European Journal of Pharmacology 654 (2011) 166–172

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Changes in adaptability following perinatal morphine exposure in juvenile and adult rats

Barbara Klausz [a], Ottó Pintér [a], Melinda Sobor [b,c], Zsuzsa Gyarmati [b], Zsuzsanna Fürst [b,d], Júlia Tímár [b], Dóra Zelena [a,*]

[a] Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
[b] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
[c] National Inst. of Pharmacy, Hungary
[d] HAS-SE, Neuropsychopharmacology Research Group, Hungary

ARTICLE INFO

Article history:
Received 5 May 2010
Received in revised form 16 November 2010
Accepted 23 November 2010
Available online 11 December 2010

Keywords:
Anxiety
Depression
Stress
ACTH
Corticosterone
Morphine
Opiate

ABSTRACT

The problem of drug abuse among pregnant women causes a major concern. The aim of the present study was to examine the adaptive consequences of long term maternal morphine exposure in offspring at different postnatal ages, and to see the possibility of compensation, as well. Pregnant rats were treated daily with morphine from the day of mating (on the first two days 5 mg/kg s.c. than 10 mg/kg) until weaning. Male offspring of dams treated with physiological saline served as control. Behavior in the elevated plus maze (EPM; anxiety) and forced swimming test (FST; depression) as well as adrenocorticotropin and corticosterone hormone levels were measured at postpartum days 23–25 and at adult age. There was only a tendency of spending less time in the open arms of the EPM in morphine treated rats at both ages, thus, the supposed anxiogenic impact of perinatal exposure with morphine needs more focused examination. In response to 5 min FST morphine exposed animals spent considerable longer time with floating and shorter time with climbing at both ages which is an expressing sign of depression-like behavior. Perinatal morphine exposure induced a hypoactivity of the stress axis (adrenocorticotropin and corticosterone elevations) to strong stimulus (FST). Our results show that perinatal morphine exposure induces long term depression-like changes. At the same time the reactivity to the stress is failed. These findings on rodents presume that the progenies of morphine users could have lifelong problems in adaptive capability and might be prone to develop psychiatric disorders.

1. Introduction

The problem of drug abuse among pregnant women causes a major concern. The main opiate of abuse is heroin, which is rapidly converted to morphine in the organism. Like many abused drugs, morphine can cross the placenta, blood–brain barrier and even exudes to the breast milk. Opiates appear to selectively accumulate in the nervous tissues of offspring presumably because of an increased permeability of the blood–brain barrier, and appear to affect the development of the central nervous system (CNS) and cause a variety of delays in ontogeny (Peters et al., 1972; Shah and Donald, 1979). Changes in somatic parameters, analgesia, sexual behavior and responsiveness to stress and stimulants have been reported in rodents exposed in utero to opiates (Castellano and Ammassari-Teule, 1984; Eriksson and Ronnback, 1989; Vathy et al., 1985).

Stress response is an array of adaptive physiological changes elicited by negative or positive stimuli. Its key neurochemical mediator is the hypothalamic–pituitary–adrenal (HPA) axis. During its activation hypothalamic secretagogues stimulate adrenocorticotropin (ACTH) release from the anterior pituitary. Then ACTH activates the adrenal cortex, and the glucocorticoid secretion helps the organism to adapt to stress. Endorphins and related peptides are found throughout the CNS and have profound effects on neuroendocrine function. Evidences from pharmacological studies suggest that the opiate system may have both inhibitory and stimulatory functions on the HPA axis (Odio and Brodish, 1990). In humans, heretofore, only the inhibitory action of endogenous opioids or orally administered opiates has been demonstrated (Allolio et al., 1987; Grossman et al., 1986; Taylor et al., 1986; Zhang et al., 2008). In rats ACTH is under tonic supression of endorphins (Kiem et al., 1995), and animals rendered tolerant to morphine failed to release ACTH and corticosterone in response either to a subsequent injection of the opiate or to a stressor. In contrast, a single injection of morphine exhibited a marked hypersecretion of ACTH and an exaggeration of the HPA response to stressor in the same species (Buckingham, 1982; Buckingham and

* Corresponding author. Institute of Experimental Medicine, Hungarian Academy of Sciences, 1083 Budapest, Szigony 43, Hungary. Tel.: +36 1 210 9400; fax: +36 1 210 9951.
E-mail address: zelena.dora@koki.hu (D. Zelena).

