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HUMAN FETAL AND ADULT LIVER METABOLISM OF ETHYLMORPHINE

RELATION TO IMMUNODETECTED CYTOCHROME P-450 PCN AND INTERACTIONS WITH IMPORTANT FETAL CORTICOSTEROIDS

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Abstract—The N-demethylation of ethylmorphine was studied in liver microsomes from human fetuses and adult patients as well as from human fetal adrenals and kidneys. Unexpectedly the reaction was catalysed at the same rate in fetal (42.3–1277.4 pmol/mg/min in 11 individuals) and adult microsomes (414–1617.8 pmol/mg/min in two individuals), which also had similar values of the apparent K_m (1.50, 1.72 mM respectively) and V_{max} (1.33, 1.81 nmol/mg/min respectively) in studies of the enzyme kinetics. There was a close correlation ($r = 0.96$) between the semiquantitative immunoblotting assessment of cytochrome P-450 HLp in fetal liver microsomes (with the use of a monoclonal antibody against pregnenolone-16-alpha-carbonitrile induced rat hepatic cytochrome P-450) and the catalytic activity. The fetal adrenal microsomal N-demethylation was only 11–30% of the hepatic activity when compared within three fetuses in which such a comparison was possible. No activity was measurable in the kidneys. Two drugs that are believed to be substrates of the cytochrome P-450 HLp were tested as inhibitors of the ethylmorphine N-demethylation in human fetal and adult liver microsomes and in rat liver microsomes. Midazolam was a potent inhibitor (100% at 0.4 mM) of the reaction in all specimens, whereas cyclosporin A inhibited the reaction clearly only in adult liver microsomes. Endogenous steroids of importance in the fetal circulation were also tested as inhibitors. Progesterone and dehydroepiandrosterone inhibited the reaction by 75–80% at a concentration of 0.4 mM, whereas pregnenolone and 17-alpha-hydroxyprogesterone were almost devoid of inhibitory potency. These results are of interest in the discussion about the physiological role of the human fetal cytochrome P-450 HLp which has an unprecedented relative abundance in the liver.

Human tissue profiles of cytochrome P-450s have been described on the basis of their catalysis of various substrate oxidations which may reflect the presence of different cytochromes [1, 2]. At least 9 cytochrome P-450 isoenzyme forms have been purified from human liver [3–5].

New possibilities to characterize these enzymes arose with the introduction of monoclonal antibodies—MAbs† [6]. By virtue of their immunoreactive and immunoinhibitory potency, they may be used in "Western blots" and/or "reaction phenotyping", i.e. the determination of the contribution of specific cytochrome P-450s to specific drug metabolic reactions [1].

Particular attention has been paid to a human adult liver cytochrome P-450 species which was described in [2] and denoted as cytochrome P-450 HLp. It seems to be specifically induced in patients treated with macrolide antibiotics or dexamethasone. We have recently described this species in human fetal liver [7] as studied in Western blots by a MAb (PCN 2-13-1/C2) against pregnenolone 16-alpha-carbonitrile (PCN)-induced rat liver cytochrome P-450 [8]. This cytochrome P-450 HLp was not detected in adrenal or renal tissues from human fetuses.

Ethylmorphine has been regarded as a specific substrate of rat PCN cytochrome P-450 [9] but previous methods for assay of its N-demethylated product have employed photometric detection of formaldehyde [10] which is less specific than the presently used new high-performance liquid chromatographic (HPLC) analysis of the N-demethylated ethylmorphine.

This study presents data to indicate that in man the fetal liver N-demethylation of ethylmorphine is catalysed by the cytochrome P-450 HLp.

The relation between the enzyme contents and the catalytic activity was studied in liver, adrenals and kidneys. In addition metabolic interactions were investigated using drugs or endogenous steroids that are known to inhibit certain cytochrome P-450 HLp catalysed reactions or which serve as alternative substrates.

MATERIALS AND METHODS

Chemicals

Ethylmorphine hydrochloride was obtained from

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† Abbreviations used: MAb, monoclonal antibody; PCN, pregnenolone 16-alpha-carbonitrile; HLp, human liver cytochrome P-450 p; HPLC, high-performance liquid chromatography; UV, ultraviolet light; EM, ethylmorphine.

the Hospital Pharmacy. Norethylmorphine was synthesized analogously to a description for norcodeine [11]. Pregnenolone 16-alpha-carbonitrile was purchased from the Upjohn Company (Kalamazoo, MI). Midazolam and cyclosporin A were obtained from Roche and Sandoz companies (Basel, Switzerland), respectively, NADP, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, progesterone, 17-alpha-hydroxy progesterone, androstenedione and dehydroepiandrosterone were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were reagent or analytical grade and obtained from common commercial sources.

Methods

The chromatographic system consisted of a Waters M45 pump connected via a Rheodyne injector to a RCM compression module equipped with a Nova Pak CN column (5 mm i.d.). The mobile phase consisted of 45% acetonitrile in 0.02 M phosphate buffer, pH 6.9 at a flow rate of 0.7 ml per min. The eluent from the column was monitored using a Hitachi 655A UV detector set at a wavelength of 210 nm.

