Mood and Neuroendocrine Response to a Chemical Stressor, Metyrapone, in Buprenorphine-Maintained Heroin Dependence

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Background: Heroin dependence is associated with a hyperactive hypothalamic-pituitary-adrenal (HPA) axis, proposed as a biological correlate of craving. Maintenance treatment with methadone normalizes HPA axis activity. Here, we examined HPA axis activity under maintenance treatment with the increasingly utilized partial opiate agonist buprenorphine.

Methods: Responses to a metyrapone challenge were compared in 20 buprenorphine-maintained heroin addicts and 20 healthy volunteers (10 received a single 50 mg naltrexone dose [NTX+] and 10 received no naltrexone [NTX−]). Patients were 16 male subjects and 4 female subjects, aged 30 to 38 years, heroin-dependent and relapse-free under buprenorphine maintenance (BUP) for a minimum of 6 months. Healthy volunteers were 9 male subjects and 11 female subjects, aged 36 to 49 years, with no history of dependence. Serial measures were obtained of plasma adrenocorticotropic hormone (ACTH) and cortisol and Profile of Mood States (POMS) ratings over time. Subjects were genotyped for the OPRM1 118A/G polymorphism.

Results: Buprenorphine maintenance showed a dampened HPA axis response to metyrapone, with OPRM1 118G carriers showing a significantly attenuated response compared with 118A carriers. The response of the NTX+ group was markedly increased. In contrast, negative affect was elevated in the BUP group but did not differ between NTX− and NTX+. Buprenorphine maintenance and NTX− groups did not differ in positive affect, whereas the NTX+ group was lower.

Conclusions: In contrast to exaggerated HPA axis responsiveness reported in untreated heroin dependence, response to metyrapone was subnormal in heroin addicts maintained on buprenorphine. Despite this, increased measures of negative affect were seen in this group. This implies a dissociation of HPA axis responsiveness and affect in heroin dependence.

Key Words: ACTH, cortisol, heroin, mood, stress

Recruitment of the hypothalamic-pituitary-adrenal (HPA) axis has been proposed as a biological substrate for craving and relapse in heroin dependence (1,2). In animal experiments, intermittent escalating morphine doses, a pattern resembling human heroin abuse, result in elevated basal adrenocorticotropic hormone (ACTH) and corticosterone levels (3). A similar basal ACTH and glucocorticoid elevation, which persists beyond the immediate withdrawal period, has been reported in human heroin addicts and was accompanied by blunted responses to emotional stimuli (4). Opioid receptors exert inhibitory control over HPA axis output, in part through inhibition of hypothalamic corticotropin-releasing hormone (CRH) (2). Repeated withdrawals rather than opiate taking per se are therefore likely to cause HPA axis dysregulation in opioid dependence. On the street, short-acting opioids such as heroin are taken intermittently, setting the scene for repeated cycles of withdrawal.

The hippocampal formation provides negative feedback control of the HPA axis, while prolonged hippocampal exposure to high glucocorticoid levels during chronic stress results in loss of hippocampal volume (5). This is initially thought to occur due to loss of dendritic arborization but ultimately may involve irreversible neuronal degeneration. Also, stress inhibits hippocampal neurogenesis in rodents and nonhuman primates (6). Loss of hippocampal volume results in a vicious circle of excessive glucocorticoid production, in which the HPA axis further escapes its normal regulation and becomes chronically hyperactive. In humans, several stress-related pathological conditions, including depression, Cushing's disease, and posttraumatic stress disorder (PTSD), are associated with hippocampal atrophy (7). It is presently unknown whether this also occurs in heroin dependence.

In addition to HPA axis recruitment, neuroadaptations encompassing extrahypothalamic CRH systems have been postulated in the pathophysiology of substance dependence (8,9). Independently of the HPA axis, CRH antagonists block excessive drug taking and stress-induced relapse to drug seeking (9), as well as stress-induced reinstatement of heroin seeking (10). Corticotropin-releasing hormone systems within the extended amygdala are likely to mediate these actions. In contrast to hypothalamic CRH, which is under negative feedback control from glucocorticoids, CRH expression and function within the extended amygdala are positively regulated by stress and glucocorticoids (11,12). Also, stressors that cause dendritic atrophy in the hippocampus cause extension of neuronal processes in the amygdala (13) and in the bed nucleus of the stria terminalis (14). Extrahypothalamic CRH is not directly involved in regulation of ACTH secretion but instead mediates behavioral stress responses. Recruitment of CRH signaling in the extended amygdala is an antireward process postulated to drive the progression from impulsive to compulsive drug use (9).

