sequential maternal and fetal blood samples were obtained over 2 hr thereafter. In a second animal, glutamate (0.8 g/kg fetal weight with 10 μc ^{14}C-glutamate) was injected through the uterus and into the fetal chest wall. The third fetus received glutamate (1.5 g/kg fetal weight with 10 μc ^{14}C-glutamate) into the umbilical vein. In these two latter instances, maternal blood was sampled at intervals after administration, but only a single fetal sample was obtained at delivery 5 and 6 hr, respectively, after administration.

Maternal-fetal aspartate transfer was studied in a total of nine animals utilizing a protocol similar to that for glutamate studies, except that the infusing solution contained aspartate with added radioactive aspartic acid. Five animals received 0.1 g/kg and two each 0.2 and 0.4 g/kg.

All blood samples were centrifuged immediately and the plasma deproteinized with sulfosalicylic acid. Simultaneous radioactivity and amino acid analysis were done by a method (10) permitting the determination of amino acid levels as well as metabolically derived compounds (both ninhydrin positive and negative). Technicon NC 1 amino acid analyzers were employed.

RESULTS AND COMMENT

Maternal-Fetal Glutamate Transfer

Figure 1 illustrates chemical levels of glutamate in the five animals given maternal infusions of 0.15 to 0.4 g/kg over 1 hr. Loads of 0.15 to 0.22 g/kg raised maternal levels 10- to 20-fold (i.e., from a base line of 5 μmoles/dl to peak values of 50 to 100 μmoles/dl). Under these conditions, fetal plasma glutamate concentration did not change, implying a lack of placental transfer. At the highest dose (0.4 g/kg), the maternal glutamate level reached 280 μmol/dl (70 times base line), apparently promoting some transfer to the fetus and raising the fetal level to 44 μmoles/dl (10 times base line).

Figure 2 presents the radioactivity profile in a representative experiment in which 0.22 g/kg was infused, providing insights into glutamate metabolism in pregnant primates. During the infusion, approximately 75% of the total radioactivity in the maternal plasma represented glutamate itself with lesser, but nonetheless appreciable, quantities present in the two ninhydrin-negative compounds glucose and lactate. Aspartate, glutamine, and alanine were represented by small amounts of radioactivity. With termination of the infusion, glutamate fell rapidly, leaving glucose and lactate as the major sources of radioactivity in maternal plasma. Quite a different radioactivity profile was present in fetal plasma, in which glucose and lactate represented over 80% of the total counts, whereas glutamate and aspartate contained essentially no radioactivity.

Figure 3 illustrates the radioactivity profile in the animal infused at 0.4 g/kg, in

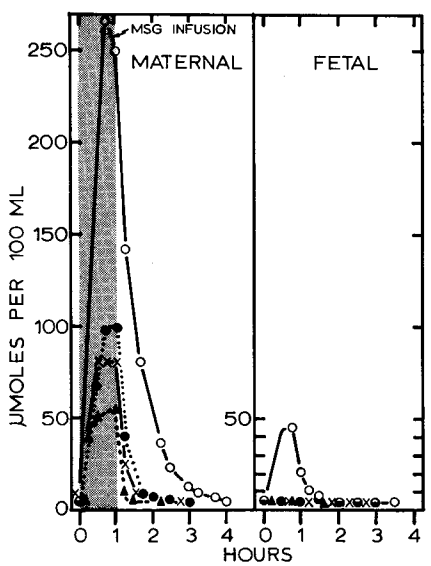

FIG. 1. Maternal and fetal plasma glutamate levels with maternal infusion of MSG at several dosage levels: ▲, 0.15; ×, 0.17 to 0.19 (mean of 2 animals); ●, 0.22; ○, 0.40 g/kg. (From Stegink et al., ref. 12, with permission.)

