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Paradoxical Effects of Chronic Morphine Treatment on the Temperature and Pituitary-Adrenal Responses to Acute Restraint Stress: A Chronic Stress Paradigm

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Key words: restraint stress, morphine, temperature, HPA axis.

Abstract

Body temperature and pituitary-adrenal responses to restraint (15 min or 4 h) stress were evaluated in nondependent and morphine-dependent rats. Male Sprague-Dawley rats were treated twice daily with increasing doses of morphine (10–100 mg/kg, s.c.) for 16 days. Transmitters were implanted in the peritoneal cavity to monitor body temperature and blood was collected for hormone assays. Acute withdrawal from chronic morphine treatment was associated with reduced body weight, increased adrenal weight and decreased thymus weight. Sixteen days after termination of chronic morphine treatment, rats had recovered normal adrenal size, but still displayed marked thymus involution and reduced body weight. Restraint-induced hyperthermia was attenuated in morphine-dependent rats that had undergone 12-h withdrawal. Sixteen days after withdrawal, rats still had not fully recovered the hyperthermic response to restraint. Chronic morphine treatment resulted in a marked elevation of basal corticosterone concentrations. Despite the negative-feedback effects of elevated basal corticosterone concentrations, morphine-dependent rats that had undergone 12-h withdrawal displayed a potentiated and prolonged corticosterone response to restraint stress. In contrast, rats that had undergone 8-day and 16-day morphine withdrawal had recovered normal basal pituitary-adrenal activity, but displayed significantly reduced and shorter adrenocorticotropic hormone and corticosterone responses to restraint. These results suggest that chronic morphine dependence is a chronic stressor, resulting in profound and long-lasting changes in the temperature and pituitary-adrenal responses to acute restraint stress in a time-dependent manner. This morphine-dependence model may be useful in understanding the role that hormonal stress responses play in the maintenance and relapse to opioid use in humans.

Epidemiological and clinical studies have suggested that exposure to stress may be related to opioid use and relapse in humans (1). In support of these observations, a number of laboratory studies have shown that exposure to stress alters the pharmacological effects of drugs commonly abused by humans, such as opioids. It has been shown that stress enhances both the reinforcing (2–4) and psychomotor (5–7) effects of opioids. Stress-induced enhancement of the pharmacological effects of morphine appears to be mediated, in part, by the hypothalamic-pituitary-adrenal (HPA) axis. Blockade of corticotropin releasing hormone (CRH) type I receptors or suppression of stress-induced release of corticosterone reduces the stimulating effects of stress on the reinforcing (8, 9) and psychomotor (6, 7, 10) properties of morphine.

It has also been shown that the temperature responses to stress are modified in animals treated with morphine. Acute administration of morphine produces hyperthermia at low doses and hypothermia at high doses (11–16). Rats chronically treated with morphine develop tolerance to the hypothermic, but not the hyperthermic, effects of morphine (13, 14). Furthermore, administration of the opioid antagonist naloxone produces a marked hypothermic response in morphine-dependent rats (16, 17). Restraint stress has been shown to produce a hypothermic response in rats treated acutely with a dose of morphine that produces hyperthermia

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in freely moving rats (18–22). Moreover, these exaggerated temperature responses to morphine in stressed rats are blocked by the synthetic glucocorticoid dexamethasone and mimicked by hypophysectomy, suggesting that activation of the HPA axis may be involved in these responses (18, 21). Wright and Katovich (23) reported that restraint does not significantly alter core body temperature in either naïve or morphine-dependent rats. On the other hand, studies in monkeys have shown that the hypothermic response to restraint in nondependent monkeys is markedly exaggerated when chronically treated with morphine (24).

Previous studies have also shown that the HPA axis responses to stress are altered in morphine-treated animals. Acute morphine administration activates the HPA axis, stimulating the release of CRH by the hypothalamus, thereby resulting in the release of adrenocorticotropic hormone (ACTH) from the pituitary and corticosterone from the adrenals in rats (25–27). Chronic administration of morphine results in tolerance to the corticosterone-stimulating effects of morphine (25, 27, 28), whereas withdrawal from morphine results in marked increases in plasma corticosterone concentration (27, 29–31). Chronic morphine treatment has also been reported to decrease hippocampal glucocorticoid receptor density (32) and increase serum corticosteroid binding globulin (33) in rats, thereby potentially influencing the negative-feedback effects of circulating corticosterone as well as the amount of free corticosterone, respectively. The effects of morphine on the HPA axis suggest that exposure to opioids may alter subsequent hormonal responses to stress. Indeed, it has been shown that acute morphine treatment increases the HPA axis responses to laparotomy and to ether vapor stress (25, 26, 34), whereas the HPA axis responses to laparotomy stress are reduced in rats rendered tolerant to morphine (25). Similarly, acute intermittent exposure both to morphine and to water restriction has been shown to decrease the ACTH response to water restriction stress alone (35).

Thus, a number of studies have investigated the interactions between stress and morphine on the temperature and HPA axis responses to these stimuli. However, few studies have evaluated the responses to stress in rats chronically treated with morphine. This is quite surprising, considering the possibility that altered physiological responses to stress may contribute to the maintenance or relapse to drug use in opioid-dependent subjects. Thus, the purpose of our investigation was to determine how the temperature and pituitary-adrenal responses to stress are modified in morphine-dependent rats maintained on morphine and those undergoing withdrawal from morphine. To this end, we examined the temperature and pituitary-adrenal responses to restraint stress in nondependent rats, in morphine-dependent rats maintained on morphine and in morphine-dependent rats that had undergone 0.5, 8 or 16 days of morphine withdrawal.

Materials and methods

Subjects

Male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN, USA), weighing 250–350 g at the beginning of experiment, were used. Rats were housed singly in Plexiglass cages in a room maintained at 20°C, 40–50% humidity with a 12 h/12 h light/dark cycle (light from 07.00 h to 19.00 h) with free access to food and water. Experiments were initiated between 07.00 h and 07.30 h and each experimental group consisted of 5–11 rats. All experimental procedures were performed according to the Guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare), and were approved by the University of Michigan Committee on the Use and Care of Animals.

Body temperature measurement

After anaesthesia with xylazine (10 mg/kg, i.m.) and ketamine (100 mg/kg, i.m.) a transmitter (model ER-4000 E-Mitter, Mini Mitter, Sunriver, OR, USA) was i.p. implanted in the rats. Surgeries were carried out at least 5 days before initiation of experimental treatments, allowing the rats to recover normal body weights. The transmitters produced temperature modulated signals, which were sent to a receiver (model ER-4000 Receiver, Mini Mitter Co.) underneath the cage. Data were collected at 5-min intervals and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co.).

Experimental procedure

On the basis of previous studies, morphine dependency was induced by subcutaneous injections of morphine (Mallinickrodt Inc., St Louis, MO, USA) twice daily at 07.00 h and 19.00 h for 16 consecutive days (24, 32). During the first 10 days, the dose of morphine was increased by 10 mg/kg per injection each day from 10 to 100 mg/kg per injection, and during the last 6 days rats were maintained on 100 mg/kg per injection of morphine (Fig. 1). As a control, nondependent rats received twice daily s.c. injections of saline for 16 consecutive days. There were six experimental groups: (i) nondependent; (ii) morphine-dependent; (iii) 12-h precipitated withdrawal; (iv) 12-h spontaneous withdrawal; (v) 8-day spontaneous withdrawal; and (vi) 16-day spontaneous withdrawal. Rats within these experimental groups were either not exposed to restraint stress or were restrained for either 15 min or 4 h in their home cages by placing them in Plexiglass restraint tubes (internal diameter 6 cm; Fisher Scientific, Pittsburgh, PA, USA) provided with numerous air holes. On day 17, nondependent rats received an s.c. injection of saline without restraint or followed immediately by either 15-min or 4-h restraint. On this day, morphine-dependent and 12-h withdrawal rats were similarly treated except that they received an injection of morphine (100 mg/kg, s.c.) or saline, respectively, either alone or immediately prior to initiation of restraint. On day 17, rats in the 12-h precipitated withdrawal group received an injection of the opioid antagonist naltrexone (1 mg/kg, s.c.) in the absence of restraint to confirm opioid dependence and withdrawal. Rats in the 8- and 16-day withdrawal groups were not manipulated on day 17 and, instead, continued to receive twice daily injections of saline for 7 days and 15 days, respectively. On day 24, the 8-day withdrawal rats received

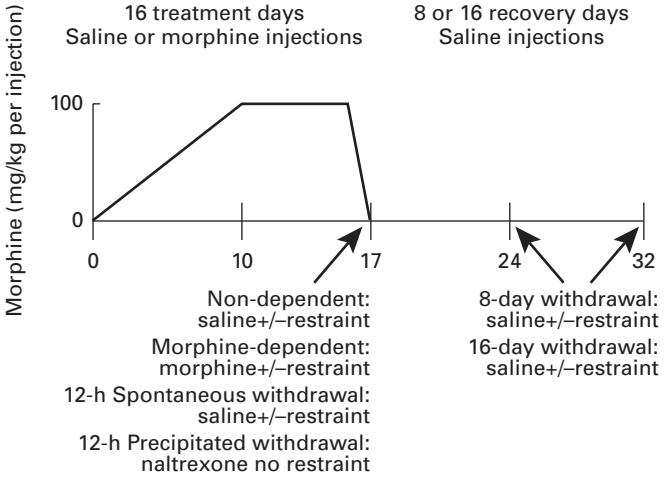


FIG. 1. Timeline of experimental procedure.

an injection of saline without restraint or followed immediately by restraint. Rats in the 16-day withdrawal group were similarly treated on day 32.

