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Chronic morphine increases the pituitary–adrenocortical response of juvenile rats to mild stress

Bruce Nock*, Theodore J. Cicero, Michele Wich

Washington University School of Medicine, Department of Psychiatry, St. Louis, MO 63110, USA
Washington University School of Medicine, Department of Anatomy and Neurobiology, St. Louis, MO 63110, USA

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Abstract

We previously reported that chronic exposure of adult male rats to morphine by pellet implantation has no effect on corticosterone secretion but causes a marked testosterone-dependent increase in CBG. In the studies reported here, we examined the effects of chronic morphine on the pituitary–adrenocortical axis of male rats prior to the developmental rise in testosterone. In contrast to adults, morphine had little effect on CBG in peripubertal males. We found nothing remarkable with regard to basal hormone levels; morphine caused only a transient increase in ACTH and corticosterone in juveniles. However, while the pituitary–adrenocortical response to mild stress was normal in adults exposed to morphine, it was markedly increased in juveniles. After 7 days of morphine exposure, the stress response was as much as 2.5 times greater than normal in morphine-treated juveniles. This exaggerated response to stress did not appear to be due to the passive withdrawal of morphine or to an additive effect of stress plus morphine. Instead, morphine may either increase the perceived severity of stressors or decrease sensitivity to the negative feedback effects of stress levels of corticosterone in juvenile males. Either way, there is a striking shift in morphine's effects on the pituitary–adrenocortical axis across development.

Keywords: ACTH; CBG; Corticosterone; HPA axis; Morphine; Opiates; Stress

1. Introduction

Chronic exposure to morphine or heroin produces a persistent glucocorticoid deficiency in humans and adult rats (Abs et al., 2000; Facchinetti et al., 1984; Garrel, 1996; Nock et al., 1997, 1998, 2000; Palm et al., 1997). In male rats, the deficit results from a naltrexone-preventable up-regulation of corticosteroid-binding globulin (CBG) in serum (Nock et al., 1997, 1998, 2000). CBG functions primarily to regulate glucocorticoid bioavailability (Breuner and Orchinik, 2002). Hormone bound to CBG cannot enter target tissues and, consequently, is physiologically inactive. Only hormone that is not bound to CBG, i.e., "free" hormone, can readily enter target tissues. After 7 days of exposure to a low dose of morphine, CBG levels ranged from 100% to 150% above normal. This in turn decreased free, i.e., physiologically active, corticosterone by as much as 90% (Nock et al., 1997, 1998, 2000).

CBG up-regulation by morphine is testosterone-dependent. Testosterone does not mediate the effects of morphine but is required for morphine effects on CBG to be fully expressed (Nock et al., 2000). Consequently, morphine does not up-regulate CBG in female rats. In females, the morphine-induced glucocorticoid deficit occurs entirely as a result of a decrease in corticosterone secretion (Nock et al., 1998).

In the studies reported here, we examined the effects of morphine on the hypothalamic–pituitary–adrenocortical (HPA) axis in male rats prior to the developmental rise in testosterone titers that begins at puberty. Our expectation was that due to the low testosterone levels, the effects of morphine on the HPA axis of peripubertal males would be similar to those that occur in adult females. Consistent with

* Corresponding author. Department of Psychiatry, Campus Box 8134, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA. Tel.: +1 314 362 2491; fax: +1 314 747 2163.
E-mail address: bruce@wustl.edu (B. Nock).

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that prediction, morphine had little or no effect on CBG in juveniles. However, in contrast to adult females, morphine had no effect on basal corticosterone secretion and markedly increased the HPA response to mild stress at this developmental stage.

2. Materials and methods

2.1. Animals

Sprague–Dawley-derived male rats (10–17/group) from Harlan Sprague–Dawley (Cumberland, IN) were used for all experiments. The rats were housed in groups of two to three per cage with lights in the colony room on from 6 a.m. to 6 p.m. Prepubertal rats were either purchased as 22-day olds or were raised in our colony from purchased pregnant females and were weaned at 20–22 days of age. We observed no systematic differences between the purchased and colony-raised juveniles in terms of the measures made in the experiments reported here. Adult rats were purchased and allowed to acclimate to our colony for at least 1 week before treatment. The experimental protocol was approved by the Washington University Animal Care and Use Committee.

Trunk blood and other tissue samples were collected after decapitation. All rats were sacrificed between 10 and 11 a.m. when corticosterone secretion is normally low in the males of our colony. For sacrifice, the rats were individually carried by hand to a procedural room that was isolated from the colony room. Rats were sacrificed within 15 s of being picked up, and no more than 60 s elapsed between the first and last rats in a cage. A number of additional precautions were taken to minimize the disturbance to the animals prior to sacrifice. First, all preparations in the procedural room and in the colony room were carried out on the preceding day. Second, the door to the colony room was baffled to minimize any sound upon opening or closing. Third, all manipulations of the cages were done as quietly as possible, and all other sounds were kept to a minimum. Fourth, routine daily maintenance in the colony room was conducted after the experiments.

