BIOCHEMICAL PHARMACOLOGY 73 (2007) 279–286

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The effect of opiates on the activity of human placental aromatase/CYP19

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ARTICLE INFO

Article history:
Received 12 July 2006
Received in revised form
17 August 2006
Accepted 18 August 2006

Keywords:
Human placenta
Opiates
Aromatase/CYP19
Estrogen formation
Pregnancy

ABSTRACT

Aromatase, cytochrome P450 19, is a key enzyme in the biosynthesis of estrogens by the human placenta. It is also the major placental enzyme that metabolizes the opiates L-acetylmethadol (LAAM), methadone, and buprenorphine (BUP). Methadone and BUP are used in treatment of the opiate addict and are competitive inhibitors of testosterone conversion to estradiol (E_2) and 16α-hydroxytestosterone (16-OHT) to estriol (E_3) by aromatase. The aim of this investigation is to determine the effect of 20 opiates, which can be administered to pregnant patients for therapeutic indications or abused, on E_2 and E_3 formation by placental aromatase. Data obtained indicated that the opiates increased, inhibited, or had no effect on aromatase activity. Their effect on E_3 formation was more pronounced than that on E_2 due to the lower affinity of 16-OHT than testosterone to aromatase. The K_i values for the opiates that inhibited E_3 formation were sufentanil, 7 ± 1 μM; LAAM, 13 ± 8 μM; fentanyl, 25 ± 5 μM; oxycodone, 92 ± 22 μM; codeine, 218 ± 69 μM; (+)-pentazocine, 225 ± 73 μM. The agonists morphine, heroin, hydromorphone, oxymorphone, hydrocodone, propoxyphene, meperidine, levorphanol, dextrorphan, and (−)-pentazocine and the antagonists naloxone and naltrexone caused an increase in E_3 formation by 124–160% of control but had no effect on E_2 formation. Moreover, oxycodone and codeine did not inhibit E_2 formation and the IC_{50} values for fentanyl, sufentanil, and (+)-pentazocine were >1000 μM. It is unlikely that the acute administration of the opiates that inhibit estrogen formation would affect maternal and/or neonatal outcome. However, the effects of abusing any of them during the entire pregnancy are unclear at this time.

1. Introduction

The human placenta assumes a crucial role in the maintenance of pregnancy as well as fetal growth and development. It provides the fetus with nutrients and oxygen and eliminates waste products. In addition, the human placenta synthesizes specific hormones that have endocrine and paracrine functions, e.g., chorionic gonadotropin, human placental lactogen, and estrogens. These hormones are vital to healthy pregnancies and mediation of parturition [1]. Therefore, the placenta serves as an interface between the maternal and fetal circulations and regulates the bidirectional transfer of metabolic intermediates.

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Abbreviations: 16-OHT, 16α-hydroxytestosterone; BSA, bovine serum albumin; BUP, buprenorphine; CYP19, cytochrome P450 19; E_2, 17β-estradiol; E_3, estriol; EDDP, 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine; LAAM, *levo*-α-acetylmethadol; norBUP, norbuprenorphine; nor- and dinorLAAM, nor- and dinor-*levo*-α-acetylmethadol

0006-2952/$ – see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.bcp.2006.08.019

Our hypothesis for the last 7 years has been that the placenta acts as a functional barrier protecting the fetus from the effects of drugs and xenobiotics. It achieves this protective role, in part, by the activity of its metabolizing enzymes and efflux transporters. Therefore, our investigations focused on placental disposition of methadone and buprenorphine (BUP) used for treatment of the pregnant opiate addict, as well as *levo*-α-acetylmethadol (LAAM), which is no longer used for treatment of this patient population. Placental disposition of administered therapeutics includes their transfer to the fetal circulation, metabolism by the tissue, and elimination. The transplacental transfer of methadone, BUP, and LAAM was investigated utilizing the dually perfused placental lobule. Placental tissue retains the three opiates to different extents; thus, a concentration gradient with the maternal and fetal circuits is formed [2–4]. Moreover, the in vitro metabolism of the three drugs by microsomal fractions obtained from term placental trophoblast tissue was investigated [5–7]. The latter investigations provided evidence that cytochrome P450 19 (CYP19), also known as aromatase, is the major enzyme responsible for the metabolism of methadone, BUP, and LAAM in term human placentas. Aromatase is a key enzyme in the conversion of androgens to estrogens in the human placenta [8]. It is also responsible for the metabolism of endogenous compounds and xenobiotics [9,10]. A recent investigation revealed that the opiates methadone, BUP, and their metabolites 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and norBUP are competitive inhibitors of androgen conversion to estrogen by placental aromatase [11]. Therefore, it became apparent that human placental aromatase could be a site for drug interactions between the opiates used for treatment of the pregnant patient and estrogen biosynthesis by the tissue. Recent reports indicated that abuse of hydrocodone and oxycodone in their various formulations is rapidly increasing in the United States [12]. In 2005, the number of prescriptions for these two medications increased by 2–2.5 times over that in 1995 while fentanyl prescriptions increased almost by 4 times. In the same period, there was no increase in the abuse of propoxyphene, codeine, meperidine, and methadone but they remain on the list of common ''nonmedically'' used drugs. It is believed that the increase in the prescriptions of these opiates resulted in their diversion and abuse.

