SINGLE DOSE OF MORPHINE INFLUENCES PLASMA CORTICOSTERONE AND GENE EXPRESSION OF MAIN NMDA RECEPTOR SUBUNIT IN THE ADRENAL GLAND BUT NOT IN THE HIPPOCAMPUS

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Objective. This study was aimed to evaluate the effects of morphine on hypothalamo-pituitary-adrenocortical (HPA) axis, namely proopiomelanocortin (POMC) mRNA and plasma corticosterone, in relation to its influence on glutamate receptor gene expression in central and peripheral sites related to HPA axis regulation. As previous data on morphine action were obtained mainly in male rats, these experiments were performed in females to see potential gender differences.

Methods. Adult female Sprague-Dawley rats were injected with a single dose of morphine (10 mg/kg s.c.) or vehicle. Blood and tissues were sampled 4 h and 24 h following the treatment. In situ hybridization was used to measure POMC mRNA concentrations, reverse transcription-polymerase chain reaction to quantify mRNA coding for N-methyl-D-aspartic acid (NMDA) receptor subunit 1 and radioimmunoassay to measure plasma corticosterone.

Results. Single dose of morphine was followed by a decrease in gene expression of glutamate receptor subunit NMDAR1 in the adrenal gland. Concentrations of mRNAs coding for NMDAR1 in the hippocampus and for POMC in the anterior pituitary remained unaffected. However, plasma corticosterone levels, which were measured at 4 and 24 h after the treatment with morphine, showed a disturbed daily variation in corticosterone release. The efficacy of morphine was confirmed by Straub tail response, one of the classical effect of this drug, in mice.

Conclusions. Present data obtained in females allow to suggest that morphine exerts some of its effects on HPA axis by POMC unrelated mechanisms seemingly in a gender specific manner. Decrease in glutamate receptor gene expression in adrenals induced by a single dose of morphine may result in a modulation of adrenal function in response to subsequent exposure to opioids and contribute to some alterations occurring during opioid drug abuse.

Key words: Morphine – Gene expression – POMC – NMDA receptor – Corticosterone – Adrenal gland

It is well known that morphine administration influences HPA axis, exerting a stimulatory effect in the rat. The stimulatory action of morphine has been repeatedly demonstrated by changes in corticosterone and adrenocorticotropic hormone (ACTH) levels (JEZOVA et al. 1982; BUCKINGHAM and COOPER 1984; ADAMSON et al. 1991; MILANES et al. 1993; KIEM et al. 1995). Less attention has been given to potential effects of morphine on ACTH production. ACTH is produced in the anterior pituitary from the precursor molecule POMC. ACTH and corticosterone release as well as the synthesis of anterior pituitary POMC are activated mainly during stress, the triggering factors being corticotropin-releasing hormone (CRH) and other neuropeptides released from the hypothalamus (VALE et al. 1981; BRUHN et al. 1984; SKULTETY-

ova and Jezova 1999). Little information is available on gene expression of POMC following administration of morphine. Limited number of studies dealing with morphine administration and POMC mRNA in the anterior pituitary failed to observe consistent changes (Hollt and Haarmann 1985; Lightman and Young 1988; Zhou et al. 1999).

Multifactorial control of HPA axis function is achieved by several neurotransmitters and neuropeptides, such as endogenous opioids. Another regulatory factor participating in the control of ACTH release is the excitatory neurotransmitter glutamate (Jezova et al. 1991; Oliver et al. 1996). Glutamate is involved in the regulation of hormone release during stress with some indications for not only central but also peripheral sites of action (Jezova et al. 1995; Schwendt and Jezova 2001).

Glutamate receptors were shown to participate also in the mechanisms of opioid actions in the central nervous system. In this respect, ionotropic N-methyl-D-aspartic acid (NMDA) receptors are of particular importance. NMDA receptors might be involved in plastic changes during long-term treatment with opioids (Mao 1999). Thus, pharmacological blockade of NMDA receptors attenuates the development of opioid tolerance and dependence as well as withdrawal signs (Mao 1999). NMDA receptors seem to play a role also in processes occurring following acute exposure to morphine as one group of authors (LeGreves et al. 1998) revealed selective changes in the gene expression of NMDA receptor subunits.

Several acute and chronic effects of opioids were found to be gender specific (D'Souza et al. 1999; Cruz and Rodriguez-Manzo 2000). Considering that most of the studies investigating interactions of opioids and glutamate receptors or HPA axis function were performed in males, it is of interest whether females would show similar or different pattern of responses.

