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

Mohinder K. SARDANA, Shigeru SASSA and Attallah KAPPAS
The Rockefeller University, New York, NY 10021, U.S.A.

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-amino-laevulinate 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 $CoCl_2$ 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 $CoCl_2$ 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 maintainance 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 $CoCl_2$-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-aminolaevulin-ate 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 $CoCl_2$. 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 $CoCl_2$. 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 $CoCl_2$ (Fisher Scientific Co., Pittsburg, PA, U.S.A.). Allylisopropylacetamide was a gift from Hoffman–La Roche (Nutley, NJ, U.S.A.).

Tissue preparation

$CoCl_2$ and allylisopropylacetamide solutions were made in 0.9% NaCl and injected subcutaneously into rats. At 12 h after administration of allyliso-propylacetamide, 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 $9000\,g$ for 20 min, and the supernatants were removed and centrifuged again at $105\,000\,g$ 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 $40\,\mathrm{mM^{-1}\cdot cm^{-1}}$ 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
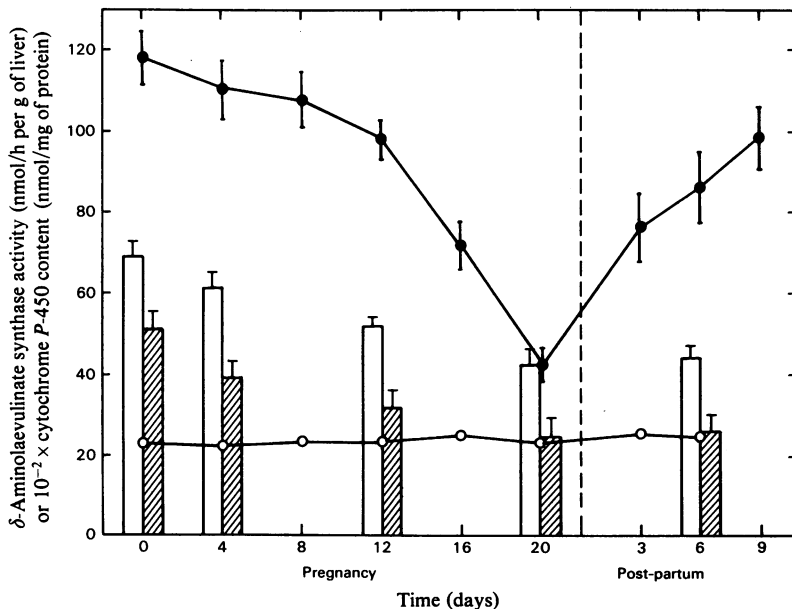

Fig. 1. *Effects of allylisopropylacetamide on δ-aminolaevulinate synthase activity and cytochrome P-450 content during pregnancy and in the post-partum period*

Normal female and pregnant Sprague–Dawley rats were starved for 12 h before administration of allylisopropylacetamide (400 mg/kg). The duration of pregnancy and the post-partum period are indicated on the abscissa. Three rats were tested in each group and animals were killed in 12 h after allylisopropylacetamide administration. Results are expressed as means ± S.E.M. The assays were performed as described in the Experimental section. ●, δ-Aminolaevulinate synthase activity, in allylisopropylacetamide-treated rats; ○, δ-aminolaevulinate synthase activity, in control animals; ▨, cytochrome P-450 content in allylisopropylacetamide-treated rats; ▢, cytochrome P-450 content in control animals.

extended into the first week of the post-partum period. The induction response to allylisopropyl-acetamide 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 12 h after the drug treatment was not significantly altered (Fig. 1). The degradation of cytochrome P-450 is known to occur during the time period 1–16 h 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-aminolaevulinate 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 allyliso-propylacetamide on the induction of δ-amino-laevulinate synthase at days 4 and 11 of pseudopreg-nancy was further studied, and the results are presented in Table 3. These time periods were selected because substantial hormonal responses

Table 1. *Effects of pregnancy on ethylmorphine N-demethylase and aniline hydroxylase activities*
The activities of ethylmorphine N-demethylase and aniline hydroxylase were determined in microsomal fractions as described in the Experimental section. Results are expressed as means ± S.E.M. for four rats.

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

Table 2. *Effect of cortisol on allylisopropylacetamide-mediated δ-aminolaevulinate 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.

