Chronic Exposure to Morphine Increases Corticosteroid-Binding Globulin

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ABSTRACT
Although it appears that corticosterone may play an important role in determining vulnerability to drugs of abuse, few studies have examined drug effects on factors that affect corticosterone efficacy. Thus, studies were carried out to assess the effects of morphine on corticosteroid-binding globulin (CBG), the major glucocorticoid binder in blood. Since CBG-bound hormone is thought to be physiologically inactive, changes in CBG levels could affect corticosterone action independently of hormone levels per se. We found that morphine caused a marked naltrexone-preventable increase (~160%) in CBG in adult male rats. Elevated levels were seen by three days and were maximal at seven days after morphine pellet (75 mg) implantation. CBG levels remained elevated while morphine was detectable in blood and returned toward normal as the drug cleared from the system. A single morphine pellet was sufficient to induce a marked increase in the concentration of CBG and two or more pellets caused maximal upregulation. Baseline and stress levels of total corticosterone (bound and unbound) were normal after chronic exposure to morphine. However, due to the elevated level of CBG, the amount of free, physiologically active hormone was dramatically reduced. These results suggest that morphine may exert potent effects on corticosterone action that are not revealed by measurement of corticosterone alone. Furthermore, the increase in CBG resulting from chronic exposure to morphine might contribute to the perpetuation of drug use and to adverse effects of drug exposure by impairing normal functions of corticosterone.

A considerable amount of evidence from epidemiological and clinical studies now exists linking life events to drug abuse. Psychosocial stresses, in particular, appear to be associated with drug use (Barnet et al., 1995; Kosten et al., 1986; McFall et al., 1992) and have been hypothesized to be contributing factors in the etiology of drug abuse (Alexander and Hadaway, 1982; Lindenberg et al., 1993; O'Doherty and Davies, 1987; O'Doherty, 1991) and in relapse after remission (Krueger, 1981).

Observations from animal studies also have linked stress to drug use and, in addition, suggest the involvement of glucocorticoids. For example, repeated exposure to stressful stimuli (Deroche et al., 1995; Leyton and Stewart, 1990; Molina et al., 1994; Shaham et al., 1995) or adverse living conditions (Deroche et al., 1993a, 1994) enhances psychostimulant and morphine psychomotor effects which may be correlated with propensity to self-administer drugs (Demi`iere et al., 1992; Deroche et al., 1993b; Piazza et al., 1989).

This stress-induced enhancement of morphine's pharmacological effects appears to be dependent upon corticosterone secretion (Deroche et al., 1993a, 1994, 1995; Piazza et al., 1994) and can be mimicked by repeated injections of corticosterone (Deroche et al., 1992). In addition, stress or adverse living conditions potentiate opiate (e.g., Alexander et al., 1981; Carroll et al., 1979; Dib, 1985; Shaham et al., 1993) and psychostimulant (Bozarth et al., 1989; Goeders and Guerin, 1994; Piazza et al., 1990; Ramsey and Van Ree, 1993) self-administration and reinstatement of self-administration after a drug free period (Shaham 1993; Shaham and Stewart, 1995). Furthermore, it appears that the propensity to self-administer is positively related to reactivity to stress and corticosterone secretion, since rats with higher locomotor and corticosterone responses to novelty have a greater propensity to self-administer morphine and psychostimulants (e.g., Deroche et al., 1993b; Goeders and Guerin, 1994; Maccari et al., 1991; Piazza et al., 1991). Considering the growing amount of evidence implicating stress and corticosterone in the development of drug-seeking behavior and in relapse after a drug-free period, it seems plausible that glucocorticoids might also play a role in reinforcing and perpetuating drug use.

Thus far, surprisingly few studies have examined the ef-

ABBREVIATIONS: ACTH, adrenocorticotropic hormone; AIDS, acquired immunodeficiency syndrome; CBG, corticosteroid-binding globulin; HIV, human immunodeficiency virus; RIA, radioimmunoassay.
fects of chronic drug exposure on glucocorticoids, and those have been limited almost entirely to effects on hormone levels in blood. Typically, acute exposure to morphine has been found to increase glucocorticoid levels in many animal species, including rodents, with tolerance developing after chronic exposure (as recently reviewed by Pechnick, 1993). Other factors that might affect the physiological efficacy of glucocorticoids have not been systematically examined. Thus, in the studies reported here, we examined the effects of chronic exposure to morphine on CBG (or transcortin) in adult male rats. Although CBG is known to be regulated by a variety of endogenous factors, including hormones (e.g., Gala and Westphal, 1966; Mataradze et al., 1992; Smith and Hammond, 1992), there have been no previous studies of the effects of drugs of abuse on this serum protein.

