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ORIGINAL ARTICLE

EXPERIMENTALLY-INDUCED EXENCEPHALY AND SPINA BIFIDA IN MICE

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Background– **An agent that many pregnant women may be exposed to is opium or one of its alkaloids such as morphine and heroin. Recent observations of central nervous system (CNS) defects such as cyclopia among the infants of addicted mothers have suggested that opiate agents might be a cause of neural tube defects. The purpose of this study was to test morphine for CNS teratogenicity in mice and to identify the primordial structures affected.**

Methods– **Timed pregnant BALB/c mice were intraperitoneally injected with 10 mg/kg morphine given in the early morning on days 10 and 11 of gestation. After dissection of fetuses and fixation in Bouin's or 10% formalin solution, CNS abnormalities were evaluated using a dissecting microscope. Data were analyzed using Fisher's exact test.**

Results– **There was a higher incidence of CNS fetal malformations including exencephaly and spina bifida in fetuses whose mothers were exposed to morphine than in control fetuses exposed to NaCl. There were defects in 33% of fetuses from mothers who received morphine on both days 10 and 11 of gestation.**

Conclusion– **Critical morphine injection times were determined to be days 10 to 11 of gestation. Our results indicate that morphine can be embryopathic during critical stages of CNS development.**

Keywords ● exencephaly ● morphine ● neural tube ● spina bifida

Introduction

It has become increasingly evident that human embryos are subjected to a variety of environmental hazards, chemical and/or physical, that might have deleterious effects on normal morphogenesis during critical stages of development.[1] A hazard that many mothers may be exposed to are opioid drugs such as morphine, which have a broad range of physiologic effects such as pain relief and a feeling of intense pleasure, which signals the start of an addiction problem.[2]

A 20 mg/kg dose of morphine in pregnant hamsters has been shown to pass placental barriers about 10 minutes after intraperitoneal administration.[3–5] The teratogenic effects of morphine, or other opium alkaloids have received very little attention until now. A limited number of animal studies have investigated the teratogenic potential of opiates and shown morphologic defects in newborn animals whose mothers received different doses of morphine during pregnancy. Using high doses (20 mg/kg/day) of morphine during gestation, abnormal structural alterations were observed in the gut, testes, adrenal gland and parts of the central nervous system (CNS).[6–8] In humans, fetal exposure to heroin or morphine has been shown to be associated with growth retardation.[9] It has also been postulated that prenatal exposure to opiates probably leads to disturbances of the CNS.

The present study was carried out to investigate the effects of a single dose of prenatal morphine administration on developing mouse embryos during critical stages of CNS morphogenesis. Our objectives were to determine: 1) the period of fetal sensitivity to morphine, i.e. the minimal maternal dose of morphine to cause detectable abnormalities; and 2) to what extent the CNS structures might be involved.

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Table. The incidence of central nervous system defects in morphine-treated mouse fetuses.

Number of fetuses	Gestational days of exposure	Frequency of defects (%)	Significance (Fisher's test) compared to control
31	8	2 (6)	0.007
22	9	2 (9)	0.004
35	10	9 (25)	0.000
26	11	6 (23)	0.000
45	10 & 11	15 (33)	0.000
30	12	1 (2)	

Materials and Methods

Forty-two virgin female BALB/c mice were mated with males of the same strain overnight and isolated in the morning upon finding a vaginal plug. This was designated as day 0 of pregnancy. Mated animals were kept in a room with controlled light and dark periods. Purina Lab Chow and tap water were provided *ad libitum*.

A single intraperitoneal injection of 1, 5, 10 and 20 mg/kg of morphine was administered on days 8, 9, 10 & 11, and 12 of gestation to 30 pregnant mice in the early morning. Because a dose of 10 mg/kg of morphine resulted in a high frequency of defects without any mortality at critical gestational days 10 and 11, most of the results reported here were taken from mice exposed. Twelve control animals were injected with an equal volume of diluent (sterile 0.9% NaCl).

All pregnant animals were anesthetized with ether and immediately sacrificed. Embryos, fetuses and newborn animals with their membranes were dissected free of the uterine wall under the dissecting microscope and placed in phosphate buffer to detect anatomical abnormalities. Body weight and crown-rump size of all fetuses were also recorded prior to fixation in Bouin's or 10% formalin solution. Selected specimens of interest were photographed using an Olympus dissecting microscope with attached camera.