This study was aimed to evaluate the effects of morphine on HPA axis function in relation to its action on glutamate receptor gene expression in central and peripheral sites related to HPA axis regulation in female rats.

Materials and Methods

Animals. Adult female Sprague-Dawley rats (250-300 g) were housed 3 per cage. They were maintained on a 12-hour light/dark cycle and given food and water ad libitum. In one series of experiments (Straub test), adult female albino mice (30-34 g) divided in groups of 5-10 were used.

Drug treatment. To reduce the interference of manipulation procedures, rats were subjected to handling and subcutaneous (s.c.) saline injections at least twice a day. After several days, the rats were injected with morphine (Morphinium chloratum, Spofa, Prague, Czech Republic) in the dose of 10 mg/kg s.c. or normal saline (0.9 % NaCl) used as vehicle. After the injections (performed at 9.00-10.00 h in the morning) rats remained in their cages until decapitation 4 h or 24 h later. Thus, at the time interval of 24 h, the blood and tissue sampling were performed in the morning (9.00-10.00h) while those at 4 h in the afternoon (13.00-14.00h).

Corticosterone analysis. Blood was collected into cooled polyethylene tubes with EDTA as anticoagulant and centrifuged immediately to separate plasma, which was stored at –20 ºC until analyzed. Plasma corticosterone was measured by RIA using ^{3}H-corticosterone (Amersham, Buckinghamshire, UK) and corticosterone antiserum raised in the Laboratory of Experimental Neuroendocrinology (INSERM U297, Marseille, France), as described previously (Jezova et al. 1994).

In situ hybridization. Pituitaries were quickly removed, frozen in isopentane at –30 ºC on dry ice and stored at –70 ºC. Coronal 12 μm sections of pituitaries were cut on a cryostat at –17 ºC and thaw-mounted onto poly-L-lysine-covered microscopic slides. The sections were hybridized according to the protocol described previously (Skultetyova et al. 1998). The probes were 48-mer oligonucleotides complementary to the bases corresponding to 102-117 of rat POMC (a gift from Dr. G. Aguilera, USA), synthesized by Synthecell (Rockville, MD, USA). The autoradiographic hybridization signal was quantified using a computerized image analysis system (Image 1.47 Wayne Rasband, NIH, USA) and the result expressed as arbitrary units. At least 6 sections/animal were analyzed.

RT-PCR. Total RNA from the hippocampus or adrenal gland was extracted by modified guanidium thiocyanate-phenol/chloroform method (Chomczynski and Sacchi 1987). Concentration and purity of RNA preparations were measured by absorption

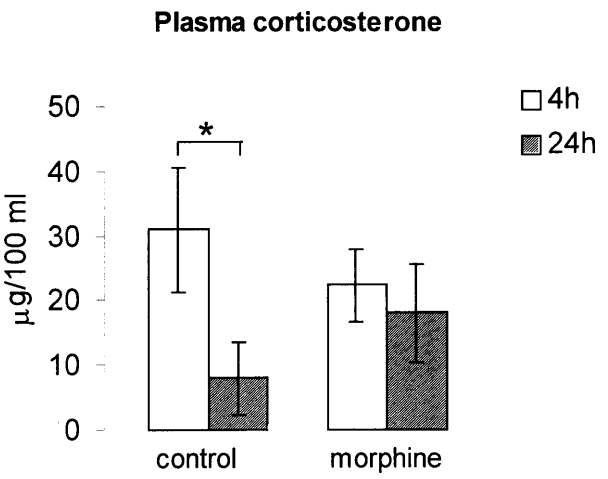

Fig. 1 Effect of acute morphine injection (10 mg/kg s.c.) on plasma corticosterone levels in the morning (24 h after the treatment) and in the afternoon (4 h after the treatment). Values represent means ± SEM (n = 6); * - P < 0.05

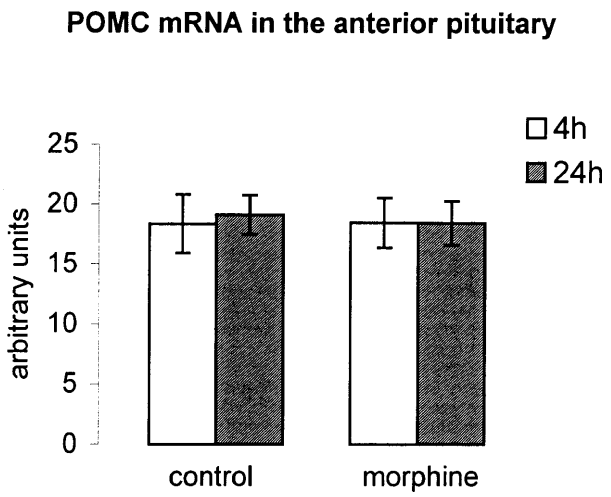

Fig. 2 Levels of POMC mRNA in the anterior pituitary 4 h (in the afternoon) and 24 h (in the morning) following a single dose of morphine (10 mg/kg s.c.). Values represent means ± SEM (n = 6).