CBG has a high affinity for corticosterone ($K_d \approx 0.5$ nM; see “Results” section) and is the major corticosterone-binding protein in blood. It is generally accepted that only unbound (free) corticosterone can gain access to target cells and initiate physiological responses (as reviewed by Mendel, 1989). CBG-bound hormone is thought to be physiologically inactive. Thus, drug-induced changes in blood levels of CBG could have a significant impact on the efficacy of circulating corticosterone, which could influence ability to cope with stress and perhaps drug seeking behavior.

**Materials and Methods**

**Animals**

Male rats purchased from Harlan Sprague-Dawley, Inc. (Cumberland, IN) or bred in our colony from Harlan Sprague-Dawley stock were used for all experiments (10–15 rats per group). The rats were housed in groups of 2 to 3 per cage with lights in the colony room on from 6 A.M. to 6 P.M. They were 60 to 90 days old at the beginning of drug treatment.

Trunk blood was collected after decapitation. All rats were sacrificed between 10 A.M. and 11 A.M. when corticosterone secretion is normally low in 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 sec of being picked up, and no more than 60 sec elapsed between sacrifice of the first and last rats in a cage.

**Drug Treatments**

Placebo, morphine (75 mg) or naltrexone (30 mg) pellets were implanted s.c. under Brevital anesthesia (40 mg/kg) and were allowed to remain in place for the duration of the experiment. All pellets were generously provided by the National Institute on Drug Abuse (Rockville, MD).

All naltrexone pellets were implanted on day 0. All morphine pellets also were implanted on day 0 except when specified otherwise. For the dose-response experiment, one or two morphine pellets were implanted on day 0 or five morphine pellets were implanted sequentially. One control group received two placebo pellets on day 0. This group served as the control for the one and two morphine pellet treatments. The group receiving one morphine pellet also received one placebo pellet to equalize the number of pellets at two for the groups. For the five pellet paradigm, morphine pellets were implanted sequentially, with one pellet implanted on day 0 and two additional pellets implanted on days 3 and 5. The control group for this treatment received the same number of placebo pellets on the appropriate days. Since there were no significant differences between the two control groups, data were combined for presentational purposes.

**Stress Paradigm**

When specified, rats were mildly stressed by i.p. injection of 0.7 ml physiological saline. Unstressed control rats were undisturbed until the time of sacrifice.

**CBG 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°C to 4°C.

**Sample preparation.** Endogenous steroids were removed from serum by charcoal absorption. A 5 μl aliquot of serum was diluted to 1 ml with buffer (30 mM Tris HCl, 1 mM disodium EDTA, 10 mM sodium molybdate, 1 mM diethiothreitol, 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.

**Binding procedures.** A 100 μl aliquot of steroid-free sample was incubated overnight at 4°C with 7.0 nM [3H]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. Bound and free [3H]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 EDTA, 10 mM sodium molybdate, 1 mM diethiothreitol, 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 pmols specific binding/ml serum or pmols specific binding/mg serum protein, both of which gave virtually identical results. Protein content was determined by the protein-dye binding method of Bradford (1976).

**Morphine Levels in Serum**

Morphine was assayed in serum using a 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 lower limit of sensitivity of the assay was 5 ng/ml and the standard curve was linear over the range 5 ng to 1,000 ng/ml. Intra- and interassay variability was less than 5%.

**Serum Corticosterone Levels**

Unless specified otherwise, corticosterone levels refer to the total amount of corticosterone in serum. Corticosterone was assayed in serum using a RIA kit purchased from Diagnostic Products Corporation (Los Angeles, CA). 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 lower limit of sensitivity of the assay was 20 ng/ml, and the standard curve was linear over the range 20 ng to 2,000 ng/ml. Intra- and interassay variability were less than 5%.

CBG-bound and unbound, i.e., free, corticosterone was calculated using Pearlman’s (1970; also see Plymate et al., 1987 and McCormick et al., 1995) modification of the mass equation:

$$ x = \frac{b}{\sqrt{b^2 - 4a/2}} $$

where $x$ is the molar concentration of CBG-bound corticosterone, $b$ is the equilibrium dissociation constant for corticosterone and CBG ($K_d$) plus the molar concentration of CBG ($B_{max}$) 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. $K_d$ and $B_{\text{max}}$ were determined experimentally in the study shown in Figure 4.