Therefore, the aim of this investigation was to determine the effect of 20 opiates that are either used therapeutically or commonly abused on the activity of placental aromatase in estrogen biosynthesis—namely the conversion of testosterone to estradiol (E_2) and 16α-hydroxytestosterone (16-OHT) to estriol (E_3).

2. Materials and methods

2.1. Chemicals

The following opiates were gifts from the National Institute on Drug Abuse drug supply unit: heroin hydrochloride (3,6-diacetylmorphine hydrochloride), oxycodone hydrochloride, LAAM and its metabolites norLAAM and dinorLAAM, (+)-α-propoxyphene hydrochloride, fentanyl hydrochloride, sufentanil citrate, meperidine hydrochloride, (+)- and (−)-pentazocine succinate.

The following opiates were also gifts: oxymorphone hydrochloride (Dupont, Wilmington, Del), levorphanol tartrate dihydrate and dextrorphan tartrate (Hoffmann La Rouche Inc. Nutley, NJ), naloxone hydrochloride (Endo Laboratories Inc. Garden City, NY).

Morphine sulphate, codeine hydrochloride, hydrocodone bitartrate, hydromorphone hydrochloride, naltrexone hydrochloride, testosterone and all other chemicals and reagents were purchased from Sigma Chemical Co., St. Louis, MO except for 16-OHT (Research Plus Inc. Bayonne, NJ) and acetonitrile HPLC grade (Fisher Scientific, Houston, TX).

2.2. Clinical material

Term human placentas were obtained immediately after delivery from women with uncomplicated pregnancies giving birth at the delivery ward of the John Sealy Hospital, Galveston, TX, according to a protocol approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston. Placentas of women under treatment for opiate dependence or with a history of drug abuse were excluded.

Villous tissue was excised from the placentas, rinsed in ice-cold saline, minced, and homogenized in 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was used to prepare crude subcellular fractions by differential centrifugation. The microsomal fraction was prepared by resuspending the 104,000 × *g* pellet in 0.1 M potassium phosphate buffer (pH 7.4), and its protein content determined by a kit using BSA as a standard (Bio-Rad, Hercules, CA). The microsomal fraction was stored in aliquots at 80 °C until used. Several pools made of microsomal preparations obtained from term placentas were used for all experiments.

2.3. Effect of the opiates on aromatase activity

The effect of agonists and antagonists of the structurally related opiates, in the different classes, on the activity of aromatase CYP19 in aromatization of its androgen substrates was investigated. Two reactions catalyzed by the aromatase of placental microsomes were used—namely the conversion of testosterone to E_2 and 16-OHT to E_3. The opiates investigated were the phenanthrenes morphine, heroin, hydromorphone, oxymorphone, hydrocodone, oxycodone, codeine, naloxone and naltrexone; the phenylheptylamines propoxyphene, LAAM (a congener of methadone) and its metabolites norLAAM and dinorLAAM; the phenylpiperidines fentanyl, sufentanil, and meperidine; the benzomorphans (+)- and (−)-pentazocine; the morphinans levorphanol and dextrorphan.

The reaction solution contained each substrate of CYP19 (testosterone or 16-OHT) in a concentration equal to its apparent K_m value [11]. The IC_{50} for each opiate was determined by its addition to the reaction solution in the range of concentrations as indicated below for each experiment. The IC_{50} value was calculated by plotting the amount of product formed in absence of the opiate (control, set as 100%) versus either the concentration of the inhibitor (opiate) or the log of its concentration. The data reported for the effect of each

opiate on estrogen formation are the mean of three to four experiments.