2.2. Drug treatments

Placebo or morphine (75 mg) pellets were implanted sc under Brevital® anesthesia (20–25 mg/kg for juveniles and 40 mg/kg for adults) and were allowed to remain in place for the duration of the experiment. Juvenile rats were 27 days old, and adults were 60 days old at the time of pellet implantation.

We chose to administer morphine by pellet implantation to avoid the corticosterone increases associated with repeatedly handling and restraining the animals for injections. We could see no reasonable way to accurately assess stress–morphine interactions if the HPA axis was repeatedly activated by the restraint stress associated with injections. This was especially important in view of a growing body of evidence indicating that repeated stress can modify the consequences of opiate exposure.

2.3. Stress paradigms

Two mild stress paradigms, which are referred to as novelty stress and injection stress, were utilized. For novelty stress, home cages were carried by hand to an adjacent colony room where the rats were transferred to an identical cage that contained fresh bedding, food and water. Rats were sacrificed at 15, 30 or 60 min after they were transferred to the new cage. For injection stress, rats were given a single i.p. injection of physiological saline and returned to their home cages. Juveniles were injected with 0.5 ml, and adults were injected with 0.7 ml of saline. Rats were sacrificed at 15 min after they were injected. For both stress paradigms, unstressed control rats were undisturbed until the time of sacrifice. Hormone levels for those groups are referred to as unstressed or basal levels.

2.4. Serum preparation

Serum was prepared by standard procedures from trunk blood. All steps were carried out at 0–4 °C. Blood was allowed to clot for approximately 45 min, and serum was decanted after centrifugation for 15 min at 1910 RCF in an IEC PR-7000 M preparative centrifuge.

2.5. Morphine levels in serum

Morphine was assayed in serum using an RIA kit purchased from Diagnostic Products Corporation (Los Angeles, CA). The antibody is highly specific for morphine with less than 0.03% cross-reactivity for morphine-3-glucuronide and less than 0.1% cross-reactivity for morphine-6-glucuronide. The standard curve was linear over the range from 5 ng to 1000 ng/ml. Intra and interassay variability was less than 5%.

2.6. Serum corticosterone levels

Unless specified otherwise, corticosterone levels refer to the total amount of corticosterone in serum. Corticosterone was assayed using an RIA kit purchased from Diagnostic Products Corporation. The antibody is highly specific for corticosterone with less than 3% cross-reactivity for 11-deoxycorticosterone and less than 1% cross-reactivity for 18-hydroxydeoxycorticosterone, cortisol, progesterone, 17α-hydroxyprogesterone, dehydroepiandrosterone, aldosterone, testosterone and estradiol. The standard curve was linear over the range from 20 ng to 2000 ng/ml. Intra and interassay variability was less than 5%.

CBG-bound and -unbound, i.e., free, corticosterone was calculated using Pearlman's (1970) modification of the mass equation $x = \left(b - \sqrt{(b^2 - 4a)}\right) \div 2$ where x is the molar

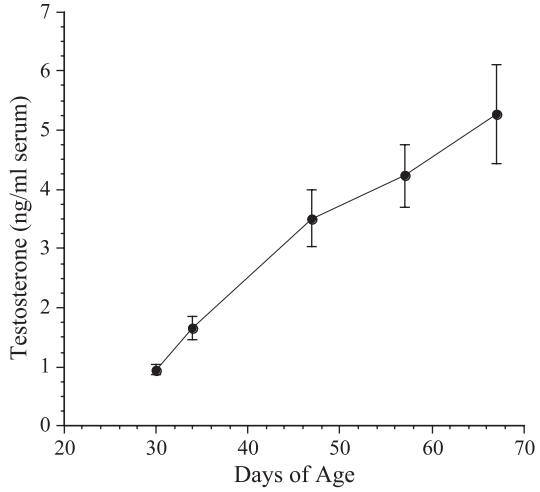

Fig. 1. Testosterone levels in untreated rats at selected ages from about the time of puberty to early adulthood.

concentration of CBG-bound corticosterone, b is the equilibrium dissociation constant for corticosterone and CBG (K_d) plus the molar concentration of CBG (binding at a saturating concentration of ligand) plus the molar concentration of corticosterone, and a is the molar concentration of corticosterone times the molar concentration of CBG. Free corticosterone is the total concentration of corticosterone minus the concentration of CBG-bound corticosterone. K_d=0.5 nM as determined previously (Nock et al., 1997).

2.7. Serum ACTH levels

ACTH was assayed using a double-antibody kit provided by the National Pituitary Hormone Program. The standard curve was linear over the range from 0.02 ng to 5.0 ng/ml. Intra and interassay variability was less than 5%.

2.8. Serum testosterone levels

Testosterone was assayed using an RIA kit purchased from Diagnostic Products Corporation. The antibody is highly specific for testosterone with less than 1% cross-reactivity for aldosterone, androstenedione, corticosterone, estradiol and progesterone The standard curve was linear over the range from 0.4 ng to 16 ng/ml. Intra and interassay variability was less than 5%.