Scanning of immunoblots (a PCN 2-13-1/C2) from an earlier study [7] was performed using an UltraScan XL laser densitometer (LKB). The areas of the absorbance peaks for every specimen were expressed as a % of the control—174 ng purified rat cytochrome P-450 (PCN)—included in each immunoblot. This was kindly supplied by Dr Joe de Pierre, Stockholm University.

Our previous experience using either rat cytochrome P-450 (PCN) or microsomes from PCN treated rats showed a good and accurate correlation between area absorbance and protein concentration electrotransferred.

Biological material

Male Sprague–Dawley rats of about 200 g weight were induced with PCN (50 mg/kg/day dissolved in corn oil) by intraperitoneal injection for 3 days. The rats were killed on the fourth day by cervical dislocation and the liver, adrenals and kidneys were excised for microsomal preparation.

Human fetal tissue specimens from 11 fetuses were obtained at legal abortions which were performed for socio-medical reasons. The abortions were induced by prostaglandin instillation. The fetal tissues (liver, adrenals, kidneys) were excised, usually within 30 min of the abortion, and frozen at −70° until assay [7]. Two human adult liver specimens were included. One was obtained *post mortem* (from the department of Forensic Medicine (H1), the other at partial liver resection of a hepatic adenoma (H2); the latter liver yielded two specimens, one biopsy from the normal (H2h) and one from the pathologic (H2s) part of the liver. The morphology of the H1 liver specimen was normal. The liver samples were frozen at −70° until assay.

The study was approved by the Ethics Committee of the University Hospital. Informed consent was obtained from patient H2.

Microsomal preparation

Isolation of microsomes from the various human and rat tissues was performed as previously described [12]. The microsomal pellets were resuspended in 50 mM Tris–HCl buffer, pH 7.4 containing 0.25 M sucrose, and stored at −70° until assay. Protein was measured according to Lowry *et al.* [13] using bovine serum albumin as a standard.

Metabolic assays

Ethylmorphine metabolism. The hepatic oxidation of ethylmorphine to norethylmorphine was studied in microsomes from different tissues. The incubation mixtures, of final volume 0.1 ml, contained 0.15 M Tris–HCl pH 7.4, 5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 0.5 mM NADP, 0.2 units of glucose-6-phosphate dehydrogenase and microsomal protein to give a final concentration of 1.0 mg per ml. The reactions were started by addition of the NADP and glucose-6-phosphate dehydrogenase and incubated at 37° for 15 min. The reaction was terminated by freezing the tubes in acetone/dry ice. Samples were stored at −70° until analysis. The enzyme kinetics experiments in the human and rat liver microsomes were carried out with ethylmorphine over the concentration range 0.1 to 10 mM. On the basis of these experiments a final concentration of 5 mM ethylmorphine was selected for use in subsequent incubations. Linearity with time and protein concentration was maintained in all assays.

Inhibition studies. Studies of the effect of potential inhibitors of ethylmorphine *N*-demethylase were carried out with different concentrations of the following compounds: midazolam, cyclosporin A, progesterone, pregnenolone 16-alpha-carbonitrile, dehydroepiandrosterone, androstenedione and 17-alpha-OH-progesterone. The drugs were dissolved in methanol. Due to the inhibitory effect of methanol on the microsomal activity (5–10, 25, 90% inhibition at final concentrations of 2.5, 5 and 20%, respectively) a final concentration of only 2% was used in all interaction studies. The various drugs were preincubated for 6–8 min at room temperature before the addition of ethylmorphine (5 mM) and the cofactors. The rest of the reaction was performed as described above at 37°. Control samples with or without solvent were always included in the analyses.

HPLC determination of norethylmorphine. The N-demethylated metabolite of ethylmorphine was quantified by a liquid chromatographic method using UV detection after organic solvent extraction of the microsomal incubation mixture. The method which was developed in our laboratory followed this procedure: The 100 μl incubation mixture was transferred from the incubation tube to a new tube. To the incubation tube was then added 1 ml of water which was combined with the already transferred mixture. The mixture was made alkaline with 1 ml of 0.5 M NaOH and extracted with 5 ml dichloromethane-2-propanol (85:15) for 20 min on a shakeboard. This extraction procedure was optimized for norethylmorphine and does not include the extraction of morphine from the incubates. After centrifugation (500 *g*, 10 min) the aqueous layer was removed and discarded. Four ml of the organic phase was transferred to a new tube and the solvent was evaporated under nitrogen. The residue was dis-

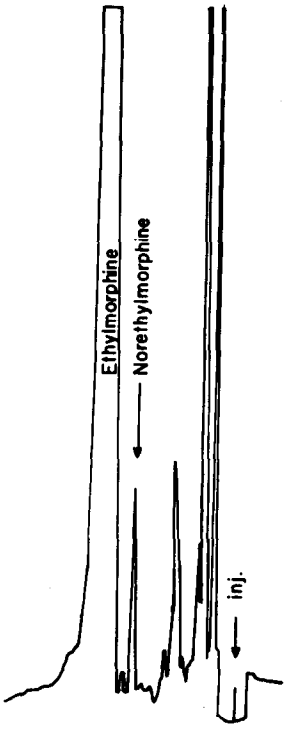


Fig. 1. Chromatogram of an incubation mixture processed through the method. The sample contains 570 μg of norethylmorphine per 100 μl incubation mixture.

solved in 250 μl of the mobile phase and an aliquot of 10–30 μl was injected into the liquid chromatograph.