Two processes might thus act in concert in heroin dependence. Initial chronic HPA axis hyperactivity may lead to an escape from hippocampal negative feedback control. This, in

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turn, may drive a feedforward of CRH activity within the extended amygdala, resulting in escalating drug seeking and taking. Of interest for this framework, maintenance treatment with the full opiate agonist methadone (MMT) can normalize HPA axis activity in heroin addicts, and this mechanism has been suggested as a possible factor contributing to its therapeutic efficacy (15). More recently, therapeutic efficacy in heroin dependence has also been demonstrated with the partial opioid agonist buprenorphine (16,17). It is presently unknown whether this compound shares with methadone an ability to dampen a hyperactive HPA axis in heroin addicts. Finally, the opioid antagonist naltrexone is also used in heroin dependence, but results in unselected patients are not encouraging (18). Inability of naltrexone to substitute for rewarding properties of heroin presumably contributes to its limited therapeutic efficacy. However, an interesting possibility is that HPA axis activation by naltrexone (19) also contributes to this.

In summary, it has been proposed that a normalization of pathological HPA axis reactivity is important for clinical efficacy in treatment of heroin dependence. The documented ability of MMT to achieve such normalization is clearly consistent with this proposition. However, the lack of other effective treatments for heroin dependence has, until recently, restricted opportunities to evaluate whether this reflects a general mechanism. Evidence now available for a clinical efficacy of buprenorphine offers an opportunity to independently examine the possible link between HPA axis normalization and clinical efficacy in heroin dependence. Here, we therefore investigated HPA axis reactivity after maintenance treatment with buprenorphine, using the metyrapone challenge (20). For comparison, HPA axis reactivity was examined after an acute dose of naltrexone. To assess a possible correlation between neuroendocrine response and negative affect, mood ratings were obtained in parallel with hormone levels. Finally, because genetic variation at the μ-opioid receptor gene (*OPRM1*) (21) is associated with heroin dependence (22) and differential HPA axis reactivity (23), we assessed the contribution of this variant to our results.

Methods and Materials

The study was approved by the Karolinska South Human Subjects Ethics Committee (Dnr 374/03). All subjects received written and oral information and gave their informed consent as approved by the ethics committee. Twenty buprenorphine-maintained heroin-dependent subjects (daily dose in all cases 16–36 mg except for one subject on 4 mg) were recruited by word of mouth at a large clinic for pharmacologically assisted treatment of heroin dependence in Stockholm. These subjects had been stable in treatment for 6 months or longer and were relapse-free as documented by negative weekly supervised urine toxicology. Urine samples were analyzed and found to be negative for illicit opiates, cannabinoids, central stimulants, and benzodiazepines. Cocaine was not routinely analyzed, since it is almost never encountered in this population. Twenty healthy volunteers with no history of any substance use disorder (with the exception of allowing presence of nicotine dependence) and not using any prescription drugs were recruited by word of mouth among health care professionals. Subjects were compensated by receiving gift certificates worth 1000 Swedish krona (SEK) (approximately $120) on completion of the study.

To assess the endocrine stress response, the standard metyrapone challenge was carried out, as described previously (20). In brief, subjects were instructed to receive nothing by mouth except water for at least 9 hours before testing. At 8:00 AM of the testing day, they arrived at the research unit, were given an intravenous (IV) line, and were allowed bed rest. At 9:00 AM, 2.25 grams of metyrapone (Metopirone, Novartis, Basel, Switzerland) was administered orally with 30 cc of an oral antacid to minimize gastrointestinal upset. At 10:00 AM, buprenorphine-maintained subjects received their regular buprenorphine dose, while 10 of the healthy volunteers who had been randomized to single dose naltrexone received 50 mg of this compound by mouth (PO) in an unblinded fashion. Subjects were allowed to eat at 11:00 AM. Subjects who were smokers were not permitted to smoke until 1:00 PM. Blood samples for plasma ACTH and cortisol levels were obtained at 9:00 AM (just before metyrapone administration) and at 1:00 PM (prior to allowing any smoking) and 5:00 PM, i.e., 4 and 8 hours later. Blood was drawn into sodium ethylenediamine tetraacetic acid (EDTA) vacutainer tubes and stored on ice for up to 40 min until centrifuged at 3000 rpm for 5 min. Plasma was then removed, aliquoted, and stored at −40°C until assayed. Samples were analyzed according to regular clinical routine by the SWEDAC accredited clinical chemistry laboratory of the Karolinska University Hospital.