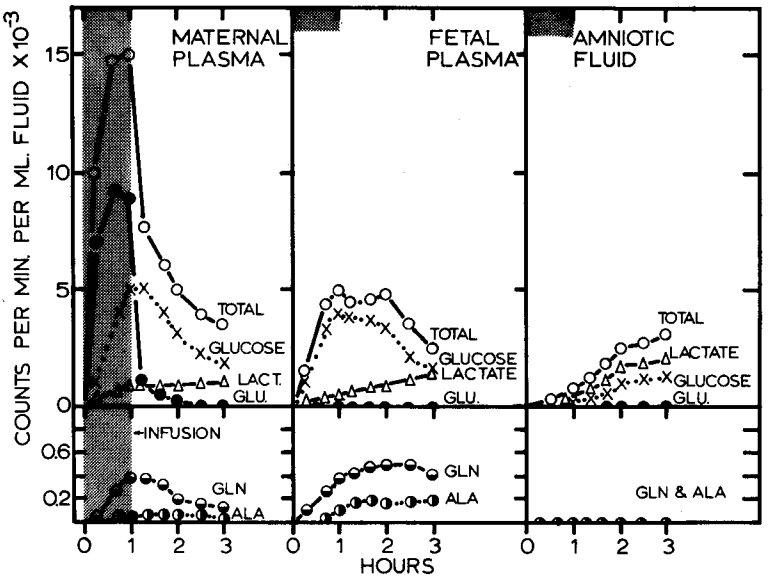

FIG. 2. Radioactivity profile of glutamate-derived metabolites in maternal and fetal plasma and amniotic fluid during and following maternal infusion of 0.22 g/kg MSG. (From Stegink et al., ref. 12, with permission.)

which it will be recalled that fetal plasma glutamate levels increased modestly in response to greatly elevated maternal levels. During the infusion, most of the maternal plasma radioactivity represented glutamate with smaller quantities present as glucose, lactate, aspartate, and glutamine. In the fetus, again almost all of the radioactivity was associated with glucose and lactate, although coincident with the maximal maternal level a small amount of radioactive glutamate was present in the fetal plasma.

From these results it is possible to construct a model describing glutamate metabolism in primate pregnancy. Most of the maternally administered glutamate circulates in the plasma as glutamate, raising maternal levels of this amino acid. However, despite plasma elevations up to 20-fold over base line, no transfer to the fetus occurs. With greater maternal loads, a limited amount is transferred to the fetus. From comparison of simultaneously determined maternal and fetal plasma levels, it appears that the "threshold" for transfer is a maternal concentration in the range of 250 to 300 μmoles/dl or 50 to 60 times the normal fasting value.

Approximately 20% of the infused glutamate is metabolized in the maternal compartment during the infusion. Glucose and lactate represent the principal metabolites, presumably resulting from the deamination of glutamate to α-ketoglutarate, which enters the tricarboxylic acid cycle and is then converted through pyruvate to glucose and lactate (11). Glucose and lactate cross the placenta readily, and their concentrations in the fetus generally mirror those in the mother.

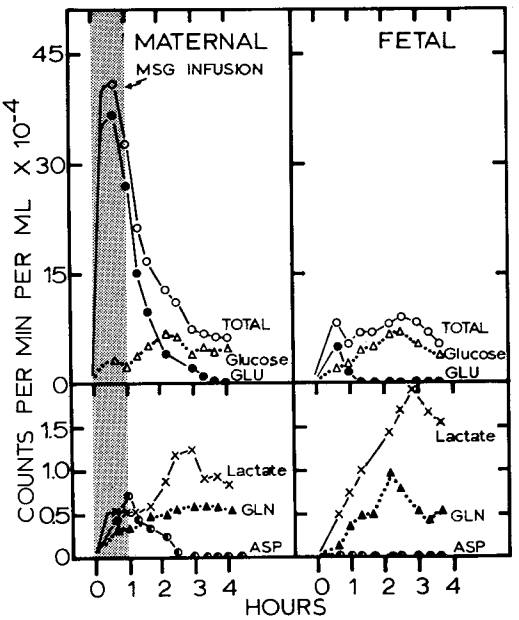

FIG. 3. Radioactivity profile of glutamate-derived metabolites in maternal and fetal plasma during and following maternal infusion of 0.4 g/kg MSG. (From Stegink et al., ref. 12, with permission.)

Small quantities of aspartate, glutamine, and alanine are also derived from glutamate. Aspartate appears to be handled in a manner similar to glutamate (i.e., it does not seem to cross the placenta), whereas glutamine and alanine reach the fetus in amounts appropriate to the normal transplacental gradient for these amino acids.

Fetal-Maternal Glutamate Transfer

In two experiments involving fetal glutamate administration by bolus injection of 1.5 and 2.4 g/kg (with added tracer radioactive glutamate), repeated analyses of maternal plasma failed to indicate any evidence of the transfer of any amino acid. However, increasing levels of radioactive glucose and lactate were found, reflecting fetal metabolism of glutamate to these two compounds, which then crossed to the maternal circulation.

Figure 4 illustrates the results of an experiment in which glutamate was infused into a fetus in an amount of 5 g/kg fetal weight (with added radioactive glutamate) over 1 hr. Fetal plasma glutamate levels rose to 2,000 μmoles/dl (400 times normal), and amniotic fluid values behaved similarly. Despite this extreme elevation, only a small amount of glutamate reached the maternal circulation. With cessation of the fetal infusion, fetal levels fell below 1,000 μmoles/dl, and there was a corresponding drop in the maternal level, implying that placental transfer had ceased.