Changes in body weight were monitored in nondependent and morphine-dependent rats. For this purpose, nondependent rats received twice daily injections of saline for 32 consecutive days. On the other hand, morphine-dependent rats received twice daily injections of morphine (10–100 mg/kg, s.c.) during the initial 16 days followed by 16 days of saline treatment. Body weight was determined daily at approximately 07.00 h, immediately prior to saline or morphine injection. Cessation of chronic morphine treatment induced a number of behavioural and physical withdrawal symptoms, including piloerection, ptosis, lacrimation, rhinorrhea, diarrhoea and body weight loss.

Blood sampling and assays

On the final experimental day for each of the six treatment groups, 30 min prior to saline or morphine injection, rats were gently wrapped in a piece of cloth while the tip of the tail of each rat (approximately 1 mm) was nicked with a scalpel (no. 10 blade) and approximately 240 µl of blood was collected in heparinized microcapillary pipettes (Fisher Scientific). During the subsequent blood collections, at 15, 30, 60, 90, 120 and 240 min after initiation of the experiment, rats were gently wrapped in a piece of cloth and the same incision was used for repeated blood (240 µl) collections. The initial studies were also used to characterize the circadian rhythm of temperature and corticosterone in vehicle- and morphine-treated rats. For these studies, blood samples were also collected 480 min and 720 min after initiation of the experiment. The tail nick method allowed rapid and repeated blood collection without causing either behavioural or hormonal distress to the rats as evidenced by the basal pituitary-adrenal hormone levels, which are within the range previously reported using tail nick and decapitation methods (27, 31, 36–39). The blood collection procedure lasted less than 2 min. Blood samples were immediately placed on ice, centrifuged at 4000 r.p.m. for 5 min at 4°C and then plasma was pipetted into 1 ml Cryovials (Fisher Scientific) and stored at −80°C until analysis.

Plasma corticosterone concentrations were determined using RIA kits purchased from Diagnostic Products Corporation (Los Angeles, CA, USA). Plasma ACTH concentrations were determined in the nondependent, 8-day and 16-day withdrawal rats using an RIA kit purchased from Nichols Diagnostics (San Juan Capistrano, CA, USA).

Sacrifice and tissue collection

For determination of adrenal and thymus weight, vehicle-treated rats not exposed to restraint stress were decapitated on day 17, 4 h after the administration of saline at approximately 12.00 h. Similarly, morphine-treated rats were sacrificed on day 17, 4 h after the administration of morphine (morphine-dependent) or saline (12-h withdrawal), or on days 24 (8-day withdrawal) or 32 (16-day withdrawal), 4 h after the administration of saline. Immediately after decapitation, thymuses and adrenals were removed and weighed.

Data analysis

Core body temperature and plasma ACTH and corticosterone concentrations are shown in raw form. Plasma ACTH and corticosterone values are also depicted as area under curve (AUC) to facilitate comparisons of stress hormone levels among the various treatment groups. Both normalized AUC (response above basal hormone concentrations) and total AUC (response including basal hormone concentrations) are shown to account for marked differences in basal hormone concentrations among the various treatment groups. Normalized AUC values are an estimate of ACTH or corticosterone release relative to basal levels, whereas total AUC values are an estimate of the total ACTH or corticosterone release, including basal concentrations of these hormones. AUC values were calculated using the trapezoidal rule (40).

Statistical analysis

Data are presented as mean ± SEM For AUC values, a square root transformation was utilized to achieve homogeneity of variances. One or two-way repeated measures ANOVA and post-hoc pairwise comparisons using the Tukey HSD test of significance ($P < 0.05$) were carried out using Statistica (v. 5.0; Statsoft, Tulsa, OK, USA).

Results

Body weight gain in vehicle- and morphine-treated rats (Fig. 2)

During the 16-day treatment period, vehicle-treated rats gained weight whereas morphine-treated rats lost weight ($P < 0.0001$ and $P < 0.01$, respectively). Furthermore, 2 days after cessation of morphine treatment, a marked and rapid decrease in body weight was observed ($P < 0.01$). Although this effect was compensated by a period of increase in body weight gain, after 16 days of withdrawal, morphine-treated rats still weighed significantly less than vehicle-treated rats ($P < 0.01$).

Adrenal and thymus weights in vehicle- and morphine-treated rats (Fig. 3)

Chronic morphine administration significantly increased adrenal weights (Fig. 3A, $P < 0.0001$). Adrenal weight was significantly increased in morphine-dependent rats maintained on morphine and those undergoing 12-h or 8-day withdrawal. Sixteen days after morphine withdrawal, the adrenal weight of morphine-treated rats was similar to that of vehicle-treated rats. Thymus weight was significantly different between vehicle- and morphine-treated rats (Fig. 3B; $P < 0.0001$). Chronic morphine administration alone did not significantly affect thymus weights. However, thymus weight was significantly decreased in rats undergoing 12-h, 8-day and 16-day morphine withdrawal. Importantly, the raw data for adrenal and thymus weights in nondependent, morphine-dependent, 12-h withdrawal, 8-day withdrawal and 16-day withdrawal rats [adrenal weight (g): 0.023 ± 0.001, 0.039 ± 0.002, 0.031 ± 0.001, 0.034 ± 0.001 and 0.028 ± 0.001;

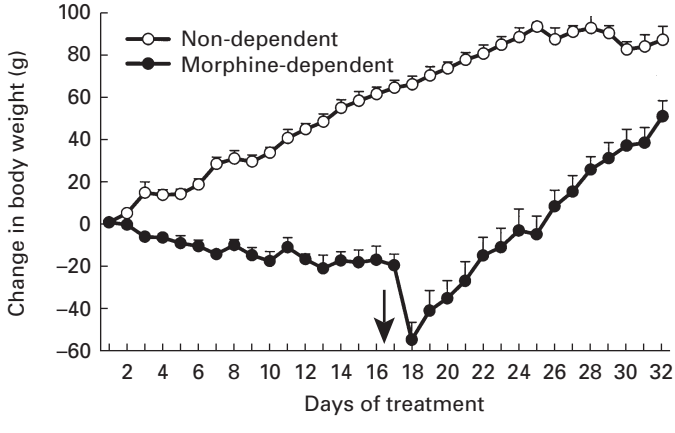


FIG. 2. Changes in body weight in vehicle- and morphine-treated rats. Non-dependent rats received twice daily injections of saline for the duration of the study, whereas morphine-dependent rats received morphine (10–100 mg/kg per injection, s.c.) during the initial 16 days and saline on the subsequent 16 days. Arrow indicates the final morphine injection in dependent rats. Body weight was determined daily at approximately 07.00 h, immediately prior to saline or morphine injection. Data are means and SEM (n = 6). Significant differences in body weight gain between nondependent and morphine-dependent groups were observed on all days except for day 1 ($P < 0.01$).