At 1, 2, 4, and 8 hours following administration of metyrapone, assessment of mood was carried out using the established Profile of Mood States (POMS) self-report instrument (24). This instrument is thought to assess transient (state-dependent) mood changes, originally divided into six dimensions: depression, tension-anxiety, anger, fatigue, confusion, and vigor.

DNA was purified from whole blood using standard methods. Genotyping of the single nucleotide polymorphism (SNP) 118A/G or Asn40Asp (rs1799971) was performed by a TaqMan allelic discrimination assay using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, California) following the manufacturer's protocol. Briefly, 10 ng DNA was amplified in a final volume of 10 μL containing .5 μL of 20X MGB probe and primers and 5 μL of 2X TaqMan Universal PCR Master Mix in a 384-well microplate format (Applied Biosystems Inc.). Amplification conditions were 50°C for 2 min then 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Fifteen percent of the samples were genotyped in duplicates as a quality check, with complete concordance.

STATISTICA 7.0 (StatSoft, Tulsa, Oklahoma) was used for all analyses. Cortisol and ACTH data were analyzed separately, in each case using the general linear model module, with repeated hormone values as a within-subjects factor, group and sex as between-subjects factors, and age, body mass index (BMI), and buprenorphine dose (the latter on analyses within buprenorphine patient group only) as covariates. Profile of Mood States scores were subjected to a factor analysis. Principal component factors were extracted and rotated using normalized varimax rotation. Repeated measures analysis of variance (ANOVA) was carried out, with factor scores on the respective factor as dependent variable, time as repeated measures/within-subjects factor, and group as between-subjects factor.

Results

Patient characteristics are shown in Table 1. The groups differed in some baseline characteristics of potential relevance, most notably age and sex composition. Baseline characteristics were therefore included in the initial analysis as specified below, as covariates or factors, but these were dropped if they did not contribute significantly or at a trend level (threshold $p = .10$). Furthermore, although the sample size was not designed with an

Table 1. Subject Characteristics

	Untreated Healthy Control Subjects	Naltrexone-Treated Healthy Control Subjects	Buprenorphine-Maintained Heroin Addicts
Male/Female	5/5	4/6	16/4
118A/A	8	9	14
118A/G	2	1	4
118G/G	0	0	2
Age (95% CI)	42.2 (35.7–48.7)	42.1 (39.5–44.7)	34.2 (30.2–38.1)
BMI (95% CI)	24.1 (22.5–25.8)	24.9 (22.2–27.7)	24.4 (23.0–25.9)
Buprenorphine Dose (95% CI)	NA	NA	20.5 mg (17.4–23.6)

BMI, body mass index; CI, confidence interval.

association study in mind, the frequency of the *OPRM1* 118G allele was 20.0% in heroin-dependent subjects but only 7.5% in healthy volunteers, yielding a trend for a statistical significance ($\chi^2 = 2.64$; $p = .10$). To evaluate whether the different 118G allele frequency contributed to the metyrapone challenge results and psychological ratings, all analyses were replicated excluding 118G carriers, and the contribution of genotype was further explored in some secondary analyses as described below.

Adequate suppression of cortisol production by metyrapone administration is shown in Figure 1. Age and BMI were not significant covariates and were therefore dropped from the final analysis. The most robust effect was the main, within-subjects effect for change of cortisol over time [$F(2,32) = 90.6$, $p < .0001$]. There was also a main affect of group [$F(2,32) = 14.8$, $p < .0001$] and of sex [$F(2,32) = 13.4$, $p < .001$], but this did not interact with the metyrapone effects, leading to the conclusion that metyrapone-induced suppression of cortisol did not affect the groups differentially. Within the buprenorphine-maintained group, the daily dose of buprenorphine was evaluated as a potential covariate and did not affect the cortisol response [$F(1,17) = .2$, $p = .7$]. The results of these analyses were very similar when repeated excluding 118G carriers (data not shown).