2.9. Corticosteroid-binding globulin levels

CBG was measured using a protein-binding assay modified from procedures described by McCormick et al. (1995). All steps were carried out at 0–4 °C.

2.9.1. Sample preparation

Endogenous steroids were removed from serum by charcoal adsorption. A 5-µl aliquot of serum was diluted to 1 ml with buffer (30 mM Tris–HCl, 1 mM disodium ethylenediaminetetraacetic acid, 10 mM sodium molybdate, 1 mM dithiothreitol, 10% glycerol) containing dextran charcoal (Norit-A; 5.0 mg/ml final). The mixture was vortexed, allowed to sit at 4 °C for 20 min and centrifuged for 15 min at 2500 RPM. Aliquots of the supernatant fluid were diluted 1:5 with buffer and assayed.

2.9.2. Binding procedures

A 100-µl aliquot of steroid-free sample was incubated overnight at 4 °C with 7.0 nM [^{3}H]corticosterone with or without 16 µM corticosterone to define specific binding (final volume=150 µl). Ethanol (1% final) was included in the incubation mixture to minimize steroid binding to glass.

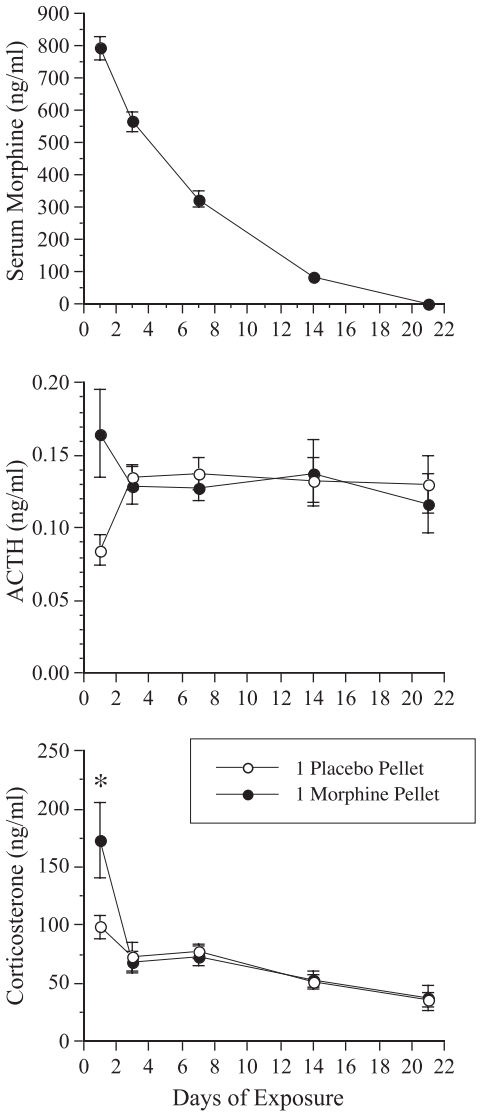

Fig. 2. Levels of morphine, ACTH and corticosterone in serum of prepubertal male rats at selected intervals after implantation of a single morphine or placebo pellet. The rats were 27 days of age when the pellets were implanted. *Indicates p<0.05 versus placebo control. N=10–13 rats/group. ACTH: $F(4,111)_{Days}$=0.2, p=0.95; $F(1,111)_{Drug}$=0.9, p=0.34; $F(4,111)_{Days\times Drug}$=2.1, p=0.08. Corticosterone: $F(4,113)_{Days}$=20, p=0.0001; $F(1,113)_{Drug}$=3.4, p=0.07; $F(4,113)_{Days\times Drug}$=3.9, p=0.006.

