Early Exposure to Morphine Influences Neurochemical and Behavioral Functions in Rats

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Introduction

A series of developmental events that correlate with the onset of central nervous system (CNS) susceptibility to hormones involved in sexual differentiation occurs during mid- to late gestation in rats. These changes include : 1) final cell division of preoptic area and other hypothalamic neurones between days 14-17 p [1]; 2) the appearance of steroid receptors in the hypothalamus including preoptic area around day 15 [2] ; and 3) the onset of gonadal steroid secretion at approximately the same time [3]. Any or all of these may serve as signal for the onset of the "critical period" of brain sexual differentiation [4].

In addition to the emergence of steroid receptors, opiate receptors begin to appear in the brain on the 14th day of fetal life or perhaps even earlier [5]. Hence, the time of appearance of opiate receptors closely parallels the timing of other developmental events which correlate with the sensitive period for brain sexual differentiation. A number of studies show that administration of drugs of abuse during the critical period of CNS development results in abnormal post-pubertal gonadal function and reproductive behavior in humans and animals [6-7].

Emergence of opiate receptors in parallel to steroid receptors leads to the possibility that perinatal opiate exposure may have implications on the organization of the brain sexual differentiation process. This is further substantiated by the fact that the highest concentrations of neural opiate receptors is found in the limbic system, thalamus and spinal cord. Simon and Miller [6] and Synder [7] have suggested that physiological mechanisms other than analgesic and pain perception may be affected by narcotics.

The effects of morphine are produced as a result of its interaction with opioid mu, kappa and sigma receptors [1-8]. Morphine is considered relatively selective agonist for mu-opioid receptors. Morphine is also known to modulate the development and transmission of noradrenergic [9,10] and dopaminergic [9,11] systems in various regions of the brain. Endogenous opiate peptides are known to be involved in regulation of the hypothalamo-pituitary-ovarian axis by suppressing noradrenergic input to the hypothalamus [9]. We have recently demonstrated that morphine exposure during gestation exerts deleterious effects on the outcome, possibly by altering the hormonal factors involved in the initiation of parturition. Changes in the catecholaminergic activity in the male offspring at the critical period of brain sexual differentiation also resulted in LH-dependent reduced testicular steroidogenesis, post-pubertally [12]. In extension to our previous study present study was meant to assess the effects of perinatal morphine exposure on post-pubertal gonadal functions in the female offspring. It was also aimed to evaluate their ability to display adult female sexual behavior in response to ovarian steroids administered in adulthood and to examine underlying hormonal and catecholaminergic changes.

Results and Discussion

Data pertaining to the effects on adult females, their pregnancy and its outcome has already been discussed elsewhere [12,14]. However, female rats perinatally exposed to morphine did not show significant difference on any measures as a function of being reared by a saline-vs morphine-treated mothers. Therefore, all data reported are combined

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from morphine or saline exposed pups raised by both morphine and saline treated mothers.

In all behavioral measures, perinatally morphine-exposed females scored 63-65% lower frequency of adult lordosis responses when compared to saline-exposed controls (Table I). Moreover, the mean quality of lordosis in morphine exposed animals was 0.4±0.2 compared to 1.7±0.2 for controls. The mean solicitation scores (the number of darts, hops and ear wiggling) were also significantly decreased in morphine exposed female rats (0.6±0.2), when compared to saline-exposed controls (3.7±0.5).

Table-I: Feminine sexual behavior as displayed by adult rats treated with morphine sulfate or saline during perinatal life on three consecutive weeks.

Treatment	n	Lordosis latency (sec)	Lordotic quotient (%) Test 1	Test 2	Test 3
Morphine sulfate	14	173±41	14.3±1.6*	17.5±1.5*	23.2±2.3*
Saline	14	29±4*	61.0±7.1	60.8±7.6	62.0±7.1

Values are group means ± SEM, * $p<0.001$

Our previous studies showed that perinatal morphine exposure causes a disruption in the brain sexual differentiation process and gonadal functions in the male offspring [16], therefore in this study we assessed neurochemical basis of behavioral alterations observed in the morphine-exposed female offspring. Changes in NE and DA concentrations in the hypothalamus and amygdala of ovariectomized female offspring born to morphine or saline-exposed offspring were measured by HPLC-ECD. Perinatal morphine exposure to the female offspring showed a mean decrease of 29% in hypothalamic NE concentration ($p<0.05$) whereas its concentration did not change significantly in the amygdala. Concentrations of DA in a hypothalamus and amygdala were not influenced by the morphine treatment (Table II).