Differential ACTH response to the metyrapone challenge is shown in Figure 2A. For the ACTH response, age and BMI were not significant as covariates and were therefore dropped from the final analysis. Similarly, sex was not a significant factor and was therefore also dropped. There was a significant response of ACTH to the metyrapone challenge [$F(2,74) = 36.7$, $p < .0001$] and main effect of group [$F(2,37) = 17.9$, $p < .0001$]. Most importantly, a differential response of ACTH between the groups was shown by a highly significant interaction term [$F(4,74) = 7.5$, $p < .0001$]. Post hoc comparison using Newman-Keuls test showed that both the naltrexone ($p = .001$) and the buprenorphine ($p = .027$) groups differed from the control group. Within the buprenorphine-maintained group, the daily dose of buprenorphine was evaluated as a covariate and did not significantly affect the ACTH response [$F(1,18) = .7$, $p = .4$].

Following exclusion of 118G carriers, response over time [$F(2,58) = 31.2$, $p < .0001$], main effect of group [$F(2,28) = 14.0$, $p = .00006$], and the interaction [$F(4,58) = 5.3$, $p = .001$] remained significant. However, on post hoc analysis, the naltrexone group still robustly differed from both untreated control subjects ($p = .002$) and buprenorphine-maintained subjects ($p = .0002$), but the buprenorphine-maintained group and untreated control subjects no longer differed ($p = .15$, ns).

The contribution of the 118G allele to the ACTH response was supported by secondary analyses within the group of buprenorphine-maintained heroin addicts. Controlling for buprenorphine dose, age, BMI, and sex, there was within this group a significant main effect of genotype [$F(1,13) = 5.6$, $p = .03$] and a highly significant interaction between genotype and the time course of ACTH response over time [$F(2,26) = 8.9$, $p = .001$]. In fact, as seen from the figure, 118G carriers simply lacked an ACTH response to the metyrapone challenge (Figure 2B).

For POMS data, a scree plot indicated that a three-factor solution best described the underlying structure in our data. These three factors together explained 46.2% of the variance. First, a factor that accounted for 17.4% of the variance received loadings from items on the vigor subscale of the POMS, which reflect elevated mood, vigor, or energy. We hereafter label it "positive affect." All results reported below were virtually identical whether scores on the traditional vigor subscale or scores on the positive affect factor were used, but the residual variance was consistently lower using the latter, which was therefore ultimately used for the analysis. Second, a factor which accounted for 17.1% of the variance received loadings from items on the depression, tension-anxiety, and anger subscales, which in our extraction were highly correlated, similar to what has been published in some other populations (e.g., 25). Given the high degree of correlation between these measures, analyzing them as independent outcomes was considered inappropriate. Instead, this composite factor was labeled "negative affect" and was used for all subsequent analyses. Third, a factor contributing 11.7% of the variance received loadings from items on both the fatigue and confusion subscales, which again were highly correlated. This factor was not of interest for the current study and was not further analyzed.

Analysis of variance of factor scores on negative affect yielded a highly significant group effect [$F(2,148) = 9.8$, $p = .0001$]. Post hoc analysis using Newman-Keuls test showed that the buprenorphine-maintained group differed from both untreated control subjects ($p = .002$) and naltrexone-treated healthy subjects ($p = .001$), which in turn were indistinguishable ($p = .98$). There was no main effect for change of scores on this factor over time [$F(3,148) = 1.4$, $p = .24$], nor was there any interaction between group and time [$F(6,148) = .14$, $p = .99$]. When this analysis was repeated excluding 118G carriers, results remained virtually identical (data not shown). Negative affect scores for the three groups over time are shown in Figure 3.

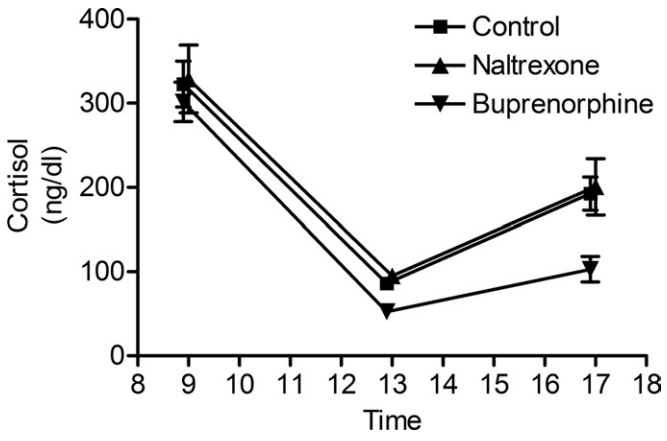

Figure 1. Effective suppression of cortisol production by a metyrapone challenge in buprenorphine-maintained heroin addicts ($n = 20$), untreated healthy control subjects ($n = 10$), and healthy control subjects treated with a single oral 50 mg dose of naltrexone ($n = 10$).

