Presence of Adenosine in the Human Term Placenta

DETERMINATION OF ADENOSINE CONTENT AND PATHWAYS OF ADENOSINE METABOLISM

By Meng Kwoon Sim and M. Helen Maguire

ABSTRACT

Adenosine, inosine, and hypoxanthine were isolated from human term placenta by paper chromatography of the dialyzates of aqueous placental homogenates and quantified by ultraviolet spectrosopy. Placentas obtained upon vaginal delivery contained 230 ± 18 nmoles adenosine/g tissue, 300 ± 29 nmoles inosine/g, and 250 ± 19 nmoles hypoxanthine/g. Three prelabor placentas obtained by cesarean section contained 44 ± 3 nmoles inosine/g and 277 ± 22 nmoles hypoxanthine/g, but adenosine was not detected in two of the placentas and was present only at 4 nmoles/g in the third. The activities of placental enzymes catabolizing ATP were determined in homogenates of term placentas. No adenylate deaminase or xanthine oxidase activity was found. Adenosinetriphosphatase, adenylate kinase, and 5'-nucleotidase were more active than were adenosine kinase, adenosine deaminase, and purine nucleoside phosphorylase. The results indicate that in the placenta both the formation of adenosine from adenosine monophosphate and the fate of adenosine, i.e., salvage or catabolism, are influenced by the level of adenosine triphosphate. Possibly, during labor, placental ischemia favors the enzymatic production of adenosine.

KEY WORDS adenosine monophosphate inosine hypoxanthine adenosine deaminase 5'-nucleotidase labor adenosine kinase adenine nucleotide degradation

In a recent study, we found that the activity of adenosine deaminase in the human fetal placenta was considerably lower than it was in the fetal placentas of the guinea pig and the cow (1). It seemed possible, therefore, that adenosine might accumulate in the human placenta, particularly since the earlier studies of Hayashi et al. (2) suggested that the human term placenta lacks adenylate deaminase and that the pathway of degradation of adenosine monophosphate (AMP) involves adenosine as an intermediate rather than inosine monophosphate (IMP). The factors which mediate the local regulation of blood flow in the placenta, a noninnervated organ, have not been elucidated, and the detection of vasoactive adenosine in the placenta could be of physiological significance. Accordingly, we attempted to extract and quantify adenosine and its breakdown products, inosine and hypoxanthine, from human term placentas obtained before and after labor. We also measured the activities of the placental enzymes responsible for the formation of AMP from adenosine triphosphate (ATP) and those responsible for AMP catabolism.

METHODS

PLACENTAS

Human placentas were obtained immediately upon normal vaginal delivery or upon delivery by...
cesarean section and processed at once. No complications were associated with the pregnancies, and all placentas were of normal size, color, and weight.

**ISOLATION OF ADENOSINE, INOSINE, AND HYPOXANTHINE**

Placentas were extracted in the following way. Three 20–25-g samples of tissue were cut from the maternal side of each placenta, one from the center and two from the periphery. The tissue was blotted on absorbent paper to remove as much blood as possible, and 60 g was homogenized in 120 ml of distilled water at 90–95°C for 2 minutes at maximum speed in a Sorvall Omnimix. The homogenate was then kept at 100°C for 2 minutes to destroy any remaining enzymatic activity and, thus, to ensure that no enzyme-catalyzed formation or breakdown of adenosine occurred during the subsequent dialysis step. The human placenta contains ATP (3, 4) which would be expected to undergo some nonenzymatic hydrolysis resulting in the formation of AMP during heating at 90–100°C. The stability of AMP during the extraction procedure was checked by paper chromatographic analysis of a 15 mM solution of sodium AMP kept at 100°C for 4 minutes. No hydrolysis of AMP was observed, in accord with the reported long half-life of aqueous solutions of the nucleotide at high temperatures (5). The homogenate was cooled and dialyzed at 4°C for 24 hours against 2 liters of distilled water. The dialyzate was then concentrated in vacuo to about 10 ml while the temperature was held below 45°C. The precipitate which separated was removed by centrifugation and washed twice with 2 ml of water. The washings were combined with the main supernatant fluid to give approximately 14 ml of a clear yellow solution. A 3-ml sample of this solution was applied to a 25 × 40-cm sheet of washed Whatman no. 3MM paper, which was developed by descending chromatography in a butanol-water (86:14) solvent system for 17 hours. Observation of the dried paper under ultraviolet light showed the presence of five absorbing bands and one fluorescent band. The chromatogram was then redissolved and dried four times; in this way the absorbing band of highest R<sub>p</sub> (band 1) was eluted from the paper, and the remaining five bands were resolved into seven discrete bands (bands 2–8), as shown in Figure 1. Each band was cut out and eluted with water. Band 1 was obtained from a second chromatogram after a single 17-hour development. The eluates were concentrated in vacuo to about 1 ml, and each was applied to a 25 × 40-cm sheet of washed Whatman no. 3MM paper. The chromatograms were developed ascendingly in a butanol-water-15N ammonia (86:14:1) solvent system for 24 hours. Bands 2, 3, and 5–7 were homogeneous in this solvent system, but band 4 was separated into three bands and band 8 was resolved into trace components which could not be detected under