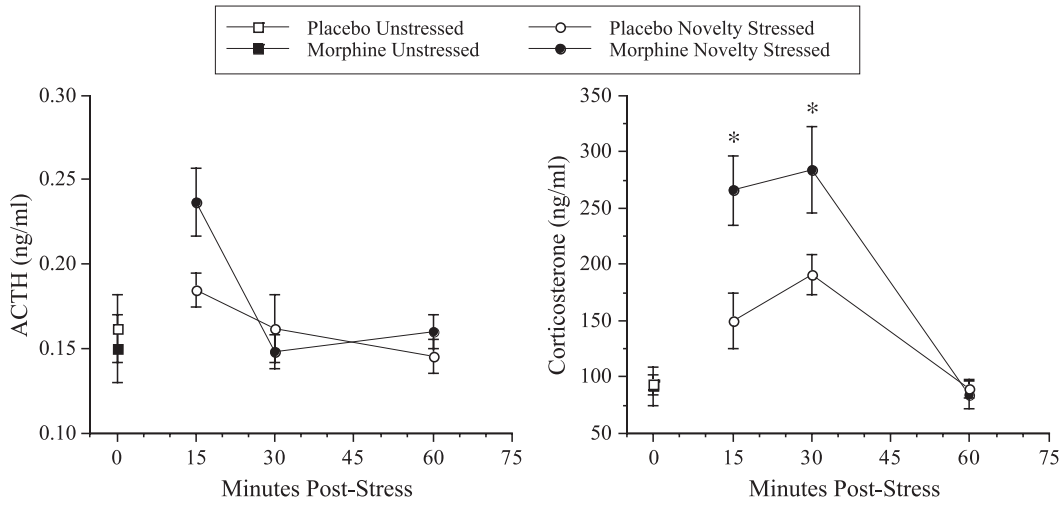

Fig. 3. Pituitary–adrenocortical hormone levels in placebo- and morphine-exposed juvenile male rats at selected time intervals after exposure to novelty stress. A single placebo or morphine pellet was implanted sc 7 days prior to exposure to the stressor. *Indicates $p<0.05$ versus placebo control. N=10–13 rats/group. ACTH: $F(3,90)_{Time}=7.0$, $p=0.0003$; $F(1,90)_{Drug}=0.9$, $p=0.34$; $F(3,90)_{Time\times Drug}=2.1$, $p=0.10$. Corticosterone: $F(3,87)_{Time}=24$, $p=0.0001$; $F(1,87)_{Drug}=10.2$, $p=0.002$; $F(3,87)_{Time\times Drug}=3.9$, $p=0.01$.

Bound and free [^{3}H]corticosterone were separated using Sephadex LH-20 columns. Columns were made from 1.0-ml plastic disposable micropipette tips stopped with a 4-mm glass bead. The column was filled with LH-20 to a height of 3.2 cm from the middle of the glass bead and equilibrated with 400 μl of buffer (30 mM Tris–HCl, 1 mM disodium ethylenediaminetetraacetic acid, 10 mM sodium molybdate, 1 mM dithiothreitol, 10% glycerol). A 100-μl aliquot of incubation mixture was placed onto the column and washed in with 100-μl buffer. Thirty minutes later, the sample was eluted using 600-μl buffer. Specific binding was expressed as picomoles specific binding/mg serum protein. Protein content was determined by the protein–dye binding method of Bradford (1976).

2.10. Statistical analysis

All data are expressed as mean±standard error of the mean. *t*-Tests were used to compare two groups; otherwise, data were subjected to analysis of variance followed by post hoc analysis. Differences between groups were considered statistically significant when the two-tailed probability that they occurred by chance was less than 0.05.

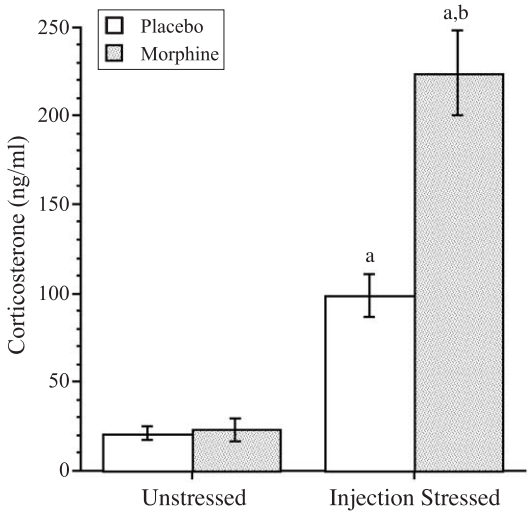

Fig. 4. Corticosterone levels in placebo- and morphine-exposed juvenile male rats at 15 min after exposure to injection stress. A single placebo or morphine pellet was implanted sc 7 days prior to exposure to the stressor. [a]Indicates $p<0.05$ versus the unstressed group with the same drug treatment. [b]Indicates $p<0.05$ versus the placebo stressed group. N=14–15 rats/group. $F(1,54)_{Stress}=95$, $p=0.0001$; $F(1,54)_{Drug}=19$, $p=0.0001$; $F(1,54)_{Stress\times Drug}=18$, $p=0.0001$.

3. Results

Serum testosterone levels were measured at selected ages from 30 days of age through early adulthood. As shown in

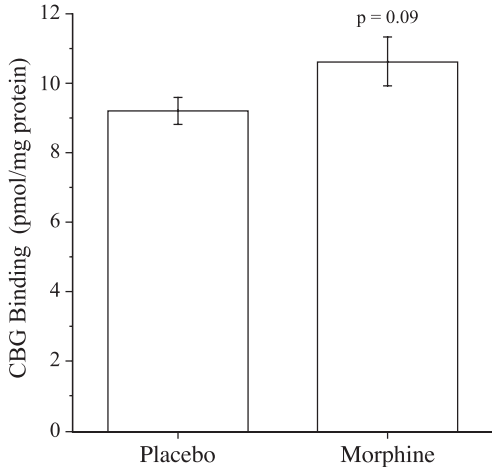

Fig. 5. Serum CBG binding in juvenile male rats. A single placebo or morphine pellet was implanted sc 7 days earlier. $t(21)=-1.8$, $p=0.09$.