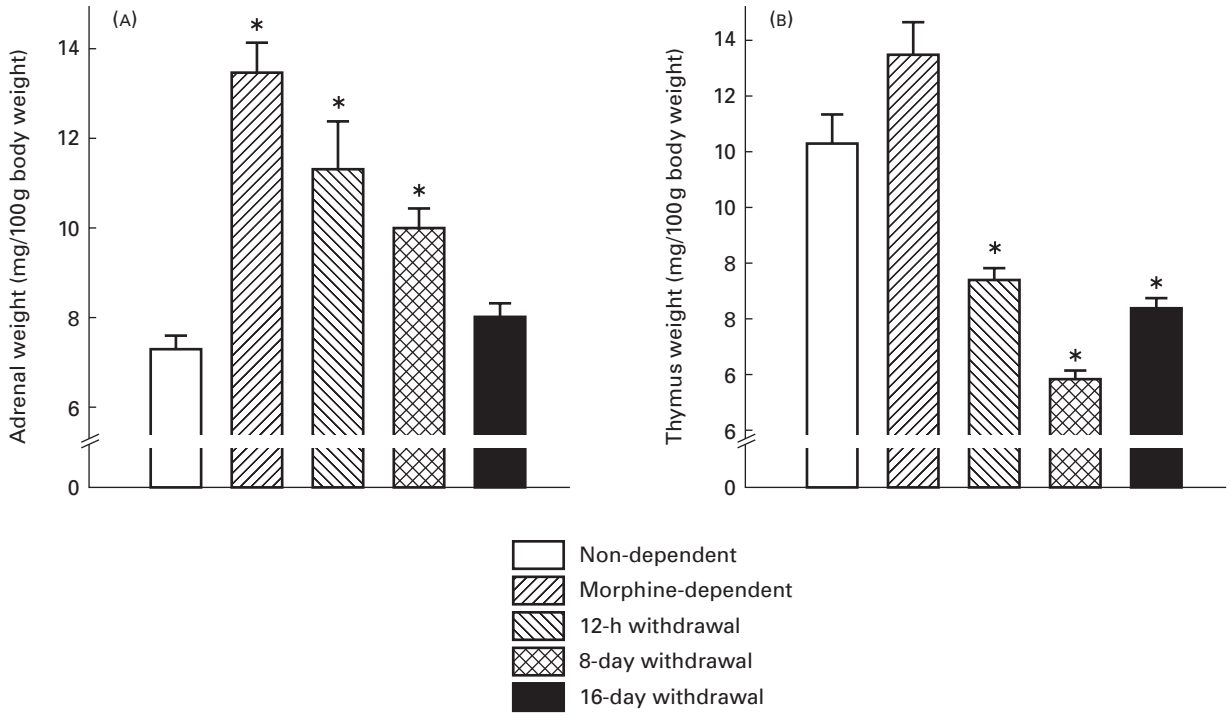


FIG. 3. Adrenal and thymus weights in vehicle- and morphine-treated rats. (A,B) Showing adrenal and thymus weights relatively to body weight in nondependent, morphine-dependent, 12-h withdrawal, 8-day withdrawal, and 16-day withdrawal rats. Data are means and SEM (n = 5–6). *$P < 0.05$ versus nondependent group.

thymus weight (g): 0.593 ± 0.058, 0.694 ± 0.066, 0.311 ± 0.018, 0.201 ± 0.019 and 0.342 ± 0.016, respectively] demonstrated a virtually identical trend.

Temperature and corticosterone responses to morphine withdrawal (Fig. 4)

Chronic morphine treatment did not alter basal body temperature (Fig. 4A,C). However, across the 12 h observation period, body temperature levels were significantly different among nondependent rats, morphine-dependent rats maintained on morphine, and morphine-dependent rats undergoing spontaneous or precipitated withdrawal ($P < 0.0001$). Morphine-treated rats displayed a marked hyperthermic response to morphine (100 mg/kg, s.c.) and a long-lasting hypothermic response to naltrexone (1 mg/kg, s.c.) administration. Body temperature was similar between nondependent rats and rats undergoing 12-h spontaneous morphine withdrawal. Furthermore, administration of naltrexone did not modify body temperature in nondependent rats (data not shown).

Basal plasma corticosterone concentrations were significantly elevated in morphine-treated rats (260.7 ± 35.5 ng/ml) compared to vehicle-treated rats (45.6 ± 13.2 ng/ml) (Fig. 4B,D; $P < 0.001$). Across the 12-h observation period, morphine-treated rats showed significantly higher plasma corticosterone concentrations compared to nondependent rats irrespective of whether they were maintained on morphine or undergoing spontaneous or precipitated withdrawal ($P < 0.0001$). This effect was observed at all time points, except at 4 h and 8 h after administration of the maintenance dose of morphine in morphine-dependent rats. Administration of naltrexone did not significantly alter plasma corticosterone concentrations in nondependent rats (data not shown).

Temperature responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats (Fig. 5)

In nondependent rats, handling rats for blood collection produced a transient and nonsignificant rise in body temperature. Exposure to both 15-min and 4-h restraint produced a rapid and persistent hyperthermic response in nondependent rats (Fig. 5A; $P < 0.001$). In morphine-dependent rats, exposure to restraint also significantly affected body temperature (Fig. 5B; $P < 0.01$). However, in these animals, morphine-induced hyperthermia was significantly enhanced only in response to 4-h, but not 15-min, restraint. In the 12-h withdrawal group, neither 15-min nor 4-h restraint significantly altered body temperature (Fig. 5C). Eight days after withdrawal from morphine, rats displayed a significant increase in body temperature in response to restraint (Fig. 5D; $P < 0.01$), with 4-h, but not 15-min, restraint producing a hyperthermic response compared with unrestrained rats. Body temperature was not significantly altered in response to restraint in rats undergoing 16-day withdrawal (Fig. 5E).

Because the purpose of this experiment was to compare the effects of restraint on body temperature between vehicle- and morphine-treated rats, basal and stress body temperature was compared among the five treatment groups and a significant