**FIGURE 1**

Descending paper chromatogram of the aqueous extract of a normally delivered term placenta. The chromatogram was photographed under ultraviolet light. Resolution of the bands was obtained by repeated development of the chromatogram in a butanol-water (86:14) solvent system, as described in the text. The absorbing bands were shown to consist of uracil (2), hypoxanthine (3), adenosine + uridine + an uncharacterized component (4), inosine (6), and guanosine (7). The fluorescent bands (5 and 8) were not identified.

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ultraviolet light. The bands were cut out and eluted with distilled water, and the compounds in the eluates were identified by their ultraviolet absorption spectrums at acid, neutral, and alkaline pH. Further confirmation of the identity of the eluted compounds was obtained by their chromatography with markers for the appropriate bases or nucleosides. Band 1 was found to be nicotinamide. Bands 2, 3, 6, and 7 were identified as uracil, hypoxanthine, inosine, and guanosine, respectively. The fluorescent bands, 5 and 8, were not characterized. Band 4 on further resolution gave adenosine, uridine, and an unidentified ultraviolet-absorbing compound. Adenosine, inosine, and hypoxanthine were quantified by their absorbance changes in 5 mM HCl buffer at the appropriate pH. At pH 7, the extinction coefficients of adenosine at 259 nm and of inosine at 248.5 nm are 15.4 x 10^3 and 12.2 x 10^3, respectively, and, at pH 6, the extinction coefficient of hypoxanthine at 247 nm is 12.0 x 10^3 (6).

The minimum quantity of adenosine that could be visualized as an ultraviolet-absorbing band on paper chromatography was determined by applying four 1-ml samples containing 13, 26, 39, and 52 nmoles, respectively, of adenosine to four 25 X 40-cm Whatman no. 3MM papers. The papers were developed by ascending chromatography in a butanol-water-15N ammonia (86:14:1) solvent system and observed under ultraviolet light. The band containing 26 nmoles of adenosine could just be detected, and no band was seen on the chromatogram of 13 nmoles. From similar experiments in which the bands were eluted and quantified by ultraviolet spectroscopy, it was ascertained that the recovery of known amounts of nucleosides after chromatography was quantitative. The limit of detection of adenosine in the procedure was 26 nmoles, equivalent to 2 nmoles adenosine/g placenta.

The amount of blood in placental extracts was determined by the cyannmethemoglobin method of Cohn (7).

ENZYME ASSAYS

Placentas from normal vaginal deliveries were used to prepare homogenates for enzyme assays. Three portions of approximately 2 g each were cut from the maternal side of the placenta as described above, freed from connective tissue, and blotted to remove as much blood as possible. A 4-5-g sample was homogenized in ten volumes of ice-cold distilled water in a Sorvall Omnimix at maximum speed for 2 minutes while the homogenizing vessel was immersed in ice. The homogenate was filtered through five layers of cheesecloth, dialyzed against 1 liter of distilled water for 3 hours at 4°C, and stored at 4°C.

Homogenates were used for enzyme assays within 5 hours of preparation.

