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Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats

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Abstract *Rationale:* Stress and one of the physiological components of most stress responses, glucocorticoid hormones (CORT), are known to influence the rewarding effects of a number of drugs of abuse. We have previously shown that an acute uncontrollable stressor (inescapable shock, IS) potentiates the rewarding effects of morphine using conditioned place preference (CPP). *Objectives:* The following experiments were conducted to determine the role of CORT in this process. *Methods:* First, the CORT response to 3.0 mg/kg morphine was measured in male Sprague–Dawley rats 24 h following exposure to IS. Second, we determined the effect of adrenalectomy (ADX) on the IS-potentiated CPP to morphine. Finally, we used the temporary CORT synthesis inhibitors metyrapone and aminoglutethimide to determine the necessity of CORT rises during either IS or morphine administration on the potentiated CPP response. *Results:* Prior IS significantly potentiated the CORT response to morphine. ADX significantly blocked the potentiated CPP to morphine produced by previous IS. However, CORT inhibition during

IS had no effect on the IS potentiation of morphine CPP, whereas inhibition during morphine administration completely blocked this potentiation. *Conclusions:* The results indicate that the CORT response to morphine is enhanced in rats that were previously exposed to an uncontrollable stressor, and that this response to the drug, not the stressor, is necessary for the stress-enhanced potentiation of morphine CPP.

Keywords Reward · Addiction · Opiate · Corticosterone · CORT · Adrenalectomy · Metyrapone · Aminoglutethimide · Inescapable shock

Introduction

There are a number of factors that may enhance the rewarding effects of drugs of abuse, leading to addictive behaviors such as dependence, withdrawal, and relapse. Stress is one such factor that influences the addictive effects of a variety of drugs. Clinical and animal studies have shown that exposure to an acute or chronic stressor leads to increased drug craving and self-administration, and stressors can induce relapse to drug craving and self-administration following periods of withdrawal (for review, see Sinha 2001; Lu et al. 2003).

We have previously demonstrated that exposure to an acute uncontrollable stressor, such as inescapable shock (IS), leads to a variety of behavioral and physiological changes that are not observed following exposure to the identical duration, intensity, and pattern of a controllable stressor (escapable shock, ES), a phenomenon that has been termed “learned helplessness” (Maier and Seligman 1976) or “behavioral depression” (Weiss et al. 1981). Behaviorally, these changes include poor escape learning and exaggerated fear conditioning (Maier 1993), as well as a change in the response to morphine (Will et al. 1998). For example, IS, but not equal amounts of ES, leads to an

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enhancement of the reinforcing effects of morphine as measured by conditioned place preference (CPP). Interestingly, this enhanced response occurs even when morphine is administered up to 7 days after IS, and in a different environment from that in which IS was administered. Glucocorticoids have been shown to be important in mediating some of the effects of stress on drug responsiveness (for review, see Piazza and Le Moal 1997; Marinelli and Piazza 2002), but the role of glucocorticoid hormones in this long lasting and trans-situational potentiation of morphine reward by IS is unknown.

Glucocorticoids, cortisol in humans and corticosterone (CORT) in rats, are released by a number of environmental challenges (Johnson et al. 1992; de Kloet 2000), including stress (Munck et al. 1984) and positively reinforcing stimuli (Piazza and Le Moal 1997). Although the precise mechanism by which stress, and CORT more specifically, alters the rewarding effects of drugs is unknown, there is abundant evidence implicating CORT as a possible mediator of the interaction between stress and drugs of abuse. For example acute administration of psychostimulants (Knych and Eisenberg 1979), opiates (Sable-Amplis et al. 1974), nicotine (Balfour et al. 1975), or ethanol (Ellis 1966), have been shown to increase plasma CORT levels in rodents. Although acute morphine treatment decreases plasma CORT levels in humans (Allolio et al. 1987), and chronic opiate and alcohol administration leads to the development of tolerance of the CORT response to these drugs in both rodents and humans (Spencer and McEwen 1990; Pechnick 1993; Kreek 1996). Nonetheless, surgical and pharmacological inhibition of CORT attenuates the reinforcing effects of cocaine (Goeders and Guerin 1996), while adrenalectomy with basal CORT replacement has been shown to block the stress-induced increase of the locomotor response to morphine and amphetamine (Deroche et al. 1992). However, it is not currently known whether the CORT response to a stressor, drug, or both, is critical for the stress-enhanced response to drugs of abuse.