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doi:10.1016/j.ejphar.2010.11.025

Cooper, 1984; Domokos et al., 2008; Gonzalvez et al., 1991). In accordance with the prolonged treatment in adults, intrauterine morphine exposure may lead to hypoactivity of the newborn rat HPA axis, and to reduced adrenal size, as well, which persist during the early postnatal life (Lesage et al., 1996).

HPA axis changes are strongly connected with the mood (Holsboer and Ising, 2010; Kammerer et al., 2006). Several investigations have shown that peripheral injection of morphine or other μ-opioid receptor agonists has anxiolytic effect (Asakawa et al., 1998; Koks et al., 1998; Zarrindast et al., 2005), while the opioid receptor antagonists induce anxiogenic response (Tsuda et al., 1996; Zhang et al., 1996). Recent investigations reported that the in utero morphine treatment increased μ-opioid receptor density in the nucleus accumbens and central amygdala (Vathy et al., 2003). These regions are involved in anxiety (Harris et al., 2006) and the loss of enjoyment in depressed individuals is linked to the very same parts of the brain. Indeed, in a widespread test of depression (forced swim) morphine induced a depressive-like behavior both after acute and prolonged treatment (Broom et al., 2002b; Molina et al., 1994; Zurita and Molina, 1999).

In our earlier study pups exposed to complete perinatal morphine exposure showed delayed habituation to a new environment two days after weaning and this effect was more characteristic in males (Timar et al., 2010). Here we tried to prove that perinatal administration of morphine leads to altered development of adaptive responses both at endocrine and behavioural levels. The actual plasma levels of the stress-hormones not always reflect long-term changes, therefore examination of the weight of so called stress-organs is required (Ulrich-Lai et al., 2006). As blood glucose level is one of the widely studied homeostatic parameter in human, which reacts to stressors, too, therefore we decided to measure this parameter. During the later development compensatory mechanism may overcome any effect therefore we examined two timepoints: right after weaning at the time of cessation the morphine treatment through mother and in adulthood that is 5 weeks after weaning.

2. Materials and methods

2.1. Animals and treatment

Nulliparous female Wistar rats, weighing 200–220 g (Charles River, Budapest) were mated with males at a ratio of 3:2. The presence of sperm in the vaginal smear was checked afterwards every morning and the sperm positive females were separated and housed individually. Females where no sperm was found were excluded after 5 days. From the day of mating the dams were treated either with saline (0.1 ml/100 g) or with morphine hydrochloride (ICN, Tiszavasvári disolved in saline) subcutaneously (s.c.) once a day (5 mg/kg on the first two days, 10 mg/kg afterwards) always at 8.00 a.m. during the whole period of gestation and lactation. This treatment protocoll was choosen not to influence the physical state of dams.

After parturition (PD1) the pups were sexed, weighed and remained with their mother in the home cage until weaning (PD21). Afterwards they were separated according to sex and housed 5 rats per cage. Half of the males were used within 4 days (juvenile) and the other half at their 8-week-old age (adult). The animals were kept at constant temperature (20–21 °C) and humidity (55 ± 5%) under a standard 12–12 h light/dark cycle (light on at 6.00 h a.m.). Water and rodent chow were available ad libitum.

The number of dams in each group was 15. All the offspring were used in one experiment only; from each litter only one male pup was put in the same group in order to avoid litter effects and to balance the possible differences in gestation (thereby treatment) period. Separate sets of animals were used for decapitation among basal conditions, for elevated plus maze and for forced swim test. All experiments were repeated twice. The number of animals was 10–14 in each group.

All studies were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Semmelweis University, Budapest, Hungary.

2.2. Experiments

On the morning of the experiments rats were transfered to an empty room in a new cage and were left there undisturbed for 3 h. This room had direct connection with the testlab and with another room where the decapitation was done. The controls were also kept in the first room for 3 h, than they were transfered one by one to the decapitation room where they were decapitated within 30 s. Body weight of the animals was measured on the day previous to testing to avoide additional stress of the measurement.

2.2.1. Measurements at rest

Blood glucose levels were measured immediately by a commercially available analyser (D-Cont Personal, 77 Elektronika Kft., Budapest). Blood samples were collected in ice cold tubes, centrifuged at 3000 *g* for 30 min. Serum was stored at −20 °C until hormone measurement. Thymus, both adrenal glands and spleen were put in preweighted tubes and measured later.