Rat	Treatment	δ-Aminolaevulinate synthase activity (nmol/h per g of liver)
Normal female	Saline	22 ± 4
Normal female	Allylisopropylacetamide	121 ± 11
20-day pregnant	Allylisopropylacetamide	46 ± 8
20-day pregnant	Allylisopropylacetamide + cortisol	49 ± 8

Table 3. *Effect of allylisopropylacetamide on δ-aminolaevulinate synthase activity in pseudopregnant animals*
Pseudopregnant rats at days 4 and 11 were injected subcutaneously with allylisopropylacetamide (400 mg/kg body wt.). After 12 h the animals were killed and δ-aminolaevulinate synthase activity was determined as described in the Experimental section. Results are expressed as means ± S.E.M. for four animals.

Rat	Treatment	δ-Aminolaevulinate synthase activity (nmol/h per g of liver)
4-day pseudopregnant	Saline	22.6 ± 3.2
4-day pseudopregnant	Allylisopropylacetamide	126.8 ± 7.1
11-day pseudopregnant	Saline	28.1 ± 3.8
11-day pseudopregant	Allylisopropylacetamide	131.5 ± 8.5

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 $CoCl_2$ 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 $CoCl_2$ 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 $CoCl_2$ is not altered.

The ability of $CoCl_2$, 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
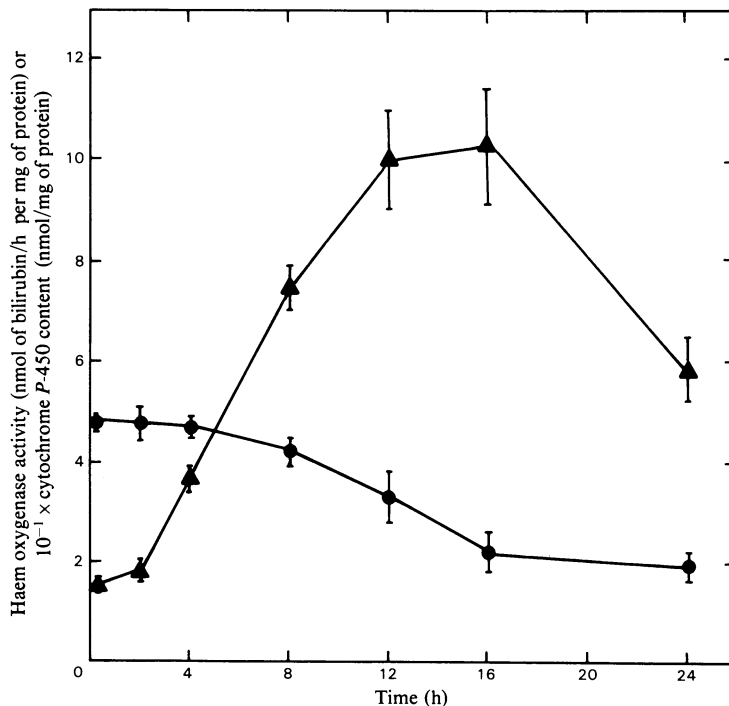

Fig. 2. *Time course of the effects of $CoCl_2$ on haem oxygenase activity and cytochrome P-450 content in late pregnancy*
Pregnant rats (20 days) were injected subcutaneously with $CoCl_2$ (200 μmol/kg) at 0 h and killed at the time intervals indicated on the abscissa. Each time point represents the mean value $\pm$ s.e.m. for three rats. The assays were performed as in the Experimental section. $\blacktriangle$, Haem oxygenase activity (nmol of bilirubin formed/h per mg of protein); $\bullet$, cytochrome P-450 content (nmol/mg of protein).
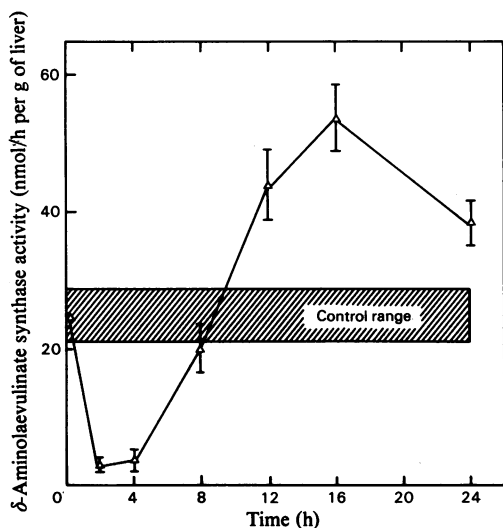
Fig. 3. *Effect of CoCl₂ on the rebound increase of δ-aminolaevulinate synthase activity in 20-day-pregnant rats*

