guanine phosphoribosyltransferase, and requiring the presence of phosphoribosyl-1-pyrophosphate. The intracellular 6-MP nucleotide, TIMP, may then subsequently, be broken down to the nucleoside, MPR, by a phosphatase. The MPR which then appears in the urine may be an indirect marker for intracellular conversion of 6-MP to TIMP. The latter pathway has been proposed as the mechanism by which allopurinol riboside has been found in the urine of a PNP-deficient patient receiving allopurinol therapy [15].

In summary, we have identified a previously unreported metabolite of 6-MP, 6-mercaptopurine riboside, in the urine of patients receiving 6-MP as a prolonged intravenous infusion. Although MPR is a minor metabolite of 6-MP, accounting for less than 2% of the administered dose of 6-MP, it may be of clinical significance. Unlike the previously reported urinary metabolites of 6-MP, MPR has known anti-neoplastic activity. In addition, if its formation results from phosphatase-mediated breakdown of TIMP, then MPR may be a marker of intracellular activation of 6-MP.

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Biochemical Pharmacology, Vol. 33, No. 24, pp. 4092–4095, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Neonatal chlordecone alteration of the ontogeny of sex-differentiated hepatic drug and xenobiotic metabolizing enzymes*

(*Received* 27 *October* 1983; *accepted* 15 *June* 1984)

The perinatal period of development in humans and rodents is recognized as a time during which critical organizational events are still taking place in the central nervous system. Organizational effects are permanent and the expression of this type of hormone regulation does occur until after the onset of sexual maturation, long after the effector has been metabolized and excreted. The fetus and newborn are, therefore, particularly susceptible to hormone imbalance that may be brought about by genetic endocrine disorders, abnormal pregnancies, drug treatment or exposure to environmental chemicals that can change the hormonal milieu during this critical period of development.

Previous work by us and others has demonstrated the role of neonatal androgen and estrogen for the determination of adult sex-differentiated hepatic metabolism [1–5]. We have shown that perinatal exposure to hormones and estrogenically-active xenobiotics can alter the ontogeny of hepatic metabolism [5–7]. Chlordecone is an estrogenically-active and toxic chemical [8–11] that selectively alters brain and pituitary endorphin levels in prepubertal and adult rats following neonatal exposure [12]. In this study we have investigated the potential of chlordecone when administered during the neonatal period to alter the ontogeny of the following drug and xenobiotic metabolizing enzyme systems: benzo[*a*]pyrene hydroxylase, glutathione *S*-transferase, UDP-glucuronyltransferase and cytochrome P-450 content.

* This project was supported in part by an Institutional Research Grant from the American Cancer Society (IN-141A) and by BRSG Grant S07 RR05386 awarded by the Biomedical Support Grant Program, Division of Research Resources, National Institutes of Health.

Materials and methods

Experiments were carried out using Sprague–Dawley CD rats (Charles River Breeding Laboratories Inc., Wilmington, MA). Animals had free access to food (Purina Lab Chow 5001) and water. The animals were housed in a controlled environment (21°; 12 hrs light–dark cycle), were weaned at 21–23 days of age, and were housed four animals per cage after weaning. Chlordecone (Kepone; 99% pure)

was purchased from Chem Service, West Chester, PA. Neonatal rats were treated subcutaneously with 1 mg chlordecone in 20 μl sesame oil on day 4 postpartum [12], while controls received 20 μl sesame oil only.

Animals were decapitated and allowed to bleed, and their livers were rapidly removed and placed on ice. Hepatic cytosol and microsomes were prepared as previously described [7]. UDP-glucuronyltransferase was assayed from freshly prepared microsomes using *p*-nitrophenol as substrate [3, 13]. Cytochrome P-450 content was determined from freshly prepared and washed microsomes by measuring the carbon monoxide difference spectra after reduction with dithionite [14]. Benzo[*a*]pyrene hydroxylase activity was determined from microsomes prepared in 50 mM Tris (pH 7.4) containing 0.25 M sucrose, frozen at −80°, and later assayed according to the method of Nebert and Gelboin [15] as modified by Yang *et al.* [16] using 3-hydroxybenzo[*a*]pyrene as product standard. Cytosolic glutathione *S*-transferase activity was assayed from frozen samples (−80°) by measuring the conjugation of glutathione with the substrate 1-chloro-2,4-dinitrobenzene (CDNB) [17]. Danner-Rabovsky and Groseclose [18] have shown that microsomal monooxygenase activities are stable for 1–2 months when frozen under these conditions, and we have confirmed this for aryl hydrocarbon hydroxylase and glutathione *S*-transferase for a period of at least 6 weeks. All enzyme activity levels are expressed as nmoles/min/mg protein while cytochrome P-450 content is expressed as nmoles/mg protein. In our experiments, activity levels in prepubertal males and females were determined separately, found not to be statistically different, and therefore reported collectively. Protein determinations were carried out by the method of Lowry *et al.* [19]. Chlordecone concentrations were determined by gas chromatography [20]; detection limits in extracted tissue were 20 ng chlordecone/g tissue.