2.2.2. Elevated plus maze

Offspring were placed into the center of the plus-maze (70 cm high, arm length: 50 cm; arm width: 15 cm; central platform: 15 × 15 cm; closed arm walls height: 40 cm) for 5 min, videotaped and analyzed later by an experimenter blind to the treatments by means of a computer-based event recorder (H77, Budapest, Hungary). Percentage of time spent in open arms and open/total (open plus closed) arm entries ratio (entry: three paws of animal in compartment) were calculated and used as measures of anxiety. The number of closed arm entries reflects the locomotion of the animal. Rats were decapitated right at the end of the test and blood samples were collected for hormone measurement.

2.2.3. Forced swim test

Rats were individually placed in a glass cylindrical tank 45 cm tall and 14 cm in diameter filled with tap water (24 ± 1 °C) at a height of 15 cm (juvenile) or 30 cm (adults). The animals were forced to swim for a 15-min period (pre-test) and 24 h later were subjected to a 5-min swimming session (test). The test section was taped and analyzed later by an observer blind to the treatments by means of a computer-based event recorder (H77, Budapest, Hungary). The percentage of time spent by the animal in typical immobile posture (floating) during the 5 min of the test session was rated as depression-like behavior. The floating is defined as a posture of the animal, keeping its head above the water by movements necessary to it. In this case animals can make certain, slight swimming movements in order to remain afloat. Climbing is defined as vigorous movements of the four limbs, with the front paws breaking through the surface of the water, against the wall of the tank (Wongwitdecha et al., 2006). Swimming differs from climbing, that the rats make coordinated and sustained movements with all four limbs, usually traveling around the interior of the cylinder, but do not break the surface of the water with forelimbs (Bravo and Maswood, 2006). During diving, the rats submerge entire head and body beneath the water (Arunrut et al., 2009). Rats were decapitated 5 min after the end of the 5 min test section and blood was collected for hormone measurement.

2.3. Hormone measurement

Plasma ACTH was measured by a radioimmunoassay (RIA) in 50 μl unextracted plasma as described earlier (Zelena et al., 1999). The intraassay coefficient of variation was 4.7%. Plasma corticosterone was

measured in 10 μl unextracted plasma from decapitated animals by a RIA using specific antiserum developed at the Institute of Experimental Medicine as described earlier (Zelena et al., 2003). The intraassay coefficient of variation was 12.3%. All samples were measured in one RIA.

2.4. Statistical analysis

Data were analysed by the ANOVA module of the Statistica software (StatSoft 8.0). Two-factor ANOVA (treatment and age) was used in case of behavioural parameters, and in basal somatic parameters. Three-factor ANOVA (treatment, age, and stress) was used when the changes in response to the certain stimulus (EPM and FST) was measured. Post hoc analysis was conducted by the Newman Keuls method. All data are expressed as means ± S.E.M and significance was set at $p<0.05$.

3. Results

3.1. Somatic parameters

The body weight of morphine rats was lower in comparison with untreated ones' ($F_{(1,40)}=30.9$, $P<0.01$), which difference consists of the 42.8% deviation observed in juvenile, and the 6.3% difference detected in adult age (Fig. 1A). Adult rats had higher body weight than juveniles ($F_{(1,40)}=3315.0$; $P<0.01$). There was no interaction between treatment and age.

Thymus weight data showed a very similar pattern to that observed in body weight (Fig. 1B). In morphine rats the thymus was significantly smaller, than in controls ($F_{(1,40)}=6.3$; $P<0.05$), which consists of the 26.3% difference between the juvenile groups, and the 4.9% bias in adults. As it was expected, the adult animals had heavier organs ($F_{(1,40)}=389.5$; $P<0.01$). There was no interaction between treatment and age.

Spleen weight of morphine rats were lower in comparison with controls ($F_{(1,40)}=5.1$; $P<0.05$) (Fig. 1C). Examining separately, in juvenile subjects the spleen weight of morphine animals were 23.4% lower, while in case of adults this difference was barely 4.4%. The adult animals had bigger spleens ($F_{(1,40)}=470.2$; $P<0.01$). There was no interaction between treatment and age.

Regarding the adrenal gland the observed difference between the treatment groups was relatively stable across ages ($F_{(1,40)}=15.5$; $P<0.01$), both in juvenile and adult subjects the morphine rats' adrenal gland were around 15% smaller, than the control ones' (Fig. 1D). As it was expected the bigger an animal was the heavier its organs were ($F_{(1,40)}=290.3$; $P<0.01$). There was no interaction between treatment and age.