Table 1
Pituitary–adrenocortical hormone levels (ng/ml serum) in placebo- and morphine-exposed juvenile male rats at 15 min after injection stress[a]

	Unstressed		Injection stressed	
	Placebo	Morphine	Placebo	Morphine
3 Days[b]				
ACTH	0.141±0.01	0.108±0.01	0.201±0.02[c]	0.186±0.03[c]
Corticosterone	73±12	68±9	216±23[c]	292±33[c]
7 Days[d]				
ACTH	0.059±0.01	0.059±0.01	0.112±0.01[e]	0.295±0.07[e,f]
Corticosterone	42±4	49±5	150±35[e]	355±41[e,f]
14 Days[g]				
ACTH	0.133±0.01	0.138±0.02	0.152±0.02[c]	0.207±0.03[c]
Corticosterone	52±5	52±8	150±21[c]	205±28[c]
21 Days[h]				
ACTH		Not determined		
Corticosterone	35±6	37±11	91±11[c]	84±12[c]

[a] One placebo or morphine pellet was implanted sc 3, 7, 14 or 21 days prior to exposure to the stressor. The rats were 27 days old when the pellet was implanted.

[b] ACTH: $F(1,44)_{Stress}=10$, $p=0.003$; $F(1,44)_{Drug}=1.2$, $p=0.28$; $F(1,44)_{Stress\times Drug}=0.2$, $p=0.68$. Corticosterone: $F(1,48)_{Stress}=73$, $p=0.0001$; $F(1,48)_{Drug}=2.7$, $p=0.11$; $F(1,48)_{Stress\times Drug}=3.5$, $p=0.07$.

[c] Denotes a main effect of stress, $p<0.05$.

[d] ACTH: $F(1,41)_{Stress}=20$, $p=0.0001$; $F(1,41)_{Drug}=8$, $p=0.007$; $F(1,41)_{Stress\times Drug}=8$, $p=0.007$. Corticosterone: $F(1,43)_{Stress}=56$, $p=0.0001$; $F(1,43)_{Drug}=14$, $p=0.0005$; $F(1,43)_{Stress\times Drug}=13$, $p=0.0009$.

[e] Denotes $p<0.05$ versus unstressed controls.

[f] Denotes $p<0.05$ versus the stressed placebo group.

[g] ACTH: $F(1,44)_{Stress}=4.8$, $p=0.03$; $F(1,44)_{Drug}=2.3$, $p=0.14$; $F(1,44)_{Stress\times Drug}=1.6$, $p=0.21$. Corticosterone: $F(1,43)_{Stress}=47$, $p=0.0001$; $F(1,43)_{Drug}=2.4$, $p=0.13$; $F(1,43)_{Stress\times Drug}=2.2$, $p=0.15$.

[h] Corticosterone: $F(1,55)_{Stress}=25$, $p=0.0001$; $F(1,55)_{Drug}=0.1$, $p=0.78$; $F(1,55)_{Stress\times Drug}=0.2$, $p=0.67$.

Fig. 1, testosterone levels increased steadily during this development period.

Fig. 2 shows the levels of morphine, ACTH and corticosterone in serum of juvenile male rats at selected intervals after implantation of a single morphine pellet. Morphine titers were highest on the day following pellet implantation and thereafter declined to nondetectable levels by day 21. Basal ACTH and corticosterone levels were elevated on the day after pellet implantation (although the difference was not statistically significant for ACTH; $p=0.08$) but did not differ from control values by day 3. The weight of the adrenal glands at 7 days after morphine exposure (30.8±1.0 mg) also did not differ from control values (29.8±0.9 mg), confirming that hormone hypersecretion persisted for only a brief period after morphine exposure.

Fig. 3 depicts pituitary–adrenocortical hormone levels in placebo- and morphine-exposed (one pellet for 7 days) juvenile male rats at selected time intervals after novelty stress. Stress elevated ACTH and corticosterone in both the placebo and the morphine-treated groups. However, the hormonal response to stress was greater in the morphine