Results and discussion

Prepubertal male and female rats have similar activity levels of drug and xenobiotic metabolizing enzymes. Eventually, some of these enzyme systems undergo sexual differentiation during the pubertal period, resulting in higher activities in one sex than in the other. The enzyme systems chosen for this study are characterized by higher activity levels in adult male rats than in adult female rats (Fig. 1). Neonatal chlordecone treatment resulted in increased hepatic cytochrome P-450 content and glutathione *S*-transferase activities in 21-day-old male and female rats but caused no changes in these 70- and 120-day-male and female rats when compared to controls (Fig. 1a and 1b). Benzo[*a*] pyrene hydroxylase activity was unchanged at all ages investigated (Fig. 1c). Neonatal chlordecone treatment had no effect on UDP-glucuronyltransferase activity in prepubertal males or females or in adult males (70- to 120-day-old) but activity levels were elevated in 70- and 120-day-old females (Fig. 1d). Whole body, liver, testes or uteri, and pituitary weights were not significantly different between treated and control animals (data not shown), suggesting at least that sex-steroid hormone levels would not be drastically affected.

We subsequently investigated the effect that an equivalent dose of chlordecone (83 μg/g body weight) and a lower dose of chlordecone (one-third, i.e. 28 μg/g body weight) would have on these enzymes when administered to adult or prepubertal female rats later than the critical age when organizational effects are initiated [21, 22]. Adult female rats treated on day 63 with chlordecone and killed 7 days later had statistically higher cytochrome P-450 content ($P < 0.01$), benzo[*a*]pyrene hydroxylase ($P < 0.01$), glutathione *S*-transferase ($P < 0.01$) and UDP-glucuronyltransferase ($P < 0.01$) activity levels than did the controls. Likewise, prepubertal female rats treated with chlordecone on day 14 postpartum and killed 7 days later also had similarly increased activity levels. However, prepubertal female rats treated on day 21 postpartum and killed on day 70 postpartum had increased cytochrome P-450 content only. Benzo[*a*]pyrene hydroxylase, glutathione *S*-transferase and UDP-glucuronyltransferase activities in these prepubertally chlordecone-treated female rats were not significantly different from that of the controls. For UDP-glucuronyltransferase this is especially significant since treatment on day 4 postpartum resulted in increased activities at 70 and 120 days (Fig. 1d).

Residual chlordecone concentrations were concomitantly determined in the liver, whole brain, and testes or uteri of prepubertal and adult animals treated neonatally with chlordecone. The highest concentrations were found in the liver while lower concentrations were found in the brain, testes and uteri (Table 1). These levels decreased as a function of age. At 120 days, chlordecone concentrations in the brain and gonads were not measurable, but chlordecone was still present in the liver. The liver half-life for chlordecone in animals treated on day 4 was calculated to be 16 days during the days 21 through 70 postpartum, and 25 days during the days 70 through 120 postpartum. Similar results were obtained by Egle *et al.* [23].

Induction. The effects of neonatal exposure to chlordecone on hepatic P-450 content and glutathione *S*-transferase activity (Fig. 1) appear to be due to the direct effect of residual concentration levels of chlordecone resulting in induction. This is supported by the fact that increases in activity levels were seen shortly after treatment only, i.e. treatment on day 4 caused increased activity levels of cytochrome P-450 and glutathione *S*-transferase in 21-day-old male and female rats but not in 70- and 120-day-old rats. Cytochrome P-450 content has been shown to be induced by chlordecone [24–26]. Our induction experiments confirm this for cytochrome P-450 and for glutathione *S*-transferse in prepubertal and adult female rats treated with chlordecone and killed 1 week later. In neonatally chlordecone-treated animals, cytochrome P-450 and glutathione *S*-transferase levels were back to normal in adult animals presumably due to deactivation, excretion, redistribution and/or decreased tissue concentrations as a function of body growth. Our data in Table 1 show that residual chlordecone concentrations from animals treated neonatally decreased as a function of age, therefore accounting for the lack of induction in adult animals.

Imprinting. UDP-glucuronyltransferase ontogeny was apparently altered by a different mechanism. Prepubertal UDP-glucuronyltransferase activities were unchanged following neonatal chlordecone treatment (day 4) but adult female rats (70 and 120 days old) had higher UDP-glucuronyltransferase activities than their respective controls. This is in spite of a decrease in residual chlordecone concentrations in adult animals, making it unlikely that induction is responsible for the altered ontogeny. As evidenced by our induction experiments, hepatic UDP-glucuronyltransferase can be induced in prepurbertal and adult female rats, but only in the presence of high residual concentrations. In those prepubertal female rats treated on day 21 and killed on day 70, there was no persistent increase in UDP-glucuronyltransferase activity.