Most studies that have examined the role of CORT during a stress-enhanced response to drugs of abuse administered the stressor and drug concurrently or very close together in time, thus making it difficult to differentiate between the importance of the animal's CORT response to the stressor and to the drug. However, the ability of stress to enhance the rewarding effects of drugs may independently rely on the hormonal response to either event. It is possible that the CORT response to IS is critical, or that IS might potentiate the CORT response to later morphine, with this exaggerated CORT response being a key mediator of IS-potentiated CPP. Of course, both could play a role. Here we examined the CORT response to morphine 24 h after IS to determine whether it is indeed exaggerated, and whether CORT blockade via adrenalectomy would attenuate the IS-enhanced CPP to morphine. Finally, CORT was pharmacologically inhibited during either the stressor or the drug administration to determine the necessity of CORT increases to each event during the IS potentiation of morphine CPP.

Materials and methods

Subjects

Adult male Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind., USA) weighing 280–400 g were housed in pairs in Plexiglas cages with food and water available ad libitum. The subjects were maintained in a climate-controlled colony room at 22°C on a 12 h light–dark cycle, and all experiments were conducted during the light phase (0600–1800 hours). All subjects were naive and allowed a minimum of 1 week adaptation prior to surgery or testing. All animal care and experimental procedures were in accord with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Apparatus

Conditioned place preference

The Plexiglas place preference apparatus measured 72×30×30 cm³ (length, width, height) and comprised two distinct conditioning environments and a neutral area. Each conditioning environment measured 30×30×30 cm³. One environment was striped horizontally with alternating 2 cm black and white electrical tape on the walls, while the other environment was striped vertically in the same manner. The floor of the apparatus was black sanded Plexiglas with 2 cm wire grid on the horizontal side and 3 mm wire mesh on the vertical side. The neutral area measured 12×30×30 cm³, was painted gray, and had no wire mesh or grid on the floor. During the conditioning phase, vertically and horizontally striped Plexiglas partition walls were inserted on the respective side of the neutral area to restrict the animals to their designated conditioning environment.

The activity of each subject was monitored by a Philips TC352A video camera (Lancaster, Pa., USA) mounted 1.5 m above the center of the CPP apparatus. The camera relayed the information of the subject's location to the Chromotrack Version 4.02 tracking software (Prototype Systems Ltd, Boulder, Col., USA) run on a PC compatible computer located in a separate room. The SA-3 tracker (San Diego Instruments, San Diego, Calif., USA) measured the subject's time spent within each of the three compartments.

Stressor

The stressor environment was a dimly lit room with dimensions of approximately 3×2.5×2.5 m³. IS occurred in Plexiglas restraining tubes which measured 17.5 cm in length and 7.0 cm in diameter. Each rat's tail extended from the rear of the tube and was taped to a Plexiglas rod measuring 4.0 cm in length. The front end of the tube was blocked by a Plexiglas plunger containing several air

holes. Unscrambled shocks were delivered by a source modeled after Grason-Stadler Model 700. Two copper strips were coated with a small amount of electrode paste, wrapped 4 cm apart around the midsection of the tail, and attached to two electrodes.

Procedure

Experiment 1: CORT response to morphine after IS

Rats were either left in their home cages (HC) or taken to a separate room where they received stressor treatment between 0900 and 1100 hours. Rats were placed in the restraining tubes and 100 1.0 mA tailshocks were administered, lasting 5 s each with a 60 s average between shocks. After IS, animals were returned to the colony room. Twenty-four hours later, at 1100 hours, serial blood sampling began in the colony room. Baseline samples were taken, immediately followed by morphine (3.0 mg/kg, SC) or vehicle administration. Samples were then taken 30, 60, and 90 min following drug/vehicle injections. Blood samples were obtained by taking the rat from its home cage, wrapping it gently in a towel, and restraining it with a Velcro strap, leaving the tail exposed. A small nick was made in the lateral tail vein and the tail was stroked until 200 μ l of whole blood was collected in a microfuge tube. This entire procedure lasted no longer than 1.5 min per rat. Blood samples were then spun in a centrifuge at 4°C and 50 μ l of plasma was aliquoted and stored at -20°C until assayed. Thus, the design was a 2 (IS versus HC) \times 2 (morphine versus vehicle) factorial. Total sample sizes were seven to ten in each group.