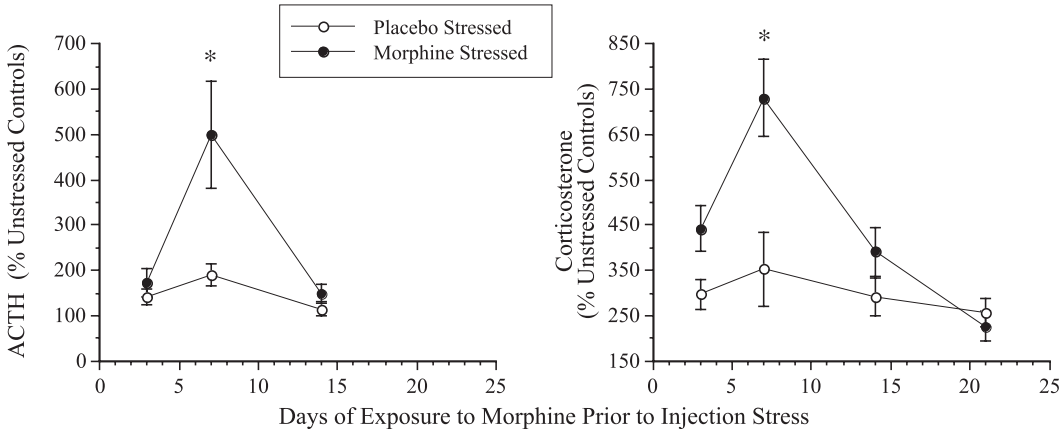

Fig. 6. Pituitary–adrenocortical hormone levels in placebo- and morphine-exposed juvenile male rats at 15 min after injection stress. One placebo or morphine pellet was implanted sc 3, 7, 14 or 21 days earlier. The figure depicts the data for the placebo-stressed and morphine-stressed groups in terms of percent of unstressed control values. The untransformed data for all four groups and the statistical analyses are shown in Table 1. *Indicates $p<0.05$ versus placebo-stressed group. N=11–16 rats/group.

group. For ACTH, this was evident at the 15-min poststress time interval when levels in the morphine group were 58% higher than unstressed control values, while levels in the placebo group were 14% above unstressed control values. However, the difference between the placebo and morphine groups did not attain statistical significance (p=0.10). By 30 min, ACTH had returned to basal levels in the morphine, as well as the placebo groups. The corticosterone response to stress was greater in the morphine group at 15 and 30 min poststress. At those time points, corticosterone levels in the morphine groups were 192% and 212% higher than unstressed control values, while levels in the placebo groups were 61% and 105% above unstressed control values. By 60 min, corticosterone levels for the stressed groups did not differ from unstressed controls (Fig. 3).

Fig. 4 shows corticosterone levels in placebo- and morphine-exposed (one pellet for 7 days) juvenile male rats at 15 min after injection stress. Exposure to morphine increased the hormonal response to this stressor (Fig. 4), as well as to novelty stress (Fig. 3). In this instance, stress levels of corticosterone in the morphine group were ≈875% higher than unstressed control values, while stress levels in the placebo group were ≈370% above unstressed controls. The concentration of CBG in serum was somewhat higher (15%) in the morphine group than in the placebo group, but the difference did not attain statistical significance (Fig. 5). When these CBG values were used to calculate free corticosterone, levels in the stressed morphine group (41±0.03 ng/ml) were approximately 135 times higher than in the stressed placebo group (0.3±0.01 ng/ml).

Table 1 shows pituitary–adrenocortical hormone levels at 15 min after injection stress in juvenile rats exposed to one placebo or morphine pellet for 3, 7, 14 or 21 days. Fig. 6 shows the data for the placebo-stressed and morphine-stressed groups in terms of percent of unstressed control

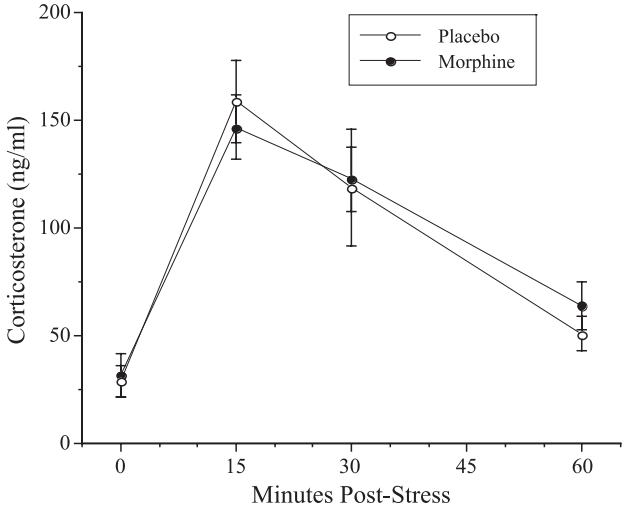

Fig. 7. Corticosterone levels in placebo- and morphine-exposed adult male rats at selected time points after injection stress. Two placebo or morphine pellets were implanted sc 7 days earlier. N=13–16 rats/group. $F(3,112)_{Time}$=31, p=0.0001; $F(1,112)_{Drug}$=0.04, p=0.85; $F(3,112)_{Time\times Drug}$=0.26, p=0.86.