Statistical method

The data represent the mean of duplicate or triplicate assays (±the standard error of the mean, SE) for every experiment or analytic assay.

RESULTS

Ethylmorphine—HPLC analysis

For this study, a high-performance liquid chromatographic assay for the analysis of ethylmorphine and its norethylmorphine metabolite was developed. The chromatograms showed no disturbing background and the norethylmorphine peak was symmetrical. In this system ethylmorphine and norethylmorphine had a retention time of 5.5 and 7 min, respectively, which was considered an adequate resolution. The coefficient of variation (CV) obtained for norethylmorphine at a level of 2.5 μg/ml was 2.3% (N = 10) and the recovery was 98%. The method could be used with good precision down to an amount of 90 pmol of ethylmorphine or norethylmorphine in the incubation (Fig. 1).

Ethylmorphine metabolism

Linearity of the ethylmorphine N-demethylation with time and protein concentration was studied in human fetal and adult (H1) liver microsomes. Linearity with time was ascertained up to 30 and 90 min in fetal and adult liver microsomes, respectively. The reaction was linear to 1 mg protein per ml incubate in fetal and 4 mg protein per ml incubate in adult liver microsomes. On the basis of these results an incubation time of 15 min was chosen for subsequent incubations. The standard protein concentration was 1 mg per ml incubate.

The N-demethylation of ethylmorphine obeyed Michaelis–Menten kinetics, both in human fetal and adult (H2n) liver microsomes (Fig. 2). The kinetics data were transformed into Lineweaver–Burk (Fig. 2a) and Eadie–Hofstee plots (Fig. 2b) which show a monophasic enzyme reaction in fetal microsomes.

The V_{max} and K_m values were estimated from Lineweaver–Burk plots of data obtained in the human fetal and adult liver microsomes, and in microsomes from Sprague–Dawley rats pretreated with PCN. There was a striking similarity between the values in adult and fetal microsomes (K_m 1.72 and 1.50 mM; V_{max} 1.81 and 1.33 nmol/mg/min, respectively). Experimental data with liver microsomes from PCN-induced rats indicated a 5-fold higher apparent affinity (K_m 0.23 mM) and a 35–48-fold higher V_{max} (63.3 nmol/mg/min).

The specific activities in the human adult and fetal liver and in human fetal adrenal and renal microsomes are given in Table 1. The fetal liver N-demethylation of ethylmorphine was conspicuously high and in the same range as in the adult liver microsomes. It should be pointed out that these levels represent the minimum activities in the fetal livers since the time interval between the fetal death and the abortion varies and is difficult to control.

Figure 3 demonstrates that the human fetal hepatic ethylmorphine N-demethylation correlated with the intensity of the MAb (2-13-1/C2) identified protein band ($r = 0.96$) as estimated by the scanning procedure.

In human fetal adrenal microsomes the activity towards ethylmorphine was generally lower. There was no measurable enzyme activity towards ethylmorphine in renal microsomes from five investigated fetuses. Similarly, there was no enzyme activity in the investigated microsomal preparations from PCN-induced rat adrenals or kidneys (Table 1).

Midazolam was a potent inhibitor of ethylmorphine N-demethylation (80% at 0.5 mM) in the PCN-treated rat liver microsomes (Fig. 4a). Enzyme kinetics studies demonstrated a competitive inhibitory effect (Fig. 4b). There was also a similar pronounced or even higher degree of inhibition of ethylmorphine N-demethylation by midazolam in the human adult liver (H2n and H2p). Similarly in four fetuses (F1, F2, F3, F6) investigated the inhibition by midazolam was complete at 0.4 mM concentration (Fig. 5a).

We also tested the interaction with cyclosporin A. This drug did not affect the N-demethylation of ethylmorphine in PCN-induced rat liver microsomes or in human fetal liver microsomes even at the maximum studied concentration of 0.5 mM (Fig. 4a and 5b). In contrast, it exerted a significant inhibitory effect (70% at 0.4 mM) on the N-demethylation in human adult liver microsomes (Fig. 5b).