The basal blood glucose levels were not altered by perinatal morphine treatment. Only the effect of age was significant with smaller blood glucose levels in adults ($F_{(1,40)}=12.4$; $P<0.01$) (juvenile: 5.5 ± 0.15 mmol/l; adult: 4.9 ± 0.1 mmol/l). The stronger stimulus (FST) was able to induce a rise in blood glucose levels. After the challenge the morphine rats revealed smaller rise ($F_{(1,40)}=5.48$; $P<0.05$), while the difference between juvenile and adult rats remained the same ($F_{(1,40)}=5.2$; $P<0.05$) (juvenile control: 7.3 ± 0.3 mmol/l; juvenile morphine: 6.5 ± 0.1 mmol/ml; adult control: 6.5 ± 0.3 mmol/l; adult morphine: 6.1 ± 0.2 mmol/ml). There was no interaction between treatment and age.

3.2. Hormonal parameters

As main effect the morphine rats had lower ACTH levels than their saline treated counterpairs ($F_{(1,121)}=6.73$, $P=0.01$) (Fig.2A). Age had

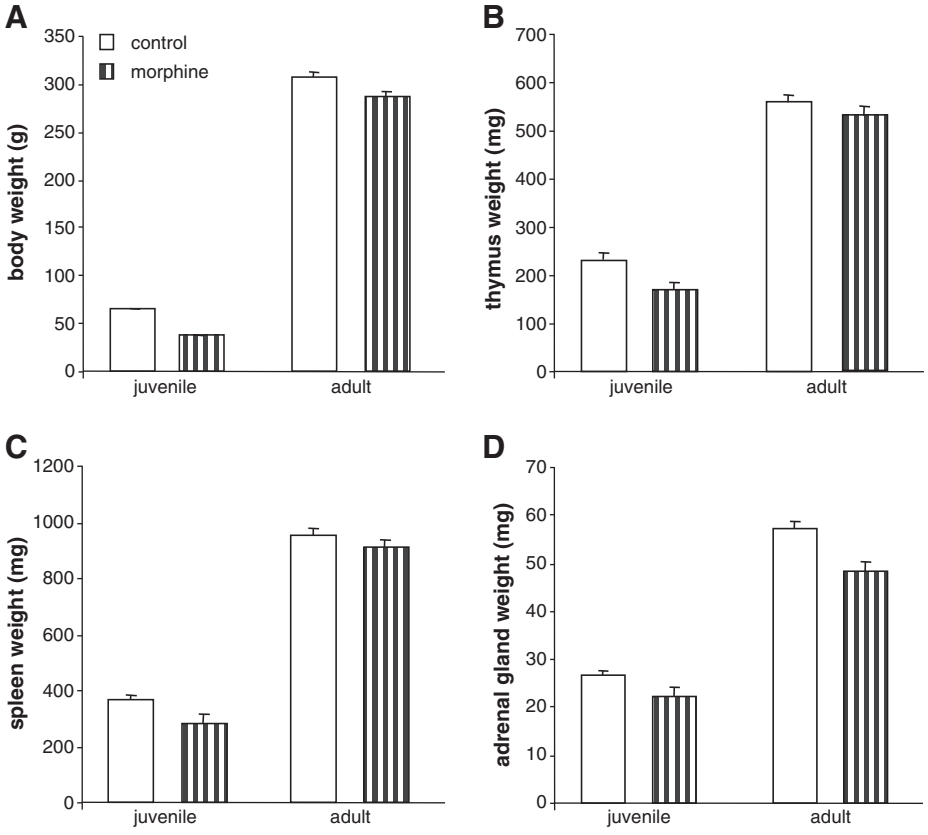

Fig. 1. The effect of perinatal morphine exposure (daily 10mg/kg sc from the beginning of pregnancy till weaning) on somatic parameters. Body weight (A), thymus (B), spleen (C) and adrenal (D) weights were measured from decapitated animals in both juvenile (25-day-old) and adult (8-week-old) age. In all cases both the effect of perinatal morphine treatment as well as the age were significant ($p<0.05$). Data are mean +/- S.E.M. $N=10–14$.