Total plasma CORT was measured by radioimmunoassay. Plasma samples (20 μ l) were diluted in 0.01 M PBS (1 ml) and heat inactivated at 75°C for 1 h. Samples and standards (25–2000 pg/tube) were then incubated overnight with antiserum (rabbit antibody B3-163; Endocrine Sciences, Inc., Tarzana, Calif., USA) and [³H] CORT (20,000 cpm/tube). Free CORT was separated from antibody-bound CORT with 500 μ l dextran-coated activated charcoal. Antibody-bound CORT was then mixed with scintillation cocktail (3 ml) and counted with a liquid scintillation counter (Packard, 1600TR). The assay sensitivity was approximately 0.5 μ g/ml for a 20 μ l plasma sample. Intraassay and interassay coefficients of variation were less than 10%.

Experiment 2: Effect of ADX on IS potentiation of morphine CPP

ADX surgery and verification Bilateral adrenalectomies (ADX) were aseptically performed under halothane anesthesia (Halocarbon Laboratories, River Edge, N.J., USA). All removed tissue was examined immediately to ensure complete removal of the adrenal gland. Sham-operated

animals received the identical procedure, except that the adrenal glands were gently manipulated with the forceps but not removed. Steroid replacement began for ADX animals immediately after surgery and continued for the remainder of the experiment. ADX animals received basal CORT (Sigma-Aldrich, St Louis, Mo., USA) replacement in their drinking water since this method has been shown to mimic the normal circadian pattern of CORT secretion (Jacobson et al. 1988). CORT was initially dissolved in ethyl alcohol (EtOH) and diluted to a final concentration of 25 μ g/ml in 0.2% EtOH. CORT-water also contained 0.9% saline. Sham animals received drinking water containing 0.2% EtOH. Rats were allowed 3–4 weeks to recover before testing.

For ADX verification, rats were briefly exposed to ether and decapitated 2 weeks after the end of behavioral testing. Whole trunk blood was collected, then spun and assayed as described above.

Behavioral testing Prior to each testing session, for tracking purposes a light assembly consisting of a red LED and two 1.5 V watch batteries encased in a 1.25 cm portion of a 15 ml conical tube was threaded into the tube cap mounted 1 week earlier on the subjects' heads. On day 1, between 1200 and 1400 hours, all subjects were individually exposed to the CPP apparatus. Subjects were initially placed in the neutral area and allowed to explore the entire preference apparatus for 20 min. This day served to assess the subjects' initial preferences and any possible box bias. Any rat which spent less than 4 min (20% of total time) in either environment was eliminated from the study. On day 2, half the rats received IS treatment, while the other half remained as non-stressed HC controls. On day 3, all subjects were weighed and given random counter-balanced assignments so that half were conditioned with morphine (3.0 mg/kg, SC) in the vertically striped side and half in the horizontally striped side. We have previously shown that a 3.0 mg/kg dose of morphine is optimal in revealing IS potentiation of CPP (Will et al. 1998). There were two conditioning trials per day, one with morphine and one with vehicle. Morning conditioning occurred between 1100 and 1200 hours, while afternoon conditioning occurred 4 h later. Half the animals received morphine conditioning in the morning and half in the afternoon. Animals were first injected and then 5 min later placed into the conditioning environment for 45 min. Subjects were conditioned with morphine to the drug-paired side and vehicle to the other side at the other time of day. On day 4, animals again were conditioned in the same manner except the order of presentation was reversed. On day 5, testing of CPP was conducted between 1200 and 1400 hours exactly as was performed on day 1. Subjects were simply placed in the neutral area of the preference apparatus and their presence in each compartment was measured for 20 min. Thus, the design was a 2 (ADX versus Sham) \times 2 (IS versus HC) factorial. Total sample sizes were seven in each group.