Several laboratories including ours have shown that sex-differentiation of hepatic steroid and xenobiotic metabolism is imprinted in the male rat during the neonatal period by release of testicular androgens [1–5]. It has been proposed that this imprinting occurs whereby testosterone is converted to estradiol in the brain target cells, and this estrogen is the active imprinting metabolite that can bind the estrogen receptor to program for a male type of metabolism [22, 23, 27]. Indeed, neonatal castration has been shown to result in feminization of hepatic metabolism; neonatal testosterone and estrogen treatment of castrate-males has been shown to reverse the effect of neonatal castration [1–5]. Furthermore, perinatal exposure of intact

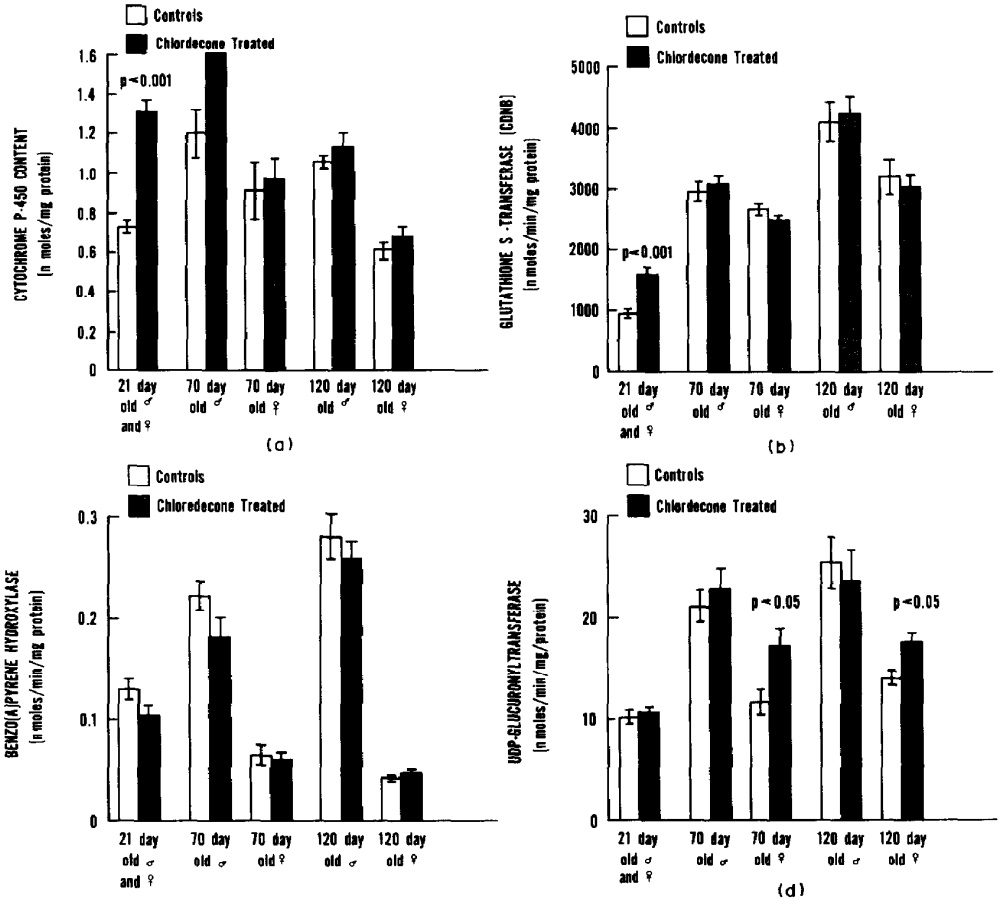

Fig. 1. Altered ontogeny of sex-differentiated hepatic enzymes following neonatal chlordecone treatment. Neonatal male and female rats were treated on day 4 postpartum with chlordecone (1 mg/rat) and killed at 21, 70 and 120 days of age, and the following activity levels were determined: (a) cytochrome P-450, (b) glutathione *S*-transferase (CDNB), (c) benzo[*a*]pyrene hydroxylase and (d) UDP-glucuronyltransferase. All values are means ± S.E. N = 8.

animals to hormones and estrogenically-active xenobiotics has been shown to alter the ontogeny of several sex-differentiated hepatic enzymes [3, 5–7, 17]. Chlordecone has also been shown to possess estrogenic activity. In immature rats, chlordecone produces an increase in uterine growth, a persistent vaginal estrous and anovulation following neonatal treatment [8]. Chlordecone has been shown to be a weak competitive binder to uterine cytoplasmic [9, 10] and nuclear [11] estrogen receptors.