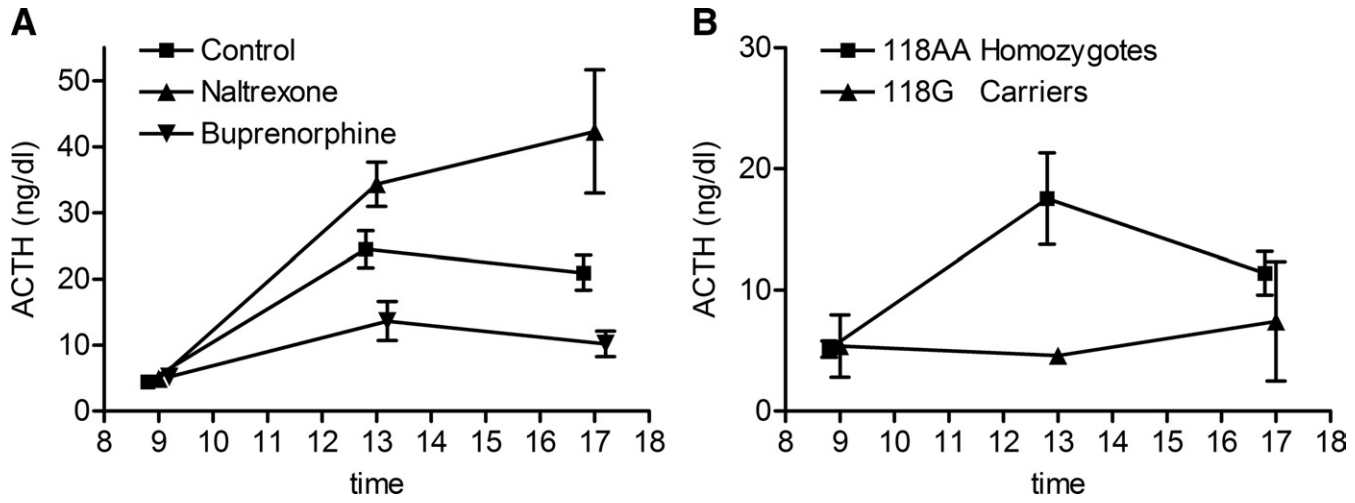

Figure 2. (A) Differential ACTH response to the metyrapone challenge in the three experimental groups. There was a highly significant main effect of group ($p < .0001$), and a differential response of ACTH between the groups was shown by a highly significant interaction between ACTH over time × group ($p < .0001$). Both the naltrexone-treated healthy volunteer group ($p = .001$) and the group of buprenorphine-maintained heroin addicts ($p = .027$) differed from the control group. The significant suppression of the ACTH response to metyrapone in buprenorphine-maintained subjects was eliminated by the removal of OPRM1 118G allele carriers ($p = .15$, ns). This indicated that 118G carriers, who were overrepresented in the heroin-dependent group, contributed to the result. This group was therefore further analyzed in panel B. **(B)** Analysis of ACTH response to metyrapone as a function of OPRM1 118A/G genotype within the group of buprenorphine-maintained heroin addicts. A significant main effect of 118A/G genotype was seen ($n = 14$ and $n = 6$, respectively; $p = .03$) and interacted highly significantly with the time course of the ACTH response ($p = .001$). Essentially, 118G carriers lacked an ACTH response to metyrapone. ACTH, adrenocorticotropic hormone.

Analysis of variance of factor scores on positive affect also showed a significant group effect [$F(2,148) = 4.7$, $p = .01$]. Here, post hoc analysis using Newman-Keuls test showed that the naltrexone group deviated from the other two groups ($p = .04$ vs. untreated control subjects, and $p = .01$ vs. the buprenorphine-maintained subjects, respectively). There was no main effect for change of scores on this factor over time [$F(3,148) = .3$, $p = .80$], nor was there any interaction between group and time [$F(6,148) = .50$, $p = .81$]. When this analysis was rerun excluding 118G allele carriers, results were similar. The main group effect was still trend-level significant [$F(2,112) = 2.3$, $p = .10$], with the post hoc comparison of the naltrexone-treated group versus untreated control subjects approaching significance at $p = .06$, while the buprenorphine-maintained group still did not differ from untreated control subjects ($p = .34$). Positive affect scores for the three groups over time are shown in Figure 4.