2.3.1. *Effect of the opiates on the conversion of testosterone to 17β-estradiol*

The effect of a range of opiate concentrations between 10 and 1000 μM on the activity of a pool of placental microsomes in the conversion of testosterone to E_2 was determined. The reaction solution (1 mL) contained 0.2 μM testosterone and 0.25 mg microsomal protein in 0.1 M potassium phosphate buffer (pH 7.4). The contents were preincubated in the presence of an opiate for 5 min at 37 °C. The reaction was then initiated by the addition of an NADPH regenerating system (NADP, 0.4 mM; glucose-6-phosphate (G-6-P), 4 mM; G-6-P dehydrogenase 1 U/mL; 2 mM $MgCl_2$) and the incubation continued for a period of 5 min at the same temperature. The reaction was terminated by the addition of 100 μL trichloroacetic acid (10%, w/v) and placed on ice. The internal standard estrone, 100 μL of (10 μg/mL solution), was added to each tube and followed by centrifugation at 12,000 × *g* for 10 min. The products of the reaction were extracted by butyl chloride (1.5 mL) added to the supernatant. The organic layer was aspirated, evaporated to dryness, and the residue resuspended in 200 μL of the mobile phase used in the HPLC system described below. The amounts of E_2 formed were determined by HPLC–UV.

2.3.2. *Effect of the opiates on the conversion of 16α-hydroxytestosterone to estriol*

The effect of the opiates on the activity of the pool of placental microsomes in catalyzing the conversion of 16-OHT to E_3 was determined under conditions similar for E_2 formation except for the following: the concentration range for fentanyl and sufentanil was between 1 and 1000 μM and the range for LAAM was between 10 and 500 μM; the concentration of the substrate (16-OHT) was 6.0 μM, the incubation period was 60 min and the internal standard was chlorimipramine (25 μL of 10%, w/v). The amounts of E_3 formed were determined in the supernatant by HPLC–UV.

2.4. *Kinetics for aromatase inhibition by opiates*

The inhibition of aromatase activity by opiates was investigated to determine whether it was competitive or noncompetitive. The inhibition constant for the opiates with IC_{50} values less than 1 mM was determined. For each opiate, three or four substrate concentrations were utilized with one equal to its apparent K_m and the others higher and lower—namely 0.1, 0.2, and 0.4 μM for testosterone and 3.0, 6.0, and 12 μM for 16-OHT (the fourth concentration, when used, was 18 μM). The concentration range used for each opiate was as follows: (1) for E_2 formation: 500–1500 μM LAAM and norLAAM, 100–500 μM dinorLAAM; (2) for E_3 formation: 50–250 μM oxycodone, 100–500 μM codeine, 25–200 μM LAAM, 100–1000 μM norLAAM, 50–500 μM dinorLAAM, 10–150 μM fentanyl, 10–50 μM sufentanil, and 200–1000 μM (+)-pentazocine. For each reaction, zero time served as blank and the control represented the activity of the microsomes in the absence of an opiate. Several opiates were dissolved in nonaqueous solvents, and a volume of the solvent was added to the control to achieve the same final concentration present in the reaction.

The type of inhibition was determined by plotting the data obtained (Lineweaver–Burk) in the absence and presence of two or three concentrations of the inhibitor. The constant of inhibition (K_i) for an opiate was determined by Dixon plots. Data reported on inhibition of estrogen formation represent the mean of three experiments.

2.5. *Analysis of 17β-estradiol and estriol formation*

The amounts of E_2 and E_3 formed were determined by HPLC–UV (using a 300 mm × 3.9 mm Bondapak C_{18} 10 μm column; Waters, Milford, MA) according to established methods with slight modification [13]. For E_2 formation, the mobile phase was made of acetonitrile:water (45:55 v/v) containing 0.1% (v/v) triethylamine and adjusted to a pH 3.0 with orthophosphoric acid. Isocratic elution was performed at a flow rate of 1.2 mL/min and monitored at a wavelength of 200 nm. For E_3 formation, the mobile phase was made of acetonitrile:water (35:65 v/v) containing 0.2% (v/v) triethylamine at pH 3.5. The flow rate was 0.5 mL/min for the first 15 min, then 1 mL/min for the remaining period, and monitored at a wavelength of 280 nm. Quantification of the E_2 and E_3 that was formed was performed in the linear range of a standard curve that was prepared for each experiment.

2.6. *Statistical analysis*

Statistical analysis of the data on the effect of the opiates on aromatase activity was carried out using ANOVA with multiple comparison analysis.

3. Results

3.1. *Effect of opiates on the activity of placental aromatase*

The therapeutic and/or commonly abused opiates were investigated. These opiates belong to the following structurally related classes: phenanthrenes, phenylheptylamines, phenylpiperidines, morphinans, and benzomorphans. The effect of each opiate on the activity of placental aromatase/CYP19 in the conversion of its substrates testosterone to E_2 and 16-OHT to E_3 was investigated using a pool of crude microsomal fractions.

