To explore the possibility that exposure to environmental agents known to induce xenobiotic metabolizing enzymes could alter the specific activities of detoxication enzymes in the bladder epithelium, groups of four rats each were treated with phenobarbital or Arochlor 1254 at doses previously shown to result in significant increases in hepatic glutathione *S*-transferase activities [23–25]. The data in Fig. 1 indicate that treatment with either Arochlor 1254 or with phenobarbital markedly increased glutathione *S*-transferase activity in rat liver. In the bladder epithelium, phenobarbital increased the specific activity of glutathione *S*-transferases while treatment with Arochlor had no significant effect. The data in Fig. 1 and Table 2 suggest that a variety of environmental agents are capable of altering the relative ability of the bladder epithelium to detoxify electrophilic carcinogenic or cytotoxic agents.

In summary, the results of this work indicate that bladder epithelial cells are fully capable of catalyzing the metabolic deactivation of proximate carcinogens through conjugation with glutathione. Moreover, glutathione and the glutathione transferases may very well play a role in modulating the cytotoxic/mutagenic actions of electrophiles formed in other tissues and excreted in the urine. In addition, these studies have demonstrated that reduced glutathione levels and the specific activity of the glutathione *S*-transferases may be altered by other environmental agents and suggest that further investigations to understand those factors which may predispose certain individuals to the deleterious effects of bladder carcinogens may prove fruitful.

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Department of Urology
City of Hope National Medical Center
Duarte, CA 91010, U.S.A.
WENDY L. WHYTE
RAYMOND S. PONG

Department of Community and Environmental Medicine and Southern Occupational Health Center
University of California, Irvine
Irvine, CA 92717, U.S.A.
ALAN R. BUCKPITT*

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* Author to whom correspondence should be addressed.

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Effect of maternal pethidine administration on neonatal brain cyclic AMP levels and ornithine decarboxylase activities

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Pethidine is widely used for the relief of pain during labor [1, 2]. After intramuscular or intravenous administration, the drug is readily transferred from the mother to the fetus [3]. This results in a depressive effect on the ventilation of newborns and in longer-lasting adverse effects on the neuromuscular physiology and behavior of the offspring [4].

Despite its wide use in obstetrics, the information available on the effects of the drug on the developing tissues in infants is rather limited.

Ornithine decarboxylase (EC 4.1.1.17, ODC), which is the rate-limiting enzyme in the polyamine biosynthetic pathway, appears to be associated with rapid cell growth [5]. It has been shown [6] that both fetal and neonatal rat

brain have a characteristic ODC developmental pattern which appears to be regulated by the adenylate cyclase system [7]. Opiates inhibit adenylate cyclase activity in homogenates or slices of brain [8] and prevent the induction of ODC by prostaglandin E_1 in neuroblastoma × glioma hybrid cells [9]. Butler and Schanberg [10] reported that ODC activities are altered in brain of pups of chronic opiate-treated rat mothers. This finding supports the hypothesis that drug administration to the pregnant mother adversely affects the biochemical maturation of the offspring.

In the present study, the effect of acute pethidine administration on the developmental pattern of brain ODC is examined, using pregnant rats and their pups as well as human material.

Materials and methods

The genetically heterogenous random bred Sabra strain of rat with a gestational period of 21 days was used. Pregnant rats of day 20 of gestation were exposed to a single, intraperitoneal dose of pethidine (50 mg/kg body wt). At different time intervals, following the injection, the pregnant rats were killed. A mid-line laparatomy through the linea alba was performed, and the rat fetuses were removed, stripped of the fetal membranes, and their brains extracted.

Fetal human brains were obtained from mid-trimester abortions performed by intra-amniotic injection of 40 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The prostaglandin was injected under ultrasonic vision. The gestational age, which has been established by ultrasonography, was 20 weeks (± 1 week). There were two groups: one control group that was divided into two subgroups: (a) patients who received no analgesics (four patients) and (b) patients who received epidural analgesia with marcain for relief of pain (two patients), and a second group (eleven patients) which received pethidine intravenously (from one to three injections, 50–75 mg per injection, between 1 and 9 hr before abortion). The brains were extracted with the appropriate buffers and kept at −80°.

Cyclic AMP levels were determined according to Gilman [11] as follows: the brain extracts were homogenized in sodium acetate buffer (pH 4.0, 50 mM), and the cells were disrupted by freezing and thawing. After centrifugation at 20,000 *g* for 10 min, aliquots of the supernatant fluids were assayed for cAMP in duplicates. Bovine adrenal cortex protein kinase served as the binding protein [12]. The pellets were dissolved in 0.1 N NaOH, and protein was assayed according to Lowry *et al.* [13]. The activity of ODC was determined as described previously [14].

Results and discussion

Our experiments dealt with an acute effect exerted by a single injection of pethidine into the pregnant rats. Figure 1 shows a decrease in cAMP levels in the brain taken from pregnant rats 30–120 min after pethidine injection. Thereafter, cAMP levels returned to normal values and were even somewhat elevated.

The activity of ODC is high in proliferating tissues and declines when cells differentiate [5]. As expected, ODC activity in brain from pregnant rats was negligible (results not shown).