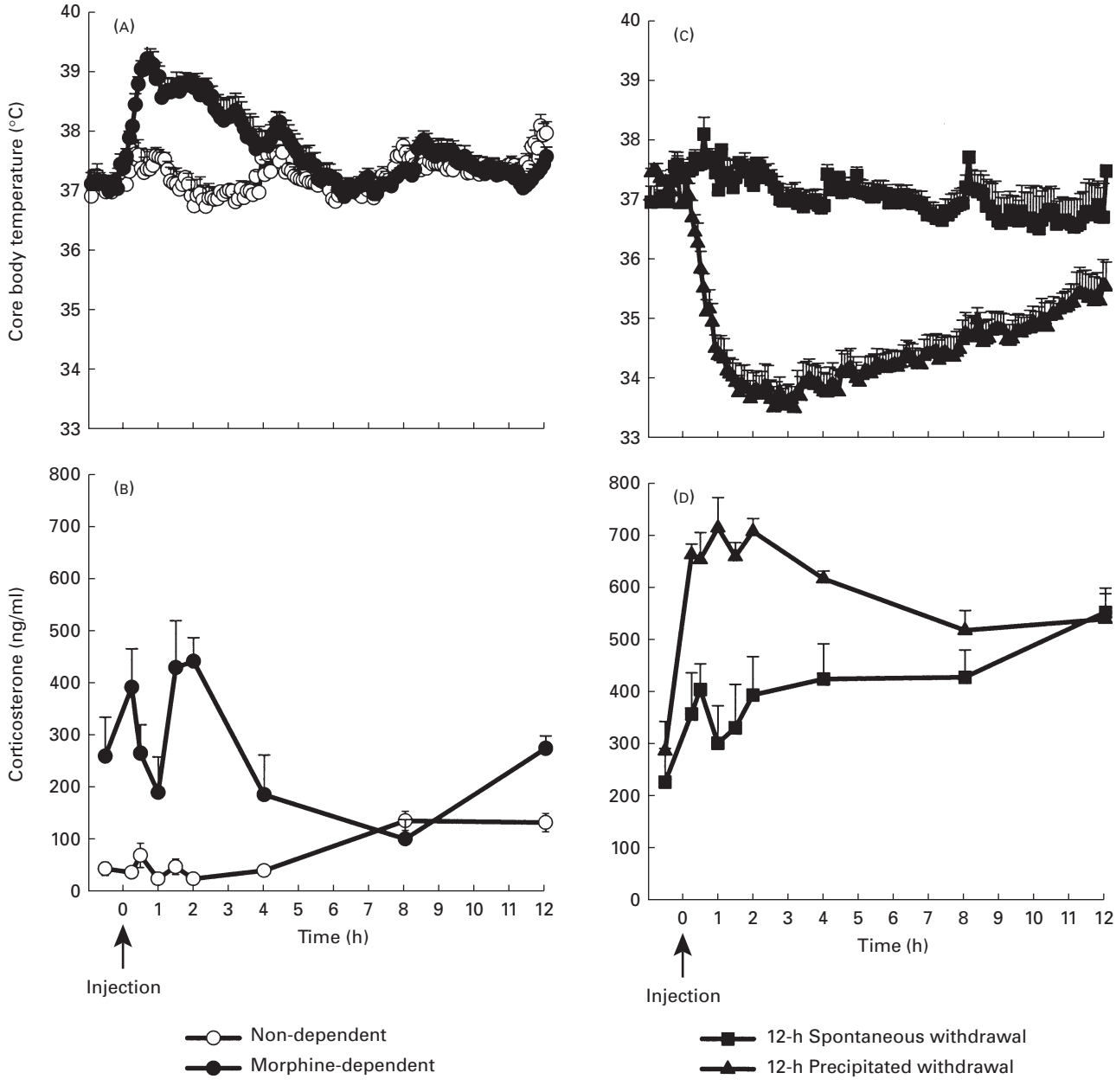
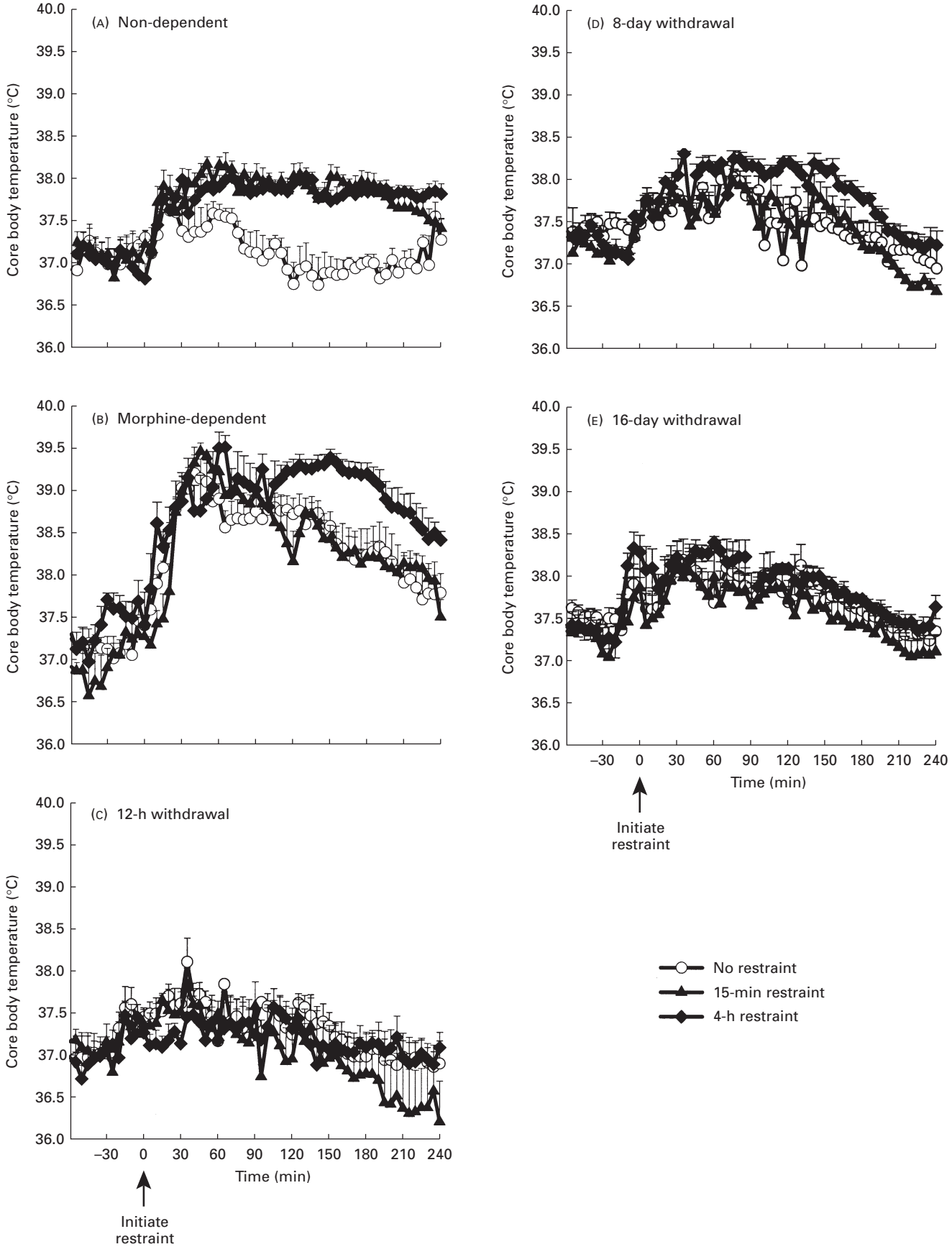


FIG. 4. Core body temperature and corticosterone responses to morphine withdrawal. (A,B) Showing core body temperature and plasma corticosterone concentrations, respectively, in nondependent and morphine-dependent rats. (C,D) Showing show core body temperature and plasma corticosterone concentrations, respectively, in 12-h spontaneous withdrawal and 12-h precipitated withdrawal rats. Arrow indicates time of injection. Data are means and SEM ($n = 5–6$). A significant hyperthermic response was observed in morphine-dependent rats from 20 min up to 3 h postinjection ($P < 0.05$). A significant hypothermic response was observed in precipitated withdrawal rats within 10 min after injection and was still evident at 12 h thereafter ($P < 0.01$). Plasma corticosterone levels were significantly elevated in morphine-dependent, spontaneous withdrawal, precipitated withdrawal groups at all time-points, except 4 h and 8 h after morphine administration ($P < 0.05$).

FIG. 5. Core body temperature responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats. (A) Nondependent; (B) morphine-dependent; (C) 12-h morphine withdrawal; (D) 8-day morphine withdrawal; (E) 16-day morphine withdrawal. Arrows indicate the time of injection and/or start of restraint stress. Data are means and SEM ($n = 5–11$). Non-dependent rats restrained for either 15 min or 4 h displayed significantly elevated body temperatures from 65 min to 240 min compared to unrestrained controls. Morphine-dependent rats restrained for 4 h displayed a significant hyperthermic response compared to both unrestrained (125–185 min) and 15-min restrained (125–95 min) rats. Eight days after withdrawal, in response to 4 h restraint, rats displayed significantly elevated body temperature levels at 20, 30, 65, 100, 100–115 and 135–150 min compared to nonrestrained rats and at 20, 35, 45, 50, 65, 79, 90, 95, 125, 130 and 175–200 min compared to 15-min restrained rats (all $P < 0.05$).


(A) Non-dependent
(B) Morphine-dependent
(C) 12-h withdrawal
(D) 8-day withdrawal
(E) 16-day withdrawal
Core body temperature (°C)
40.0
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
–30
0
30
60
90
120
150
180
210
240
Time (min)
Initiate restraint
No restraint
15-min restraint
4-h restraint

interaction between morphine treatment and stress exposure was observed ($P < 0.0001$). In the absence of restraint, morphine-dependent rats displayed significantly elevated body temperatures compared to nondependent rats. Furthermore, 12-h withdrawal rats displayed a reduced hyperthermic response to 15-min and 4-h restraint, whereas morphine-dependent rats displayed an augmented hyperthermic response to 4-h restraint compared to nondependent rats.

Corticosterone responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats (Fig. 6)

In nondependent rats, repeated blood collections did not significantly alter basal plasma corticosterone concentrations. Although exposure to both 15-min and 4-h restraint significantly increased plasma corticosterone concentrations in nondependent rats (Fig. 6A; $P < 0.0001$), the corticosterone response to 4-h restraint was significantly prolonged compared to 15-min restraint.

Basal corticosterone concentrations in morphine-dependent rats were approximately seven-fold higher than that observed in nondependent rats. Administration of morphine reduced basal plasma corticosterone concentrations in morphine-dependent rats to levels observed in nondependent rats. Exposure to 4-h, but not 15-min, restraint immediately after administration of morphine significantly elevated plasma corticosterone concentrations in these rats (Fig. 6B; $P < 0.05$). Furthermore, the corticosterone response to 15-min restraint was significantly reduced compared to the response to 4-h restraint in morphine-dependent rats.

Although basal corticosterone concentrations in 12-h withdrawal rats were already markedly elevated compared to nondependent rats, omission of a single morphine injection produced further significant increases in plasma corticosterone concentrations during the next 4 h (Fig. 6C; $P < 0.01$). In rats undergoing 12-h withdrawal, plasma corticosterone concentrations were significantly increased in response to restraint ($P < 0.01$). Although exposure to both 15-min and 4-h restraint significantly elevated corticosterone concentrations in 12-h withdrawal rats, the corticosterone response to 4-h restraint was significantly prolonged compared to the response to 15-min restraint.