In the control reactions, i.e., in the absence of an opiate, the activity of the pool of microsomes in converting testosterone to E_2 varied between preparations and ranged between 67 and 124 pmol mg protein^{-1} min^{-1} and for the conversion of 16-OHT to E_3 between 14 and 25 pmol mg protein^{-1} min^{-1}. The rates for E_2 and E_3 formation in the absence of an opiate (control) were set as 100% and data obtained on the effect of an opiate was calculated and represented as percent of control (Fig. 1A–D).

3.1.1. *Phenanthrenes*

The phenanthrenes investigated were the opiate agonists morphine, codeine, heroin, hydromorphone, oxymorphone, hydrocodone, oxycodone and the antagonists naloxone and

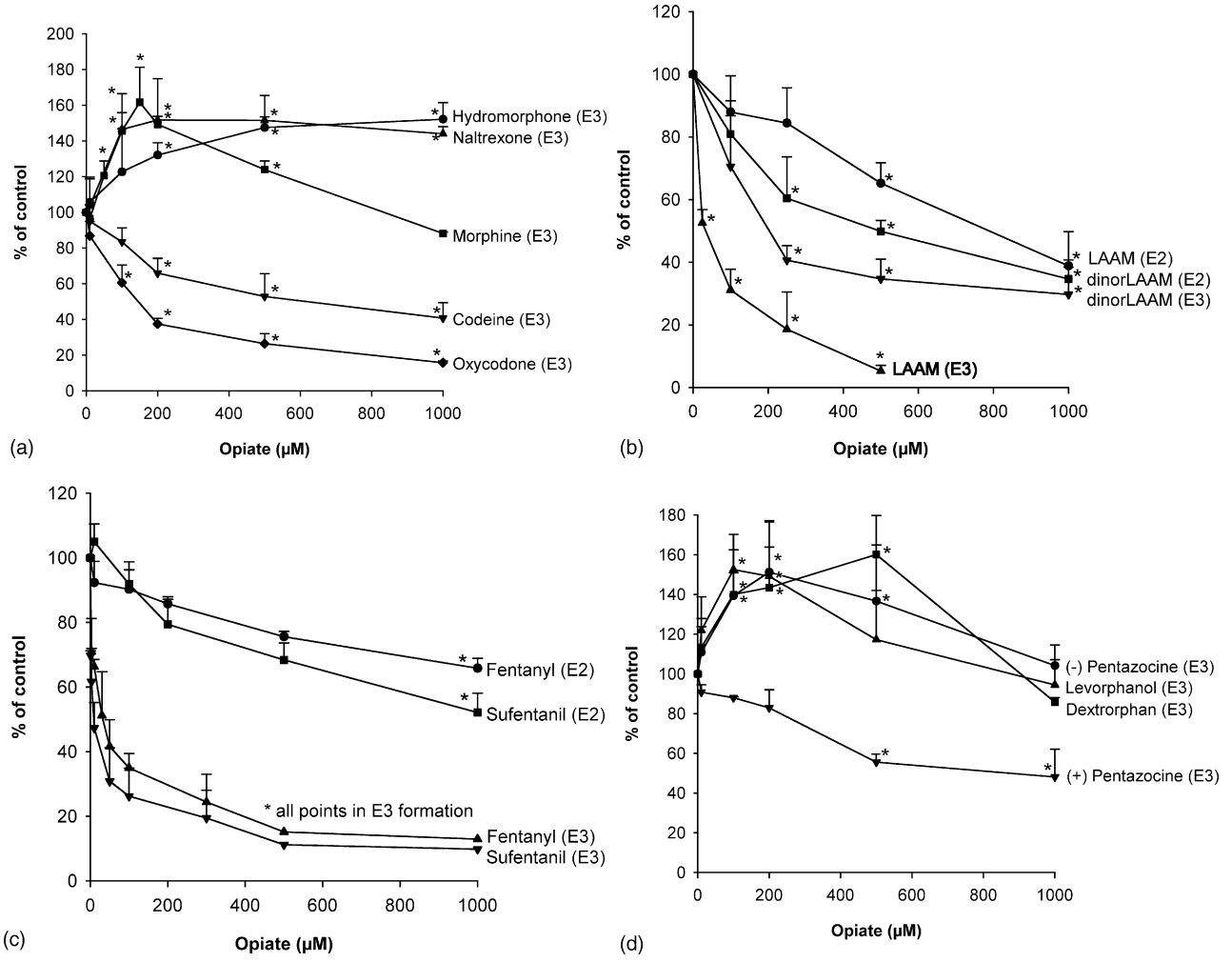

Fig. 1 – The effects of increasing concentrations of opiates (A) phenanthrenes, (B) phenylheptylamines, (C) phenylpiperidines and (D) morphinans and benzomorphans on 17β-estradiol (E_2) and estriol (E_3) formation. The opiates were preincubated with the steroid substrates, as described in Section 2. The rates of estrogen formation are expressed as a percent of control (set at 100%). Each data point represents the mean ± S.D. of three experiments. *Statistical significance of $P < 0.05$.

naltrexone. These phenanthrenes had no effect on E_2 formation but either activated or inhibited that of E_3. Morphine caused an activation of E_3 formation and plots of the data revealed a bell-shaped curve, which, at the drug concentration of 150 μM, reached a maximum of 162% that of control (Fig. 1A).