spectroscopy. The quality of RNA was judged from the pattern of ribosomal RNA after gel electrophoresis. Total RNA (1-2 μg) was reverse-transcribed to cDNA and subjected to polymerase chain reaction (PCR) procedure according to manufacturer instructions (Promega, WI, USA). Separate PCR reactions for NMDAR1 and β-actin were performed in the presence of gene-specific, upstream/downstream primers (yielding the products of following size): NMDAR1: CTCATCTCTAGCCAGGTCTA / TCGCATCATCTCAAACCAGAC (225 bp); β-actin: TACAACCTCCTTGCAGCTCC / ACAATGCCGTGTTCAATGG (280 bp). PCR reactions were carried out for 35 cycles, with thermocycle profile: denaturation at 94 ºC/1 min, primer annealing at 55 ºC/ 1 min and primer extension at 72 °C/1 min. Amplified products were separated on 1.8 % agarose gel, stained with ethidium bromide and photographed under UV illumination with gel-documentation system (KODAK DS 1D, Kodak, MD, USA). All RT-PCR experiments included no-template and no-RT controls that remained negative. A 100 bp marker (Promega, WI, USA) was used as a size standard.

Straub tail response. Positive Straub tail response was considered as a persistent elevation of the tail at an angle more than 45 º. Experiments were performed under sound-attenuated conditions to minimize the effect of noise on the Straub tail response. Mice were injected with a single dose of saline or morphine (2.5, 5, 10, 20 mg/kg s. c.).

Statistical evaluation. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Fisher PLSD test and expressed as ± S.E.M.

Results

Plasma corticosterone. Single morphine injection failed to induce significant changes in plasma corticosterone levels at 4 h and 24 h folloving the treatment. However, the results indicate a disruption of the daily rhythm of corticosterone secretion in morphine treated animals. In the control, saline treated groups, plasma corticosterone levels were significantly higher in the afternoon (391 %), compared to those in the morning. This difference was absent in morphine treated animals (Fig. 1).

POMC mRNA in the anterior pituitary. Acute injection of morphine did not modify POMC mRNA levels in the anterior pituitary investigated 4 h and 24 h after the drug administration (Fig. 2).

NMDAR1 mRNA in the hippocampus. Levels of mRNA coding for NMDAR1, the main subunit of NMDA glutamate receptor, in the hippocampus remained unchanged at both 4 h and 24 h following acute administration of morphine (Fig. 3).

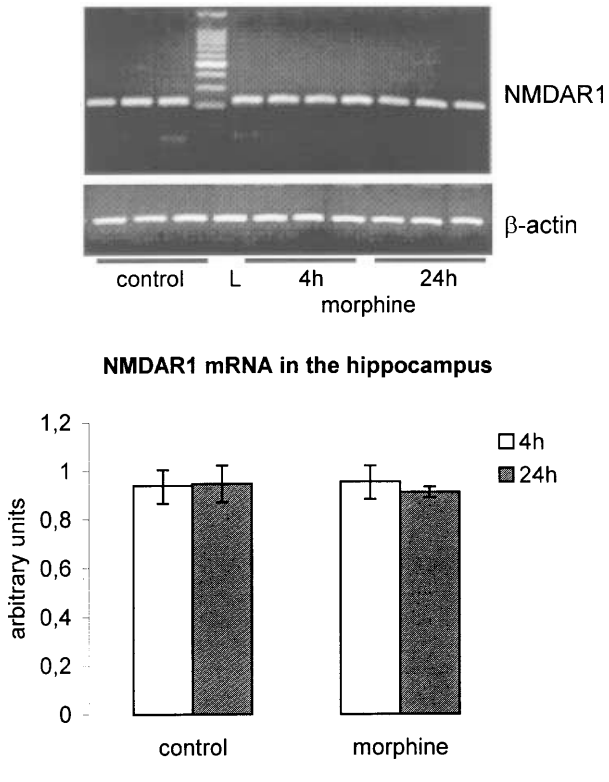

Fig. 3 Levels of mRNA coding for NMDAR1 receptor subunit in rat hippocampus at 4 h and 24 h after the administration of morphine (10 mg/kg s.c.). Values represent means ± SEM (n = 6).

