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Counteraction by Morphine of Stress-Induced Inhibition of Growth Hormone Release in the Rat (41923)

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Abstract. The purpose of this study was to determine the effect of morphine (MOR) administration on pituitary growth hormone (GH) release during stress in the male rat. Circulating GH levels were significantly decreased following a brief (2 min) exposure to ether, during repetitive etherization coupled with blood withdrawal, and during continuous immobilization. Under all three stress conditions, systemic administration of MOR resulted in a significant increase in plasma GH levels compared to the vehicle-treated group. These results indicate that the pathway for opiate-induced stimulation of GH release is functional during stress, and suggest that the suppressive effect of stress does not involve a blockade of opiate receptor stimulation of GH. Thus, the present findings, taken together with reports that the overall activity of central opioid neurons is enhanced during stress, support the view that the decline in GH is due to the overriding inhibitory influence of an independent nonopioid mechanism. However, MOR can apparently increase opiate receptor stimulation sufficiently to counteract this inhibitory signal, implying that stress and the opiates may influence GH release via separate mechanisms.

The effects of stress on pituitary GH release have been investigated extensively, with several studies reporting that stressed rats show a decline in plasma GH levels (1–3). In contrast, stress in humans has been observed to promote an increase in circulating GH (4, 5). Although the mechanism(s) underlying these hormonal responses is not completely understood, Arimura *et al.* (6) and Terry *et al.* (7) have reported that passive immunization against growth hormone release-inhibiting factor (somatostatin or SRIF) can prevent the stress-induced decrease in plasma GH in the rat. These findings suggest that the suppressive effect of stress on GH release in this species may be mediated, at least in part, by an alteration in release of hypothalamic SRIF. Alternatively, stress may modify hypothalamic release of growth hormone-releasing factor (GRF), but this possibility has not yet been explored.

In the rat, stress enhances the overall activity of central opiatergic neurons, as suggested by changes in both hypothalamic and whole brain opiate content (8–10), and a decrease in endogenous receptor availability (11, 12). Stress also results in an increase in release of pituitary β-endorphin (13, 14). There is good evidence that endogenous opiate receptors are present within central neural pathways that stimulate GH secretion. In both the rat and man, administration of morphine or the endogenous opioid peptides (EOPs) results in an increase in basal circulating GH levels (15–19). While the EOPs are apparently not involved in the regulation of basal GH release, there is evidence that they may stimulate the increased release of GH in response to specific stimuli. Naloxone, a specific opiate antagonist, has no effect upon basal GH levels (20–23), but can prevent the elevation of circulating GH induced by suckling (24) or 2-deoxy-D-glucose injection in rats (25), or exercise in humans (23). It is generally believed that opiate action on pituitary GH secretion occurs at the level of the hypothalamus, since neither morphine nor the EOPs alter pituitary release of GH *in vitro* (19).

Since stress in the rat results in a decrease in plasma GH despite an overall increase in endogenous opiate activity, it is possible that the stimulatory effect of the EOPs on GH release is overridden by an independent inhibitory signal initiated by stress. On the

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other hand, EOP action on GH release may be blocked by stress. In the present study, we examined the effect of morphine administration on circulating GH levels during stress to determine if opiate/receptor stimulation can counteract the suppressive effect of stress on GH release.

Methods and Materials. *Animals and chemicals.* Young adult male Sprague–Dawley rats were purchased from Charles River Breeding Laboratories (Portage, Mich.) and maintained in our animal facilities for at least 2 weeks prior to experimental use. Animals were housed in groups of four to five per cage under a lighting schedule of 14 hr light:10 hr dark, and received food and water *ad libitum.* Morphine sulfate (MOR) was provided by Mallinckrodt, Inc. (St. Louis, Mo.). MOR was weighed out and dissolved in sterile saline (0.9%) immediately before use.

Experiment 1: Effect of MOR on acute ether stress-induced reduction in plasma GH levels. At the onset of the experiment, animals (300–350 g body wt) were divided into the following five groups (eight animals per group): groups 1 through 4 were assigned to undergo acute ether stress, while group 5 was designated as the unstressed controls. Immediately prior to stress, animals received a subcutaneous (sc) injection of either MOR (5 mg/kg body wt) (groups 1 and 2) or the vehicle (SAL, groups 3 and 4). Unstressed controls (group 5) were also injected with SAL at this time. Groups 1 through 4 were then subjected to saturated ether vapor for 2 min. Animals were sacrificed by decapitation and trunk blood collected at 30 (groups 1, 3 and 5) and 90 min (groups 2 and 4) after initiation of stress.