Various effects of steroid hormones on the N-demethylation were also studied. Figure 6 depicts

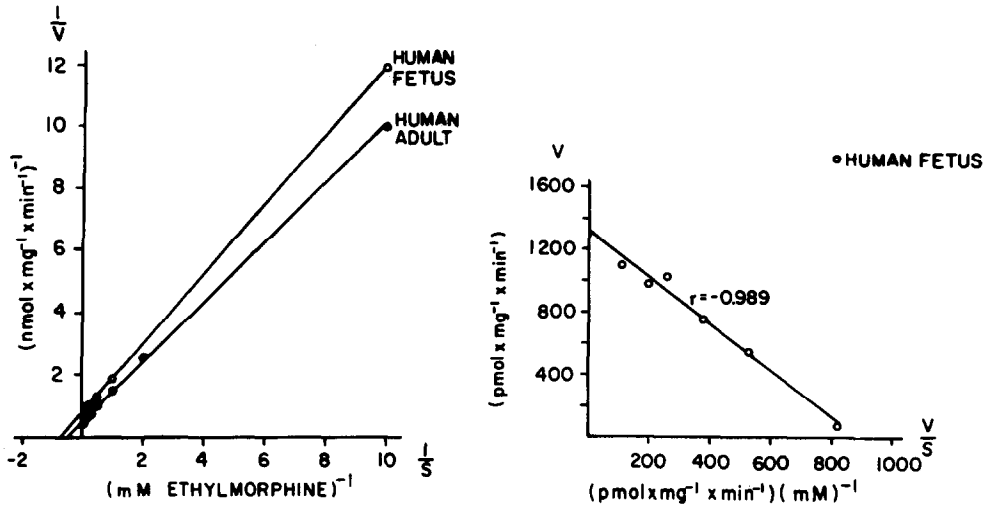


Fig. 2. Ethylmorphine N-demethylation at various substrate concentrations in liver microsomes from fetus (F1) and from an adult patient H2h. The concentration of ethylmorphine ranged from 0.1 to 10 mM, incubation time was 15 min and protein concentration 1 mg/ml. The data were transformed into a Lineweaver–Burk (a) or Eadie–Hofstee plot (b). The estimated K_m was 1.81 and 1.72 mM and the V_{max} 1.33 and 1.81 nmol/mg/min, for fetus 1 and H2 normal part biopsy, respectively. Data are the mean of appropriate duplicates.

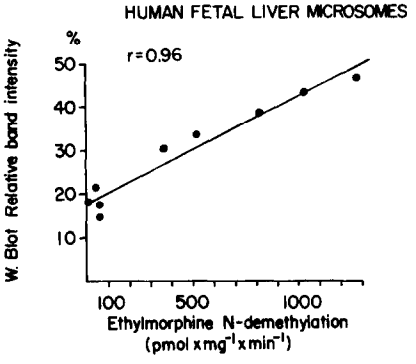


Fig. 3. Correlation between the rate of ethylmorphine N-demethylation in fetal liver microsomes and their corresponding immunoreactive cytochrome P-450 to a monoclonal antibody against PCN-induced rat liver cytochrome P-450 (PCN 2-13-1/C2). Western blot scanned values are calculated as % of the control = (100% area) of absorbance for rat PCN cytochrome P-450 form (174 ng) included on the blots [7].

the inhibitory potency of the various steroids in PCN-treated rat liver microsomes at three different steroid concentrations (0.01, 0.1, 0.5 mM). Progesterone and dehydroepiandrosterone were the most potent inhibitors whereas pregnenolone-16-alpha-carbonitrile and 17-alpha-hydroxyprogesterone were almost devoid of inhibitory capacity. Androstenedione caused an apparent enhancement of the enzyme activity.

Comparative inhibition studies were carried out in fetal liver microsomes at two concentrations (0.05 mM and 0.4 mM) of the inhibitors (Fig. 7). Progesterone and dehydroepiandrosterone (0.4 mM) exerted a high inhibitory effect (80%, 70–80% respectively) in all fetuses (F1, F2, F3) investigated. This inhibitory effect is in the same range as that of midazolam shown in Fig. 5a. A lack of inhibition is observed for pregnenolone-16-alpha-carbonitrile and 17-alpha-hydroxyprogesterone. Addition of androstenedione caused an apparent increase in the metabolic rate to 150–200% of control values.

DISCUSSION

The human counterpart of the rat cytochrome P-450 that is inducible by PCN, an anti-glucocorticoid substance [14] and glucocorticoids [15] is denoted as cytochrome HLp [2]. In man this cytochrome is one of the major forms in the cytochrome P-450 family and its concentration is particularly high in subjects treated with macrolide antibiotics or dexamethasone [2]. We have previously found that human fetal liver consistently contains large amounts of cytochrome P-450 HLp [7]. We now demonstrate that the ethylmorphine N-demethylation activity in human fetal liver is in the same range as in human adult liver (Table 1). Such a high ratio of fetal/adult drug oxidation rate is unexpected since most fetal enzymes have only 5–40% of the activity of the corresponding adult enzymes [10, 16]. No other oxidation reaction with xenobiotics have been shown so far to be fully developed in the fetal mid-gestational liver.