Heroin and hydrocodone (10 μM) caused a slight increase (17–24%) in E_3 formation (Table 1) and their effect was also bell shaped. On the other hand, oxycodone and codeine were the only phenanthrenes that inhibited the conversion of 16-OHT to E_3. The respective IC_{50} values for oxycodone and codeine were 141 ± 45 μM and 613 ± 55 μM. At their equimolar concentration of 1 mM, oxycodone inhibited E_3 formation by 85% and codeine by 60%. This data indicates that oxycodone is a more potent inhibitor of aromatase than codeine (Fig. 1A).

The opiate agonists hydromorphone and oxymorphone and the antagonist naloxone at their concentration of 100 μM caused a slight increase in E_3 formation but the effect of the antagonist naltrexone was more pronounced and caused the significant increase of 150% that of control (Table 1). Hydromorphone, 500 μM, caused a 150% increase in E_3 formation (Fig. 1A).

3.1.2. *Phenylheptylamines*

The effect of the following phenylheptylamines on the activity of aromatase was investigated: propoxyphene, LAAM, and its metabolites, norLAAM and dinorLAAM.

LAAM and its two metabolites inhibited both E_2 and E_3 formation. However, all three compounds were more potent inhibitors of E_3 than E_2 formation. At equimolar concentrations of LAAM and its two metabolites, dinorLAAM was the most potent inhibitor of E_2 formation (Fig. 1B), while LAAM and norLAAM had similar effects as revealed by their IC_{50} values (Table 2). On the other hand, for E_3 formation, LAAM was a more potent inhibitor of aromatase activity than its two metabolites with an IC_{50} value of 29 ± 1.2 μM. Moreover, LAAM, at a concentration of 250 μM, caused an 80% inhibition of E_3 formation (Fig. 1B). The metabolites norLAAM and dinorLAAM, though equipotent, were weak inhibitors of E_3 formation as revealed by their IC_{50} values (Table 2).

Table 1 – The opiates that caused an increase in estriol formation

Class	Opiate	Estriol formation as % of control		Maximal level of estriol formation	
		Concentration (10 μM)	Concentration (100 μM)	Concentration (μM)	% of control
Phenanthrenes	Morphine	96 ± 8	146 ± 21	150	162 ± 20
	Heroin	117 ± 10	124 ± 14	100	124 ± 14
	Hydromorphone	105 ± 14	123 ± 24	500	147 ± 18
	Oxymorphone	110 ± 3	123 ± 15	200	131 ± 12
	Hydrocodone	117 ± 6	124 ± 5	100	124 ± 5
	Naloxone	100 ± 10	116 ± 5	200	128 ± 3
	Naltrexone	103 ± 15	146 ± 9	200	152 ± 2
Phenylheptylamine	Propoxyphene	102 ± 11	111 ± 10	200	135 ± 9
Phenylpiperidine	Meperidine	105 ± 9	114 ± 16	200	126 ± 8
Morphinans	Levorphanol	122 ± 17	152 ± 18	100	152 ± 18
	Dextrorphan	113 ± 10	140 ± 23	500	160 ± 20
Benzomorphans	(−)-Pentazocine	111 ± 17	139 ± 12	200	151 ± 25

Propoxyphene, at its concentrations between 10 and 1000 μM, had no effect on E_2 formation (Table 1). However, its effect on E_3 formation was bell shaped. The opiate at a concentration of 200 μM activated aromatase and caused a 135% increase in the amount of the hormone.

3.1.3. *Phenylpiperidines*

The phenylpiperidines fentanyl and sufentanil inhibited both E_2 and E_3 formation. However, they were weak inhibitors of E_2 as revealed by their IC_{50} being >1 mM (Fig. 1C). On the other hand, the two opiates were potent inhibitors of E_3 formation with IC_{50} values of 35 and 12 μM, for fentanyl and sufentanil, respectively and K_i values of 25 and 7 μM. This data indicates that sufentanil, among the 20 investigated opiates, is the most potent inhibitor (Table 2).

Meperidine, similar to most of the investigated opiates, did not affect E_2 formation; however, at a concentration of 200 μM, it caused a 25% increase in formation of E_3.