Experiment 2: Effect of MOR on plasma GH levels during repetitive blood sampling under ether anesthesia. Animals were divided into two groups (eight animals per group), bled by orbital sinus puncture under light ether anesthesia and injected sc immediately thereafter with either 5 mg MOR/kg body wt (group 1) or SAL (group 2). Serial blood samples were obtained from each animal by orbital puncture at 15, 30, and 60 min after injection.

Experiment 3: The effect of MOR on restraint stress-induced decrease in plasma GH release. Two days prior to restraint stress, animals were implanted with Silastic intraatrial cannulae under light ether anesthesia and housed in individual cages. On the day of the experiment, animals were divided into two groups. Prior to onset of immobilization, two blood samples, of 0.7 ml each, were obtained from each animal via cannula. At time 0, animals in both groups were subjected to continuous restraint stress, according to a method previously used in our laboratory (26). Concomitant with the onset of restraint, animals in group 1 ($n = 7$) were injected sc with 5 mg MOR/kg body wt, while group 2 ($n = 6$) received a sc injection of SAL. Subsequent blood samples (0.7 ml) were obtained from both groups at 15, 30, and 60 min after onset of stress. Throughout the experiment, each blood sample was immediately centrifuged to separate plasma from cells. The cells were then resuspended in sterile saline and returned to the appropriate animal.

Hormone analysis and statistical procedure. Plasma GH levels were determined by radioimmunoassay, using an NIAMDD GH kit. GH values were expressed as nanogram per milliliter in terms of NIAMDD-GH-RP-1. Analysis of variance and Duncan's multiple range test were used to analyze the data. Differences were considered significant if $P < 0.05$.

Results. As shown in Fig. 1, 30 min after acute ether exposure, plasma GH concentrations were significantly depressed when compared to the unstressed, SAL-injected controls. By 90 min, GH levels were still low, but not statistically different from control values. MOR injection prior to etherization prevented the stress-induced decline in plasma GH. At both 30 and 90 min after stress, MOR-treated animals showed a significant elevation in plasma GH relative to the vehicle-treated group, and a return of GH levels to nonstress baseline values.

The effects of serial bleeding under light ether anesthesia on plasma GH are summarized in Table I. SAL-injected animals undergoing repetitive blood sampling showed a progressive decline in plasma GH over time. MOR treatment after the first sample reversed this trend, resulting in an elevation of circulating GH that was significantly different

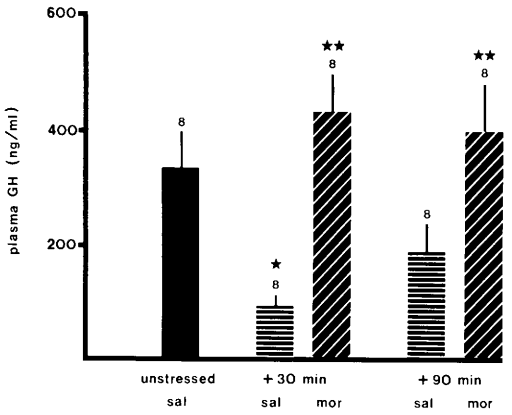

FIG. 1. Effect of MOR on acute ether stress-induced changes in plasma GH. Bars represent mean plasma GH values, while vertical lines show SEM. Numerals above bars indicate number of animals per group. Animals were injected sc with either SAL or MOR (5 mg/kg body wt) and, except the controls (solid bar), subjected to 2 min of etherization. Animals were sacrificed either 30 or 90 min later. One star = $P < 0.05$, compared to unstressed controls. Two stars = $P < 0.05$, compared to the stressed, SAL-treated group.

from the vehicle-treated group at the +60-min time point.

As shown in Table II, continuous immobilization stress resulted in a significant reduction in plasma GH from prestress levels at all time points examined. Animals injected with MOR at the onset of restraint stress showed significantly elevated GH levels as compared to the vehicle-treated group at both 30 and 60 min of stress.

Discussion. The present findings are in agreement with previous reports showing that plasma GH levels are suppressed in rats following acute ether exposure and during repetitive etherization coupled with blood withdrawal, or continuous immobilization (3). In the present study, the systemic administration of MOR to rats stressed by any one of these means resulted in a significant increase in circulating GH to levels comparable to or greater than nonstress control values. These results indicate that stimulation of endogenous opiate receptors can promote an increase in GH release despite the suppressive influence of stress, and show that the pathway for opiate-induced GH release is functional during stress. The present findings suggest that the decline in GH release during stress does not result from a blockade of opiate receptor stimulation of GH, but rather may be due to the overriding inhibitory effect of an independent nonopioid mechanism.