Results of all experimental and control specimens were analyzed using Fisher's exact test and statistical software SPSS (version 8) and EPI6.

Results

Details of morphine exposure and results of its teratogenic effects are presented in Table. The incidence of abnormalities increased when a single injection of 10 mg/kg of morphine was given during days 10 and 11 of gestation. However, the percentage of defects when morphine injections were administered on both days 10 and 11 was higher than that of each day alone. Single morphine injections (all tested doses) before day 10 and after day 11 of gestation did not produce any CNS abnormalities (Figure 1). CNS defects included different degrees of exencephaly. It was

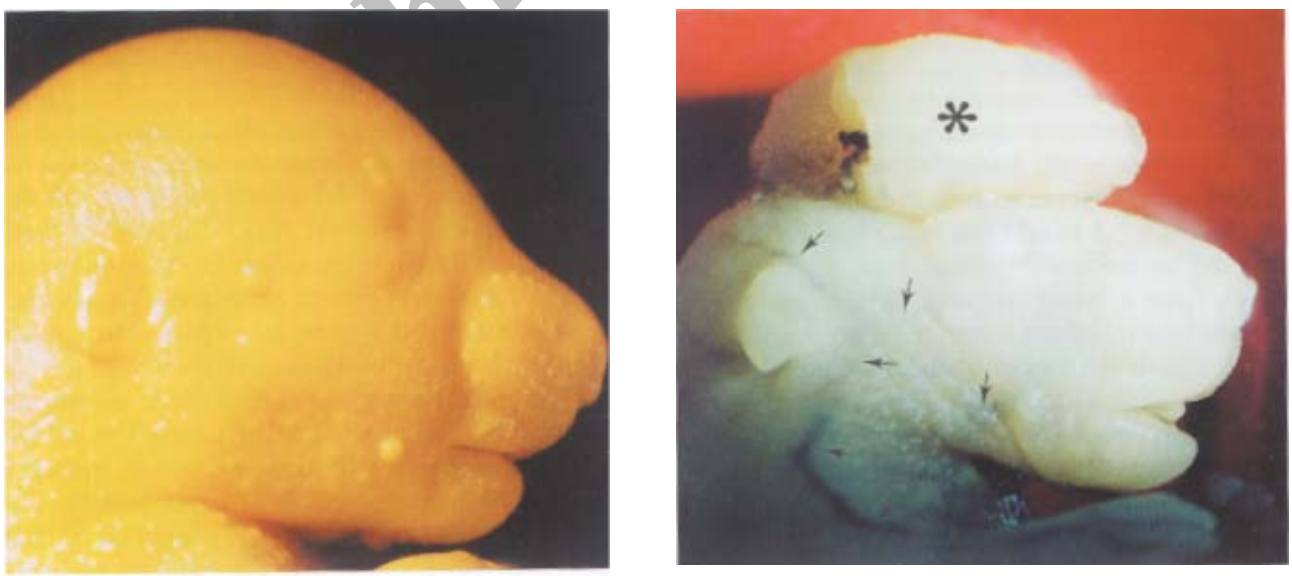

Figure 1. Lateral views of a normal (left) and experimental newborn mouse with severe exencephaly (right) exposed to 10 mg/kg morphine on gestational day 10 (asterisk). The eyes are very prominent, the eyelids have not formed, and the facial vessels are dilated (arrows). Both were fixed in Bouin's fluid.

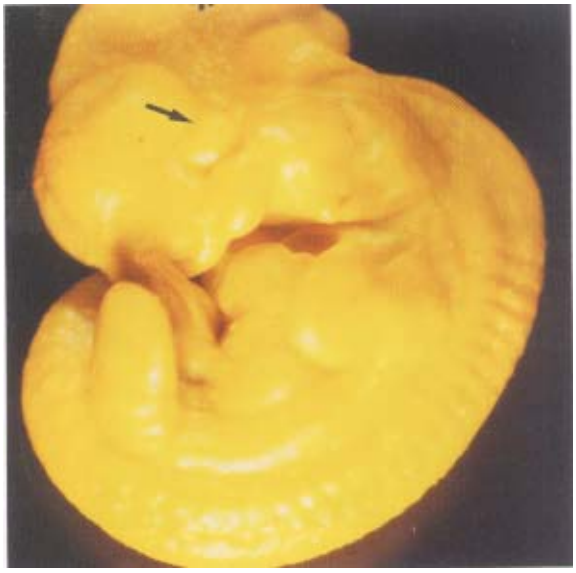

Figure 2. Lateral view of a morphine-exposed 13-day-old mouse embryo with unusual cranial region. The exposed brain is marked with an asterisk; the large, abnormal eye with an arrow.