Experiment 3: Effect of temporary CORT inhibition on IS potentiation of morphine CPP

Behavioral testing One day prior to testing, a 2×2 cm² piece of reflective tape was attached to a rat collar (BAS, West Lafayette, Ind., USA), which was then loosely collared around each rat's neck for detection by the tracking system. The reflective tape eliminated the need for additional surgery to implant the LED assembly used in experiment 2. The CPP procedure was similar to experiment 2, except that metyrapone (100 mg/kg, SC) and aminoglutethimide (100 mg/kg, SC) (MA) were administered 2.5 h and 1.5 h, respectively, before initiation of either IS or morphine conditioning trials. Metyrapone, an inhibitor of 11-β hydroxylase, and aminoglutethimide, an inhibitor of cholesterol side-chain cleavage, were used in conjunction to be assured of complete suppression of endogenous CORT levels. The administration timepoints, doses, and volumes were taken from Plotsky and Sawchenko (1987) to also ensure low toxicity, and altered for use with our CPP protocol. Thus one IS and one HC group received MA before these treatments and vehicle before each conditioning trial. In the first pilot study, rats received either MA or vehicle before initiation of IS, and CORT suppression significantly blocked the glucocorticoid response to the stressor ($P<0.05$) (Fig. 1a). A second set of rats received vehicle before IS or HC treatment and MA before each conditioning trial. In the second pilot study, rats received either MA or vehicle in the morning, followed by morphine in the morning or afternoon. These timepoints are identical to the times during which rats would receive MA and morphine injections at some point in the CPP study. MA, administered once in the morning, was sufficient to block the CORT response to both morning and afternoon morphine injections ($P<0.05$) and would probably block the CORT response to saline during alternate conditioning trials as well (Fig. 1b). This was done so that any rewarding or aversive effects of MA itself would be equally associated with the two environmental contexts. A final set of IS and control groups received vehicle at each point in the experiment. Thus, the design was a 2 (IS versus HC)×3 (MA before IS/HC, MA before conditioning, MA before neither) factorial. Total sample sizes were seven to eight in each group.

Drugs

Morphine sulfate (NIDA) was dissolved in 0.9% sterile saline and injected subcutaneously at a dose of 3.0 mg/kg. Injection volume of morphine and saline was 1.0 ml/kg body weight. Metyrapone and aminoglutethimide (Sigma-Aldrich) were dissolved in propylene glycol. Injection volume of metyrapone was 0.5 ml/kg body weight, whereas injection volume of aminoglutethimide was 1.0 ml/kg body weight.

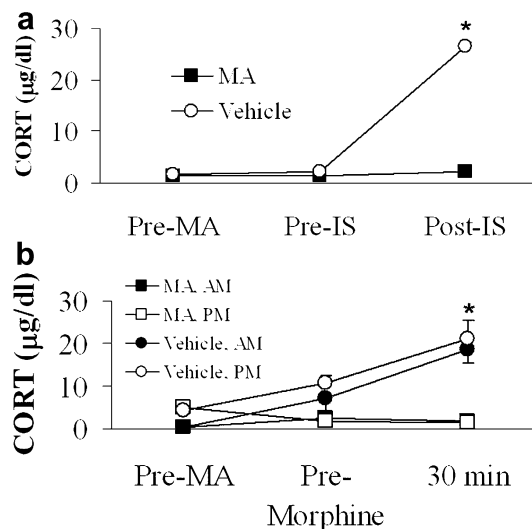


Fig. 1 **a** Corticosterone (CORT) response (mean±SEM, µg/dl) to inescapable shock (IS) following treatment with 100 mg/kg SC metyrapone and 100 mg/kg SC aminoglutethimide (MA) or vehicle. Serial blood samples were taken immediately before MA or vehicle injections (*Pre-MA*), immediately before initiation of IS (*Pre-IS*), and immediately after termination of IS (*Post-IS*). **b** CORT response (mean±SEM, µg/dl) to 3 mg/kg SC morphine. Rats were administered MA or vehicle in the morning, followed by morphine in the morning (*AM*) or 4 h later in the afternoon (*PM*). Serial blood samples were taken immediately before MA or vehicle injections (*Pre-MA*), immediately before morphine administration (*Pre-Morphine*), and 30 min after morphine administration (30 min) to assess the CORT response to morphine during the morning and afternoon CPP conditioning trials. *Different from MA injected rats. $P<0.05$

Statistical analysis

Data from the CORT response in experiment 1 were analyzed using a 2×2 ANOVA between stressor (IS versus HC) and drug administration (morphine versus vehicle) for the baseline samples and a 2×2×3 repeated measures ANOVA between stressor (IS versus HC), drug administration (morphine versus vehicle), and time (30, 60, and 90 min) for the remaining samples. The CPP experiment involving ADX was analyzed using a 2×2 ANOVA between stressor (IS versus HC) and surgery (ADX versus Sham), while the CPP experiment involving MA was analyzed using a 2×3 ANOVA between stressor (IS versus HC) and time of MA administration (before IS/HC versus before conditioning versus neither). Each ANOVA was followed by post hoc Newman-Keuls tests (alpha set at 0.05). During CPP testing, the dependent variable for measuring each subject's preference score was expressed as the difference in time (s) spent in the drug-paired environment between the pre-conditioning and post-conditioning test sessions. Positive scores indicate an increase in preference during the post-conditioning test for the previously morphine-paired environment.

