Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Brain Research 159 (2005) 12 – 17

DEVELOPMENTAL BRAIN RESEARCH

www.elsevier.com/locate/devbrainres

Research Report

Effects of maternal oral morphine consumption on neural tube development in Wistar rats

Shiva Nasiraei-Moghadam[a], Hedayat Sahraei[b,c,*], Hossein Bahadoran[d], Mehrangiz Sadooghi[a], Seyed Hossein Salimi[e], Gholam Reza Kaka[d], Hossein Imani[d], Hossein Mahdavi-Nasab[d], Hossein Dashtnavard[d]

[a]*Department of Biology, AZAD University, North Branch, Tehran, Iran*
[b]*Department of Physiology and Biophysics, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[c]*Department of Behavioral Sciences Research Center (BSRC), Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[d]*Department of Anatomy, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[e]*Department of Psychology, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran*

Accepted 1 June 2005
Available online 27 July 2005

Abstract

Opiate abuse during pregnancy may result in abnormal nervous system function. In order to evaluate the effects of morphine on the development of the nervous system, the present study focused on the effects of maternal morphine consumption on neural tube development in Wistar rats.

Female Wistar rats (250–300 g) were crossed with male rats and coupling time was recorded (embryonic day 0–E0). Experimental groups received 0.1, 0.05, and 0.01 mg/ml of morphine in drinking water daily (14 ml water for each rat). Control group received tap water. On embryonic day 9.5 (E9.5), the animals were anesthetized and the embryos were surgically removed. The embryos were fixed in 10% formalin for 1 week. After this time, weights and lengths (antero-posterior axis—A-P) of the embryos were determined and then tissues were processed, sectioned, and stained in hematoxylin and eosin (H&E). The sections were investigated for neural tube development by light microscope and MOTIC software.

The decrease in "A-P" length and embryonic weight for the group that received 0.01 mg/ml morphine was significant. It seems that daily consumption of morphine sulfate could delay neural tube development. In addition, administration of 0.01 mg/ml of morphine led to damage to the regulated neuro-ectoderm layer and its thickness.

This study showed that oral morphine consumption leads to neural tube defects, as indicated in the morphometric change and also reduction in weight and length of the embryos. These defects might affect the behavior of the animals.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Opioids: anatomy, physiology and behavior

Keywords: Development; Neural Tube; Addiction; Morphine; Rat

1. Introduction

The prevalence of opioid abuse is high worldwide. Several studies have shown that opioid abuse may affect the embryos of pregnant women. In this regard, it has been shown that opioid administration during pregnancy caused delay in embryonic development, reduced birth weight, and neural tube defects such as spina bifida [12,14,16,21]. In

* Corresponding author. Department of Physiology and Biophysics, Baqiyatallah (a.s.) University of Medical Sciences, PO Box 19395-6558, Tehran, Iran. Fax: +98 21 2281561.
E-mail address: h.sahraei@bmsu.ac.ir (H. Sahraei).

0165-3806/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.devbrainres.2005.06.012

addition, neonatal abstinence was common in the infants of opioid dependent mothers [2]. It is also found that these children had several behavioral abnormalities including hyperactivity, lower Mental Development Index (MDI), and Lower Motor Development Index (PDI) [12,14,21] which may be due to the delay in the development of central nervous system (CNS). Animal studies have indicated that daily morphine injections might lead to reduced activity in chicks [17]. This study indicated that morphine tolerance and dependence may be produced in chick embryos by injecting morphine (2.5, 5, and 10 mg/kg) into the airspace of the egg daily for 4 days beginning on incubation day 12 [17]. The results were dose-dependent with 2.5 mg/kg produced a lesser degree of tolerance than that produced by 5 and 10 mg/kg [17]. The researchers found that there were no differences between the responses of the 5 with 10 mg/kg groups to morphine challenge [17]. Moreover, morphine administration reduced the weight of the brain, liver, and kidney of the embryos in rabbits [16], as well as reduction in their cranio-rump length. Others have shown that prenatal morphine administration also affects the ovarian cycle and sexual receptivity in rats [3]. Moreover, morphine readily crosses the placenta and affects embryonic cells [1,8].