There were no differences in basal plasma corticosterone concentrations between 8-day withdrawal and nondependent rats (Fig. 6D). Plasma corticosterone concentrations in the 8-day withdrawal rats were significantly, and similarly, increased in response to both 15-min and 4-h restraint ($P < 0.0001$).

Basal corticosterone concentrations were also similar between 16-day withdrawal and nondependent rats. Exposure to restraint significantly increased corticosterone concentrations 16 days after withdrawal (Fig. 6E; $P < 0.001$). Although both 15-min and 4-h restraint significantly increased plasma corticosterone concentrations in 16-day withdrawal rats, the corticosterone response to 4-h restraint persisted longer than that to 15-min restraint.

In order to compare corticosterone concentrations in the absence and presence of restraint stress among the various treatment groups, the data were reanalysed and expressed as both normalized and total AUC to account for differences in basal corticosterone concentrations (Table 1). Normalized and total corticosterone AUC were significantly changed as a result of exposure to stress and chronic morphine treatment (both $P < 0.0001$). Total corticosterone AUC in the no-restraint, 15-min, and 4-h restraint conditions was significantly elevated in both morphine-dependent and 12-h withdrawal rats compared to nondependent rats. On the other hand, total corticosterone AUC in response to 4-h restraint was significantly reduced in the 8-day and 16-day withdrawal rats compared to nondependent rats.

Adrenocorticotropic hormone responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats (Fig. 7)

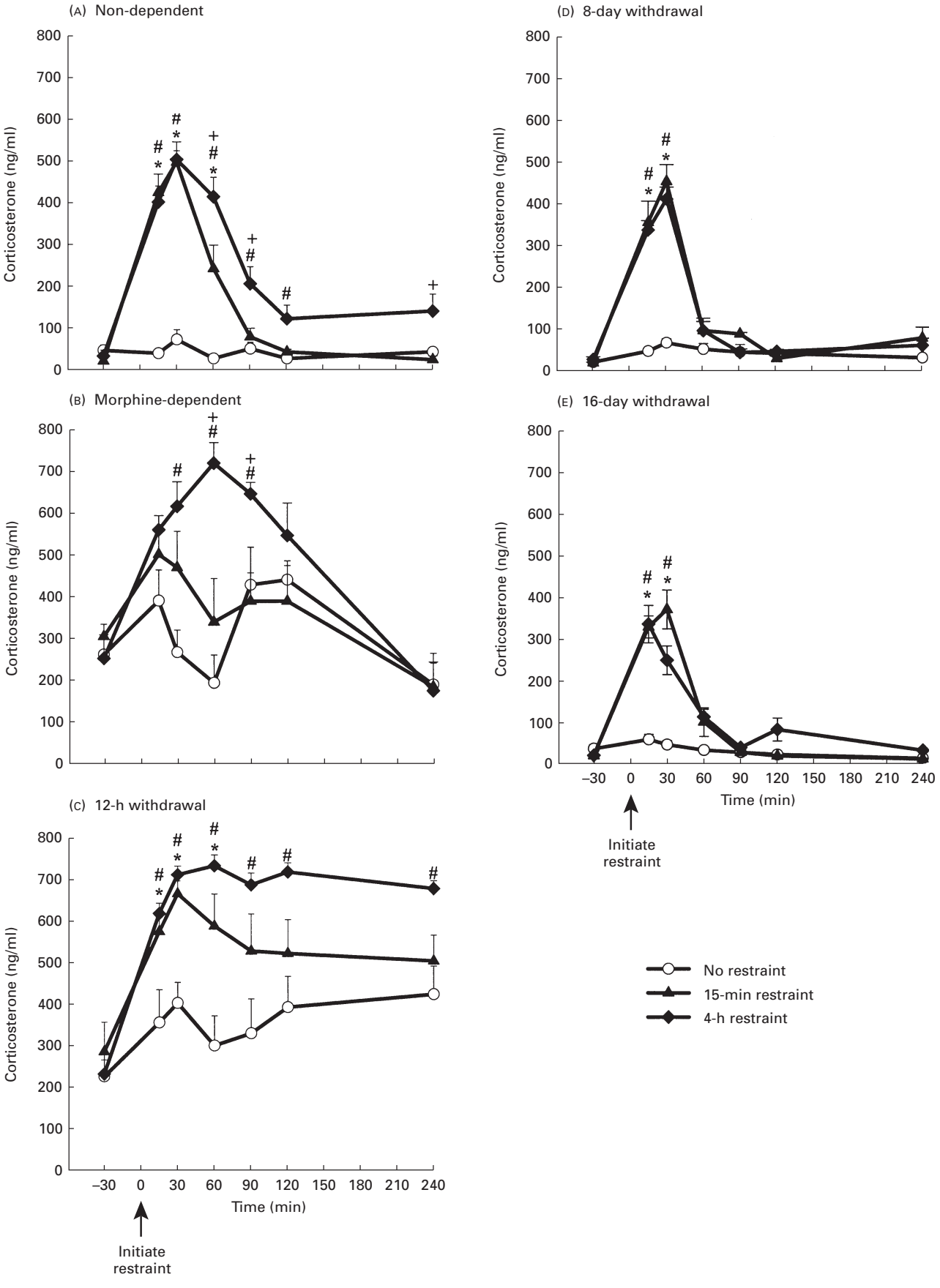
In nondependent rats, repeated blood collections did not significantly alter basal plasma ACTH concentrations. Exposure of nondependent rats to both 15-min and 4-h restraint produced significant increases in plasma ACTH concentrations with a similar time course (Fig. 7A; $P < 0.05$).

Basal ACTH concentrations in 8-day withdrawal rats (4.5 ± 1.5 pg/ml) were significantly reduced compared to nondependent rats (16.8 ± 5.0 pg/ml) (Fig. 7B; $P < 0.05$). Exposure to restraint produced a slight, but significant increase in plasma ACTH concentrations in these rats ($P < 0.01$). Although only 15-min, but not 4-h, restraint produced a significant increase in plasma ACTH concentrations in 8-day withdrawal rats, the differences in the ACTH responses to 15-min and 4-h restraint did not reach significance.

Basal ACTH concentrations were similar between nondependent and 16-day withdrawal rats. However, 16 days after withdrawal, rats continued to display a dramatically reduced ACTH response to restraint compared to nondependent rats (Fig. 7C). Exposure to 15-min, but not 4-h, restraint significantly increased plasma ACTH concentrations in 16-day withdrawal rats ($P < 0.01$). Despite greater ACTH responses to 15-min restraint compared to 4-h restraint, significant differences between these two treatment groups were not observed.

In order to compare ACTH concentrations in the absence and presence of restraint stress among the various treatment groups, the data were reanalysed and expressed as AUC (Table 1). Both normalized and total ACTH AUC in response to restraint were significantly affected by exposure to stress and chronic morphine treatment (both $P < 0.0001$).

FIG. 6. Plasma corticosterone responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats. (A) Nondependent; (B) morphine-dependent; (C) 12-h morphine withdrawal; (D) 8-day morphine withdrawal; (E) 16-day morphine withdrawal. Arrow indicates the time of injection and/or start of restraint stress. Data are means and SEM ($n = 5$–9). *$P < 0.05$, 15-min restraint versus no restraint. #$P < 0.05$, 4-h restraint *vs* no restraint. †$P < 0.05$, 15-min restraint versus 4-h restraint. See Table 1 for comparisons across groups.


(A) Non-dependent
(B) Morphine-dependent
(C) 12-h withdrawal
(D) 8-day withdrawal
(E) 16-day withdrawal
Corticosterone (ng/ml)
0
100
200
300
400
500
600
700
800
–30
0
30
60
90
120
150
180
210
240
Time (min)
Initiate restraint
No restraint
15-min restraint
4-h restraint

Normalized ACTH AUC in response to 4-h restraint, and total ACTH AUC in response to both 15-min and 4-h restraint were significantly reduced in 8-day withdrawal rats compared to nondependent rats. Furthermore, both normalized and total ACTH AUC in response to 15-min and 4-h restraint were significantly blunted in the 16-day withdrawal group compared to the nondependent group.

Discussion

In this study, we have shown that chronic morphine dependence induces physiological responses comparable to those observed after chronic stress: reduced body weight, increased adrenal weight, decreased thymus weight and elevated basal corticosterone concentrations. In addition, we have shown that chronic morphine treatment affects temperature regulatory systems so that rats are unable to show the normal hyperthermic response to restraint stress as late as 16 days after the final morphine injection. Most interestingly, we have demonstrated that chronic morphine treatment has a paradoxical effect on the pituitary-adrenal system, resulting in potentiated and prolonged corticosterone responses to restraint in rats undergoing acute withdrawal and reduced and shorter ACTH and corticosterone responses to restraint in rats undergoing chronic morphine withdrawal. The results presented here may be relevant to altered stress responses in opioid-dependent subjects, which may be involved in the maintenance or relapse to opioid use.