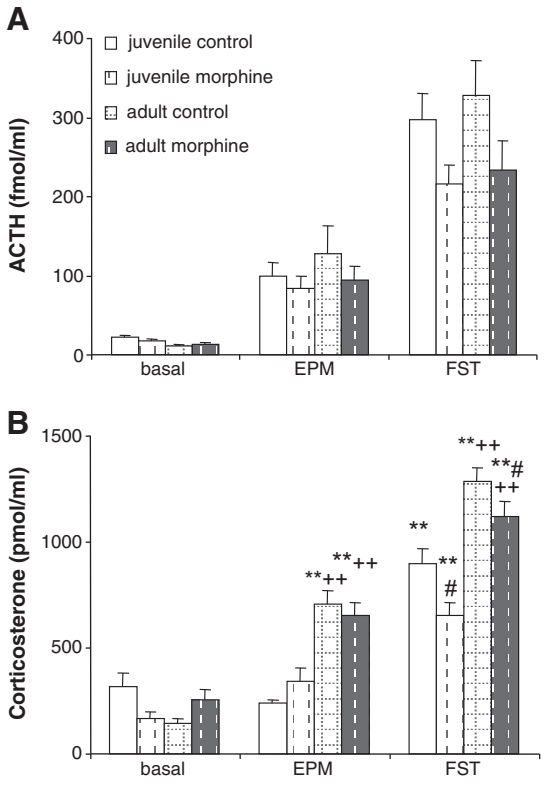

Fig. 2. HPA axis changes in prenatally morphine exposed (daily 10mg/kg sc from the beginning of pregnancy till weaning) juvenile (25-day-old) and adult (8-week-old) rats among rest (basal), after a mild (5 min. EPM- elevated plus maze) and a sever challenge (5 min. FST- forced swim test). There were signficant morphine and stress effects as well as morphine × stress interaction in serum ACTH (fmol/ml) (A) levels. The coricosterone (pmol/ml) (B) levels were influenced by morphine treatment, age, stress as well as by the interaction of all three cathegories. Data are mean +/- S.E.M. $N = 10–14$ Significant differences: **<0.01 vs basal #$p<0.05$, ##$p<0.01$ vs saline treated, ++$p<0.01$ vs juvenile.

no effect but different challenges (EPM and FST) was able to induce significant elevations ($F_{(2,121)} = 102.3$, $P<0.01$). Implicitly the ACTH levels was increased exponentially from the level measured under basal condition, through the level measured in response to a mild stressor (EPM), until the level which was measured after FST, as a sever stressor. Both stresses were able to induce smaller elevation in morphine rats than in controls by about 24.2% (treatment × challenge: $F_{(2,121)} = 3.08$, $P<0.05$). No other interaction was significant.

The perinatal morphine treatment had significant effect on the corticosterone levels ($F_{(1,121)} = 4.26$, $P<0.05$), however the direction of differences varies through tests and ages (Fig. 2B). In contrast to ACTH, here appeared a significant age effect, too, with higher levels in adults ($F_{(1,121)} = 62.0$, $P<0.01$). As it was expected the challenges (EPM and FST) induced remarkable elevation of the corticosterone levels ($F_{(2,121)} = 182.5$, $P<0.01$). Similarly to ACTH, the milder stress (EPM) induced only a small rise, while severe stressor (FST) was able to induce a 5-times elevation. Juvenile rats were less sensitive (age × challenge: $F_{(2,121)} = 20.7$, $P<0.01$) with only a 3.33-times elevation compared to the 6.66-times elevation in adulthood. Morphine rats were also less sensitive to different stimuli compared to controls (treatment × challenge: $F_{(2,121)} = 4.88$, $P<0.01$). There was a general interaction between all studied groups, too (treatment × age × challenge: $F_{(2,121)} = 3.56$, $P<0.05$).

3.3. Behavior

During the 5 min of EPM challenge both adult and juvenile morphine rats tended to spend less time in open arms, however the difference failed to be significant ($F_{(1,39)} = 3.0$ $P<0.1$) (Fig. 3A). Likewise no statistically significant differences were observed in open/total frequencies ratio between the treated and control groups neither in juvenile nor in adult animals (data not shown), demonstrating no impact of perinatal exposure to morphine on anxiety level measured on EPM. Number of closed arm entries pointing the overall activity of the animals also did not differ (Fig. 3B). There were no significant difference between juvenile and adult rats in any of the studied EPM parameters. Moreover there was no interaction between treatment and age.