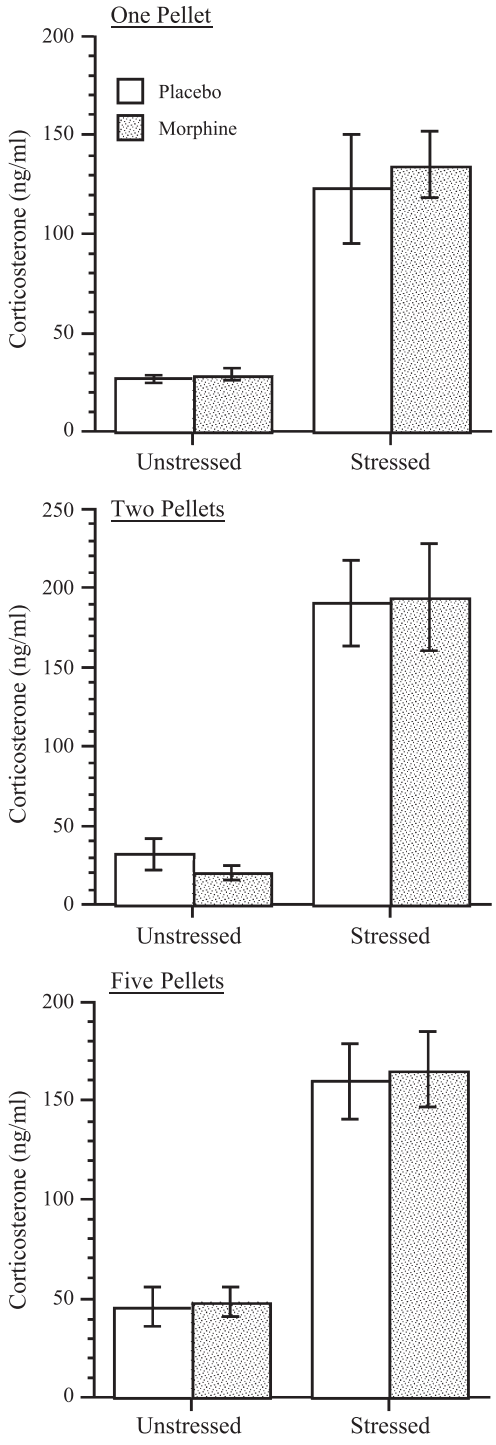

Fig. 8. Corticosterone levels in placebo- and morphine-exposed adult male rats at 15 min after injection stress. Rats were exposed to morphine (one, two or five pellets) for 7 days. For the five-pellet paradigm, one pellet was implanted on day 0 and two additional pellets were implanted on days 3 and 5. N=10–17 rats/group. One pellet: $F(1,47)_{Stress}$=36, p=0.0001; $F(1,47)_{Drug}$=0.2, p=0.68; $F(1,47)_{Stress\times Drug}$=0.1, p=0.77. Two pellets: $F(1,46)_{Stress}$=53, p=0.0001; $F(1,46)_{Drug}$=0.03, p=0.86; $F(1,46)_{Stress\times Drug}$=0.1, p=0.72. Five pellets: $F(1,52)_{Stress}$=56, p=0.0001; $F(1,52)_{Drug}$=0.1, p=0.78; $F(1,52)_{Stress\times Drug}$=0.01, p=0.91.

values. At 3 days after pellet implantation, stress levels of ACTH and corticosterone for the morphine and placebo groups did not differ, although corticosterone levels in the morphine group tended to be higher than in the placebo group (p=0.07). At 7 days after pellet implantation, stress levels of both ACTH and corticosterone were significantly higher in the morphine group than in the placebo group. At 14 and 21 days after pellet implantation when serum morphine levels were very low and undetectable, respectively (Fig. 2), stress levels of ACTH and corticosterone for the morphine and placebo groups did not differ statistically (Table 1; Fig. 6).

In contrast to juveniles, the hormonal response of adult males to mild stress was not significantly affected by chronic exposure to morphine. For the study shown in Fig. 7, adult males were implanted with two placebo or morphine pellets, and 7 days later, corticosterone was measured at selected time intervals after injection stress. In a direct comparison, morphine levels in blood of adults at 7 days after implantation of two morphine pellets (554±44 ng/ml) were similar to those seen in juveniles (480±28 ng/ml) at 7 days after implantation of a single pellet. Corticosterone levels were elevated at 15 and 30 min after injection stress and had returned to near basal levels by 60 min poststress. There were no significant differences between the placebo and morphine groups.

For the study shown in Fig. 8, one or two placebo or morphine pellets were implanted into adults on day 0, or 5 pellets were implanted sequentially, with one on day 0 and two additional pellets on days 3 and 5. None of these morphine treatment paradigms affected corticosterone levels at 15-min postinjection stress. We previously reported that all of these morphine treatments maximally or near maximally increase the concentration of CBG in serum (Nock et al., 1997). In the present study, CBG levels were determined only in the groups that were implanted with two pellets. As in the previous study, morphine increased the concentration of CBG in serum (Fig. 9). When these CBG values were used to calculate free corticosterone, levels in the stressed morphine group (0.22±0.02 ng/ml) were 99% lower than in the stressed placebo group (27±0.03 ng/ml).

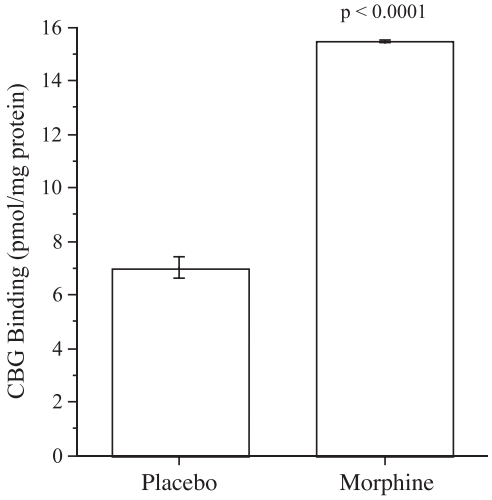

Fig. 9. Serum CBG binding in adult male rats. Two placebo or morphine pellets were implanted sc 7 days earlier. $t(22)=-12.0$, p=0.0001.