Table-II: Effect of perinatal morphine on catecholamine concentrations in the hypothalamus and amygdala of female rats

	Norephinephrine		Dopamine	
Treatment	Hypothalamus	Amydgala	Hypothalamus	Amygdala
Saline	36.0 ± 4.0 (18)	12.6 ± 2.0 (18)	8.7 ± 0.9 (18)	7.5 ± 0.8 (18)
Morphine sulfate	25.4 ± 2.5* (14)	11.7 ± 1.7 (14)	8.4 ± 1.1 (14)	9.0 ± 0.9 (14)

Catecholamine concentrations are given in pg/mg protein. Values are mean ± SEM; number of offspring studied is given in parentheses. *$p<0.05$ vs. saline-exposed animals.

Despite the escalating number of studies on maternal substance of abuse, our knowledge about the effects of prenatal drug exposure on adult neuroendocrine functions and behaviors is still limited. In a previous study, we reported the long-term effect of morphine treatment to adult female rats. Female rat offspring given morphine during pregnancy and neonatal life profoundly offsetted the timing of occurrence of sexual maturation and impaired their ability to exhibit estrus behavior in response to the appropriate ovarian steroids administered in the adult life [14]. Dorsiflexion of the back of the female rat in response to male mounting, the lordosis reflex is used as a clear expression of sexual behavior in the female rat [15].

Throughout the treatment period, an incremental dose regimen of morphine was given to the adult female rats in order to cover the effects of tolerance and physical dependence against the morphine treatment. It resulted in irregularity of estrus cyclicity as 52% of animals exhibited prolonged diestrus and a reduced fertility since only 7 of 16 females (44%) became pregnant. This suggests that morphine treatment might have resulted in gonadotrophin release. Dose regimen of morphine was able to induce LH-dependent changes in estrus cyclicity. However it is well documented that morphine inhibits LH release in adult rats of both sexes affecting noradrenergic projection to preoptic area in the hypothalamus [6,7].

Perinatal morphine exposure severely affected the adult female sexual behavior. The frequency of lordosis responses was reduced but execution was complete inspite of low scores for soliciting behavioral measures such as darting, hopping and ear wiggling. This suggests impairment of sensory processing of stimuli that regulate copulation without affecting the execution of estrus behavior in female rats. These results are in agreement with findings of Vathy *et al* [16-18] who related such behavioral deficit in morphine exposed females to opiate-mediated increase in the circulating androgens or estrogens during the critical period of brain sexual differentiation. Another likely possibility of morphine-induced alternation of estrus behavior in female rats exposed to morphine during development results from alterations in NE concentrations in the hypothalamus. Our study indicates decreased NE levels in the hypothalamus of female offspring of the treated mothers as shown in Table II.

In fact prenatal morphine treatment can alter NE content and turnover in male and female differently in sexually dimorphic area of the hypothalamus in the rat [19]. Monoaminergic neurones extensively innervate the hypothalamus where gonadotrophin releasing hormone (GnRH) and endogenous opioid peptides-containing neurones display overlapping localisation [9,20,21]. It is well reported that opiates and opioid peptides inhibit LH release in intact and gonadectomized rats whereas naloxone, opiate receptor antagonist, stimulates LH release in intact and in steroid-primed gonadectomized rats [20·21]. Therefore the possibility that the toxic effects of morphine on reproducitve functions are exerted upstream on the pathway, i.e. by altering noradrenergic activity at the hypothalamic level, cannot be ruled out. Morphine treatment also affects the development of noradrenergic system as it decreases the tyrosine hydroxylase, NE uptake and survival of NE neurones. Such an effect is thought to be mediated through modulation of adenylylcyclase cascade [24].

In summary we have shown that alterations induced in the opiate receptors pattern due to morphine exposure during perinatal life reduced estrus responsiveness to the presence of stimulus males. This is probably by modulating the interactions amongst the gonadal steroids, noradrenergic systems and endogenous steroids.