Results

Experiment 1: CORT response to morphine after IS

The mean CORT responses are shown in Fig. 2. An ANOVA on the baseline measure revealed a main effect of stress [$F(1,29)=6.474$, $P<0.05$], indicating that rats receiving IS had elevated basal CORT levels relative to HC controls. The 3.0 mg/kg dose of morphine resulted in a slight elevation of CORT (13.7 ± 2.0 $\mu\text{g}/\text{dl}$) at the 30-min timepoint relative to vehicle controls. CORT returned to control levels 60 min following morphine administration and remained stable thereafter. More importantly, the CORT response 30 min after morphine nearly doubled in rats that received IS 24 h earlier (25.4 ± 1.1 $\mu\text{g}/\text{dl}$). CORT levels were decreasing, but still elevated, 60 min after morphine administration and returned to control levels 90 min after the injection. A repeated measures ANOVA revealed a main effect of stressor treatment [$F(1,29)=16.727$, $P<0.001$]. Although the overall interaction nearly approached significance ($P=0.11$), post hoc tests were conducted based on our a priori predictions and revealed that IS rats receiving morphine had significantly elevated CORT levels relative to IS rats receiving saline and HC rats receiving morphine 30 min after drug administration.

Experiment 2: Effect of ADX on IS potentiation of morphine CPP

Mean CPP scores are shown in Fig. 3. For animals receiving sham surgery, HC controls showed a modest increase in preference for the morphine-paired environment, and IS significantly potentiated this response. ADX completely blocked the IS enhancement of morphine CPP while having no effect in controls. An ANOVA revealed a main effect of stressor treatment [$F(1,24)=4.976$, $P<0.05$]. While the interaction nearly approached significance

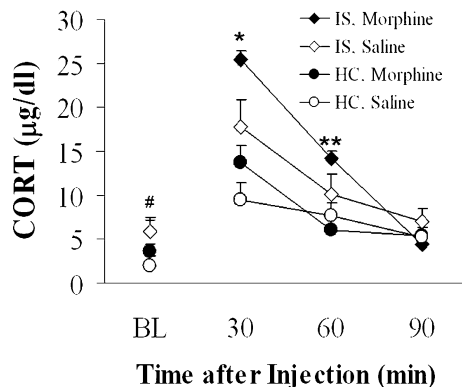


Fig. 2 CORT response (mean \pm SEM, $\mu\text{g}/\text{dl}$) to 3 mg/kg SC morphine or saline 24 h after exposure to inescapable shock (IS) or home cage (HC) treatment. #IS different from HC. $P<0.05$. *Different from IS rats injected with saline and HC rats injected with morphine. $P<0.05$. **Different from HC rats injected with morphine. $P<0.05$

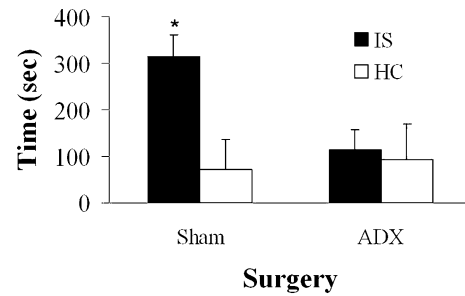


Fig. 3 Effect of adrenalectomy (ADX) on the inescapable shock (IS)-enhanced conditioned place preference (CPP) to morphine. Data are expressed as the difference in time (s) spent in the drug-paired environment between the pre-conditioning and post-conditioning test sessions. Positive scores indicate an increase in preference during the post-conditioning test for the previously morphine-paired environment. *Different from ADX-IS and Sham-HC groups. $P<0.05$

($P=0.07$), post hoc tests were conducted and revealed that IS rats receiving sham surgery showed significantly greater preference for the morphine-paired environment than non-stressed controls and IS rats receiving ADX surgery. ADX verification revealed that the CORT response to ether stress was normal in sham-operated animals and completely blunted in ADX animals [$F(1,28)=23.64$, $P<0.0001$].