Initial studies characterized the effects of chronic morphine treatment on body weight gain, temperature regulation and HPA axis activity as indices of morphine dependence and withdrawal. Consistent with previous reports, we found that chronic morphine treatment reduced body weight gain and cessation of morphine treatment produced a marked decrease in body weight (31). Administration of a high dose of morphine produced a marked hyperthermic response in dependent rats, suggesting that tolerance had developed to the hypothermic, but not the hyperthermic effects of morphine (14, 15, 41). Spontaneous withdrawal from morphine did not alter body temperature levels in dependent rats, whereas naltrexone-precipitated withdrawal induced a marked hypothermic response (31). However, both spontaneous and precipitated morphine withdrawal were associated with marked increases in plasma corticosterone concentrations, suggesting that the HPA axis and temperature responses to withdrawal are mediated through different mechanisms.

Interestingly, chronic morphine treatment increased basal plasma corticosterone concentrations. This finding is in contrast to previous reports that basal corticosterone concentrations are not modified in morphine-dependent rats (27, 29, 30). This discrepancy is likely due to differences in morphine doses and treatment durations used to induce dependence, which were both greater in the present study compared to previous studies. Elevation of basal plasma corticosterone concentrations in chronically dependent rats and the ability of acute morphine administration to inhibit basal corticosterone concentrations suggests that corticosterone hypersecretion in dependent rats may be due to morphine withdrawal. Alternatively, basal corticosterone

TABLE 1. Cumulative Release (Area Under Curve, AUC) of Plasma Corticosterone and Adrenocorticotropic Hormone (ACTH) in the Absence of Restraint or in Response to 15-min or 4-h Restraint in Vehicle- and Morphine-Treated Rats.

	Corticosterone (μg/min/dl)		ACTH (pg/min/ml)	
Treatment	Normalized AUC	Total AUC	Normalized AUC	Total AUC
Non-dependent				
No restraint	−179 ± 635	2353 ± 426	2306 ± 2848	9465 ± 3298
15-min restraint	11 000 ± 1252[a]	12 126 ± 1502[a]	34 556 ± 8052[a]	50 041 ± 10058[a]
4-h restraint	14 468 ± 1236[a]	16 246 ± 1412[a]	51 485 ± 7931[a]	55 380 ± 6403[a]
Morphine-dependent				
No restraint	2840 ± 4137	17 321 ± 1300[b]	ND	ND
15-min restraint	5210 ± 2047	22 940 ± 2847[b]	ND	ND
4-h restraint	17 382 ± 3322[a]	32 546 ± 3244[a,b]	ND	ND
12 h withdrawal				
No restraint	6979 ± 1413	19 654 ± 3052[b]	ND	ND
15-min restraint	14 406 ± 2230	30 374 ± 3083[a,b]	ND	ND
4-h restraint	23 133 ± 3696[a]	35 758 ± 812[a,b]	ND	ND
8 day withdrawal				
No restraint	1446 ± 334	2437 ± 286	−625 ± 1568	2974 ± 1081
15-min restraint	8742 ± 837[a]	9718 ± 899[a]	14 648 ± 2287[a]	16 591 ± 2256[a,b]
4-h restraint	7617 ± 777[a]	9088 ± 678[a,b]	8055 ± 1849[b]	9570 ± 1851[b]
16 day withdrawal				
No restraint	−115 ± 462	1812 ± 101	1017 ± 1443	8110 ± 638
15-min restraint	6637 ± 881[a]	7823 ± 1040[a]	7803 ± 3498[b]	13 382 ± 3042[b]
4-h restraint	6708 ± 816[a]	7603 ± 960[a,b]	6615 ± 1388[b]	8766 ± 2090[b]

ND, not determined. [a]$P < 0.05$ compared to no restraint within the same treatment group. [b]$P < 0.05$ compared to same stress exposure in nondependent group.

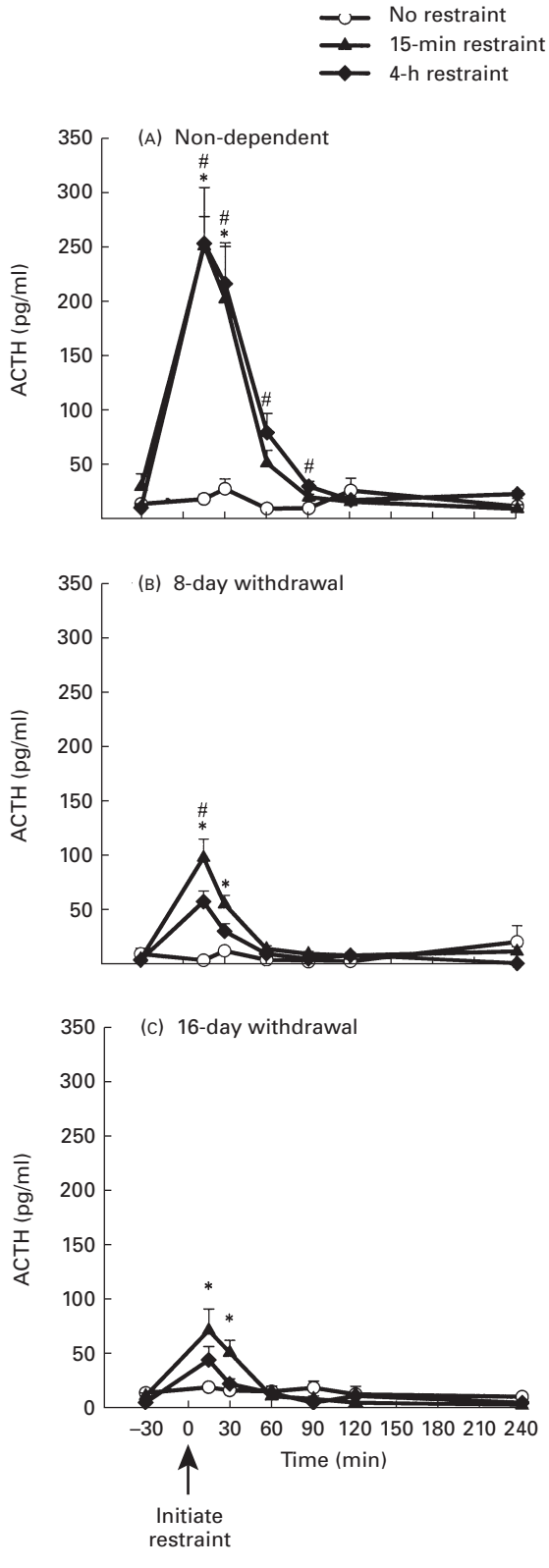


FIG. 7. Plasma ACTH responses to 15-min and 4-h restraint stress in vehicle- and morphine-treated rats. (A) Nondependent; (B) 8-day morphine withdrawal; (C) 16-day morphine withdrawal. Arrow indicates the time of injection and/or start of restraint stress. Data are means and SEM (n = 5–6). *P < 0.05, 15-min restraint versus no restraint. #P < 0.05, 4-h restraint versus no restraint. See Table 1 for comparisons across groups.

hypersecretion in morphine-dependent rats may, in part, result from enhanced sensitivity of these rats to experimental manipulation, which may be reduced with morphine. Nonetheless, it should be noted that acute morphine administration only briefly reduced basal plasma corticosterone concentrations in dependent rats, and that corticosterone was almost continuously hypersecreted in morphine-dependent rats. In unstressed rats, basal activity of the HPA axis during the peak, but not the trough, of the circadian rhythm is dependent on hypothalamic CRH secretion (42). Therefore, elevated basal corticosterone concentrations in morphine-dependent rats might be related to increased hypothalamic secretion of CRH. Additionally, reduced sensitivity of negative-feedback systems to circulating glucocorticoids and/or increased sensitivity of the adrenals to ACTH may be involved in corticosterone hypersecretion. Interestingly, elevated basal plasma corticosterone concentrations have been observed in animals chronically exposed to stress (43, 44), suggesting that chronic morphine dependence is a chronic stressor.