All assays were carried out in duplicate at 30°C with volumes of homogenate that yielded a reaction velocity linearly related to homogenate concentration. When enzyme activity was measured spectrophotometrically, absorbance changes were followed by a Unicam monochromometer coupled to a Gilford absorbance indicator and a Varian 2000 recorder. Initial reaction rates were recorded immediately after the addition of the homogenate to the assay mixture contained in a cuvette thermostatically maintained at 30°C; all absorbance changes were linear for at least 3 minutes. The incubation time chosen for enzyme assays by other techniques was such that the dependence of reaction rate on incubation time was linear. When coupled enzyme assays were used, the auxiliary and indicator enzymes were always present in excess so that an additional increase in the concentration of these enzymes did not increase the overall reaction rate.

One unit of enzyme activity is, in all cases, the amount of enzyme which catalyzes the conversion of 1 μmole substrate/min at 30°C under the conditions of the relevant assay. ADENOSINEMETHOSPHATASE.-The activity of adenosinemethosphatase (ATPase) was determined spectrophotometrically by coupling the production of adenosine diphosphate (ADP) to the oxidation of reduced nicotinamide adenine dinucleotide (NADH) via the pyruvate kinase and lactate dehydrogenase reactions. The assay mixture contained 0.05M Tris-HCl, pH 8.8, 0.01M MgSO4, 0.1X KCl, 0.15 mM ATP, 0.1 mM phosphoenolpyruvate, 0.1X NADH, 10 units of pyruvate kinase, and 15 units of lactate dehydrogenase in a total volume of 1.48 ml. A cuvette with a path length of 0.5 cm was used. The reaction was started by adding 20 μliters of placental homogenate, and the resulting increase in absorbance at 340 nm was recorded. One unit of ATPase activity is the amount of enzyme which results in the oxidation of 1 μmole NADH/min. The molar absorption change for the oxidation of NADH is 6.22 x 10^3 cm^-1 M^-1 (8).

ADENYLATED KINASE.-The rate of conversion of ADP to AMP was measured by coupling the reaction with excess adenylate deaminase and following the decrease in absorbance at 265 nm as AMP was deaminated to IMP (9). The conversion of 1 μmole of ADP resulted in the formation of 0.5 μmoles of AMP, and the factor used to convert the change in absorbance per minute to micromoles of ADP converted was 4.43 x 10^3 cm^-1 M^-1, i.e., half the molar absorbance change for the deamination of AMP (10). Assays were carried out in cuvettes with a path length of 0.5 cm, containing 0.05M Tris-HCl, pH...
7.5, 0.13 mM MgSO\(_4\), 0.13 mM KCl, 0.13 mM ADP, and 20 units of adenylic deaminase in a volume of 1.45 ml. The reaction was started by adding 50 \(\mu\)l of placental homogenate to the preincubated assay mixture.

**Alkaline Phosphatase and Acid Phosphatase.**

The activities of alkaline and acid phosphatase were determined spectrophotometrically by using phenylphosphate as the substrate and following the increase in absorbance at 285 nm and 268 nm, respectively, associated with the hydrolysis of phenylphosphate to phenol. The assay medium for the measurement of alkaline phosphatase activity contained 0.5M sodium carbonate buffer, pH 10.2, 0.1 mM MgSO\(_4\), and 0.15 mM disodium phenylphosphate in a total volume of 2.9 ml. Cuvettes with a path length of 1 cm were used, and the reaction was started by adding 0.1 ml of placental homogenate to the preincubated mixture. The protocol for the measurement of alkaline phosphatase was similar except that the buffer was 0.1M citrate, pH 4.7. The molar absorbance changes for the conversion of phenylphosphate to phenol were 1.64 cm\(^{-1}\) M\(^{-1}\) at 285 nm and pH 10.2 and 1.1 cm\(^{-1}\) M\(^{-1}\) at 268 nm and pH 4.7.