**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 probability that they occurred by chance was less than .05.

**Results**

Figure 1 shows morphine, total corticosterone and CBG levels in serum at selected time-intervals after implantation of a single morphine pellet. Morphine levels were highest on Day 1 following pellet implantation and, thereafter, declined to relatively low levels by Day 14. The concentration of corticosterone in serum did not differ from control levels at any interval following morphine pellet implantation (fig. 1). CBG levels did not differ from control values at one day after morphine pellet implantation. However, at three and seven days after morphine pellet implantation, CBG levels were markedly elevated with values for the morphine exposed rats 66% (Day 3) and 97% (Day 7) higher than for controls. At 14 days after pellet implantation, CBG levels in the morphine exposed group remained 20% above control values but that difference was not statistically significant ($P = .19$). However, when a second morphine pellet was implanted on Day 7 to maintain higher morphine levels, CBG remained significantly elevated (107%) through Day 14 (fig. 1).

Although only crude dose-response relationships can be established with pellets, the magnitude of morphine’s effects on CBG appears to be dose-dependent. Figure 2 shows CBG levels in serum of untreated rats and of rats implanted with morphine or placebo pellets. The concentration of CBG in the placebo group did not differ from that of untreated rats. However, as in the time-response study described above, one morphine pellet caused a significant increase in CBG. Two morphine pellets produced even higher CBG levels, but five pellets (implanted sequentially as described under “Materials and Methods”) caused no additional increase. None of the treatments affected the total amount of protein in serum (untreated, placebo, one, two and five pellets = 6.5 ± 0.2, 6.1 ± 0.1, 6.2 ± 0.2, 6.2 ± 0.2 and 6.4 ± 0.1 ng/ml of diluted, steroid-free serum, respectively) or corticosterone levels at the time of sacrifice (untreated, placebo, one, two and five pellets = 59 ± 16, 45 ± 11, 43 ± 12, 33 ± 10 and 40 ± 15 ng/ml, respectively).

One, two and five morphine pellets resulted in serum morphine levels of 650 ± 106, 881 ± 98 and 1515 ± 38 ng/ml, respectively, on day 7. When added directly into the in vitro assay system, morphine had little or no effect on CBG binding across that range of concentrations (fig. 3). Thus, the presence of morphine in the assay system itself does not appear to affect CBG binding.

Figure 4 shows Scatchard plots of CBG binding isotherms for pooled serum taken seven days after implantation of two morphine or placebo pellets. Scatchard analysis indicated that morphine had little or no effect on the affinity of CBG for corticosterone but increased the number of binding sites by 140%.

![Fig. 1. Serum morphine (top), total corticosterone (middle) and CBG (bottom) levels at selected time intervals after implantation of one placebo or 75-mg morphine pellet (data shown by round symbols and solid lines). For the data shown by the square symbols and dotted lines, rats were implanted with an additional placebo or morphine pellet on Day 7 to maintain elevated morphine levels in blood. The figure shows that morphine had little or no effect on corticosterone but markedly increased the concentration of CBG in serum. Implantation of a supplemental morphine pellet on Day 7 prevented the decline in CBG levels between Days 7 and 14. N = 12–13 rats/group. * indicates $P < .05$ vs. the placebo group.](image-url)
trexone prevented the increase in serum CBG caused by morphine (fig. 6).

Figure 7 shows total (top graph) and free (bottom graph) corticosterone in serum of unstressed or mildly stressed rats implanted with two morphine or placebo pellets seven days earlier. The data indicate that exposure to morphine had little or no effect on baseline (unstressed) or stress levels of total corticosterone. However, the amount of free corticosterone, i.e., corticosterone not bound to CBG, was markedly lower in morphine-treated rats under both baseline and stress conditions.

**Discussion**

Chronic exposure to morphine caused a marked, time- and dose-dependent increase in the concentration of CBG in serum of adult male rats. Elevated levels were seen by three days and appeared to be maximal at seven days after morphine pellet implantation. Thereafter, CBG levels returned
toward normal as morphine cleared from the general circulation. Thus, morphine effects on CBG do not appear to persist significantly beyond the period when the drug is present in the body. However, when a supplemental pellet was implanted to maintain morphine levels, the concentration of CBG in serum remained elevated through 14 days, the longest time interval examined. Morphine effects on CBG, therefore, appear to continue for as long as the drug is detectable in blood.

A single morphine pellet was sufficient to cause a marked increase in the concentration of CBG. Two pellets caused what appeared to be a maximal upregulation since no further increase was seen with five pellets. None of the pellet implantation procedures caused a lasting change in baseline corticosterone secretion. By the day after implantation of one morphine pellet, corticosterone levels were normal. Corticosterone levels were also normal at seven days after implantation of two pellets and at three days after sequential implantation of five pellets. Thus, chronic exposure to morphine appears to affect CBG but has little effect on baseline corticosterone levels.