In response to a 5 min forced swim test the morphine animals spent more time with floating, than their untreated fellows ($F_{(1,39)} = 8.2$; $P<0.01$), expressing signs of extended depression-like behavior (Fig. 3C). In juvenile subjects the morphine rats floated 20.2% more, than the control ones, in adults this difference was 30.2%. The very same—although opposite—pattern was observed in case of the time spent with climbing (Fig. 3D). Morphine animals spent less time with climbing than controls ($F_{(1,39)} = 9.5$; $P<0.01$). Both in juvenile and adult subjects the morphine rats climbed 51% less, than controls. There were no significant difference between juvenile and adult rats in any further forced swim parameters. There were no interaction between treatment and age, too.

3.4. Correlations

The correlation analysis was aimed to reveal potential relationship between the anxiety- and depression-like behavior and HPA axis activity, particularly corticosterone levels. However, no correlations were observed between the open-arm time in EPM, or floating time in FST and hormone release (ACTH and corticosterone levels) (data not shown). Significant correlations were found only between the time spent with climbing and ACTH and CS levels (ACTH: $r = 0.40$, $P<0.05$; CS: $r = 0.38$, $P<0.05$).

4. Discussion

The present report investigated the effects of maternal morphine treatment applied during the whole gestation and lactation period on adaptive abilities of their progeny in different postnatal ages. We have found smaller stress marker organs, hypoactivity of the HPA axis and an enhanced depression-like behavior. These changes were present at both examined ages. Although the magnitude of differences between control and morphine groups was slightly smaller in adults, but the age × treatment interaction never reach the level of significance. We might conclude that the adaptive capability of the animals exposed perinatally to morphine is damaged for life.

Our findings confirm that the perinatal morphine exposure has long term influence on the responsiveness of the HPA axis, which might seen not only in the smaller stress-induced elevation of ACTH and corticosterone levels, but also in the relative stable lower adrenal gland weight through the life. Recently a dampened ACTH secretion was found in in utero morphine treated adults after restraint stress (Rimanoczy et al., 2003). Another work demonstrated HPA axis hypoactivity and smaller adrenal glands in in utero morphine exposed newborn rats, although this effect did not persist until adulthood (measured after ether inhalation) (Laborie et al., 2005). Our results overlap with the first one and are also in concordance with the fact, that behavioral sensitization to repeated morphine administration is accompanied by a blunted rather than an enhanced ACTH response in mice (Jezova et al., 2004). The partial discrepancy may be due to sensitivity of different strains or different type of stressors used. As a possible background mechanism morphine treatment during lactation impairs the maternal behaviour of the mothers, and thereby influencing the HPA axis of the offspring (Champagne, 2008; Levine, 2005; Sobor et al., 2010; Weaver et al., 2004). Another possibility is that chronic morphine treatment induces a chronic stress state in the mother (Domokos et al., 2008). Hyperactivity of the maternal HPA