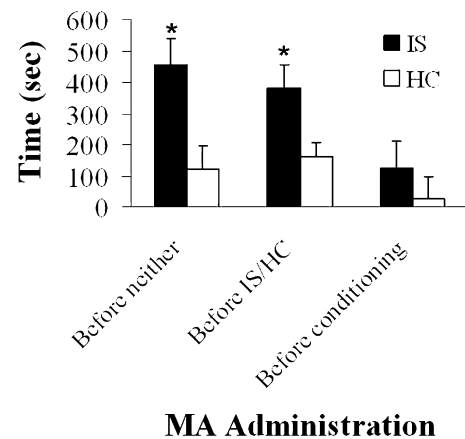


Fig. 4 Effect of temporary corticosterone (CORT) suppression during either inescapable shock (IS) or morphine conditioning on the IS-enhanced conditioned place preference (CPP) to 3 mg/kg SC morphine. The CORT response to either IS (before IS/HC) or morphine (before conditioning) was suppressed, or vehicle was administered throughout the experiment (before neither). CORT suppression was achieved by injecting each rat with 100 mg/kg SC metyrapone and 100 mg/kg SC aminoglutethimide (MA) before either IS or morphine administration. Data are expressed as the difference in time (s) spent in the drug-paired environment between the pre-conditioning and post-conditioning test sessions. Positive scores indicate an increase in preference during the post-conditioning test for the previously morphine-paired environment. *Different from the IS rats that received MA before conditioning and respective HC groups. $P<0.05$

Experiment 3: Effect of temporary CORT inhibition on IS potentiation of morphine CPP

Mean CPP scores are shown in Fig. 4. HC rats showed a modest increase in preference for the morphine-paired environment, and IS significantly potentiated this response. Inhibiting the CORT response to IS, but not to morphine, did not affect the potentiated CPP response. Rats receiving IS, but unable to produce a CORT response to the stressor, still exhibited an enhanced CPP to morphine. However, inhibiting the CORT response to morphine, but not to IS, completely blocked the potentiated CPP response. Therefore, rats receiving IS, but unable to produce a CORT response only to morphine, showed only a modest increase in preference, similar to non-stressed controls. An ANOVA revealed a main effect of CORT suppression [$F(2,41) = 5.254, P < 0.01$] and a main effect of stressor treatment [$F(1,41) = 12.996, P < 0.001$]. Post hoc tests revealed that IS rats with a suppressed CORT response to morphine showed significantly less preference for the drug-paired environment compared to the other two IS groups.

Discussion

The present findings confirm previous reports that IS enhances morphine reward as measured by CPP, even though the morphine is administered at a time removed from the stressor exposure and in a different environment (Will et al. 1998). Here we have demonstrated that a previous uncontrollable stressor enhances the CORT response to morphine as well, and that this response is necessary for the potentiation of morphine's rewarding effects as measured by CPP. It was already known that acute morphine elevates CORT levels in rodents (Sable-Amplis et al. 1974), and given the low dose of morphine used here, it is not surprising that CORT was only slightly elevated following drug administration. However, the results from the first experiment demonstrate that an acute uncontrollable stressor, such as IS, potentiates the CORT response to the same dose of morphine. Elevated CORT has been shown to increase dopamine (DA) levels in the shell of the nucleus accumbens (NAc) in a state-dependent manner (Piazza et al. 1996), a response that has been positively correlated with the reinforcing effects of a number of stimuli, including drugs of abuse (for review, see Robinson and Berridge 1993; Di Chiara et al. 1999; Koob 2000). Most drugs of abuse elevate NAc DA originating from midbrain ventral tegmental area cells (Koob and Nestler 1997), and CORT in response to a stressor may facilitate this rise. This could conceivably be a mechanism by which stress enhances the rewarding effects of abused drugs, although the specific interaction between CORT and DA is unknown. One possibility is that stress, along with a number of abused drugs, acts to increase synaptic strength at excitatory synapses on midbrain DA neurons (Saal et al. 2003). This effect of stress has been blocked by the glucocorticoid antagonist RU486, suggesting that the CORT response to stress may strengthen synapses on cells known to

mediate the reinforcing effects of drugs, thereby enhancing these effects.