These observations indicate that the placental "threshold" for glutamate is even higher with fetal-maternal than with maternal-fetal transfer. It probably lies above 1,000 μmoles/dl.

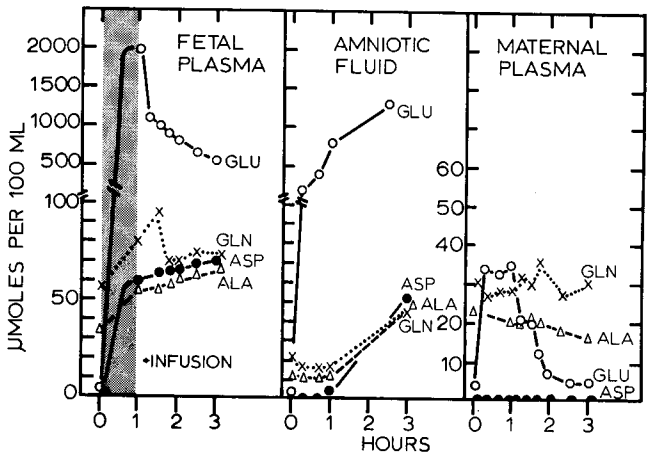

FIG. 4. Amino acid levels in fetal and maternal plasma and amniotic fluid during and following fetal infusion of MSG (5 g/kg fetal weight). (From Stegink et al., ref. 12, with permission.)

Maternal-Fetal Aspartate Transfer

Figure 5 summarizes the results of aspartate loading in pregnant monkeys. Infusion of 0.1 g/kg raised the maternal plasma levels approximately 150-fold to a mean value of 65 μmoles/dl without significantly altering fetal levels. Infusion of 0.4 g/kg produced maternal values more than 1,000 times base line, and under these

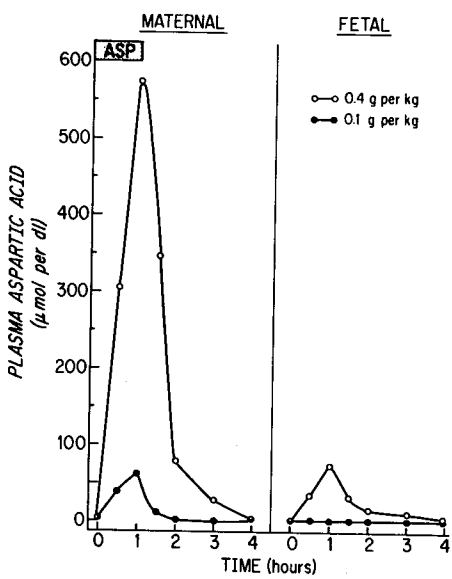

FIG. 5. Mean maternal and fetal plasma aspartate levels with maternal aspartate infusion at 0.1 g/kg (●) (5 experiments) and 0.4 g/kg (○) (2 experiments). (From Pitkin, ref. 8, with permission.)

conditions some aspartate was transferred to the fetus, but only in amounts sufficient to raise the fetal levels 200-fold.

Radioactivity studies confirmed the chemical measurements and indicated a metabolic pattern essentially identical to that of glutamate. During the infusion, most of the radioactivity in the maternal plasma involved aspartate itself with smaller quantities representing glucose and lactate, and only under conditions of very high maternal loading did labelled aspartate reach the fetal circulation.

Comparison of maternal and fetal blood levels indicates that the placental "threshold" for aspartate transfer occurs at approximately 100 μmoles/dl or 300 to 500 times base line. Below this level, no transfer to the fetus occurs, whereas above it relatively small quantities are transported.

SUMMARY

These studies have clarified the metabolism and distribution of the two dicarboxylic amino acids, glutamate and aspartate, in pregnant primates. The primary effect of maternal administration of either is to raise the circulating levels of the respective amino acid to an extent proportional to the dose. Approximately 20%, at least acutely, is metabolized, mainly to glucose and to a lesser extent to lactate. There is some interconversion between glutamate and aspartate, and small quantities of other amino acids are also formed.

The hemochorial placenta is virtually impermeable to glutamate and aspartate at maternal plasma levels less than 200 μmoles/dl (40 to 50 times fasting) and 100 μmoles/dl (300 to 500 times fasting), respectively. Above these threshold levels, some degree of transfer takes place. From a practical point of view, it should be emphasized that maternal concentrations of these magnitudes are never approached under physiologic conditions and can only be attained with intravenous infusion of large amounts.

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