Discussion

We have performed an experimental study of the HPA axis response to a metyrapone challenge in a group of 20 buprenorphine-maintained heroin addicts compared with 20 healthy volunteers, half of whom received an acute dose of 50 mg naltrexone. During the metyrapone provocation, subjects were

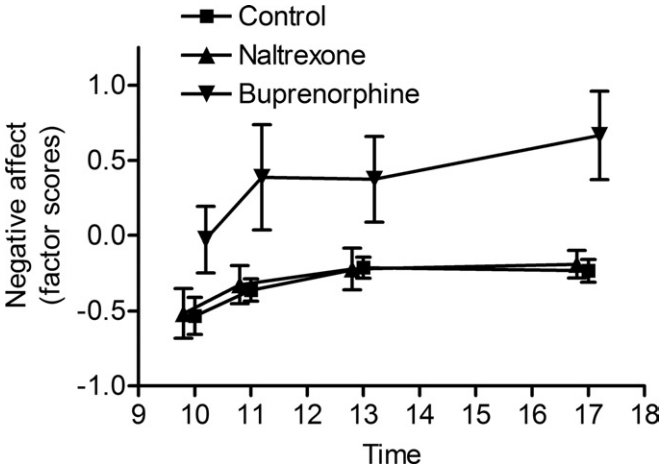

Figure 3. Negative affect scores on Profile of Mood States (POMS) for the three groups over time. There was a highly significant main effect of group ($p = .0001$). The buprenorphine-maintained group differed from both untreated control subjects ($p = .002$) and naltrexone-treated healthy subjects ($p = .001$), which in turn were indistinguishable ($p = .98$). When this analysis was repeated excluding 118G carriers, results remained essentially identical. POMS, Profile of Mood States.

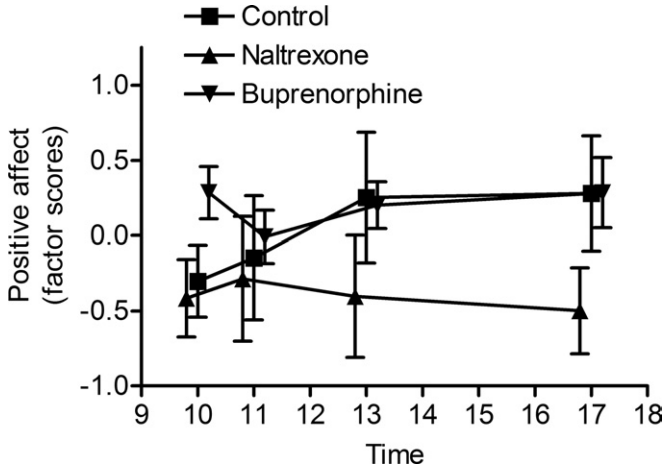

Figure 4. Positive affect scores for the three groups over time. There was significant main effect of group ($p = .01$). The naltrexone group deviated from the other two ($p = .04$ vs. untreated control subjects and $p = .01$ vs. the buprenorphine-maintained subjects, respectively). When this analysis was rerun excluding 118G allele carriers, results were similar.

reporting psychological ratings to investigate a potential link between neuroendocrine response and mood. The buprenorphine-maintained heroin addicts showed an attenuated HPA axis response to metyrapone compared with control subjects, whereas the response of the group that received naltrexone was markedly increased. In contrast, negative affect did not differ between the untreated control group and the group of healthy volunteers given naltrexone but was more intense in the buprenorphine group. The buprenorphine and the control groups did not differ in positive affect, whereas naltrexone-treated healthy volunteers reported lower scores on this dimension.