The heat stability of each phosphatase was ascertained by heating the homogenate at 65°C for 30 minutes prior to assay. Alkaline phosphatase was heat stable, as has been reported (11), but acid phosphatase was heat labile. The contribution of acid and alkaline phosphatase to nonspecific phosphatase activity at pH 7.2 was determined using both heat-treated and untreated homogenate in the standard assay protocol in 0.05M Tris-HCl, pH 7.2; the absorbance change at 285 nm was followed.

**5'-Nucleotidase.**

The activity of 5'-nucleotidase was measured spectrophotometrically using AMP as the substrate by coupling the reaction with excess adenosine deaminase and following the fall in absorbance, indicating that the combined activities of ATPase and adenylate kinase did not interfere. The low level of adenosine deaminase present in placenta was 0.1\(\mu\)M Mg\(^{2+}\) in the assay. To reduce interference by ATPase and adenylate kinase, Mg\(^{2+}\) was not added to the assay; evidence for the optimizing effect of Mg\(^{2+}\) on adenosine kinase activity varies (16, 17), but high concentrations of Mg\(^{2+}\) are known to inhibit the enzyme (18, 19). Control assays performed in the absence of adenosine resulted in a negligible fall in absorbance, indicating that the combined activities of ATPase and adenylate kinase did not interfere with the assay. No detectable change in absorbance occurred when blank assays were carried out in the absence of ATP, indicating that the low level of adenosine deaminase present in the homogenate did not affect the measurement of adenosine kinase activity.

**Adenosine Deaminase and Adenylate Deaminase.**

The activity of adenosine deaminase was measured as described previously by determining the amount of ammonia released when 1 ml of homogenate was incubated at 30°C for 15 minutes with 2 ml of 0.1M sodium \(\beta\), \(\beta\)-dimethylglutarate, pH 7, containing 5 mM adenosine (1). A 1-ml sample was taken for the estimation of ammonia by the Conway microdiffusion method (15). The protocol for the determination of adenylic deaminase activity was similar except that 0.75 ml of homogenate was added to 0.75 ml of 0.1M Tris-HCl, pH 7.2, which contained 3 mM AMP and 5 mM ATP. ATP stimulates adenylic deaminase (10), and it was included in the assay medium to facilitate the detection of low levels of the enzyme. Assays for adenylic deaminase activity were also carried out using a more concentrated homogenate prepared by homogenizing placental tissue in three volumes of water.

**Adenosine Kinase.**

Adenosine kinase activity was measured spectrophotometrically by coupling the reaction with excess adenylic deaminase and following the fall in absorbance at 285 nm as the AMP formed by the action of adenosine kinase was deaminated to IMP. The reaction was started by adding 0.1 ml of placental homogenate to 1.4 ml of assay medium containing 0.05M Tris-maleate, pH 5.8, 1.3 mM KCl, 0.1 mM adenosine, 0.1 mM ATP, and 10 units of adenylic deaminase incubated at 30°C in a 0.5-cm cuvette. The molar absorbance change for the conversion of AMP to IMP in Tris-maleate buffer, pH 5.8, was 8.1 \(\times\) 10\(^8\) cm\(^{-1}\) M\(^{-1}\).

The dialyzed homogenate contained 0.6 mM Mg\(^{2+}\) (determined by atomic absorption spectrometry) which gave 40 \(\mu\)M Mg\(^{2+}\) in the assay. To reduce interference by ATPase and adenylic kinase, Mg\(^{2+}\) was not added to the assay; evidence for the optimizing effect of Mg\(^{2+}\) on adenosine kinase activity varies (16, 17), but high concentrations of Mg\(^{2+}\) are known to inhibit the enzyme (18, 19). Control assays performed in the absence of adenosine resulted in a negligible fall in absorbance, indicating that the combined activities of ATPase and adenylic kinase did not interfere with the assay. No detectable change in absorbance occurred when blank assays were carried out in the absence of ATP, indicating that the low level of adenosine deaminase present in the homogenate did not affect the measurement of adenosine kinase activity.