Morphine exerts its effects by activating opioid receptor subtypes including mu, kappa, and delta receptors [13,18,19]. Activation of these receptors leads to reduced cyclic-adenosine-mono-phosphate (cAMP) production, increased potassium efflux, and decreased calcium influx [13,18,19].

However, in previous studies, the animals were injected with morphine through an injecting syringe or minipump only on distinct days (i.e., embryonic day 9–embryonic day 12), which may not model drug consumption by humans. It has also been shown that injection may apply stress to the animals [7]. In order to avoid these problems, we gave morphine in the tap water for the animals. This mode of morphine administration is more similar to human dependence and addiction, because the animals adjust the amount of drug received during the experiments [7]. Since normal development of the neural tube has an essential role in nervous system development [5–7] and perhaps behavioral functions, in the present study, the effects of oral morphine consumption on neural tube development in Wistar rats were investigated.

2. Materials and methods

2.1. Animals

Female Wistar rats (250–300 g, Pasture Institute, Tehran, Iran) were used (6 rats/groups). The animals were housed 6 per cage with 12/12 h light-cycle with ad-lib food and water available. The animals were randomly allocated to different groups of the experiment. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 82/10, Jan 18, 2003).

2.2. Experimental procedure

The female rats were kept with adult male rats in the same cage overnight (20:00 to 08:00) for mating. Observation of animals coupling and vaginal plug was considered as embryonic day 0 (E0). Then, the animals were divided into four groups. Group I received tap water as control. Group II received morphine solution (0.01 mg/ml of water). Group III received the dose 0.05 mg/ml of morphine in their water and the forth group (IV) received morphine (0.1 mg/ml) in their water [7]. Treatment was from E0 to E9.5 for all groups. During the experiments, the animals (experimental groups) were restricted to drink from their morphine containing water. The amount of drink was recorded for each animal in each day and the results were not significantly different among the groups.

On E 9.5, the pregnant animals were anesthetized with chloroform and the embryos were taken out surgically. The embryos were cleaned and immersed in formalin 10% for 7 days for fixation. On the 8th day of fixation, fixed embryos were sectioned using the paraffin embedded method. Before the staining procedure, the weight and length of the embryos were measured by a digital balance (0.0001 g) and a caliper (0.05 mm). For this purpose, each embryo was separated from its placenta under dissection microscope (20×) and fixed in a position that its antero-posterior axis could be indicated. In this position, the length of the embryos was measured by a caliper.

Fixed embryos were sectioned as described earlier [20] and serial sections (thickness = 5 μm) were made. The

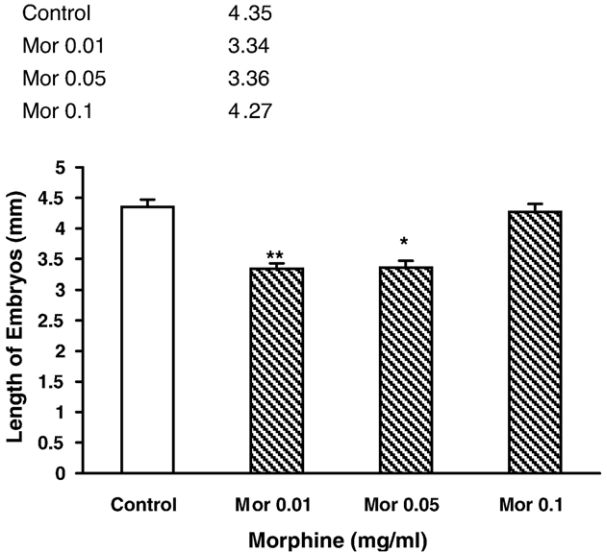

Fig. 1. Effects of maternal oral morphine consumption on embryonic antero-posterior (A-P) length. The E9.5 embryos were fixed in formalin 10% for 7 days. The A-P length of fixed embryos was recorded. Each point shows mean ± SEM of 6–9 embryos, *$P < 0.05$, **$P < 0.01$ different from the control group.