The effect of maternal pethidine administration on fetal rat brain was studied next. Figure 2A shows that maternal pethidine administration led to a decrease in fetal brain cAMP levels. A significant decrease was observed throughout the first 2 hr after pethidine injection into the pregnant rats. This was followed by an increase well above the initial values. Fetal brain ODC activity was also affected by pethidine. It is evident from Fig. 2B that treating pregnant rats with pethidine resulted in a decrease in fetal brain ODC activity. It should be noted that changes in ODC activity lagged behind the reduction in cAMP levels; the

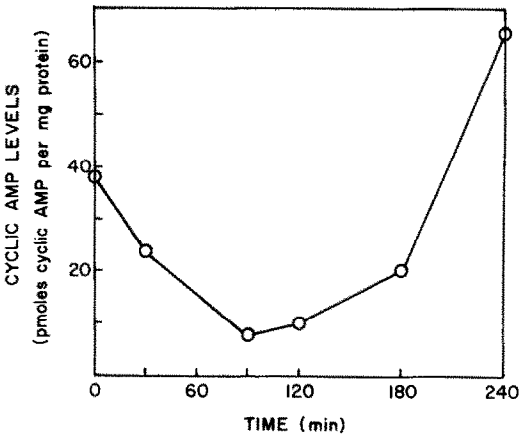

Fig. 1. Effect of pethidine on maternal rat brain. At different times after pethidine injection, the pregnant rat brains were extracted and the level of cAMP was determined.

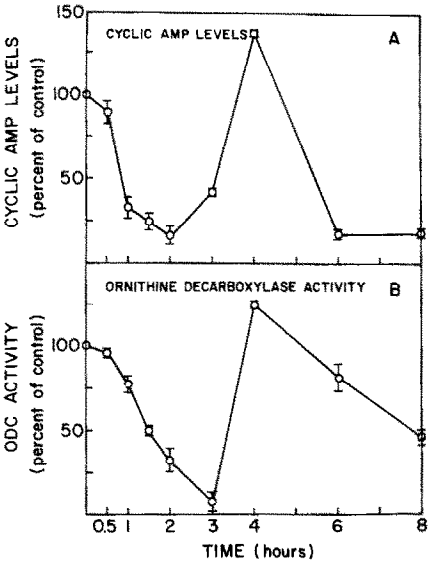

Fig. 2. Effect of pethidine on fetal rat brain. (A) At different times after pethidine injections, the pregnant rats were killed, and the fetal brains were extracted for determination of cAMP levels. (B) The experimental conditions were as given above but ODC activity was measured in the fetal brain.

lowest activity was observed after 3 hr. Again, this decrease was followed by an increase in ODC activity (Fig. 2B), but by 6 and 8 hr after the injection, the cAMP levels and the ODC activity had decreased below the control values. The increases in cAMP levels and in ODC activity are easily explained by a compensatory mechanism that leads to an over-production of adenylate cyclase and ODC in order to counteract with inhibition caused by pethidine.

A similar reduction in ODC and a subsequent elevation of activity are known to be associated with delayed cellular development [15]. Recent data [16] indicate that exposure of rats to volatile anesthetics for 2 hr results in a similar transient reduction in ODC activity. Maximal effects are noticed 4 hr after exposure. The induction of ODC is thought to provide a sensitive index for the initiation of cell proliferation and, therefore, may reflect tissue damage in adult animals [17] or, alternatively, the initiation of hypertrophy.

Table 1. Effect of maternal pethidine administration on cAMP levels and ODC activity in fetal human brain*

	cAMP levels (pmoles cAMP/mg protein)	ODC activity (pmoles CO_2/mg protein/hr)
Control	454	118
	157	121
	317	36.5
	142	114
	97	93
	151	117
Pethidine	28	56
	32	22
	55	12
	42.8	17
	20	6.3
	71.7	25.3
	89.2	40.2
	6	2.1
	10	3.7
	10	3.4

* cAMP levels and ODC activity were determined in brain from human fetuses obtained after mid-trimester abortion. The control group consisted of fetuses whose mothers did not receive pethidine at delivery. The pethidine group included fetuses whose mothers received pethidine at different times (from 1 to 9 hr before delivery).

The high levels of ODC obtained in the fetal rat brain 4 hr after injecting pethidine into the pregnant mothers (Fig. 2B) appear as a consequence of the effect caused by pethidine. These perturbations may be explained either by brain damage and a subsequent repair mechanism or, alternatively, by brain hypertrophy. Both possible changes are accompanied by a temporary increase in ODC activity.

After showing that pethidine induced changes in cAMP levels and ODC activity of fetal rat brain, human fetuses were also examined. Table 1 shows that cAMP levels and ODC activity also decreased in the human fetal brain after injecting pethidine into the mothers several hours prior to abortion. It should be noted, however, that both cAMP and ODC activity remained low, up to 9 hr after drug administration, while in fetal rat brain a subsequent increase was observed (Fig. 2B). These differences may be due to the different route of injection and the time of brain sampling.

ODC levels reflect cell proliferation in each brain region. Pethidine is widely used for the relief of pain during the first stage of labor. The drug is readily transferred to the fetus and causes a decrease in cAMP levels and ODC activity. This decline seems to be reversible, at least in the rat pups, after 4 hr. These findings suggest that, in addition to its effect on offspring ventilation [4], pethidine might affect brain development. (It appears that, at least in the systems studied, pethidine causes a delay in cellular development.) Further studies have to be carried out to define the physiological-developmental consequences of these perturbations.

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*Department of Obstetrics and Gynecology and
†Department of Molecular Biology
Hebrew University-Hadassah Medical School
Jerusalem, Israel

EMMANUEL PERSITZ*
DONNA BENALAL†
URIEL BACHRACH†‡
SHLOMO MOR-YOSEPH*

‡ Author to whom correspondence should be addressed.

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