Groups of pregnant rats (20 days) were injected with $CoCl_2$ ($200\,\mu$mol/kg) at 0 h and enzyme assays were performed at the time intervals indicated on the abscissa. Each value represents the mean ± S.E.M. for three rats. △, $CoCl_2$-mediated δ-aminolaevulinate synthase activity in pregnant animals; ▨, δ-aminolaevulinate synthase activity in saline-treated pregnant animals.

δ-aminolaevulinate synthase induction response to allylisopropylacetamide. The late rebound increase of δ-aminolaevulinate synthase after the administration of $CoCl_2$ 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 $CoCl_2$ 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, 16 h 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 $CoCl_2$ 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 $CoCl_2$ 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.

References

Alvares, A. P. & Mannering, G. J. (1970) *Mol. Pharmacol.* **6**, 206–212

DeFeo, V. J. (1966) *Endocrinology* **79**, 440–442

De Matteis, F. (1970) *FEBS Lett.* **6**, 343–345

De Matteis, F. (1971) *Biochem. J.* **124**, 767–777

Drummond, G. S. & Kappas, A. (1980) *Biochem. J.* **192**, 637–648

Imai, Y., Ito, A. & Sato, R. (1966) *J. Biochem. (Tokyo)* **60**, 417–428

Kappas, A. (1968) *N. Engl. J. Med.* **278**, 378–384

Kappas, A., Song, C. S., Sassa, S., Levere, R. D. & Granick, S. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 557–564

Klinger, W. & Müller, D. (1980) *Acta Biol. Med. Ger.* **39**, 107–112

Lim, L.-K., Srivastava, G., Brooker, J. D., May, B. K. & Elliot, W. H. (1980) *Biochem. J.* **190**, 519–526

Maines, M. D. & Kappas, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4293–4297

Maines, M. D. & Kappas, A. (1975) *J. Biol. Chem.* **250**, 4171–4177

Marver, H. S., Collins, A. & Tschudy, D. P. (1966) *Biochem. J.* **99**, 31c–33c

Matsuoka, T., Yoda, B. & Kikuchi, G. (1968) *Arch. Biochem. Biophys.* **126**, 530–538

Nash, T. (1953) *Biochem. J.* **55**, 416–421

Neale, M. G. & Parke, D. V. (1969) *Biochem. J.* **113**, 12P–13P

Neale, M. G. & Parke, D. V. (1973) *Biochem. Pharmacol.* **22**, 1451–1461

Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239**, 2379–2385

Ortiz de Montellano, P. R., Yost, G. S., Mico, B. A., Dinizo, S. E., Correa, M. A. & Kumbara, H. (1979) *Arch. Biochem. Biophys.* **197**, 524–533

Paul, S., Bickers, D. R., Levere, R. D. & Kappas, A. (1974) *FEBS Lett.* **41**, 192–194

Rabi, J. & Kragt, C. L. (1972) *Proc. Soc. Exp. Biol. Med.* **141**, 359–362

Rifkind, A. P., Sassa, S., Markatz, I. R., Winchester, R., Herber, L. & Kappas, A. (1974) *J. Clin. Invest.* **53**, 1167–1177

Sardana, M. K., Sassa, S. & Kappas, A. (1980) *J. Biol. Chem.* **255**, 11320–11323

Sassa, S. & Kappas, A. (1977) *J. Biol. Chem.* **252**, 2428–2436

Sassa, S., Kappas, A., Bernstein, S. E. & Alvares, A. P. (1979) *J. Biol. Chem.* **254**, 729–735

Schenkman, J. B., Frey, I., Remmer, H. & Estabrook, R. W. (1967) *Mol. Pharmacol.* **3**, 516–525

Tenhunen, R., Marver, H. S. & Schmid, R. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61**, 748–755

Tenhunen, R., Marver, H. S. & Schmid, R. (1969) *J. Biol. Chem.* **244**, 6388–6394