3.1.4. *Morphinans*

The morphinan levorphanol and its pharmacologically inactive isomer dextrorphan had no effect on E_2 formation. However, both compounds caused an increase in E_3 formation and their effect in the concentration range between 10 and 1000 μM was bell shaped (Fig. 1D). Levorphanol, at a concentration of 100 μM, caused a maximum increase in aromatase activity of 152%. Dextrorphan at a concentration of 500 μM, caused a maximum increase in aromatase activity of 160%. The effect of both compounds declined to the control level at their concentration of 1 mM (Fig. 1D and Table 1).

3.1.5. *Benzomorphans*

The pharmacologically active (+) and inactive (−)-stereo isomers of pentazocine were investigated. (+)-Pentazocine was a weak inhibitor of E_2 and E_3 formation with IC_{50} values >2 mM and 785 ± 49 μM, respectively (Table 2). (−)-Pentazocine did not affect E_2 formation but increased the formation of E_3 (151%) at a concentrations of 200 μM (Fig. 1D and Table 1).

3.2. *Kinetics for aromatase inhibition by opiates*

The type of inhibition caused by the opiates, competitive or noncompetitive, was determined using Lineweaver–Burk plots (Fig. 2). The inhibition constant (K_i) for the opiates that had IC_{50} values <1 mM was determined by Dixon plots as shown for sufentanil as an example (Fig. 3).

Table 2 – The inhibition constants of the opiates

Class	Opiate	Inhibition of testosterone conversion to 17β-estradiol		Inhibition of 16α-hydroxytestosterone conversion to estriol	
		IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)
Phenanthrenes	Oxycodone	No inhibition[a]	No inhibition[a]	141 ± 45	92 ± 22
	Codeine	No inhibition[a]	No inhibition[a]	613 ± 55	218 ± 69
Phenylheptylamines	LAAM	750 ± 206	381 ± 17	29 ± 1	13 ± 8
	norLAAM	1003 ± 107	408 ± 80	215 ± 96	83 ± 44
	dinorLAAM	490 ± 106	178 ± 33	227 ± 93	88 ± 33
Phenylpiperidines	Fentanyl	>2000	ND	35 ± 6	25 ± 5
	Sufentanil	1004 ± 111	ND	12 ± 7	7 ± 1
Benzomorphans	(+)-Pentazocine	>2000	ND	785 ± 49	225 ± 73

Data represented are mean ± S.D. of three experiments. ND indicates not determined.
[a] No inhibition in the concentration range tested.

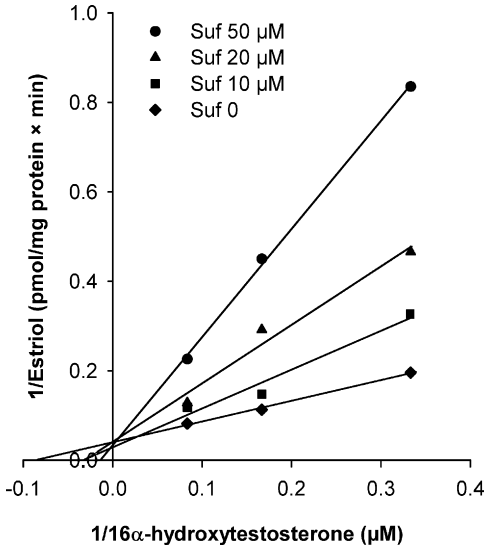

Fig. 2 – Effect of sufentanil on the conversion of 16α-hydroxytestosterone (16-OHT) to estriol (E_3). The type of inhibition was determined by a Lineweaver–Burk plot, i.e. the reciprocal of the rate of E_3 formation vs. the reciprocal of the substrate (16-OHT) concentration in the presence and absence of the inhibitor sufentanil. The data revealed a competitive type of inhibition.

Analysis of the data indicated that the following opiates were competitive inhibitors of 16-OHT conversion to E_3: the phenanthrenes oxycodone and codeine, the phenylpiperidines fentanyl and sufentanil, and the benzomorphan (+)-pentazocine. The phenylheptylamine LAAM and its metabolites, norLAAM and dinorLAAM were competitive inhibitors of both testosterone and 16-OHT.

All determined K_i values were lower for the conversion of 16-OHT to E_3 than for testosterone conversion to E_2 (Table 2).