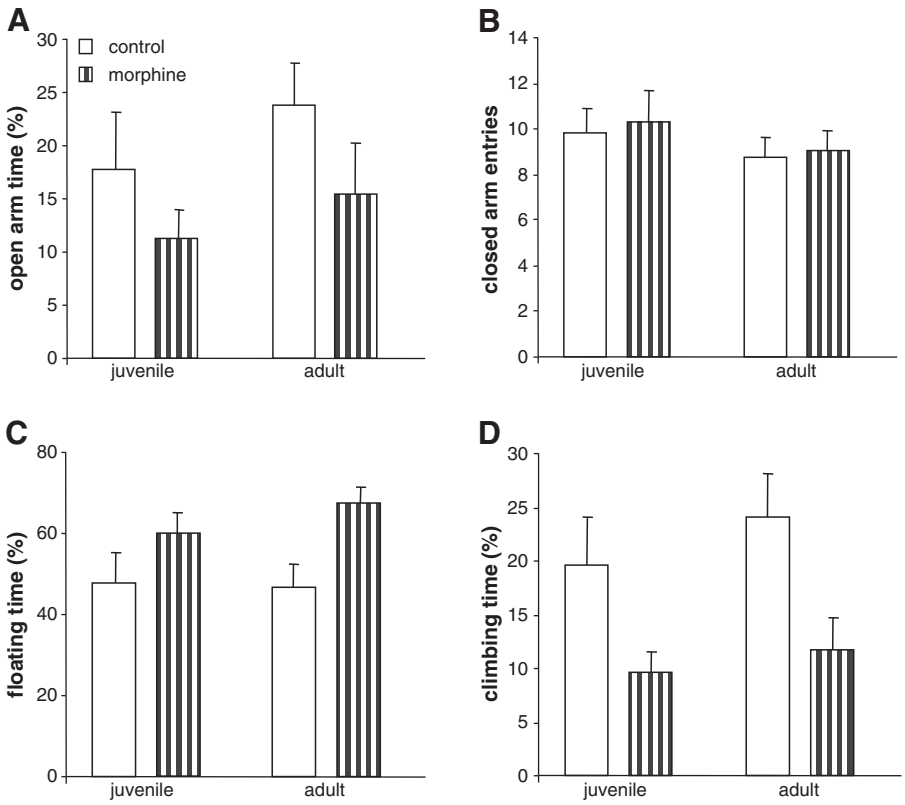

Fig. 3. Behavioral consequences of perinatal morphine treatment (daily 10mg/kg sc from the beginning of pregnancy till weaning) in both juvenile (25-day-old) and adult (8-week-old) rats. Anxiety was measured on the elevated plus maze (EPM) (A,B) but there were no significant differences between the groups either in the main anxiety parameter (open arm time, A) or in locomotion (closed arm entries, B). The depression-like changes were followed on the forced swim test (FST). There was a significant effect of morphine treatment both on time spent with floating (C) and climbing (D) ($p<0.01$). Data are mean +/- S.E.M. $N=10–14$.

axis can reprogram the development of the offspring's HPA axis together with subsequent brain functions and behavior (Francis et al., 2002; Plotsky and Meaney, 1993; Weaver et al., 2004). Nevertheless prolonged rather than reduced corticosterone secretion is more common in the offspring of perinatally stressed mothers (Maccari et al., 2003).

We measured the weight of the so called stress marker organs, too (Hara et al., 1980). Prolonged stress can activate the HPA axis to induce stress hormone production leading to adrenal gland hypertrophy (the place of enhanced synthesis), thymus (as a 'barometer of stress') and spleen involution (Hashimoto et al., 2001; Kvetnansky and Mikulaj, 1970). The smaller adrenal glands do not reflect enhanced HPA activity, but coherent with the lower levels of corticosterone in morphine rats (Lesage et al., 1996; Lesage et al., 1998). Therefore we assume that the overall smaller organ weights indicate a direct morphine effect on the development of the offspring (Vathy, 1995) and could be explained by early gestational treatment (Couret et al., 2009a; Couret et al., 2009b). Some authors could not observe any effect of perinatal morphine treatment in adulthood and in our hands most of the differences were less pronounced in adult age, too (Laborie et al., 2005; Zagon and McLaughlin, 1992). So, some but not full restoration of the developmental disturbance may occure with time.

Neurons and red blood cells utilize glucose almost exclusively, therefore appropriate level of blood glucose is an important marker of the homeostatic regulation. It is commonly known that environmental alterations may elevate blood glucose levels, but the relatively brief, controlled conditions are ineffective (Baum and Porte, 1980; Gonder-Frederick et al., 1990; Wing et al., 1985). In our experiments the basal blood glucose levels were not changed by perinatal morphine treatment or even by the weak stress, EPM. After a strong challenge (FST), however, the blood glucose levels were elevated significantly, but morphine rats revealed smaller rise. This reflects a disturbed adaptation to an exhausting challenge. The explanation might be, that adrenal insufficiency often comes along with lower blood glucose response to acute severe physical stress (Tempel and Leibowitz, 1994).