Our study indicates that the exaggerated HPA axis response to metyrapone previously reported in untreated heroin dependence is normalized by 6 months of successful buprenorphine maintenance. The buprenorphine finding is in agreement with previous data indicating a similar ability of methadone to achieve such a normalization (26). Unexpectedly, the HPA axis response of our buprenorphine-maintained subjects to metyrapone was actually subnormal, a pattern that is somewhat different from what has typically been reported with methadone-maintained individuals, although some studies have reported subnormal HPA axis responsiveness also in this population (27–29). The suppressed metyrapone response in buprenorphine-maintained subjects was found despite increased negative affect, as discussed below. It has previously been reported that high neuroticism, a personality trait characterized by increased propensity for negative affect, results in an HPA axis response to naloxone challenge indicative of adrenal hypertrophy. This is likely to reflect an integrated history of excessive HPA axis activation in response to emotional stimuli (30). Although not directly comparable, our data indicate that buprenorphine maintenance is capable of suppressing this type of excessive endocrine stress responses despite the fact that abnormal levels of negative affect persist. Importantly, carriers of the *OPRM1* 118G allele contributed disproportionately to the dampening of ACTH response in the buprenorphine-maintained group and basically lacked a response to the challenge. The *OPRM1* 118A/G variation has previously been shown to confer differential HPA axis response to naltrexone (23). The suggestive finding of a more potent HPA axis suppression by buprenorphine in 118G carriers is in general agreement with these data and can be thought of as their mirror image.

A single dose of the opiate antagonist naltrexone predictably increased the activity of the HPA axis in healthy volunteers. When designing the study, this group was included as a positive control group. We did not investigate the effect of naltrexone in heroin addicts because of the risk that a potentiation by naltrexone of metyrapone-induced HPA axis activation may lead to increased craving with subsequent increased risk for relapse. However, the ability of naltrexone to activate the HPA axis was established early on (19). This is presumably the result of removing inhibitory tone of endogenous opioid peptides acting to suppress HPA axis activity both at a pituitary and hypothalamic level (2). To the extent that exaggerated activation of the HPA axis constitutes a risk factor for relapse, these data suggest caution in treating heroin dependence with naltrexone. Additional caution may be prompted by the present finding that naltrexone administration suppressed positive mood. This may in part be related to an ability of naltrexone to block μ-opioid receptors in the ventral tegmental area (VTA), leading to a decrease in dopaminergic tone (31,32), although other dopamine independent mechanisms may also be involved (33,34). Irrespective of mechanism, reduction of positive mood by naltrexone presumably is an undesirable effect and may be related to known compliance issues with this medication.

A main finding of this study is an unexpected double dissociation of endocrine response and negative mood following metyrapone challenge. Thus, despite normalized HPA axis response, heroin-dependent subjects reported significantly higher intensity of negative mood, while naltrexone-challenged normal subjects, despite their excessively activated HPA axis, did not differ in this parameter from untreated control subjects. This pattern was independent of genotype. Two things are important to note with regard to the mood data. First, the mood effects observed are likely to reflect group differences that were independent of the metyrapone challenge, since they were in both cases detected as main effects of group in the absence of a significant group × time interaction over the course of the challenge session. Secondly, corresponding data are to our knowledge not available from methadone-maintained heroin addicts. The following discussion is therefore restricted to the dissociation between mood state and metyrapone responses found under buprenorphine treatment.

We did not directly assess measures of craving, because this would not be meaningful in healthy volunteers without a history of opiate abuse. However, we measured negative affect, which can be assessed in heroin addicts and healthy control subjects alike and which in the former population has been shown to correlate with craving for opiates and relapse (35–37). Furthermore, recruitment of negative affect has recently been postulated as an important antireward mechanism in the development of addiction (9). Our data suggest that a direct contribution of the HPA axis to negative affect and craving related to this mood state is unlikely. However, additional components of craving are known to exist, which are unrelated to negative affect (38,39) and could still be linked to HPA axis function. An alternative or complementary possibility is that negative affect in heroin-dependent subjects is primarily the result of increased activity in extrahypothalamic stress circuits that initially may result from but over time become relatively independent of the exaggerated HPA axis activity in these subjects. In particular, a recruitment of CRH signaling within the extended amygdala is a candidate antireward mechanism that might initially be driven by a hyperactive HPA axis (11,12) but subsequently become largely autonomous and contribute to a transition from impulsive to compulsive drug use. Dysregulation of this circuitry has been hypothesized as being particularly slow to return to homeostasis and may confer a negative affective state that persists long into stable maintenance treatment (9). In addition to CRH, serotonergic neuroadaptations could contribute to this process (40).

Regardless of the relation between increased HPA activity and craving for heroin, we have shown that buprenorphine does normalize the otherwise hyperactive HPA axis in heroin dependence. Given the potential for chronic HPA axis hyperactivity to cause hippocampal and other pathology, this would appear to be an important aspect of buprenorphine's therapeutic properties.

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