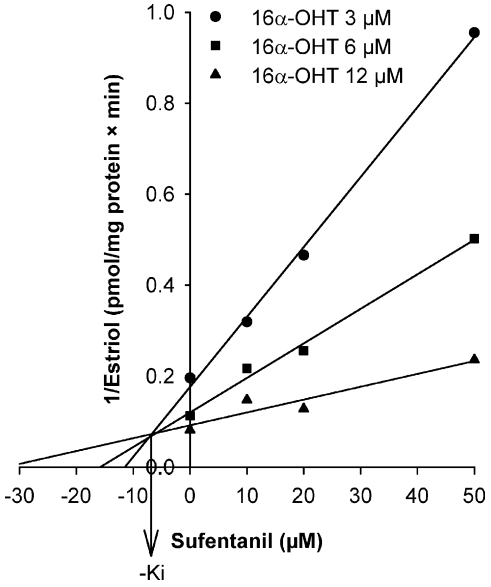

Fig. 3 – The inhibition constant for sufentanil. Dixon plot of the data obtained was used to determine the K_i for the opiate (sufentanil). The reciprocal for the velocity of estriol (E_3) formation is plotted against the concentration of the opiate at three substrate concentrations (1/2, 1, and $2K_m$). The experimental conditions are identical to those described in Fig. 2.

4. Discussion

Aromatase/CYP19 has long been recognized as a key enzyme in the metabolic pathway for the aromatization of androgens and their conversion to estrogens in the human placenta and other tissues. However, recent reports provided evidence that microsomal fractions obtained from term placental trophoblast tissues catalyzed the metabolism of BUP to norBUP, methadone to EDDP, and LAAM to norLAAM. The major enzyme responsible for the *N*-dealkylation of these three opiates in term human placenta was identified as aromatase/CYP19 [5–7]. Moreover, BUP and methadone were competitive inhibitors of aromatase substrates – namely testosterone and 16-OHT – but BUP was more potent than methadone [11]. Recently, CYP19 was also identified as the major enzyme responsible for the metabolism of methadone by preterm placentas, i.e., <34 weeks of gestation [14]. These findings suggested that aromatase could be a site for drug interactions in pregnant women under treatment for opiate dependence with either methadone or BUP. If true, then the administration of BUP or methadone to the pregnant opiate addict for treatment could affect the activity of CYP19 and consequently the levels of E_2 and E_3, respectively. Indeed, lower levels of estrogens were associated with women enrolled in methadone maintenance programs [15]. Therefore, the goal of this investigation was to determine the effect of opiates, which have therapeutic indications or have been known to be abused, on the activity of placental aromatase in its conversion of testosterone to E_2 and 16-OHT to E_3.

In this report, the opiates investigated were grouped according to their known classes of structurally related compounds. However, it should be emphasized that this investigation was not designed to examine the relation between the structure of these classes of compounds and the activity of aromatase but to determine the effects of these opiates on the formation of estrogens (E_2 and E_3) by term human placentas. The investigated 20 compounds included agonists and antagonists of the three opiate receptor types—namely mu, delta, and kappa. Data cited here revealed that the investigated opiates, irrespective of their chemical classification or as ligands of the three opiate receptors, had no effect, inhibited, or increased the rates of estrogen formation by aromatase. The effect of all the opiates on E_3 formation was more pronounced than that on E_2 for the reasons discussed below.

The investigated phenanthrene compounds included seven agonists and the two antagonists naloxone and naltrexone. All nine phenanthrenes affected E_3 formation but not E_2. Oxycodone and codeine inhibited the conversion of 16-OHT to E_3; their K_i values were 92 ± 22 and 218 ± 69 μM, respectively, and they are considered weak inhibitors (Table 2). A plausible explanation of the difference in K_i values could be based on the affinity of the two substrates

testosterone and 16-OHT to aromatase. The apparent K_m values for testosterone and 16-OHT are 0.2 and 6.0 μM, respectively. Therefore, the affinity of testosterone to aromatase is 30 times that of 16-OHT and could result in the opiates being more potent inhibitors of E_3 than E_2 formation.

On the other hand, the five remaining phenanthrene agonists and the two antagonists increased E_3 formation over control. This activation was either modest (20–30% over control) as observed for hydrocodone, heroin, oxymorphone, and naloxone, or pronounced (approximately 50% or more over control) as observed for morphine, hydromorphone, and naltrexone (Table 1). It should be noted that under our experimental conditions, the de-acylation of codeine and/or heroin to morphine cannot be ruled out. The increase in the activity of CYP isozymes, in vivo and/or in cell line cultures, is attributed to their induction. For example, tobacco smoke, or more specifically the benzopyrenes nicotine and cotinine induce CYP 1A1, 2A1, and 1B1 [16,17]. However, the activation of aromatase reported here was determined in vitro and cannot be attributed to induction. Nevertheless, several investigators reported on the in vitro stimulation of CYP isozymes, e.g., 3A4, 2C9, 17A, and 4A7. The proposed mechanism for the activation was either enhanced electron flow via cytochrome b_5 or, in the absence of electron transfer, a conformational change occurred [18]. Moreover, several organophosphorus compounds increased the levels of the metabolites of 16-OHT and 2β-hydroxytestosterone upon their preincubation with human liver microsomes (CYP3A4). However, preincubation of these compounds with the same substrates had no effect on CYP19 [19]. An investigation of the structure–activity relationship revealed that the metabolism of phenanthrene by CYP3A4 is activated by 7,8-benzoflavone [20]. The authors investigated 13 derivatives of 7,8-benzoflavone and reported that 6 of the compounds activated the enzyme. The authors suggested that both the activator and the substrate are bound to the enzyme's active site, which results in a ''narrower'' site, but they also did not rule out their binding to different sites. Three dimensional modeling and site-directed mutagenesis offered an explanation for the activation of CYP3A4 by α-naphthoflavone [21]. The authors suggested that the substrate and up to two molecules of α-naphthoflavone can fit in the active site thus explaining the homotropic enzyme stimulation observed. The orientation of the substrate, bound in the active site, can decrease enzymatic activity due to an increase in substrate mobility or it can increase the activity by stabilizing substrate mobility in a given binding orientation.

