Differential responses to inducers of δ-aminolaevulinate synthase and haem oxygenase during pregnancy

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The responses of hepatic δ-aminolaevulinate synthase and microsomal haem oxygenase to inducers were examined in pregnant rats. 2-Allyl-2-isopropylacetamide-mediated induction of δ-aminolaevulinate synthase was greatly decreased during pregnancy and in the early post-partum period. Administration of allylisopropylacetamide to pseudopregnant rats induced δ-aminolaevulinate synthase normally. Treatment of pregnant rats with cortisol failed to restore the drug-mediated induction of δ-aminolaevulinate synthase. Microsomal cytochrome P-450 content and the activities of drug-metabolizing enzymes such as aniline hydroxylase and ethylmorphine. N-demethylase were significantly lowered during pregnancy. In contrast with the greatly impaired induction of δ-aminolaevulinate synthase, the induction of haem oxygenase in response to CoCl₂ remained unaltered in pregnant rats. The normal perturbations of δ-aminolaevulinate synthase, consisting of an initial inhibition followed by a rebound increase in the enzyme activity associated with CoCl₂ treatment, were observed during pregnancy. These findings indicate that hormones and metabolic factors associated with gestation exert significant but differential controls on the induction patterns of δ-aminolaevulinate synthase and haem oxygenase.

A variety of hormones have been implicated in the maintenance and the inducibility of haem and haemoproteins in the liver (Schenkman et al., 1967; Kappas, 1968; Matsuoka et al., 1968; Sassa & Kappas, 1977). More recently, adrenalectomy has been shown to enhance significantly the CoCl₂-mediated induction in rat liver of haem oxygenase (Sardana et al., 1980), the rate-limiting enzyme of haem catabolism (Tenhunen et al., 1968, 1969). This endocrine ablation, on the other hand, is known to impair markedly the inducibility of δ-aminolaevulinic synthase, the rate-limiting enzyme of haem formation (Marver et al., 1966). Cortisol reverses these effects of adrenalectomy.

In further exploring the role of endocrine factors in regulating hepatic haem metabolism, we have examined the influence of pregnancy on the inducibility of δ-aminolaevulinate synthase by 2-allyl-2-isopropylacetamide and of haem oxygenase by CoCl₂. The results of this study indicate that, as in adrenalectomized animals, markedly different effects on the induction responses of these enzymes can be identified in gestation. These findings provide further evidence of the complex influences that endocrine status can exert on haem metabolism in the liver.

Experimental

Materials
Pregnant and non-pregnant female Sprague–Dawley rats used in this study were obtained from Holtzman Co. (Madison, WI, U.S.A.). The female rats were made pseudopregnant by mechanical stimulation of the cervix as described by DeFeo (1966). The animals were housed in individual cages and were deprived of food for 12 h before treatment with allylisopropylacetamide or CoCl₂. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), except glucose 6-phosphate dehydrogenase (Boehringer, Indianapolis, IN, U.S.A.) and CoCl₂ (Fisher Scientific Co., Pittsburg, PA, U.S.A.). Allylisopropylacetamide was a gift from Hoffman–La Roche (Nutley, NJ, U.S.A.).

Tissue preparation
CoCl₂ and allylisopropylacetamide solutions were made in 0.9% NaCl and injected subcutaneously into rats. At 12 h after administration of allylisopropylacetamide, the animals were killed by decapitation. Livers were perfused in situ with ice-cold 0.9% NaCl until free from haemoglobin;
they were then homogenized in 3 vol. of 0.1 M potassium phosphate buffer, pH 7.4, in a glass Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates were centrifuged at 9000g for 20 min, and the supernatants were removed and centrifuged again at 105 000g for 1 h to obtain the microsomal pellet.

Enzyme assays

Microsomal fractions were resuspended in 0.1 M potassium phosphate buffer, pH 7.4, to yield a final concentration of 10–12 mg of protein/ml. Microsomal haem oxygenase activity was determined as described previously (Maines & Kappas, 1975). The bilirubin concentration produced during the haem oxygenase assay was calculated by using an absorption coefficient of 40 mM⁻¹·cm⁻¹ for the difference between absorbance at 464 nm and 530 nm. The activities of aniline hydroxylase and ethylmorphine N-demethylase were assayed as described by Imai et al. (1966) and Alvares & Mannering (1970) respectively. The formaldehyde produced in the ethylmorphine N-demethylase assay was measured by the method of Nash (1953). Microsomal cytochrome P-450 content was determined by the method of Omura & Sato (1964). Determination of δ-aminolaevulinate synthase activity in the nuclear/mitochondrial preparation and protein concentration, as well as statistical analyses of data, were performed as described previously (Sassa et al., 1979).

Results

Fig. 1 depicts the effect of allylisopropylacetamide on hepatic δ-aminolaevulinate synthase activity 12 h after drug administration in non-pregnant female rats (day 0) and at eight subsequent time points during gestation and the immediate post-partum period. In non-pregnant animals, allylisopropylacetamide produced a substantial increase in δ-aminolaevulinate synthase activity, as expected. With the initiation of gestation, however, the induction response of the enzyme was lessened, and inhibition of induction became marked by day 16 of gestation; this inhibition of enzyme induction
Haem synthesis and degradation in pregnancy

extended into the first week of the post-partum period. The induction response to allylisopropylacetamide increased thereafter towards the normal adult range, but was still impaired at day 9 after birth (Fig. 1).