**Purine Nucleoside Phosphorylase.**

The coupled xanthine oxidase method of Kalkar (9) was used to assay this enzyme. The reaction was started by adding 50 \(\mu\)l of placental homogenate to 2.95 ml of preincubated medium containing 0.05M phosphate buffer, pH 7.5, 0.15 mM ADP, and 20 units of adenylate deaminase in a volume of 1.45 ml. The reaction was commenced by adding 50 \(\mu\)l of homogenate to the preincubated assay mixture. The molar absorbance change for the deamination of adenosine is 8.1 \(\times\) 10\(^8\) cm\(^{-1}\) M\(^{-1}\) (12).
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mM inosine, and 0.1 units of xanthine oxidase, and the activity was measured by following the increase in absorbance at 293 nm as hypoxanthine was formed and oxidized to uric acid. The molar absorbancy change was $12.5 \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$.

Xanthine Oxidase.—A more concentrated homogenate was employed to assay for xanthine oxidase activity. The reaction medium contained 100 mM Tris-HCl, pH 8.5, 0.6 mM 8-14C-xanthine (specific activity 1.61 mc/mmole), and 0.6 mM methylene blue in 0.1 ml, and the reaction was started by adding 0.1 ml of homogenate containing 0.2 g placenta/ml. A control assay was carried out using boiled homogenate. After 15 minutes, the reaction was terminated by heating at 100°C for 3 minutes. Then, 50 µg of xanthine and 40 µliters of a saturated solution of uric acid in 1N ammonia were added, precipitated protein was separated by centrifugation, and 50-µliter samples were applied to Whatman no. 3 MM papers and chromatographed in a descending solvent system of butanol and water (86:14) for 24 hours. Uric acid spots were visualized under ultraviolet light, cut out, and counted in Bray's solution in a Packard scintillation counter.

Assay of Enzymes in Blood.—An aqueous solution of venous blood (1:10) was prepared and used to assay for ATPase, adenylate deaminase, adenosine kinase, adenosine deaminase, 5'-nucleotidase, purine nucleoside phosphorylase, and acid and alkaline phosphatase activity as described for the placental homogenate.

MATERIALS

Adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), and adenosine 5'-monophosphate (AMP) as the sodium salts, hypoxanthine, NADH, and phosphoenolpyruvate were obtained from Sigma. Adenosine and inosine were Fluka products. 8-14C-xanthine was from ICN. Pyruvate kinase (specific activity 420 at 37°C and pH 7.6), lactate dehydrogenase (specific activity 400 at 37°C and pH 7.5), and xanthine oxidase (specific activity 0.3 at 25°C and pH 7.5) were from Sigma. Adenosine deaminase was purified from bovine placenta, as described previously (20), to a specific activity of 252 at 30°C and pH 7. Adenylate deaminase was purified from rat skeletal muscle by the method of Smiley et al. (21) and had a specific activity of 62 when assayed at 30°C in 0.05M Tris-HCl, pH 7.0.

Results

ISOLATION OF ADENOSINE, INOSINE, AND HYPOXANTHINE

The levels of adenosine, inosine, and hypoxanthine in placentas obtained upon vaginal delivery after 16-20 hours of labor and in prelabor placentas are shown in Figure 2. Adenosine was not detected in two of the three prelabor placentas and only 4 nmoles/g was found in the third. In contrast, placentas obtained after labor contained 230 ± 18 nmoles/g adenosine. The inosine content of prelabor and postlabor placentas also differed significantly, being 44 ± 3 and 300 ± 29 nmoles/g, respectively, but the hypoxanthine levels were similar in both cases. Adenosine, at 27 and 87 nmoles/g, was found in two placentas obtained by cesarean section 3 and 9 hours, respectively, after the commencement of labor. The inosine content in these placentas was 70 and 245 nmoles/g, respectively. The hypoxanthine contents were of the same order of magnitude as the values shown in Figure 2 for prelabor and normal-delivery placentas. No adenosine or inosine was detected in one placenta that was stored at room temperature for 3 hours prior to

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Adenosine, inosine, and hypoxanthine concentrations in human term placenta obtained before the commencement of labor (n = 3) and after normal vaginal delivery (n = 4). Vertical bars indicate the sd.
Estimation of hemoglobin in the placental extracts showed that they contained 10 ± 1.4% blood. Normally, there is no free adenosine or inosine in plasma or in the cellular components of blood, but concentrations of hypoxanthine of 0.04 mM and 0.01 mM have been reported (22, 23) in normal human plasma. Blood present in the placental extracts would contribute approximately 4 nmoles hypoxanthine/g placenta, which in comparison with the measured hypoxanthine levels shown in Figure 2 is negligible.