The phenylheptylamine LAAM and its metabolites, norLAAM and dinorLAAM, were more potent inhibitors of E_3 than E_2 formation. Moreover, LAAM was at least 10 times more potent than its metabolites in inhibiting E_3 formation (Table 2), most likely for the same reasons mentioned above for the affinity of the natural substrates to aromatase. Currently, LAAM is not used for treatment of the opiate dependency. However, if LAAM is used in the future, it is unlikely to affect the rates of E_3 formation in vivo for at least two reasons: (1) its rapid metabolism, and hence its classification as a ''prodrug'' to its pharmacologically active metabolites norLAAM and dinorLAAM [22,23] and (2) the metabolites are weaker inhibitors than the parent compound. Propoxyphene is structurally related to methadone but its potency is very weak (half that of codeine) with little therapeutic value and abuse potential. It did not affect the activity of aromatase except at its concentration of 200 μM, which caused slight activation of E_3 formation; thus, it is unlikely to have any in vivo effect on aromatase.

The phenylpiperidines fentanyl and sufentanil are potent opiate analgesics and are used as adjuncts to anesthetics because of their sedative effects. Similar to LAAM, both fentanyl and sufentanil were potent inhibitors of E_3 formation with K_i values of 25 ± 5 and 7 ± 1 μM, respectively. However, both fentanyl and sufentanil were very weak inhibitors of E_2 formation (Table 2). Moreover, both opiates were competitive inhibitors of 16-OHT (Fig. 2) and the most potent of all the opiates investigated. The therapeutic use of these two opiates is for short durations and it is unlikely to cause a significant effect on estrogen formation. However, the prolonged abuse of fentanyl, which is on the rise, during pregnancy might cause in vivo adverse effects. Meperidine caused negligible activation of E_3 formation.

The morphinan levorphanol and its isomer dextrorphan, which does not cause analgesia, resulted in a significant increase (140–160% of control) in E_3 formation and had no effect on E_2. It is unlikely that the use of levorphanol transiently during pregnancy could affect estrogen formation by the placenta.

The benzomorphan pentazocine (Talwin) was widely abused in the 1980s. Mixed with the antihistamine pyribenzamine, pentazocine was also known as T's and Blues. The pharmacologically active enantiomer (+)-pentazocine had no effect on E_2 but was a weak inhibitor of E_3 formation. On the other hand, its enantiomer (−)-pentazocine increased E_3 formation at high concentrations. It is unlikely that pentazocine abuse would reoccur due to its current formulation with naloxone. The prolonged use of (+)-pentazocine during pregnancy may cause a slight or no decrease in estrogen formation by the placenta.

In summary, data sited here indicate that the investigated 20 opiates, which were selected on the basis of their therapeutic use and their abuse, varied in their effects on placental aromatase activity, and these effects were not related to their pharmacological activity as analgesics. The therapeutic use of these opiates is usually for a short duration and apparently does not have adverse effects on estrogen formation during pregnancy. Moreover, the K_i values for the most potent inhibitors of aromatase (e.g., fentanyl and sufentanil) and their expected concentrations in plasma indicate that the risk of an effect on aromatase is remote. However, the reported increase in oxycodone and fentanyl abuse is alarming, and it is unclear whether their chronic administration could affect placental biosynthesis of estrogens. At this time, it is also unclear whether a decrease in estrogen formation by the placenta could adversely affect maternal or neonatal outcome.

Acknowledgements

The project described was supported by grant number DA13431 from NIDA to M.S. Ahmed. Its contents are solely

the responsibility of the authors and do not necessarily represent the official views of NIDA. The authors appreciate the assistance of the medical staff, the Perinatal Research Group, and the Publication, Grant & Media Support area of the Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas.

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