The opiate antagonist naltrexone completely blocked the morphine-induced increase in CBG, indicating that morphine affects CBG through activation of opioid receptors. We made no attempt in the current studies to identify the critical opioid receptor subtype. In addition, our data do not establish whether the opioid receptors are located in liver where CBG is synthesized or in some other tissue of the body. Moreover, we have little basis for speculating about how opiate receptor activation might increase CBG. One of the major CBG regulatory factors is corticosterone, which exerts a negative influence on CBG levels (Feldman et al., 1979; Hsu and Kuhn, 1988). Thus, a reduction in corticosterone leads to an increase in CBG. However, acute morphine stimulates corticosterone secretion in rats (as recently reviewed by Pechnick, 1993), and chronic morphine had no apparent effect on corticosterone levels in the present study. Therefore, the morphine-induced increase in CBG does not appear to be secondary to effects on corticosterone.

Although naltrexone blocked the morphine-induced increase in CBG, chronic exposure to the opioid antagonist alone for up to 21 days did not affect CBG levels in serum. It seems likely that the doses tested were sufficient to antagonize the actions of endogenous opioids, since we (Giordano et al., 1990) previously found them to induce a maximal rate of opioid receptor up-regulation—an indication that the activity...
of endogenous opioids was completely blocked. Thus, endogenous opioids do not appear to be a significant factor in the regulation of CBG. Whether they contribute to CBG regulation under other circumstances remains to be established.

In addition to normal baseline levels of total corticosterone, the corticosterone response to a mild stressor appeared normal at seven days after exposure to morphine. That is, morphine did not appear to affect stress levels of total corticosterone. This finding was unexpected for two reasons. First, it is at a time when the morphine-induced increase in CBG is maximal. Accordingly, one would expect the elevated levels of CBG to decrease the amount of physiologically active hormone and, thereby, restrict negative feedback, causing a hyper, rather than a normal, response to stress. Jacobson et al. (1988) reported a finding that may be analogous to ours.

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The second reason for not expecting to see normal stress levels of total corticosterone in morphine exposed rats is that several previous studies reported that chronic exposure to morphine reduced (Paroli and Melchiorri, 1961) or abolished the pituitary-adrenocortical response to stress (Briggs and Munson, 1955; Buckingham and Cooper, 1984). There are a number of procedural differences between those studies and ours that conceivably might be important. For example, in the studies by Briggs and Munson and by Buckingham and Cooper rats were anesthetized when the stress was applied. Furthermore, in all of the studies that found an inhibition of the stress response (e.g., Briggs and Munson, 1955; Buckingham and Cooper, 1984; Paroli and Melchiorri, 1961) morphine was administered at high doses by daily injections whereas we implanted morphine pellets.

In any event, although baseline levels and the response to a mild stressor appeared normal in terms of total corticosterone, the amount of free, physiologically active hormone estimated using Pearlman’s modification of the mass equation was markedly reduced under both conditions as a result of the morphine-induced increase in CBG. This was particularly apparent at the peak of the stress response when the amount of free corticosterone was 90% lower in morphine treated rats. In essence, the heightened level of CBG dramatically blunted the response to stress. Our results then suggest that morphine might, exert effects on endocrine physiology through CBG that are not apparent from hormone levels in blood.

At this point, we can only speculate about how a morphine-induced increase in CBG might contribute to drug seeking behavior. A number of possibilities seem feasible. First, in view of studies showing that stress can potentiate the initiation of opiate self-administration (e.g., Alexander et al., 1981; Carroll et al., 1979; Dió, 1985; Shaham et al., 1993), it seems plausible that an increase in CBG might contribute to the perpetuation of drug use by compromising a primary mechanism for coping with stress. Thus, a positive cascade can be envisioned where stress potentiates the use of drugs, which in turn compromises the individual’s ability to cope with stress and, thereby, perpetuates drug use. Second, considering that corticosterone appears to enhance psychomotor effects of opiates (Deroche et al., 1995; Leyton and Stewart, 1990; Molina et al., 1994; Shaham et al., 1995), it is conceivable that the increase in CBG and concomitant decrease in physiologically active corticosterone with chronic drug exposure would blunt the psychomotor effects of morphine and, thereby, lead to a compensatory increase in drug use. Thus, an increase in CBG might contribute to the perpetuation of drug use at multiple levels.

Finally, it seems appropriate to mention that a morphine-induced increase in CBG might contribute to some adverse affects of drug use. In particular, over the past several years, it has become increasingly clear that glucocorticoids are necessary for the development and maintenance of normal immunity (as reviewed by Jefferies, 1991). Thus, a morphine-induced increase in CBG might impair immunity and contribute to the heightened susceptibility of opiate addicts to infectious diseases, including HIV, and the development of AIDS (acquired immunodeficiency syndrome; Donahoe and Falek, 1988; Horsburgh et al., 1989; Lazzarin et al., 1984; Rouveix, 1992).

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References


GOEDERS, N. E. AND GUERRI, G. F.: Non-contingent electric footshock facilitates


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