**ENZYME ASSAYS**

The activities of the placental enzymes which catalyze the formation of AMP from ATP and the catabolism of AMP are listed in Table 1 together with the activities of acid and alkaline phosphatase. Alkaline phosphatase retained its activity at pH 7.2 and was responsible for more than 50% of the AMP-hydrolyzing activity present in the placental homogenate (Fig. 3). Acid phosphatase was not active at pH 7.2, and all the heat-sensitive phosphohydrolase activity at this pH was due to 5'-nucleotidase. Placental 5'-nucleotidase was inhibited by ATP; 75 μM ATP in the assay for 5'-nucleotidase caused a 25% reduction in 5'-nucleotidase activity. No xanthine oxidase or ATP-stimulated adenylate deaminase activities were detected.

The assays for ATPase, adenylate kinase, adenosine kinase, 5'-nucleotidase, and acid and alkaline phosphatase in blood found no measurable activity, indicating that the levels of these enzymes in blood were too low to contribute to the activities of the enzymes determined in the placental homogenates. Purine nucleoside phosphorylase activity in blood was equivalent to 0.12 units/g placenta and, thus, did not make a significant contribution to the amount of the enzyme, 0.9 ± 0.1 units/g placenta, found in the placental homogenates.

![Graph](http://circres.ahajournals.org/)

**FIGURE 3**

Hydrolysis of AMP by placental homogenate preheated at 65°C for 30 minutes (•) and by homogenate which was not heat treated (o). Heat-stable phosphohydrolase activity was due to alkaline phosphatase and heat-labile activity to 5'-nucleotidase. Assays were performed in 0.05 M Tris-HCl, pH 7.2, in the presence of added adenosine deaminase, and enzyme activity was measured by following the decrease in absorbance at 265 nm as adenosine was converted to inosine.

**TABLE 1**

Activities of Enzymes Mediating the Catabolism of ATP and Activities of Acid and Alkaline Phosphatase in the Human Term Placenta

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (units/g placenta ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATPase</td>
<td>16.5 ± 1.3 (4)</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>7.2 ± 0.3 (4)</td>
</tr>
<tr>
<td>Adenyate deaminase</td>
<td>UD (4)</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>2.2 ± 0.3 (4)</td>
</tr>
<tr>
<td>Adenosine kinase</td>
<td>0.2 ± 0.1 (4)</td>
</tr>
<tr>
<td>Adenosine kinase</td>
<td>0.47 ± 0.1 (4)</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>0.9 ± 0.1 (4)</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>UD (3)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>13.4 ± 1.13 (3)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>6.6 ± 1.0 (3)</td>
</tr>
</tbody>
</table>

Activities were measured as described in the Methods; 1 unit is the amount of enzyme which catalyzes the conversion of 1 μmole substrate/min at 30°C under the conditions of the relevant assay. The figures in parentheses are the numbers of placentas assayed. UD = undetected.
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Discussion