Experimental

Adult female Wistar rats weighing 180-220 g were verified for regular cyclicity by examining cell types in consecutive 4 or 5 days estrus cycles. Animals were randomly assigned to a morphine sulfate-treated experimental group (n = 16) and a saline-treated control group (n = 6). The treatment and experimental design were similar to that previously reported [12]. Accordingly, adult rats during adulthood received morphine i.p. daily for 40 days. The dose of morphine was progressively increased at 10 day intervals from 5, 7.5, 10 to 15 mg/kg body weight until day 40. These rats were mated between days 38 and 45. The administration of morphine at the dose rate of 20 and 30 mg/kg continued during pregnancy. This dose was further increased to 40 mg/kg for 10 days post-natally.

Animals were observed for the length of gestation, average litter size, body weight of each pup and incidence of stillbirths. Part of the data in this regard has already been reported elsewhere [12]. Surviving offspring were weaned from their mothers on day 25, weighed and segregated. As the morphine treatment was already discontinued to the dams, therefore, other than moderate sign of irritability upto 2 weeks, no other gross symptoms of withdrawal were discernable. Three to five animals of the same sex were housed in each cage and maintained on the same temperature and light-controlled environment as described previously [12] with food and water accessible freely.

At 75-80 days of age both experimental and control animals were bilaterally ovariectomized under ether anaesthesia. Following two weeks of recovery period each female was given a s.c. injection of estradiol benzoates (5 mg in 0.05 ml corn oil) for two days, followed 24 h later by a s.c. injection of progesterone (500 μg in 0.05 ml corn oil). 4-5 h post-progesterone administration, each female was tested for its ability to display feminine sexual behavior when placed with a vigorous stud male. Each male was allowed to adapt to the testing arena for at least 5 min. prior to the introduction of an experimental female. Males were permitted to mount female rats for 5 min. and the number of lordosis responses as well as the quality of each lordosis was recorded. A lordosis quotient (LQ, number of lordosis/number of mounts x 100) was derived as a measure of estrus responsiveness. In addition, solicitation behavior including darting, hopping and ear-wiggling was recorded whenever it occurred throughout the 5-min. mount test. Each female was tested for 3 consecutive weeks.

Immediately after the final behavioral test female rats were killed by decapitation. After sacrifice, the brains were removed instantly, placed on a glass plate over ice and the hypothalamus and the amygdala isolated as described by Baron *et al.* [13]. Average weights of the hypothalamus and amygdala were 35.1±2.3 mg and 48.5±3.3 mg, respectively. At any given age in a particular group the size discrepancy in removing the specific brain region varied only from 0.2 to 2.0%.

High performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) was used to measure catecholamine content in the hypothalamus and the amygdala in adult animals. Tissues were homogenized in 0.1 mol/l HCl

containing 3,4 dihydroxy benzylamine (DHBA, 50 pg) as an internal standard. The homogenate were centrifuged at 500 g for 5 minutes at 4°C and 50µl samples of the resulting supernatant were used for simultaneous measurement of norepinephrine (NE) and dopamine (DA) by HPLC-ECD. A working standard solution containing norepinephrine bitartrate, dopamine hydrochloride and the internal standard was used to calibrate the chromatography system. The mobile phase contained sodium acetate (0.1 mol/l), citric acid (0.1 mol/l), glacial acetic acid (0.019 mol/l), Na EDTA (0.2m mol/l), 5% methanol and 0.3 mM octyl sodium sulfate.

To ensure complete ionization of catecholamine molecules pH was adjusted to 4.9 - 5.1. The elution times at a solvent flow rate of 1ml/min were 2.7 min for NE, 3.9 min for DA and 5.6 min for DHBA. The working electrode was set at a potential of + 0.70 vs Ag/AgCl reference electrode. The limit of detection was approximately 10 pg. The intra - and inter-assay coefficients of variance were 6.1% and 8.0%, respectively.

Statistical analysis

All behavioral and biochemical measures were analysed by one-way analysis of Variance followed by Duncan's multiple range t-test. Differences were considered statistically significant if $p<0.05$.

Acknowledgements

This study was supported by the National Scientific and Research Development Board, Islamabad. We thank the Health and Excise Departments, Government of Sindh, for allowing and providing the morphine sulfate used in this study. Secretarial assistance of Mr. Hassan Ali Samnani and Mr. Hilary Fernandes are also gratefully acknowledged.

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