Microsomal cytochrome P-450 content declined gradually during gestation (to approx. 40% at day 20), but the fraction of cytochrome P-450 degraded by allylisopropylacetamide at 12h after the drug treatment was not significantly altered (Fig. 1). The degradation of cytochrome P-450 is known to occur during the time period 1–16h after administration of allylisopropylacetamide in normal animals (De Matteis, 1970, 1971).

Since cytochrome P-450 contents showed a steady decrease during pregnancy, the cytochrome P-450-dependent ethylmorphine N-demethylase and aniline hydroxylase activities were observed as early as 4 days after pregnancy was established (Table 1). Ethylmorphine N-demethylase activity decreased further at 12 and 20 days of pregnancy, to less than 30% of the initial activity. Aniline hydroxylase activity, on the other hand, did not show further decreases at 12 and 20 days of pregnancy (Table 1).

Table 2 shows that cortisol did not restore the induction response of δ-aminolaevulinic synthase to normal in 20-day-pregnant animals, although the same dose of cortisol is known to restore the inducibility of this enzyme in adrenalectomized animals to normal (Marver et al., 1966). The endocrine–metabolic state of the animal in pregnancy can be mimicked to some extent by the activation of luteal progesterone secretion and by the increase in prolactin produced during pseudopregnancy. Therefore the effect of allylisopropylacetamide on the induction of δ-aminolaevulinic synthase at days 4 and 11 of pseudopregnancy was further studied, and the results are presented in Table 3. These time periods were selected because substantial hormonal responses

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Table 1. Effects of pregnancy on ethylmorphine N-demethylase and aniline hydroxylase activities

<table>
<thead>
<tr>
<th>Duration of pregnancy (days)</th>
<th>Ethylmorphine N-demethylase activity (μmol of formaldehyde formed/h per mg of protein)</th>
<th>Aniline hydroxylase activity (nmol of p-aminophenol formed/h per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (normal female)</td>
<td>0.39 ± 0.04</td>
<td>40.91 ± 2.15</td>
</tr>
<tr>
<td>4</td>
<td>0.30 ± 0.02</td>
<td>29.79 ± 1.90</td>
</tr>
<tr>
<td>12</td>
<td>0.12 ± 0.01</td>
<td>32.90 ± 4.35</td>
</tr>
<tr>
<td>20</td>
<td>0.11 ± 0.01</td>
<td>30.09 ± 3.07</td>
</tr>
</tbody>
</table>

Table 2. Effect of cortisol on allylisopropylacetamide-mediated δ-aminolaevulinic synthase induction in pregnancy

Allylisopropylacetamide was injected subcutaneously at a dose of 400 mg/kg body wt. Cortisol was administered intraperitoneally (5 mg/kg) 30 min before the administration of allylisopropylacetamide. Results are expressed as means ± S.E.M. for four rats.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Treatment</th>
<th>δ-Aminolaevulinic synthase activity (nmol/h per g of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal female</td>
<td>Saline</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Normal female</td>
<td>Allylisopropylacetamide</td>
<td>121 ± 11</td>
</tr>
<tr>
<td>20-day pregnant</td>
<td>Allylisopropylacetamide</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>20-day pregnant</td>
<td>Allylisopropylacetamide + cortisol</td>
<td>49 ± 8</td>
</tr>
</tbody>
</table>

Table 3. Effect of allylisopropylacetamide on δ-aminolaevulinic synthase activity in pseudopregnant animals

Pseudopregnant rats at days 4 and 11 were injected subcutaneously with allylisopropylacetamide (400 mg/kg body wt.). After 12h the animals were killed and δ-aminolaevulinic synthase activity was determined as described in the Experimental section. Results are expressed as means ± S.E.M. for four animals.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Treatment</th>
<th>δ-Aminolaevulinic synthase activity (nmol/h per g of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-day pseudopregnant</td>
<td>Saline</td>
<td>22.6 ± 3.2</td>
</tr>
<tr>
<td>4-day pseudopregnant</td>
<td>Allylisopropylacetamide</td>
<td>126.8 ± 7.1</td>
</tr>
<tr>
<td>11-day pseudopregnant</td>
<td>Saline</td>
<td>28.1 ± 3.8</td>
</tr>
<tr>
<td>11-day pseudopregnant</td>
<td>Allylisopropylacetamide</td>
<td>131.5 ± 8.5</td>
</tr>
</tbody>
</table>
have been shown to occur at these times in pseudopregnancy, a condition that is not maintained for the full 20–21 days of normal gestation in the rat (Rabi & Kragt, 1972). Allylisopropylacetamide administered to pseudopregnant rats caused a normal induction response of δ-aminolaevulinate synthase, indicating that the limited endocrine alterations associated with the pseudopregnant state do not account for the marked impairment of enzyme induction observed in normal pregnancy.