Given that the IS-induced potentiation of CORT in response to morphine may mediate the reinforcing effects of the drug, suppression of CORT would then be expected to attenuate the stress-enhanced behavioral response to morphine. In fact, ADX completely blocked the IS potentiation of morphine CPP. However, the role of CORT on the stress-induced reinforcing and reinstating effects of drugs is not entirely clear. For example, ADX or metyrapone have been shown to reduce the reinforcing effects of cocaine in non-stressed rats as measured by self-administration (Goeders and Guerin 1996). This effect is partially reversed in rats receiving basal CORT replacement. However, ADX with basal CORT replacement blocks the enhanced locomotor response to both morphine and amphetamine by restraint stress (Deroche et al. 1992) and food restriction (Deroche et al. 1993). CORT suppression with basal replacement also blocks the enhanced locomotor response to morphine following social isolation (Deroche et al. 1994). The locomotor response to drugs of abuse has been argued to positively correlate with elevated DA within the NAc (Wise and Bozarth 1987). This suggests that basal CORT levels are necessary to observe the reinforcing and psychomotor effects of abused drugs, but stress-enhanced levels may be necessary to observe the potentiated rewarding effects due to stress. In the present study, a decrease in morphine CPP was likely not observed in non-stressed animals that received ADX treatment due to the effect of basal replacement. Contrary to these studies, other reports have concluded that blocking CORT synthesis has no effect on cocaine self-administration in rhesus monkeys (Broadbear et al. 1999) or the subjective reinforcing effect in humans (Ward et al. 1998). It should also be noted that CORT either plays no role (Le et al. 2000) or only a permissive role (Shaham et al. 1997; Erb et al. 1998; Shalev et al. 2003) in stress-induced reinstatement of drug-taking behavior. Only one other study has explored the effect of ADX on morphine CPP in non-stressed animals, citing an increase in preference for the morphine-paired environment compared to sham-operated controls (Suzuki et al. 1995). However, animals in this study did not receive basal CORT replacement and only one dose was tested with both ADX and sham-operated rats. Although the mechanism underlying this interaction remains unknown, the present results suggest that CORT is necessary for the stress-enhanced reinforcing effects of morphine.

Interestingly, nearly all of the studies that have examined the role of CORT on stress-enhanced drug reward used ADX to block the CORT response, which clearly blocks the CORT response to both stressor and drug exposure. What remained unclear was whether the CORT response to stress, drug, or both was critical for the stress-enhanced behavioral response to morphine. To address this question, the final experiment involved the use of temporary CORT synthesis inhibitors, metyrapone and aminoglutethimide, during either stressor or drug administration to determine the necessity of CORT rises to either

IS or to morphine in the production of IS-enhanced morphine reward. Only the CORT response to morphine, but not to IS, was critical for the development of an enhanced CPP in previously stressed animals. This is not entirely surprising given that both IS and ES elevate CORT to similar levels (Maier et al. 1986), yet ES fails to potentiate morphine CPP (Will et al. 1998). This suggests that elevated CORT levels in response to IS may not mediate the later behavioral response to morphine, as was confirmed in our study.

One interesting trend here is that non-stressed rats that had a suppressed CORT response to morphine seem to show lower CPP levels when compared to other non-stressed groups, possibly indicating that CORT suppression blocks the acute rewarding effects of morphine and not just the IS-enhancing effects. However, this may not be the case given that ADX with basal replacement in non-stressed animals failed to decrease CPP levels. Whether or not this is truly the case here remains unknown. The short conditioning procedure and low dose of morphine used allow for only a minimal CPP in non-stressed animals that is optimal for observing a potentiated response, rather than an attenuated one. Finally, MA during morphine treatment is likely not creating an aversive state that would influence CPP responding since CORT synthesis is being suppressed during both morphine and saline conditioning trials and the order of morphine and saline administration was counter-balanced following MA administration.