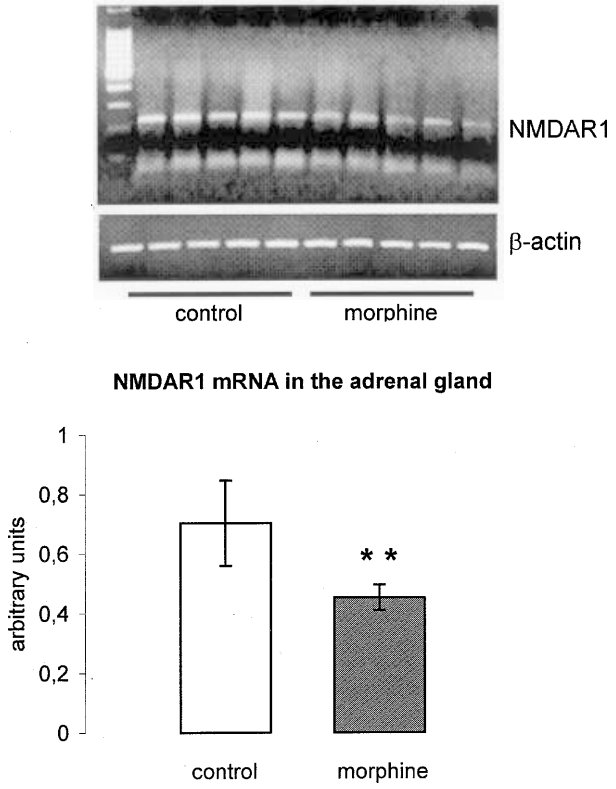

Fig. 4 Decrease in NMDAR1 receptor subunit mRNA levels in rat adrenal gland 24 h following a single dose of morphine (10 mg/kg s.c.). Values represent means ± SEM (n = 6). ** P < 0.01 compared to control.

NMDAR1 mRNA in the adrenal gland. Administration of morphine resulted in a significant decrease (35,5 % vs control) in concentrations of NMDAR1 mRNA in the adrenal gland 24 h following the treatment (Fig. 4).

Straub tail response. As several negative effects of morphine were noticed, its effectivity was verified by Straub tail test, which is one of the main determinants in the testing of opioid activity in mice. Morphine injection induced the Straub tail response in a dose dependent manner at 30 min after the treatment (Fig. 5). The percentage of mice showing the positive response increased from 17 % to 100 % following the lowest (2.5 mg/kg) and the highest dose (20 mg/kg) used. Maximal effects were observed at 15-45 min.

Discussion

Results of the present study demonstrate that single administration of morphine is followed by a decrease in gene expression of glutamate receptor subunit NMDAR1 in the adrenal gland. Concentrations of mRNAs coding for NMDAR1 in the hippocampus and for POMC in the anterior pituitary remained unaffected. However, plasma corticosterone levels measured at late time intervals after the treatment with morphine (4 and 24 h) suggest a disturbed daily variation in corticosterone release.

Lack of a significant rise in plasma corticosterone several hours after single morphine injection was an expected finding, as morphine was shown to induce ACTH and corticosterone release much earlier, namely within 30 – 60 min following the administration (Jezova et al. 1982; Hayes and Stewart 1985; Adamson et al. 1991). Secretion of corticosterone exerts a circadian rhythm with higher values in the afternoon compared to those in the morning (Krieger 1972). Plasma corticosterone levels measured in the present experiments corresponded to presumed daily variation in control but not in mor-

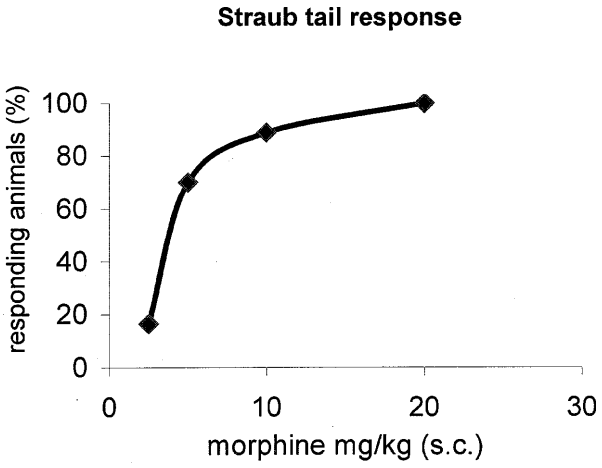

Fig. 5 Straub tail response in mice after administration of morphine (2.5, 5, 10 and 20 mg/kg, s.c.). Data are expressed as a percentage of animals showing a positive response at 30 min.