In addition to elevated basal corticosterone concentrations, morphine-treated rats resemble chronically stressed rats in a number of other ways. It has previously been shown that prolonged activation of the HPA axis as a result of chronic exposure to stress (42, 43), or chronic treatment with CRF (45), results in reduced body weight, decreased thymus weights and increased adrenal weights. In our study, morphine-treated rats demonstrated a loss of body weight. Furthermore, morphine-dependent rats that had received their last morphine injection 16-h earlier displayed dramatically reduced thymus weights. Although the difference in thymus weights between morphine-dependent and 12-h withdrawal rats is quite striking, a similar magnitude of difference within a 12-h period has been previously reported (46). In our study, similarly to chronic stress exposure, chronic morphine treatment resulted in adrenal gland hypertrophy. Thymus involution and adrenal hypertrophy are characteristic signs of tonic hyperactivity of the HPA axis, and clearly support the suggestion that chronic morphine dependence acts as a chronic stressor (44).

Because it has been suggested that chronic stress exposure modifies the activity of systems involved in temperature regulation (47), the temperature responses to acute restraint stress were examined in chronically morphine-dependent rats. Consistent with previous studies, we found that exposure to restraint stress produced a hyperthermic response in nondependent rats (47–49). However, exposure of rats undergoing acute (12-h) morphine withdrawal to restraint did not alter body temperature levels, and 16 days after termination of chronic morphine treatment, rats still had not recovered a normal hyperthermic response to restraint stress. Previously, it was reported that restraint does not significantly alter body temperature in either nondependent or morphine-dependent rats (23). It was also reported that exposure to restraint produced an exaggerated hypothermic response in morphine-dependent monkeys undergoing withdrawal (24). The differences between our results and those reported previously may be attributed to differences in morphine and stress procedures. Thus, this is the first time that chronic morphine dependence has been shown to produce marked and long-lasting changes in the temperature responses to

an acute stressor in the rat. However, further studies are required to identify the mechanisms implicated.

The results presented herein also demonstrate the first evidence that chronic morphine treatment produces dramatic and long-lasting changes in the pituitary-adrenal responses to an acute stressor and that these responses vary markedly depending on the duration of opioid withdrawal. Despite basal plasma corticosterone concentrations that were approximately seven-fold higher than those observed in nondependent rats, morphine-dependent rats undergoing acute (12-h) morphine withdrawal displayed potentiated and prolonged corticosterone responses to restraint stress. Morphine-dependent rats that had received an injection of morphine demonstrated a slightly less dramatic stress response compared to acutely withdrawn rats, suggesting that morphine reduces the psychological and/or physiological stress response in dependent rats. The exaggerated corticosterone response to restraint observed in rats chronically treated with morphine resembles the facilitated HPA axis response to an acute stressor in chronically stressed rats (37, 38, 42, 50). Chronically stressed rats display an exaggerated HPA axis response to an acute stressor, despite the negative-feedback effects of elevated basal levels of corticosterone (37, 44, 51). It has been shown that elevation of corticosterone concentrations by either repeated stress or exogenous corticosterone administration significantly reduces glucocorticoid receptor number in the hippocampus (52, 53). Similarly, it has been demonstrated that chronic administration of morphine results in glucocorticoid receptor downregulation in the rat hippocampus (32). Thus, the exaggerated and prolonged corticosterone responses to stress observed in morphine-dependent rats, as in rats chronically exposed to stress, may in part be as a result of decreased sensitivity of negative-feedback mechanisms to circulating corticosterone. However, other mechanisms may mediate corticosterone hypersecretion in morphine-dependent rats in response to restraint. Whether these mechanisms include increases in hypothalamic secretion of CRH and/or AVP, CRH/AVP-induced ACTH secretion or adrenal sensitivity to ACTH (54), remains to be determined.

In marked contrast to the potentiated and prolonged corticosterone responses to restraint observed in rats undergoing acute morphine withdrawal, rats tested 8 days and 16 days after morphine withdrawal displayed reduced and shorter ACTH and corticosterone responses to restraint stress. The reduced HPA axis responses to restraint stress in rats undergoing extended morphine withdrawal suggest increased sensitivity of negative-feedback systems to corticosterone. Such an enhanced efficiency in suppression of the HPA axis responses to stress has previously been observed in rats exposed to brief periods of handling for the first few weeks of life, and has been associated with glucocorticoid receptor upregulation as well as reduced hypothalamic CRH activity (55). Thus, reduced ACTH and corticosterone responses to stress in 8-day and 16-day withdrawal rats may be due to reduced hypothalamic secretion of CRH, attenuated sensitivity of the pituitary to CRH, or enhanced responsivity of the brain and/or pituitary to corticosterone negative-feedback (54). The reduced ACTH: corticosterone ratio in 8-day and 16-day withdrawal rats also suggests that the adrenals of these animals may be hyperresponsive to ACTH, thereby resulting in almost normal corticosterone responses to restraint in the face of a dramatic reduction of the ACTH responses to this stressor.

Another interesting possibility to consider is the recent suggestion that increased activity of the posterior paraventricular nucleus of the thalamus (pPVTH) reduces the HPA axis responses to an acute novel stressor in chronically stressed animals (50). It is possible that the increased activity of pPVTH is present in rats undergoing both acute and chronic morphine withdrawal, and that these effects become unmasked in 8-day and 16-day withdrawal rats as the glucocorticoid negative-feedback systems recover over time. The results presented herein provide us with a chronic stress model which can result in both hyper- and hyporesponsive pituitary-adrenal responses to stress separated as a function of time. Further studies should be carried out to determine the mechanisms mediating changes in HPA axis function in dependent rats undergoing acute and chronic withdrawal.

In conclusion, this is the first study in which the effects of stress on body temperature and pituitary-adrenal responses to stress have been evaluated in morphine-dependent rats maintained on morphine and at various times after morphine withdrawal. The results of this study show profound and long-lasting changes in the temperature and pituitary-adrenal responses to restraint in rats chronically treated with morphine. In the future, it will be extremely interesting to determine the mechanisms underlying the altered temperature and HPA axis responses to stress in morphine-treated rats. Further investigation of the mechanisms mediating the altered stress responses in morphine-treated rats may shed light on physiological changes associated with opioid use and relapse in humans.

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References

1 Kreek MJ, Koob GF. Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Depend* 1998; **51:** 23–47.
2 Shaham Y, Alvares K, Nespor SM, Grundberg NE. Effect of stress on oral morphine and fentanyl self-administration in rats. *Pharmacol Biochem Behav* 1992; **41:** 615–619.
3 Shaham Y. Immobilization stress-induced oral opioid self-administration and withdrawal in rats: role of conditioning factors and the effect of stress on 'relapse' to opioid drugs. *Psychopharmacology* 1993; **111:** 477–485.
4 Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. *J Neurosci* 1996; **16:** 1957–1963.
5 Deroche V, Piazza PV, Casolini P, Maccari S, Le Moal M, Simon H. Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. *Brain Res* 1992; **598:** 343–348.
6 Deroche V, Piazza PV, Casolini P, Le Moal M, Simon H. Sensitization to the psychomotor effects of amphetamine and morphine induced