As shown here the human fetal ethylmorphine N-demethylation activities correlated to a high extent with the intensity of the MAb PCN 2-13-1/C2 immunoidentified cytochrome P-450 HLp bands in Western blots. Our results strongly suggest but do not prove that ethylmorphine is a substrate of the cytochrome P-450 HLp in human fetal liver just as has been shown earlier in adult rat liver [17]. This

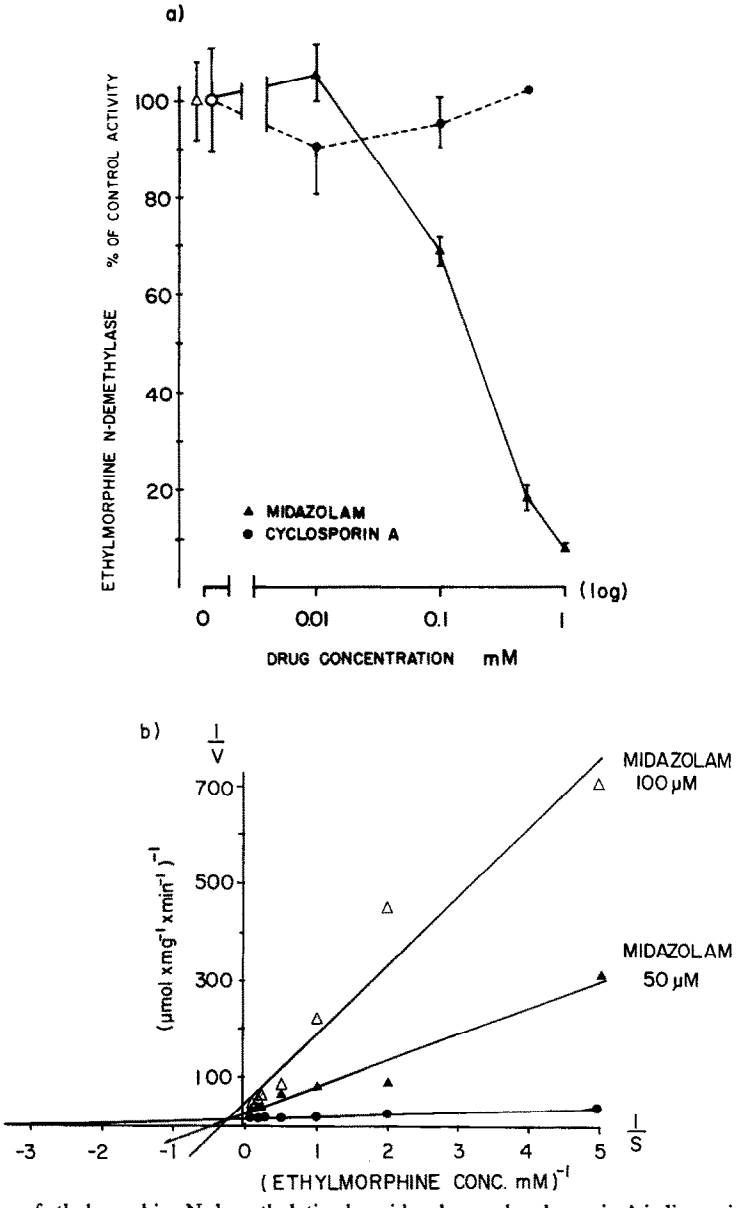


Fig. 4. Inhibition of ethylmorphine N-demethylation by midazolam and cyclosporin A in liver microsomes from PCN treated rats. (a) Midazolam inhibition from 0.01 mM to 1 mM and cyclosporin A inhibition from 0.01 mM to 0.5 mM. Higher concentrations of either drug were not dissolved well in the aqueous incubation. Data are triplicates of one of two repeated experiments. (b) Lineweaver–Burk plots of incubations with 0.2 to 10 mM ethylmorphine in absence and presence of 0.05 to 0.1 mM midazolam. Data are appropriate duplicates of one experiment. Protein concentration was 0.02 mg/ml. For all drug concentrations and control methanol 2% was the final solvent concentration in incubates.

contention is supported indirectly by the low or negligible ethylmorphine N-demethylation activity in adrenal microsomes which lack the cytochrome P-450 HLp band in Western blots [7]. The catalysis of the ethylmorphine N-demethylation by the cytochrome P-450 PCN could only be proved with the use of an inhibitory MAb, which is presently not available.

The specific activity of the N-demethylation was not only in the same range in human fetal as adult liver microsomes, the enzyme kinetics constants were also similar to those found in the adult liver specimens.

The cross-reactivity of the anti rat PCN 2-13-1/C2 MAb with the human enzyme demonstrates a certain degree of similarity at the antigenic cytochrome P-450 epitope between man and rat. In contrast, the anti-rat P-450 (PCN) MAb 2-13-1/Cl which reacts with microsomes from PCN-induced or non-induced rats and with phenobarbital treated rats (cytochrome P-450 PB-4) [8], did not cross-react with human adult or fetal liver microsomes [7].