characterized by an imperfect cranium (Figures 1, 2 and 3) and in severe cases, the brain was located outside the skull (Figure 1).

Spina bifida was also observed in the upper thoracic section (Figure 4). Most fetuses and newborns with CNS defects also exhibited eye abnormalities like cataract, exophthalmia and orbit abnormalities. The average body weight and size of the experimental fetuses and newborns (1.0 g and 1.85 cm, respectively) was significantly less than the control animals (1.3 g and 2.10 cm, respectively, $p < 0.001$).

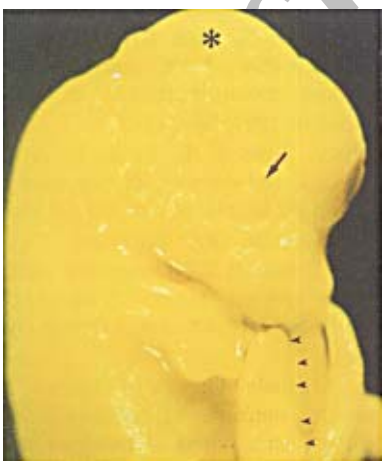

Figure 3. Lateral view of an experimental 15-day-old mouse embryo with an exencephaly (asterisk) and prominent eyes. Upper limb development was behind normal time and the lower limb had an unusual shape (arrowheads).

Discussion

In these experiments, morphine, a principal constituent of opium, was administered to timed pregnant mice during critical stages of embryonic development. Doses of 10 mg/kg/day of morphine specifically on gestational days 10 and 11 produced a high incidence of CNS defects including exencephaly and spina bifida. These results emphasize the fact that there are critical stages in CNS development during which even one dose of morphine may exert its effects. It has been shown that the effects of morphine insults during these critical periods are usually irreversible.[1,10] Teratologists believe that critical temporal periods *per se* may not exist at all, but rather a series of critical developmental events such as specific macromolecular interactions essential to normal development which are highly susceptible to derangement by environmental agents.[11–14]

In cases of morphine-induced CNS defects, we should focus on the critical events of development that are particularly amenable to analysis of mechanism(s) of morphine teratogenesis. The mechanism underlying the effects of a single or two doses of morphine exposure on the developing CNS is not easily explained. However, several possibilities exist. First, morphine might act through early embryonic opiate receptors and compete with endogenous opioid peptides, which are believed to be necessary for normal morphogenesis. [15–17] These receptors regulate pain as well as other functions in the adult brain.[2] Mechanism(s) of these interactions during early embryonic period (days 8–14 of gestation in the mouse) is not yet clear. However, in the late fetal stage (days 15–birth) and later, postnatally, opioids mediate their effects by interacting with specific receptors, which are located on the surface of certain cells in the CNS.[18–21]

Second, morphine might interfere with normal production of the extracellular matrix (ECM). It is well known that ECM functions as an important determinant during development and cell differentiation.[12, 22] Components of this matrix may serve to control cell to cell or tissue to tissue interactions.[23] In addition, regional composition differences in the ECM may also provide the stimulus for other developmental phenomena such as proliferation, differentiation, migration[24,25], programmed cell death[26,27] and also controlling morphogenetic movements such as those which act on neural tube fusion.[28]