Stress and stress hormones are important pathogenetic factors in psychiatric diseases, like depression and anxiety (Szabo, 2010). It has been reported that both intraperitoneal and intraamygdaloid injections of morphine possess anxiolytic-like properties (Glover and Davis, 2008; Rezayof et al., 2009; Zarrindast et al., 2005). In contrast, chronic morphine intake, through an effect on learning and memory, may lead to high prevalence of anxiety disorders in opiate addicts (Gu et al., 2008). Vathy's work suggested that exposure to morphine during mid-to-late gestation produces long-term alterations in the brain and behavior of adult progeny (Vathy, 1995). It was also shown that late-term prenatal morphine exposure has long-lasting effects on adult learning and memory and response to stress (Slamberova et al., 2001a), however the anxiety-related behaviour was not yet profoundly investigated. EPM is a useful test to investigate anxiogenic effects, but locomotor side effects may counteract with the results. Therefore it was important to demonstrate that perinatal morphine treatment had no effect on locomotion reflected by the constant closed arm entries between groups. In accord with our results, offspring of prenatally stressed animals was reported to show an anxiogenic tendency on the EPM (Zuena et al., 2008). In contrast, Byrnes was able to find even transgenerational anxiogenic effect (Byrnes, 2005). Perhaps the moderate dose we used was not that strong stimulus. However, higher doses (e.g. 30 mg/kg (Siddiqui et al., 1997)) may lead to direct disturbances of the pregnancy, thereby it would make no sense to study later consequences. Although an altered endocrine response could lead to changes in anxiety-like behavior (Holsboer and Ising, 2010), we were unable to find a correlation between stress hormone levels and behavioural parameters. The supposed anxiogenic impact of perinatal exposure with morphine needs more focused examination.

It is commonly investigated and clinically proved that opiate treatment has an antidepressant effect (Gold et al., 1982). The high concentration of opioid receptors and endorphins in limbic and hypothalamic regions, and their interaction with noradrenerg and dopaminerg systems, suggests the involvement of endorphins and related peptids in depression, too. Indeed, depressed patients were reported to display a deficiency of endogenous opioid activity (Pickar et al., 1980). Preclinical data are contradictory. Low dose morphine was found to enhance depression-like behavior, while higher dose was uneffective (Amir, 1982; Broom et al., 2002a). In our hands perinatal morphine exposure lead to enhanced depressive-like changes both right after cessation of the treatment (at weaning) and in adulthood. The same schedule of perinatal morphine exposure was published to enhance the sensitivity to the reinforcing effect of morphine in adult offspring, which is in good harmony with our present results (Timar et al., 2010).

When we summarise the results, there is an apparent contradiction between enhanced depression-like behavior, and the decreased ACTH and corticosterone levels in morphine rats. It is commonly recognised that hypersecretion of cortisol is associated with depression in major part of the patients (Dinan, 2001; Parker et al., 2003). One explanation could be that since ACTH and beta-endorphin (endogenous opioid) have a common precursor (Eipper and Mains, 1978; Mains et al., 1977), so the higher level of endorphins may exert a feedback inhibition on ACTH, thereby also on cortisol secretion (Taylor et al., 1983). Further explanation could be the heterogenity of depression. In melancholia the stress response seems to be hyperactive, while there are several lines of evidence of a down-regulated HPA axis in atypical depression (Gold and Chrousos, 2002; Gold et al., 2002).

The question aroses whether all these findings are the results of the direct morphine effect on the developing organism, or the changes are unspecific and rather due to the deficiency of the maternal care, or even to a general stress effect (Francis and Meaney, 1999; Meaney, 2001). A direct effect could be supposed, as it was shown that fetal and/or early neonatal exposure to opiates may cause an overall inhibition of brain growth and development due to the inappropriate neural response to hormones and neurotrophic signals during the critical period of CNS development (Hammer et al., 1989; Tempel et al., 1988; Tsang and Ng, 1980). On the other hand, opiates are known to influence various components of maternal behavior, too, like maternal motivation or the time of nursing (Byrnes et al., 2000; Nelson and Panksepp, 1998; Panksepp et al., 1994; Slamberova et al., 2001b; Sobor et al., 2010). We cannot rule out the effect of this factor, and similarities can be drawn with the human situation, too (maternal neglect among morphine users). The HPA axis activation is another important aspect, as plainly the drug (morphine), or even the morphine withdrawal among the single administrations or after weaning could perform as a stressor (Domokos et al., 2008). In many other studies the prenatally stressed rats showed higher emotional reactivity, higher levels of anxiety, and depression-like behavior, independently from the nature of the stress (Bhatnagar et al., 2005; Estanislau and Morato, 2005; Morley-Fletcher et al., 2003). However contribution of this effect could not be very important as prolonged rather than reduced corticosterone secretion is more common in the offspring of perinatally stressed mothers (Maccari et al., 2003).

Based upon our rodent study we might suggest that the offspring of morphine users are prone to develop psychiatric disorders and they might face with lifelong adaptational problems due to a complex array of changes.

Acknowledgements

This study was supported by Hungarian grants OTKA NN71629, OTKA K60999, and ETT-441/2006.

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