Table 1. N-demethylation of ethylmorphine (EM) in microsomes from human adult and fetal liver, fetal adrenals and kidneys and liver, kidneys and adrenals from rats induced by pregnenolone 16-alpha-carbonitrile

Fetal specimen (no.)	Gestational age (weeks)	Norethylmorphine formed (pmol/min/mg protein) Liver		Adrenals		Kidneys	
F1	14	1277.4 ± 66.8	(3)*	—		—	
F2	18	502.5	(2)	—	—	—	
F3	19	1020.9	(2)	285.0	(2)	—	
F4	18	817.2	(2)	107.6 ± 17	(3)	—	
F5	24	69.03	(2)	57.9	(2)	—	
F6	20	361.8	(2)	40.1 ± 2.2	(3)	—	
F7	15	—		—		0	(2)
F8	15	42.3	(2)	90.2	(2)	0	(2)
F9	18	0	(2)	—	0	(2)	
F10	21	0	(2)	—	0	(2)	
F11	17	56.8	(2)	—	0	(2)	
Adult specimen							
H1		414 ± 71	(3)	—		—	
H2h		1617.8	(2)	—		—	
H2s		2012 ± 64	(3)	—		—	
PCN-treated rat specimen		58086 ± 1938	(3)	0	(3)	0	(3)

Data are from duplicates or triplicates of assay incubations performed as described in Materials and Methods. In all cases the protein concentration was 1 mg/ml except for rat-PCN-liver microsomes (0.2 mg/ml), reaction time was 15 min.

* Number in parenthesis is the number of assays; 0, undetected levels; dash indicates that no assay was performed.

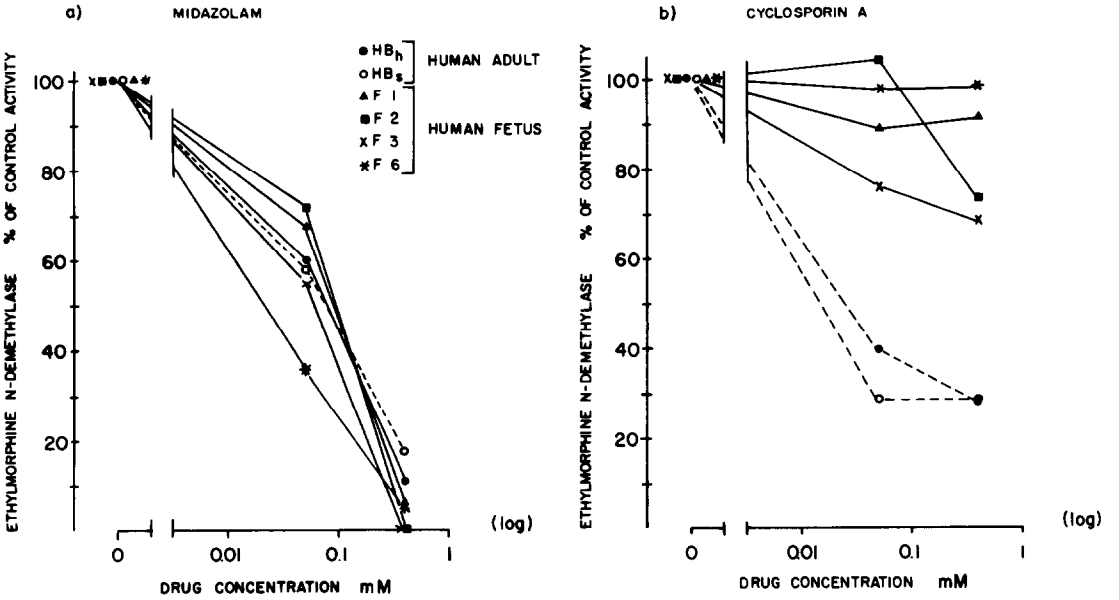


Fig. 5. Midazolam and cyclosporin A inhibition of ethylmorphine N-demethylation in human adult and fetal liver microsomes. Inhibition studies in four fetal (F1, F2, F3, F6) and adult human liver specimens (H2h, H2s). Protein concentration was 1 mg/ml and incubation time was 15 min. The solvent methanol concentration was 2% final concentration in all incubations including the control incubates (=100% activity). Drug concentrations assayed were 0.05 mM and 0.4 mM.