The presence of adenosine, inosine, and hypoxanthine in the human term placenta and the significant differences in the levels of adenosine and inosine in prelabor and postlabor placentas can be considered in terms of the placental enzymes concerned with the degradation of ATP and in terms of the response of these enzymes to possible physiological changes induced by labor. The present study shows that the human term placenta contains high levels of ATPase and adenylate kinase, which ensure that placental ATP can be readily hydrolyzed to ADP and that 2 moles of ADP can be readily equilibrated to 1 mole each of AMP and ATP. In confirmation of an earlier observation (24), no adenylate deaminase was detected; consequently, the only catabolic pathway for AMP is via phosphohydrolysis to adenosine. Both alkaline phosphatase and 5'-nucleotidase are present in the placental homogenates and catalyze the hydrolysis of AMP. The contribution made by the nonspecific phosphatase to adenosine formation in vivo depends on the subcellular distribution of the enzyme and on the subcellular location of AMP in the placental trophoblast. Ultrastructural studies have indicated that alkaline phosphatase is disposed mainly on the outer surface of the trophoblastic villi (25, 26). Since the formation of AMP is probably intracellular, it is not likely that alkaline phosphatase plays an important role in the in vivo hydrolysis of AMP. ATP is known to be a potent inhibitor of mammalian 5'-nucleotidase (12), and ADP has been shown to inhibit the enzyme more strongly than does ATP (14, 27). The presence in the human placenta of a 5'-nucleotidase which is inhibited by ATP signifies that the formation of adenosine in the placenta may be regulated by placental ATP levels and possibly also by ADP levels. Thus, a decrease in the concentration of ATP should facilitate an increase in both the concentration of AMP formed via the action of adenylate kinase on ADP and the expression of 5'-nucleotidase activity, resulting in the conversion of AMP to adenosine. The fate of adenosine in the placenta is also probably influenced by placental ATP concentrations. The activity of adenosine kinase is twice that of adenine deaminase, so, when ATP levels are high, any adenosine formed is more likely to be rephosphorylated to the nucleotide by ATP and adenylate kinase than it is to be deaminated by adenine deaminase. When the ATP concentration is low, an increased rate of breakdown of AMP may result in the accumulation of adenosine. The phosphorolysis of inosine by placental purine nucleoside phosphorylase yields hypoxanthine, which is the end product of ATP degradation in the human placenta. The pathways involved in placental ATP catabolism are shown in Figure 4.

Although the presence of adenosine and inosine in the human placenta has not been reported previously, low levels of the two nucleosides have been found in normally oxygenated canine and rat myocardium, and these levels increase with periods of ischemia concomitantly with a decrease in the tissue ATP levels (28-30). In the rat heart, the concentration of adenosine reaches a peak of 500 nmoles/g after 20 minutes of ischemia and then decreases with longer ischemic periods (29). The activity of adenosine deaminase in the rat heart is 0.58 units/g (31), twice that in the human placenta. The rat myocardium contains low levels of adenylate deaminase and xanthine oxidase and shows minimal adenosine kinase activity (31) but, like the human placenta, has high levels of ATPase, adenylate kinase, and 5'-nucleotidase (Maguire, unpublished observation). It has been suggested that the release of adenosine by the myocardium is involved in the regulation of coronary blood flow (32). The present hypothesis is that in conditions of hypoxia the breakdown of myocardial ATP via ADP to AMP results in lowered ATP levels and increased AMP levels, which favor the expression of 5'-nucleotidase activity rather than adenylate deaminase activity and lead to the formation of adenosine. The released adenosine then dilates the coronary vessels, facilitating coronary blood flow and enabling the
oxygen requirements of the myocardium to be met (30).

Adenosine dilates the peripheral as well as the coronary vascular beds and constricts the kidney vasculature (33). No definitive study of the action of adenosine on placental blood vessels has been reported, but both dilator and constrictor responses have been observed in human placentas perfused with nonoxygenated Locke's solution (34). The present study shows that the enzymatic complement of the human placenta facilitates adenosine formation under ischemic conditions. The powerful uterine contractions of labor are known to decrease blood flow through the endometrial spiral arteries into the intervillous spaces of the maternal side of the placenta (35) and may thus cause localized ischemic areas in the placenta where adenosine production would be favored. The greatly increased levels of adenosine found in the postlabor placentas may thus be due to ischemia resulting from the repeated uterine contractions. Adenosine formation in the placenta, like that in the myocardium, appears to be an active process dependent on the reserves of ATP in the tissue. The absence of adenosine in the postlabor placenta stored for 3 hours prior to extraction is comparable with the fall in the myocardial adenosine concentration observed after prolonged ischemia (29). The mammalian placenta is not innervated, and the control mechanisms responsible for the local regulation of blood flow are not understood. The isolation of vasoactive adenosine from the human placenta and the elucidation of a regulated pathway for its formation could be of physiological significance and warrant further investigation of the actions of adenosine on placental blood vessels and of the relationship between placental adenosine concentration and the duration of labor.

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