Even through chlordecone has been found to be 10,000 times weaker than estradiol in its biological effects or in its affinity for estrogen receptors, it does have a long biological half-life, approximately 20 days during the neonatal period [23], and could possibly bind hypothalamic estrogen receptors during the critical period of brain sexual maturation.

Accordingly, this estrogenically-active xenobiotic may bind hypothalamic estrogen receptors and consequently promote transcriptional, translational and posttranslational processes to result in defeminization of the hypothalamus and consequently interfere with normal maturational processes. These alterations are permanent and expressed postpubertally via the hypothalamic-pituitary axis to result in altered ontogeny of UDP-glucuronyltransferase.

In summary, neonatal administration of chlordecone

Table 1. Residual concentrations of chlordecone in the developing rat following neonatal exposure*

	Chlordecone concentrations (μg/g tissue)					
	21-day-old		70-day-old		120-day-old	
	Males	Females	Males	Females	Males	Females
Liver	23.5 ± 3.9	27.3 ± 4.9	4.7 ± 0.8	3.8 ± 1.0	0.9 ± 0.2	1.1 ± 0.3
Brain	8.3 ± 1.7	11.1 ± 1.0	0.3 ± 0.02	0.3 ± 0.05	Not detectable	
Testes or uteri	8.9 ± 1.7	2.6 ± 0.5	0.3 ± 0.01	0.6 ± 0.11	Not detectable	

* Chlordecone (1 mg) was administered subcutaneously on day 4 postpartum in 20 μl sesame oil. All values are means ± S.E. (N = 3–4). Detection limits were 20 ng chlordecone/g tissue.

resulted in the altered ontogeny of sex-differentiated drug and xenobiotic metabolizing enzymes. Neonatal chlordecone altered the imprinting of UDP-glucuronyltransferase (defeminization) and caused induction of cytochrome P-450 content, benzo[*a*]pyrene hydroxylase and glutathione *S*-transferase.

Acknowledgements—We gratefully acknowledge the technical assistance provided by John Herman, Steven Moore and Chuen-Mao Yang and the typing of Charise Harper.

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Biochemical Pharmacology, Vol. 33, No. 24, pp. 4095–4098, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Structure–activity evidence against opiate receptor involvement in Leu^5-enkephalin-induced pulmonary vasoconstriction

(*Received* 20 *January* 1984; *accepted* 9 *June* 1984)

The recognition that endogenous opioids may play an important role in the cardiovascular system [1] has led to attempts to identify the receptors mediating such actions. Opioid-mediated cardiovascular responses usually are blocked by naloxone [1–4], thereby implying opiate receptor involvement. More recent evidence suggests that these receptors may be of the δ subtype [5–7]. However, there are some instances in which naloxone is ineffective, thus raising the possibility that non-opiate receptors may be involved. Dean *et al.* [8] have shown that inhibition of nicotine-induced catecholamine release from adrenal chromaffin cells by Leu^5-enkephalin is not antagonized by naloxone. Similarly, neither morphine-induced tachycardia in conscious squirrel monkeys [9] nor systemic hypertension induced in conscious dogs by des-Tyr^1-D-Ala^2-Leu^5-enkephalinamide is blocked by naloxone [10]. We have demonstrated recently that pulmonary vasoconstriction provoked by Leu^5-enkephalin in isolated rat lungs is not blocked by either naloxone or naltrexone, nor is it mimicked by morphine [11]. To more firmly establish the non-opiate nature of the receptor mediating Leu^5-enkephalin-induced pulmonary vasoconstriction, this report examines in isolated rat lungs the pulmonary vasoactivity of several cogeners of Leu^5-enkephalin as well as a number of smaller fragments of the pentapeptide. In addition, we have evaluated the inhibitory effects of diprenorphine on responses to Leu^5-enkephalin in an effort to rule out a role for δ opiate receptors.

Methods

Leu^5-enkephalin, Met^5-enkephalin, D-Ala^2-D-Leu^5-enkephalin, bestatin and captopril were purchased from the Sigma Chemical Co. Diprenorphine was obtained from Reckett & Colman, Ltd. The peptide fragments Gly-Gly-L-Phe-L-Leu, L-Phe-L-Leu, L-Tyr-Gly and L-Tyr-Gly-Gly were synthesized as described previously [12–15], and their structures were confirmed by elemental and amino acid analysis, proton magnetic resonance spectrometry and GLC-mass spectrometry [16].

Pulmonary vasoactivity of Leu^5-enkephalin and cogeners