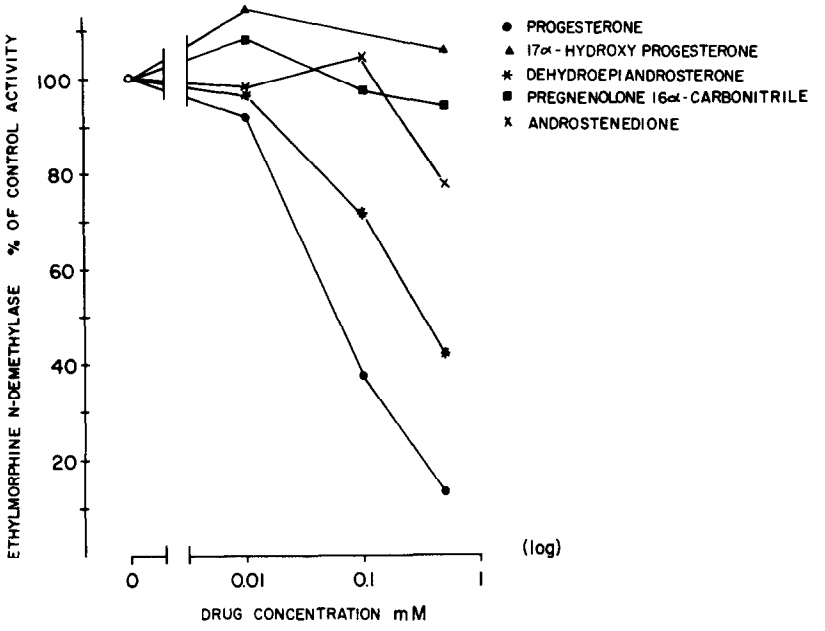


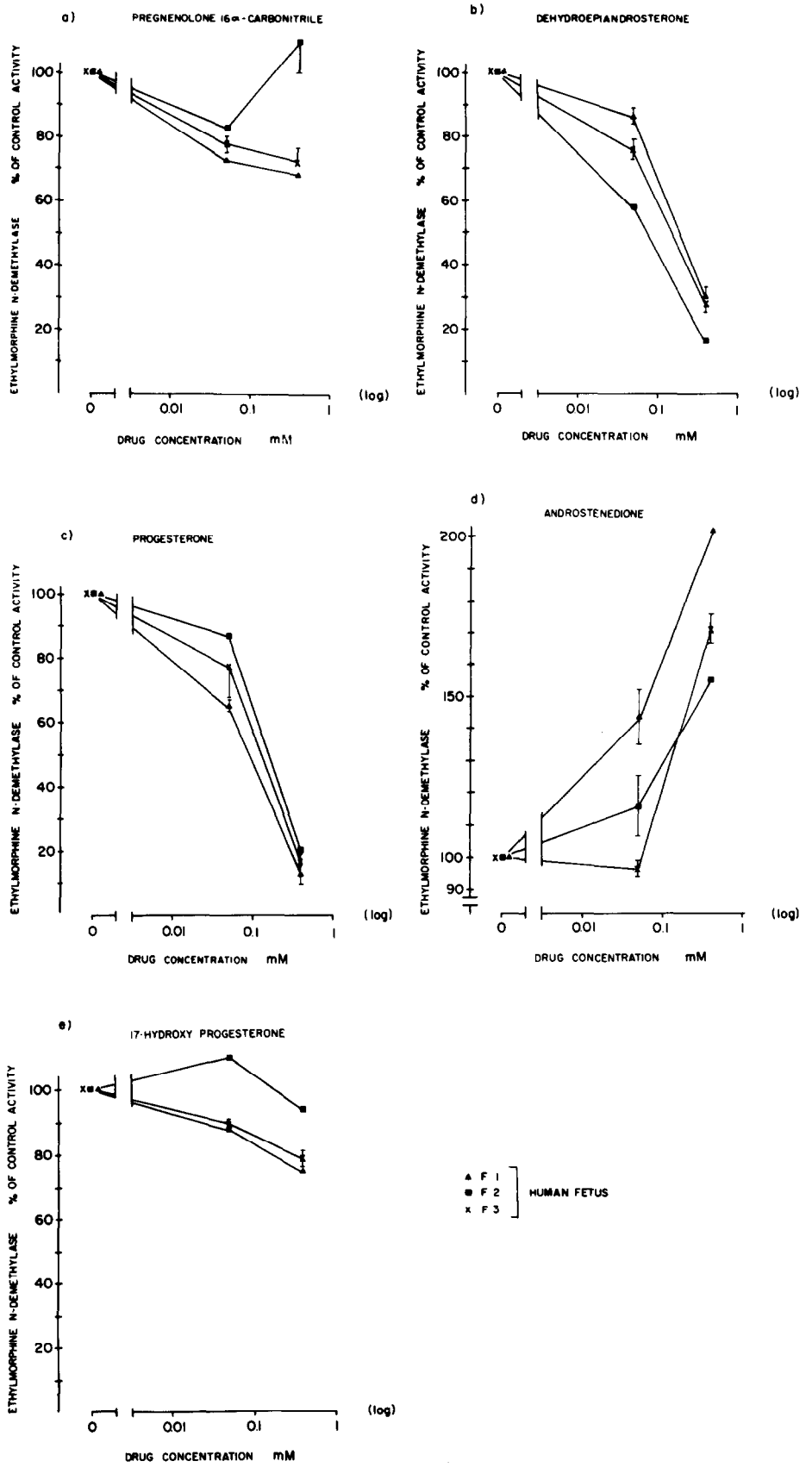
Fig. 6. Steroidal inhibition of ethylmorphine N-demethylation in rat-PCN-liver microsomes. Steroid inhibition at 0.05, 0.1 or 0.5 mM concentration (dissolved in methanol, final concentration 2% in incubate) was assayed in rat PCN liver microsomes (0.02 mg/ml). Controls contained 2% solvent. Data show appropriate duplicates of one of two experiments with same results.