4. Discussion

Chronic exposure of peripubertal male rats to morphine by pellet implantation markedly increased the pituitary–adrenocortical response to two mild stressors, which we refer to as novelty and injection stress. The exaggerated stress response was evident by 3 days and was greatest at 7 days after exposure to morphine. At 7 days, the magnitude of the stress response was as much as 2.5 times greater in the morphine rats than in the placebo rats. Moreover, free corticosterone levels in the morphine rats were calculated to be ≈135 times higher than normal at the peak of the stress response. By 14 days after pellet implantation, morphine levels in serum were relatively low, but the magnitude of the stress response remained somewhat greater in the morphine rats. By 21 days, morphine was no longer detectable in serum, and the hormonal response to stress was similar in the morphine and placebo rats. Thus, the exaggerated response to stress appears to dissipate as morphine clears from the general circulation.

Consistent with studies reported by Little and Kuhn (1995), chronic exposure of juveniles to morphine caused only a transient increase in basal ACTH and corticosterone levels. By the 3rd day after pellet implantation, basal hormone levels in the morphine rats did not differ from those in the placebo rats. The exaggerated response to stress therefore occurred when basal ACTH and corticosterone levels were normal. Consequently, the higher stress levels in the morphine rats cannot be attributed to an additive effect of morphine plus stress. Rather, chronic exposure to morphine appears to increase the pituitary–adrenocortical response of juvenile rats to mild stress.

In contrast to juveniles, we previously reported that chronic exposure of adult male rats to morphine did not affect the magnitude or duration of the corticosterone response to a mild stressor (Nock et al., 1997). We confirmed that finding in the present study. Specifically, the stress response was normal at 7 days after implantation of two morphine pellets, which produced morphine levels in blood that were similar to those produced in juveniles by a single pellet. In addition, the stress response of adults was normal after exposure to a single morphine pellet and after sequential implantation of five pellets. The differential effect of morphine in juveniles versus adults, therefore, does not appear to be dose-dependent.

It may be significant that the exaggerated stress response of juveniles occurred during the period when morphine levels in blood were declining. However, we can discern no evidence indicating that it is attributable to the

passive withdrawal of morphine. Most importantly, as discussed above, basal corticosterone levels, which are a sensitive index of withdrawal (Kishioka et al., 1994), were normal when the exaggerated response occurred. In addition, no behavioral nor physical signs of withdrawal, such as wet dog shakes or diarrhea, were evident in the morphine-treated rats at the time of sacrifice. Furthermore, the stress response of adult males was normal under virtually identical conditions, i.e., when morphine levels in blood were declining (Nock et al., 1997). Thus, the increased pituitary–adrenocortical response to stress seen in juveniles does not appear to be attributable to passive withdrawal of morphine and the expression of latent withdrawal symptoms.

Alternatively, morphine may either increase the perceived severity of stressors or decrease sensitivity to the negative feedback effects of corticosterone in juvenile males. At this point, there is no firm basis for excluding either possibility. However, because morphine typically induces antinociception and euphoria, it seems unlikely that it would increase the perception of stressor severity. It is more likely that it affects feedback sensitivity, which is principally determined by the level of intracellular corticosteroid receptors (e.g., see De Kloet et al., 1991; Jacobson and Sapolsky, 1991; Ordyan et al., 2001; Shrimpton and Randall, 1994; Welberg et al., 2001). Two types of corticosteroid receptors mediate negative feedback, mineralocorticoid (MR; also called the type I corticosteroid receptor) and glucocorticoid receptors (GR; also called the type II corticosteroid receptor). MR appears to maintain low basal activity of the HPA axis during the circadian trough. GR, with facilitation by MR, constrains HPA activity during the circadian peak and acute stress (Cole et al., 2000; Jacobson and Sapolsky, 1991; Reul et al., 1987). Since morphine affects stress but not basal hormone levels, it may affect GR and not MR density in juvenile males.

In addition to the age-related effects of morphine on corticosterone secretion, effects on CBG are also age-dependent. At 7 days after morphine pellet implantation, CBG levels were more than 100% higher than normal in adults. On the other hand, morphine had little or no effect on CBG levels in peripubertal males. Since morphine effects on CBG are testosterone-dependent in adult males, the marginal effect in juveniles is probably due to the low testosterone levels at this stage of development (see Introduction). Whether the differential response of CBG to morphine contributes to the age-related effects on ACTH and corticosterone secretion remains to be determined.

In summary, there appears to be a striking shift in morphine's effects on the pituitary–adrenocortical axis across development. In juvenile males, chronic exposure to morphine has only a small effect on CBG and a transient effect on basal ACTH and corticosterone secretion. However, it markedly increases the hormonal response to stress. In adult males, morphine markedly increases CBG but has no apparent effect on hormone secretion.

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