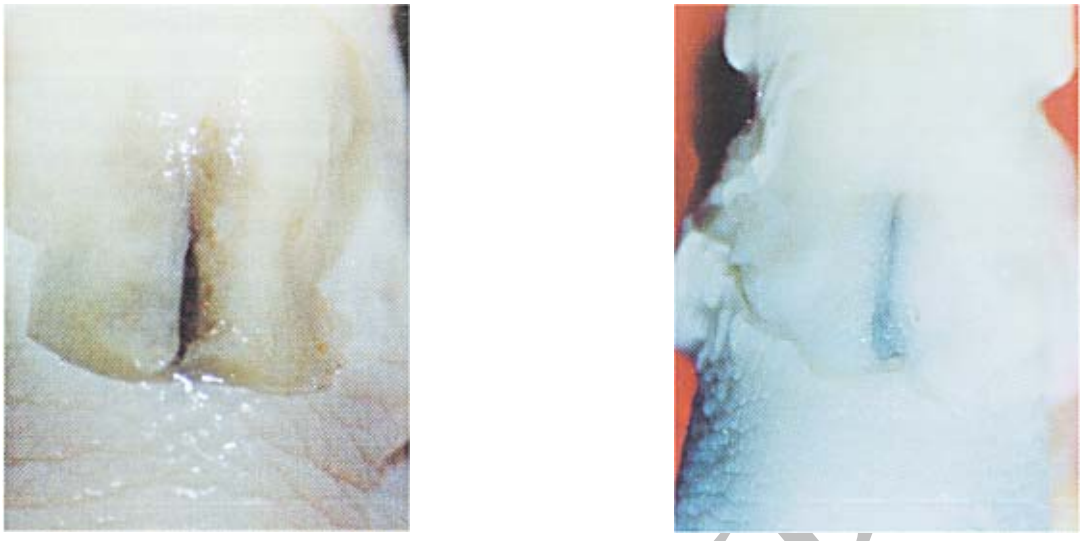

Figure 4. Two different appearances of spina bifida in the upper thoracic level of morphine-exposed mouse embryos. The spina bifida was covered by a thin membrane (right).

The failure of either mesenchymal or epithelial cells to synthesize specific components of the extracellular matrix and cell surface glycol-conjugates that are necessary for cell interactions particularly during sensitive times,[14,23] could result in abnormal development such as exencephaly and spina bifida. [29–31] One important aspect of our studies lies in the location of spina bifida in the high thoracic level of the spinal cord, which is a very unusual place to produce spina bifida experimentally. Perhaps morphine has a direct effect on the selected region and cell population along the developing neural tube and/or somehow increases the rate of the programed cell death in the upper thoracic and rostral neural tube.

Histochemical and lectin histochemical studies are currently underway to address whether certain chemicals, such as cell surface glycoconjugates of the effective areas, are subject to change after exposure of pregnant mice to morphine.

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References

1 Shepart T. Human teratology. In: Reed GB, Claireaux AE, eds. *Diseases of the Fetus and Newborn: Pathology, Imaging, Genetics, and Management.* 2nd ed. New York: Chapman and Hall Medical; 1995: 83–93.

2 Simon E, Hiller J. Opioid peptides and opioid receptors. In: Siegel GJ, Agranoff BW, eds. *Basis Neurochemistry: Molecular, Cellular, and Medical Aspects*. 5th ed. New York: Raven Press; 1994: 321–339.

3 Dygalo NN, Korani L, Feltsegi K, et al. Effects of morphine and naloxone administered to pregnant rats on the adrenal glands and testes of the offspring. *Biull Eksp Biol Med*. 1992; **113:** 58–60. [In Russian]

4 Eriksson PS, Ronnback L, Effects of prenatal morphine treatment of rats on mortality, bodyweight and analgesic response in the offspring. *Drug Alcohol Depend*. 1989; **24:** 187–94.

5 McMillen IC, Deayton JM. Effects of morphine and naloxone on plasma prolactin concentrations in the fetal sheep and pregnant ewe during late gestation. *Neuroendocrinology*. 1989; **49:** 286–90.

6 Siddiqui A, Haq S, Shah BH. Perinatal exposure to morphine distrupts brain norepinephrine, ovarian cyclicity, and sexual receptivity in rats. *Pharmacol Biochem Behav*. 1997; **58:** 243–8.

7 Silvestre MA, Lucena JE, Roxas R, et al. Effects of timing, dosage, and duration of morphine intake during pregnancy on the amount of morphine in meconium in rat model. *Biol Neonate*. 1997; **72:** 112–7.

8 Geber WF, Schramm IC. Congenital malformations of the central nervous system produced by narcotic analgesics in the hamster. *Am J Obstet Gynecol*. 1975; **123:** 705–13.