The functional similarities and dissimilarities between human fetal and adult liver microsomes and the PCN-treated rat liver microsomes was further studied with the use of known substrates of the human hepatic cytochrome P-450 HLp such as midazolam [18] and cyclosporin A [19]. These drugs were tested as potential inhibitors of the ethylmorphine N-demethylation reaction in human fetal and adult liver microsomes and in rat liver microsomes. Midazolam was a potent inhibitor of the reaction in all preparations. In contrast, cyclosporin A inhibited the ethylmorphine N-demethylation only in human adult liver microsomes. In a previous publication with rat liver microsomes [20] cyclosporin A was not found to inhibit the ethylmorphine N-demethylation *in vitro*. However, the K_m and V_{max} are strongly affected in liver microsomes of rats pretreated with cyclosporin A. On the other hand, in mice, cyclosporin A has been shown to inhibit the N-demethylation of aminopyrine and the hydroxylation of benzo(*a*)pyrene [21]. This drug seems to exert different effects in microsomes from various species. The inconsistencies illustrate the complexity of the cyclosporin A effect on the metabolic reaction. On the basis of our own results it is conceivable, however, that the enzyme and/or its microenvironment changes during ontogenesis in man.

Administration of PCN induces the cytochrome P-450 PCN in experimental animals [17]. This suggests that steroid hormones may play an important role in the early development of this enzyme in fetal life. High concentrations of placental hormones (progesterone and pregnenolone) are circulating in the feto-placental unit. There are also considerable levels of dehydroepiandrosterone and androstenedione that are synthesized in the fetal adrenals and in the placenta [22]. Due to the deficient 3-beta-hydroxysteroid dehydrogenase activity in fetal adrenals, it is believed that progesterone or pregnenolone from the mother serve as precursors of dehydroepiandrosterone and androstenedione, respectively. Progesterone can also be used in the synthesis of cortisol. Waterman *et al.* [23] reported high activity of 17-alpha-hydroxylase in fetal bovine adrenals. This enzyme catalyses the formation of androgenic compounds from progesterone or pregnenolone.

Since the cytochrome P-450 PCN enzyme may be involved in the intermediary steroid metabolism we tested a series of gestagens and corticosteroids with

Fig. 7. Inhibitory effects of steroids in human fetal liver microsomes. Fetal liver microsomes from F1, F2, F3 were incubated with pregnenolone 16-alpha-carbonitrile (a), dehydroepiandrosterone (b), progesterone (c), androstenedione (d) and 17-alpha-hydroxyprogesterone (e); dissolved at two concentrations, 0.05 mM and 0.4 mM in methanol. In all cases the solvent concentration was 2%. Controls also contained 2% of methanol. Protein concentration was 1 mg/ml. Data correspond to appropriate duplicates or triplicates of one experiment.


a) PREGNENOLONE 16α-CARBONITRILE
b) DEHYDROEPIANDROSTERONE
c) PROGESTERONE
d) ANDROSTENEDIONE
e) 17-HYDROXY PROGESTERONE
ETHYLMORPHINE N-DEMETHYLASE % OF CONTROL ACTIVITY
DRUG CONCENTRATION mM
(log)
▲ F 1
● F 2
x F 3
HUMAN FETUS

regard to their effect on the N-demethylation. The tested endogenous steroids of placental and fetal adrenal origin are present in the human fetal circulation [22] and therefore may play a physiological role in the early development of the fetus. Progesterone and dehydroepiandrosterone were more effective inhibitors than the other tested agents. Pregnenolone and 17-alpha-OH-progesterone were almost devoid of inhibitory potency. The inhibition by the different agents may reflect their physicochemical character, e.g. lipophilicity, but it may also in part reflect their affinity for the binding site of ethylmorphine or another site which allosterically affects the ethylmorphine binding site.

Our results indicate that dehydroepiandrosterone may be a physiological substrate for the fetal P-450 HLp because of its pronounced inhibitory effect. This is consistent with a recent report [5] that antibodies against a purified human fetal form (HFLA) of cytochrome P-450 [24] strongly inhibited the dehydroepiandrosterone 16-alpha-hydroxylase in human fetal liver.

The mechanism of the conspicuous enhancement of the N-demethylation by androstendione is not known but may be related to an allosteric effect on the enzyme. The identity of the nor-ethylmorphine formed in these experiments was repeatedly confirmed with the use of a Photodiode Array Detector system (Waters Millipore SA).

The high activity of ethylmorphine N-demethylase in fetal livers and its inhibition by steroids has not been reported before. A maternal endocrine influence on the fetal hepatic-adrenal steroid metabolism may be postulated. The extensive synthesis of certain steroids in the human fetal adrenals may play a role as a trigger of the development of the hepatic cytochrome P-450 HLp in the fetus. This enzyme may participate in the metabolism of the steroids that originate in the fetal adrenals. Current studies in our laboratory aim to answer these questions.

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