Fig. 2 shows the effect of a single dose of CoCl2 on the induction of hepatic haem oxygenase at various time intervals after administration of the metal salt in pregnant (20 days) rats. The activity of the enzyme increased rapidly between 2 and 4 h and reached a maximum at 16 h (approx. 8-fold greater than normal); the time course and extent of this induction response were equivalent to those produced in normal adult rats (Maines & Kappas, 1975). Contents of cytochrome P-450 also decreased to the same extent observed in non-pregnant animals (Maines & Kappas, 1975).

As depicted in Fig. 3, in 20-day-pregnant animals CoCl2 treatment also produced the early transient decline and the late rebound increase of δ-aminolaevulinate synthase activity (approx. 3-fold greater than normal at 16 h) characteristically observed in normal animals after treatment with the metal salt (Drummond & Kappas, 1980).

Discussion

The results of the present study indicate that the hormonal and other metabolic alterations associated with pregnancy differentially influence the induction responses of hepatic δ-aminolaevulinate synthase and haem oxygenase to chemicals. Specifically in late pregnancy the induction of δ-aminolaevulinate synthase by allylisopropylacetamide is markedly impaired (Paul et al., 1974), whereas the induction of haem oxygenase by CoCl2 is not altered.

The ability of CoCl2, in 20-day-pregnant animals, to elicit both the transient early depression and the late rebound induction of δ-aminolaevulinate synthase observed in normal animals is of considerable interest in view of the marked impairment of the
δ-aminolaevulinate synthase induction response to allylisopropylacetamide. The late rebound increase of δ-aminolaevulinate synthase after the administration of CoCl₂ probably involves a de-repression of enzyme synthesis resulting from perturbations of cellular haem balance (Maines & Kappas, 1974, 1975). The normal occurrence of this rebound phenomenon in pregnancy thus suggests that the nature of the rebound increase after CoCl₂ treatment is different from that characterizing the induction of δ-aminolaevulinate synthase by allylisopropylacetamide, or that compensatory increases in δ-aminolaevulinate synthase can occur in late pregnancy within certain, perhaps physiological limits (compare Fig. 1, day-20 point, and Fig. 3, 16h point), even though a full induction response to a foreign chemical such as allylisopropylacetamide is blocked.

The mechanism of the inhibition of δ-aminolaevulinate synthase induction by allylisopropylacetamide in late gestation and the early post-partum period is not known. The fact that the blockade phenomenon disappears after parturition (Fig. 1) indicates that it must be dependent directly on the hormonal or metabolic events accompanying gestation, and may be related to either maternal host factors or the foetal–placental unit, or both. We have previously described the occurrence of humoral substances in human plasma during late pregnancy and the post-partum period that appear to be proteins (Kappas et al., 1969; Rifkind et al., 1974) and have a potent ability to block allylisopropylacetamide, steroid and other chemical-mediated inductions of δ-aminolaevulinate synthase in cultured chick-embryo liver cells. Comparable substances may also be produced during gestation in the rat and perhaps other species.

The fact that δ-aminolaevulinate synthase induction by allylisopropylacetamide and not the induction of haem oxygenase nor the rebound increase of δ-aminolaevulinate synthase by CoCl₂ is impaired in gestation implies a selectivity for the inhibition mechanism that may extend, not only to specific haem-pathway enzymes as shown in the present study, but possibly also to a specific chemical species of inducers of such enzymes. Thus the response of δ-aminolaevulinate synthase to different chemical inducers may be differentially affected during gestation.

For allylisopropylacetamide it has been shown (De Matteis, 1970, 1971; Ortiz de Montellano et al., 1979) that metabolic transformation of the drug leads to the destruction of cytochrome P-450 haem. This decrease in cytochrome P-450 haem has been implicated in the increase in δ-aminolaevulinate synthase (De Matteis, 1971; Lim et al., 1980) produced by the drug. The results of the present study indicate that, at least in pregnant rats, allylisopropylacetamide-mediated destruction of cytochrome P-450 is not necessarily associated with induction of δ-aminolaevulinate synthase activity (Fig. 1). It has also been demonstrated that low doses of allylisopropylacetamide can increase δ-aminolaevulinate synthase activity without causing a decline in cytochrome P-450 content in rat liver (Klinger & Müller, 1980). The metabolites formed in the biotransformation of allylisopropylacetamide in pregnant rats, however, are not known and may differ from those formed in non-pregnant rats. In contrast with the impaired induction of δ-aminolaevulinate synthase by allylisopropylacetamide, the administration of CoCl₂ to pregnant rats showed a normal induction pattern of haem oxygenase, accompanied by the typical perturbations in δ-aminolaevulinate synthase produced by this metal ion.

Increases in haem oxygenase are generally associated with a decrease in cytochrome P-450-dependent drug metabolism. Thus an impairment in the inducibility of δ-aminolaevulinate synthase, while haem oxygenase remains readily inducible during pregnancy, would be expected to result in alterations in haem, haemoproteins and cytochrome P-450.
dependent chemical metabolism (Neale & Parke, 1969, 1973) in response to foreign chemicals or drugs that could prove to have deleterious consequences.

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References
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