phine treated rats. It should be noted however that previous studies using acute morphine administration were performed mainly in male rats. Gender differences in corticosterone secretion are well known (Jezova et al. 1996). Moreover, in a pilot experiment in female rats, no significant increase was observed in corticosterone levels measured 45 min after morphine injection (unpublished data). Thus, gender differences in morphine action on corticosterone secretion are possible and our data highlight the need to verify them.

It might be suggested that the action of morphine on HPA axis function would be manifested by changes in regulatory peptide gene expression. However, POMC mRNA levels in the anterior pituitary were found to be unchanged up to 24 h following single administration of morphine. A similar observation was reported by Lightman and Young (1988). On the other hand, morphine seems to modify POMC mRNA levels in some regions of the hypothalamus, which are not directly related to the control of HPA axis activity (Wardlaw et al. 1996). Some authors did not observe changes in anterior pituitary POMC mRNA or hypothalamic CRH mRNA levels even after chronic treatment with morphine (Lightman and Young 1988; Zhou et al. 1999). Thus, the possibility that morphine exerts some of its effects on HPA axis by other central or peripheral mechanisms should be considered. Indeed, corticosterone release from the adrenal cortex may occur independently of pituitary function (Müller et al. 2001).

As several behavioral and neuroadaptive effects of morphine have been associated with activation of NMDA receptors (Mao 1999; Trujillo 2000) and glutamate has been shown to participate in the control of stress hormone release (Jezova et al., 1995), this excitatory amino acid may play a role also in morphine-induced changes of HPA axis. Chronic treatment with morphine was found to induce long-term changes of glutamatergic system involving alterations in gene expression of glutamate receptors in various regions of the brain (Bhargava et al. 1995; Zhu et al. 1999). In the present study in female rats, no changes in NMDAR1 mRNA levels in the hippocampus, a limbic structure involved in HPA axis regulation, were observed following a single morphine injection. This is in contrast to the data reported by LeGreves et al. (1998) also performing a single injection of morphine but in male rats. Thus, the discrepancy may be attributed to potential gender differences in morphine action on glutamate receptor gene expression. Indeed, several gender dependent effects of morphine have been observed and male rodents have been found more sensitive to morphine-induced analgesia than female animals (Ali et al. 1995).

An interesting finding of the present study is the decrease in gene expression of NMDAR1, the main subunit of NMDA glutamate receptor, in rat adrenals 24 h following a single morphine injection. These data expand our recently published results providing the evidence for NMDAR1 gene expression in rat adrenal medulla and cortex, and showing a modulation of this expression after stress exposure (Schwendt and Jezova 2001).

Opioid receptors were found to be widely expressed in several peripheral tissues, including adrenals, which possess opioid receptors of μ and κ type (Dumond and Lemaire 1984; Kapas et al. 1995; Wittert et al. 1996). Nevertheless, the studies on functional relevance of these receptors have brought controversial results. In relation to corticosteroids, an in vitro study showed that opioid peptides stimulated aldosterone and corticosterone release (Kapas et al. 1995). However, the action of morphine and its antagonist naloxone on corticosterone release in vivo was proven only in intact but not in hypophysecto-

mized or dexamethasone treated animals (Jezova et al. 1982). Another possibility is that opioids cooperate with ACTH (Heybach and Vernikos 1981) and other stimulatory factors.

The present findings are in favour of an interaction between glutamatergic and opioid systems at the level of the adrenal gland. Observed down-regulation of NMDAR1 mRNA levels is in agreement with the hypothesis that transient facilitation of NMDA receptor function by morphine may be later followed by decreased expression of NMDA receptor subunits (Bespalov et al. 2001), and probably by changes in the number of NMDA receptors in some susceptible regions. Changes in glutamate receptor gene expression induced by a single dose of morphine may result in a modulation of adrenal function in response to subsequent exposure to opioids and may contribute to some alterations occurring during opioid drug abuse.

Acknowledgements

The authors acknowledge A. Zemánková, L. Žilavá and M. Lániová for technical help. This study was supported by grant from VEGA 2/6084 and ICA1-CT-2000-70008 of EC.

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