Despite the inhibitory actions of metyrapone on the synthesis of CORT, it should also be noted that this compound can exert other effects both centrally and peripherally. Most notably, metyrapone has been argued to act as a stressor because it increases plasma ACTH and glucose levels, both markers of the stress response (Rotllant et al. 2002), and decreases feeding, a behavioral consequence of stress, via a CRH-dependent mechanism (Jain et al. 1993). Presuming that the animals receiving metyrapone before morphine were being stressed, one might then expect metyrapone to enhance, not attenuate, the rewarding effects of morphine. In fact, metyrapone reinstates extinguished self-administration of heroin (Shaham et al. 1997), suggesting that the CORT synthesis inhibitor may also act as a pharmacological stressor. However, in the present study, metyrapone appears to have exerted its effects despite its stressor properties, not because of them. In addition, although it can be argued that while ACTH and glucose are markers of the stress response, their presence does not simply imply that the animal is experiencing a stressful event. Even with regard to *c-fos* induction, immunoreactivity was observed in many, but not all, stress-sensitive brain areas. Also, given the study by Rotllant et al. (2002), the effects of metyrapone on ACTH and glucose would be decreased and back to baseline, respectively, at the time of the morning conditioning session, suggesting that both of these values would be at baseline during the afternoon conditioning session. Again, given the fact that morphine and saline conditioning sessions were counterbalanced between the morning and afternoon, there should not be any side effects of metyrapone specifically during morphine

administration. Metyrapone has also been used to block liver metabolism of morphine (Ohno et al. 1988), but if this altered any central effects of the drug, one might again expect the rewarding effects of morphine to be enhanced, as was not the case here. Despite these possible limitations of the use of metyrapone, ADX would have an even more severe limitation here, namely that the CORT response to the stressor and to the drug could not be blocked selectively. Thus, along with their widespread use in studies examining the various effects of adrenal steroids, synthesis inhibitors proved to be the most useful option to temporarily suppress glucocorticoid release in the present study.

Previous studies examining the role of CORT in stress-enhanced drug reward were unable to demonstrate this effect of temporary CORT suppression due to the fact that ADX was performed before both stressor and drug administration, making it impossible to determine the importance of the hormonal response to each event. Deroche et al. (1994) did perform ADX following 6 days of social isolation, but before morphine administration, and stress-enhanced locomotor activity was suppressed. However, it is not known whether the specific CORT response to social isolation is necessary for this effect. In one other case, the CORT response to a stressor and drug administration has been differentiated, and metyrapone treatment before exposure to a stressor or cocaine attenuated stress-induced sensitization of psychomotor activity (Rouge-Pont et al. 1995). However, chronic metyrapone was administered throughout 8 days of food restriction, a stressor that is not known to produce the behavioral effects of learned helplessness and therefore might not produce the same potentiation of morphine reward observed here. Along these lines, an uncontrollable stressor such as IS fails to enhance the rewarding effects of psychostimulants, including amphetamine (Will et al. 1998) and cocaine (unpublished data) when testing is separated in time from IS and conducted in a different environment. This may be because the long-term trans-situational facilitation of morphine CPP by IS involves mediation by sensitized serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN). IS is known to selectively activate (Maswood et al. 1998; Grahn et al. 1999) and sensitize (Amat et al. 1998a,b) DRN 5-HT cells. Morphine also activates DRN 5-HT neurons (Jolas and Aghajanian 1997), and in an animal that has experienced IS in the recent past, this activation leads to exaggerated 5-HT release in projection regions of the DRN (Bland et al. 2003). This has been shown to enhance extracellular DA in the NAc shell (Bland et al. 2004) and may be the basis for the enhanced behavioral response to the drug. In fact, pharmacological inhibition of DRN 5-HT cells during IS or morphine administration completely blocks the enhanced DA response (Bland et al. 2004) and CPP (Will et al. 2004) to morphine. However, cocaine and amphetamine do not activate DRN 5-HT neurons, and so the fact that these neurons are sensitized is without influence. Thus, the studies of Rouge-Pont et al. (1995) would not be expected to have yielded data that parallels those reported here.

It is possible that DRN 5-HT sensitization is required in response to IS, along with a sensitized CORT response to morphine, in order for IS to potentiate the rewarding effects of morphine. The exact mechanism by which 5-HT and CORT may interact to mediate the enhanced behavioral response to morphine is unknown. Perhaps 5-HT and CORT both influence mesolimbic DA transmission. 5-HT has been shown to partly mediate enhanced DA metabolism in the NAc in response to morphine (Spampinato et al. 1984), and ADX can suppress extracellular DA in the NAc in response to morphine, an effect that is reversed by glucocorticoid replacement (Barrot et al. 2000). A determination of exactly how these two factors interact must await further research.

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