by food restriction depends on corticosterone secretion. *Brain Res* 1993; **611:** 352–356.
7 Deroche V, Piazza PV, Le Moal M, Simon H. Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. *Brain Res* 1994; **640:** 136–139.
8 Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. *J Neurosci* 1997; **17:** 2605–2614.
9 Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* 1998; **137:** 184–190.
10 Deroche V, Marinelli M, Maccari S, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *J Neurosci* 1995; **15:** 7181–7188.
11 Hermann JB. The pyretic action on rats of small doses of morphine. *J Pharmacol Exp Ther* 1942; **76:** 309–315.
12 Lotti VJ, Lomax P, George R. Temperature responses in the rat following intracerebral microinjection of morphine. *J Pharmacol Exp Ther* 1965; **150:** 135–139.
13 Cox B, Ary M, Chesarek W, Lomax P. Morphine hyperthermia in the rat: an action on the central thermostats. *Eur J Pharmacol* 1976; **36:** 33–39.
14 Gunne LM. The temperature response in rats during acute and chronic morphine administration: a study of morphine tolerance. *Arch Int Pharmacodyn* 1960; **129:** 416–428.
15 Adler MW, Geller EB. The opioid system and temperature regulation. *Annu Rev Pharmacol Toxicol* 1988; **28:** 429–444.
16 Katovich MJ, Simpkins JW, O'Meara J. Effects of opioid antagonists and their quaternary analogs on temperature changes in morphine-dependent rats. *Life Sci* 1986; **39:** 1845–1854.
17 Bhargava HN, Matwyshyn GA, Gerk PM, Bozek PS, Bailey MD, Ko KH, Simko RJ, Thorat SN. Effects of naltrexone pellet implantation on morphine tolerance and physical dependence in the rat. *Gen Pharmacol* 1994; **25:** 149–155.
18 French ED. Dexamethasone blocks morphine-induced hypothermia in restrained rats. *Life Sci* 1979; **25:** 1583–1590.
19 Martin GE, Lapp NL. Effect on core temperature of restraint after peripherally and centrally injected morphine in the Sprague-Dawley rat. *Pharmacol Biochem Behav* 1979; **10:** 313–315.
20 McDougal JN, Marques PR, Burks TF. Restraint alters the thermic response to morphine by postural interference. *Pharmacol Biochem Behav* 1983; **18:** 495–499.
21 Appelbaum BD, Holtzman SG. Characterization of stress-induced potentiation of opioid effects in the rat. *J Pharmacol Exp Ther* 1984; **231:** 555–565.
22 Ushijima I, Tanaka M, Tsuda A, Koga S, Nagasaki N. Differential effects of morphine on core temperature in stress and non-stressed rats. *Eur J Pharmacol* 1985; **112:** 331–337.
23 Wright BE, Katovich MJ. Effect of restraint on drug-induced changes in skin and core temperature in biotelemetered rats. *Pharmacol Biochem Behav* 1996; **55:** 219–225.
24 Holtzman SG, Villarreal JE. Morphine dependence and body temperature in rhesus monkeys. *J Pharmacol Exp Ther* 1969; **166:** 125–133.
25 Buckingham JC, Cooper TA. Differences in hypothalamo-pituitary-adrenocortical activity in the rat after acute and prolonged treatment with morphine. *Neuroendocrinology* 1984; **38:** 411–417.
26 Buckingham JC, Cooper TA. Interrelationships of opioidergic and adrenergic mechanisms controlling the secretion of cortictrophin releasing factor in the rat. *Neuroendocrinology* 1987; **46:** 199–206.
27 Ignar DM, Kuhn CM. Effects of specific mu and kappa opiate tolerance and abstinence on hypothalmo-pituitary-adrenal axis secretion in the rat. *J Pharmacol Exp Ther* 1990; **255:** 1287–1295.
28 Guaza C, Torrellas A, Borrell J, Borrell S. Effects of morphine upon the pituitary-adrenal system and adrenal catecholamines: a comparative study in cats and rats. *Pharmacol Biochem Behav* 1979; **11:** 57–63.
29 Eisenberg RM. Effects of chronic treatment with diazepam, phenobarbital, or amphetamine on naloxone-precipitated morphine withdrawal. *Drug Alcohol Depend* 1985; **15:** 375–381.
30 Milanes MV, Laorden ML, Chapleur-Chateau M, Burlet A. Alterations in corticotropin-releasing factor and vasopressin content in rat brain during morphine withdrawal: correlation with hypothalamic noradrenergic activity and pituitary-adrenal response. *J Pharmacol Exp Ther* 1998; **285:** 700–706.
31 Fukunaga Y, Nishida S, Inoue N, Miyamoto M, Kishioka S, Yamamoto H. Time course of morphine withdrawal and preproenkephalin gene expression in the periaqueductal gray of rats. *Mol Brain Res* 1998; **55:** 221–231.
32 Budziszewska BB, Jaworska L, Lason W. Repeated morphine administration down-regulates glucocorticoid, but not mineralocorticoid, receptors in the rat hippocampus. *Psychoneuroendocrinology* 1995; **20:** 75–81.
33 Nock B, Wich M, Cicero TJ. Chronic exposure to morphine increases corticosteroid-binding globulin. *J Pharmacol Exp Ther* 1997; **282:** 1262–1268.
34 Buckingham JC, Cooper TA. Effects of naloxone on hypothalamo-pituitary-adrenocortical activity in the rat. *Neuroendocrinology* 1986; **42:** 421–426.
35 Zhou Y, Spangler R, Maggos CE, Wang XM, Han JS, Ho A, Kreek MJ. Hypothalamic-pituitary-adrenal activity and pro-opiomelanocortin mRNA levels in the hypothalamus and pituitary of the rat are differentially modulated by acute intermittent morphine with or without water restriction stress. *J Endocrinol* 1999; **163:** 261–267.
36 Akana SF, Dallman MF. Feedback and facilitation in the adrenocortical system: unmasking facilitation by partial inhibition of the glucocorticoid response to prior stress. *Endocrinology* 1992; **131:** 57–68.
37 Vernikos J, Dallman MF, Bonner C, Katzen A, Shinsako J. Pituitary-adrenal function in rats chronically exposed to cold. *Endocrinology* 1982; **110:** 413–420.
38 Bhatnagar S, Mitchell JB, Betito K, Boksa P, Meaney MJ. Effects of chronic intermittent cold stress on pituitary adrenocortical and sympathetic adrenomedullary functioning. *Physiol Behav* 1995; **57:** 633–639.
39 Garcia A, Marti O, Valles A, Dal-Zotto S, Armario A. Recovery of the hypothalamic-pituitary-adrenal response to stress. *Neuroendocrinology* 2000; **72:** 114–125.
40 Tallarida RJ, Murray RB. *Manual for Pharmacologic Calculations with Computer Programs.* New York: Springer-Verlag, 1987.
41 Ary M, Lomax P. Influence of narcotic agents on temperature regulation. In: Loh HH, Ross DH, eds. *Neurochemical Mechanisms of Opiates and Endorphins.* New York: Raven Press, 1979: 429–451.
42 Dallman MF, Akana SF, Scribner KA, Bradbury MJ, Walker CD, Strack AM, Cascio CS. Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *J Neuroendocinol* 1991; **4:** 517–526.
43 Scribner KA, Akana SF, Walker CD, Dallman MF. Streptozotocin-diabetic rats exhibit facilitated adrenocorticotropin responses to acute stress, but normal sensitivity to feedback by corticosteroids. *Endocrinology* 1993; **133:** 2667–2674.
44 Blanchard RJ, Nikylina JN, Sakai RR, Mckittrick C, McEwen B, Blanchard C. Behavioral and endocrine changes following chronic predatory stress. *Physiol Behav* 1998; **63:** 561–569.
45 Buwalda B, de Boer SF, Van Kalkeren AA, Koolhaas JM. Physiological and behavioral effects of chronic intracerebroventricular infusion of corticotropin-releasing factor in the rat. *Psychoneuroendocrinology* 1997; **22:** 297–309.
46 Jacobson L, Akana SF, Cascio CS, Scribner K, Shinsako J, Dallman MF. The adrenocortical system responds slowly to removal of corticosterone in the absence of concurrent stress. *Endocrinology* 1989; **124:** 2144–2152.
47 Bhatnagar S, Dallman M. The paraventricular nucleus of the thalamus alters rhythms in core temperature and energy balance in a state-dependent manner. *Brain Res* 1999; **851:** 66–75.
48 Long NC, Morimoto A, Nakamori T, Murakami N. The effect of physical restraint on IL-1B and LPS-induced fever. *Physiol Behav* 1991; **50:** 625–628.
49 Terlouw EM, Kent S, Cremona S, Dantzer R. Effect of intracerebroventricular administration of vasopressin on stress-induced hyperthermia in rats. *Physiol Behav* 1996; **60:** 417–424.
50 Bhatnagar S, Dallman M. Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience* 1998; **84:** 1025–1039.
51 Akana SF, Hanson ES, Horsley CJ, Strack AM, Bhatnagar S, Bradbury MJ, Milligan ED, Dallman MF. Clamped corticosterone (B)

reveals the effect of endogenous B on both facilitated responsivity to acute restraint and metabolic responses to chronic stress. *Stress* 1996; **1:** 33–49.

52 Sapolksy RM, Krey LC, McEwen BS. Stress down-regulates corticosterone receptors in a site-specific manner in the brain. *Endocrinology* 1984; **114:** 287–292.

53 Sapolsky RM, Krey LC, McEwen BS. Down-regulation of neural corticosterone receptors by corticosterone and dexamethasone. *Brain Res* 1985; **339:** 161–165.

54 Sapolsky RM, Plotsky PM. Hypercortisolism and its possible neural bases. *Biol Psychiatry* 1990; **27:** 937–952.

55 Meaney MJ, Bhatnagar S, Diorio J, Larocque S, Francis D, O'Donnel D, Shanks N, Sharma S, Smythe J, Viau V. Molecular basis for the development of individual differences in the hypothalamic-pituitary-adrenal stress response. *Cell Mol Neurobiol* 1993; **13:** 321–347.