9 Ornoy A, Michailevskaya V, Lukoshov I, et al. The developmental outcome of children born to heroin-dependent mothers, raised at home or adopted. *Child Abuse Negl*. 1996; **20:** 385–96.

10 Sulik KK, Sadler TW. Postulated mechanisms underlying the development of neural tube defects. Insights from *in vitro* and *in vivo* studies. *Ann N Y Acad Sci*. 1993; **678:** 8–21.

11 Sastry BV. Placental toxicology: tobacco smoke, abused

drugs, multiple chemical interactions, and placental function. *Reprod Fertil Dev*. 1991; **3:** 355–72.

12 Hay ED. *Cell Biology of Extracellular Matrix*. 2nd ed. New York: Plenum Press; 1991.

13 Lodish H, Berk A, Lawrence S. Cell interaction in development. In: Lodish H, ed. *Molecular Cell Biology*. 4th ed. New York: WH Freeman; 2000: 1004–48.

14 Nasrallah I, Golden JA. Brain, eye, and face defects as a result of ectopic localization of Sonic hedgehog protein in the developing rostral neural tube. *Teratology*. 2001; **64:** 107–13.

15 Zagon IS, Wu Y, McLaughhlin PH. Opioid growth factor and organ development in rat and human embryos. *Brain Res*. 1999; **839:** 313–22.

16 Leslie FM, Chen Y, Winzer-Serhan UH. Opioid receptor and peptide mRNA expression in proliferative zones of fetal rat central nervous system. *Can J Physiol Pharmacol*. 1998; **76:** 284–93.

17 Ray SB, Wadhwa S. Mu opioid receptors in developing human spinal cord. *J Anat*. 1999; **195:** 11–8.

18 Zhu H, Barr GA. Opioid withdrawal during development: are NMDA receptors indispensable? *Trends Pharmacol Sci*. 2001; **22:** 404–8.

19 Tempel H, Habas J, Paredes W, et al. Morphine-induced down regulation of mu-opioid receptors in neonatal rat brain. *Brain Res*. 1998; **469:** 129–33.

20 Petrov ES, Varlinskaya E, Smotherman WP. Endogeneous mu-opioid systems and perioral responsiveness in the rat fetus. *Physiol Behav*. 1997; **62:** 31–7.

21 Zagon IS, Verderame MF, Zimmers WE, et al. Molecular characterization and distribution of the opioid growth factor receptor (OGFr) in mouse. *Brain Res Mol Brain Res*. 2000; **84:** 106–14.

22 Ekblom P. Extracellular matrix in animal development. Role of extracellular matrix in animal development-an introduction. *Experimentia*. 1995; **51:** 851–2.

23 Pines J, Toldo L, LaFont F. Cell-to-cell contact and extracellular matrix. Web Alert. *Curr Opin Cell Biol*. 1999; **11:** 535-6.

24 Wilkinson D. Meeting report. Neural developments. *Mech Dev*. 1998; **77:** 3–7.

25 Harland R. Neural induction. *Curr Opin Gen Dev*. 2000; **10:** 357–62.

26 DeSesso JM, Scialli AR, Holson JF. Apparent lability of neural tube closure in laboratory animals and humans. *Am J Med Genet*. 1999; **87:** 143–62.

27 Goswami R, Dawson SA, Dawson G. Cyclic AMP protects against staurosporine and wortmannin-induced apoptosis and opioid-enhanced apoptosis in both embryonic and immortalized (F-II kappa 7) neurons. *J Neurochem*. 1998; **70:** 1376–82.

28 Hackett DA, Smith JL, Schoenwolf GC. Epidermal ectoderm is required for full elevation and or convergence during lending of the ovian neural plate. *Dev Dyn*. 1997; **210:** 397–406.

29 MacDonald KB, Juriloff D, Harris M. Developmental study of neural tube closure in a mouse stock with a high incidence of exencephaly. *Teratology*. 1989; **39:** 195–13.

30 Mines JH, Cunningham J, Sanes JR. Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentopathy in mice lacking the laminin alpha5 chain. *J Cell Biol*. 1998; **143:** 1713–23.

31 Harris M, Juriloff D. Mini-review: toward understanding mechanisms of genetic neural tube defects in mice. *Teratology*. 1999; **60:** 292–305.