It is of interest at this point to examine the possible mechanism(s) by which stress decreases GH secretion in the rat. There is some evidence tht hypothalamic release of somatostatin may mediate the suppressive effect of stress on GH. It has been reported that prior immunization of rats against somatostatin can prevent the decline in circulating GH levels during stress (6, 7). Furthermore, there is evidence that somatostatin can attenuate opiate-induced stimulation of GH release, as indicated by reports that somatostatin can blunt the increase in plasma GH levels observed after morphine administration (27). Thus, these findings would suggest that the decline in GH release during stress may be due to the release of hypothalamic SRIF in sufficient quantities to override the stimulatory effect of the EOPs on GH. On the other hand, stress may also result in an attenuation of opiate release by the specific opioid neurons that stimulate GH. While several studies indicate that overall endoge-

TABLE I. EFFECT OF MOR ON PLASMA GH LEVELS DURING REPETITIVE ETHERIZATION PLUS BLOOD WITHDRAWAL

	Plasma GH (ng/ml)			
Treatment at t^0	t^0	+15 min	+30 min	+60 min
Saline (n = 7), sc	137.8 ± 56.0	30.0 ± 10.3[a]	13.3 ± 5.7[a]	4.7 ± 1.8[a]
Morphine (n = 8), 5 mg/kg body wt, sc	176.7 ± 92.9	84.1 ± 42.4	132.8 ± 71.4	96.7 ± 24.0[b]

[a] $P < 0.05$, compared to t^0.
[b] $P < 0.05$, compared to saline-treated group.

TABLE II. EFFECT OF MORPHINE SULFATE ON RESTRAINT STRESS-INDUCED CHANGES IN PLASMA GH

Treatment at t^0	Plasma GH (ng/ml)				
	−60 min	−40 min	+15 min	+30 min	+60 min
Restraint + saline (n = 6)	44.6 ± 24.8	23.2 ± 13.2	3.6 ± 1.8[a]	2.6 ± 0.5[a]	0.2 ± 0.2[a]
Restraint + morphine 5 mg/kg body wt (n = 7)	44.5 ± 9.2	16.5 ± 4.2	13.3 ± 4.4	163.6 ± 43.8[b]	99.9 ± 31.8[b]

[a] $P < 0.05$, compared to prestress values.
[b] $P < 0.05$, compared to saline-treated group.

nous opiate activity within the hypothalamus is increased during stress, these findings do not reveal the functional status of individual opioid neuronal pathways and may mask an opposite trend within an isolated pathway. Thus, the possibility exists that the decline in GH may be due, at least in part, to a decrease in stimulation of the specific opiate receptors associated with GH release.

It is probable that the endogenous opiates stimulate whereas stress inhibits GH release via independent neuroendocrine pathways. Stress apparently depresses GH release by stimulating the activity of the inhibitory SRIF pathway, while MOR acts to prevent this decline in GH by sufficiently increasing activity within the excitatory pathway to override this inhibitory signal. It is interesting to speculate whether MOR's action on GH involves hypothalamic release of GRF. If the opiates act to increase GH secretion by elevating GRF release, and stress depresses GH secretion by increasing somatostatin release, then the effect achieved by concurrent stress and opiate administration on GH release would depend on the relative concentrations of GRF and somatostatin in the hypophysial portal circulation.

There is little evidence to suggest that the suppressive effect of stress on GH is mediated by an effect upon hypothalamic neurotransmitters known to influence GH. It is well established that norepinephrine, dopamine, and serotonin all stimulate GH secretion (28). However, acute exposure to stress has been observed to increase noradrenergic, dopaminergic, and also serotonergic activity within the hypothalamus (26, 29–32), suggesting that none of these neuronal systems play a role in the decline in GH. While acetylcholine has been reported to stimulate GH release (28), the effect of stress on central cholinergic activity is not clear. Similarly, there is little evidence that the stimulatory effect of the opiates on GH are mediated via a catecholaminergic mechanism. Studies have shown that opiates have an inhibitory effect upon both hypothalamic norepinephrine and dopamine activity (33–36), suggesting that other mechanisms are involved. There is some evidence that the opiates stimulate hypothalamic serotonin activity (37, 38). Since serotonin is known to increase GH (28), it is possible that opiate effects on GH are mediated, at least in part, via this neurotransmitter. It is also possible that EOP effects on GH release are mediated via cholinergic pathways. While the results of these turnover studies suggest that various neurotransmitters may or may not be involved in stress or opiate effects on GH, it should be kept in mind that measurement of turnover within the whole hypothalamus may obscure an opposite trend within the individual pathway influencing GH release.

In conclusion, the present findings suggest that any possible increase in EOP activity induced by stress is insufficient of itself to overcome the inhibitory mechanism(s) responsible for the stress-induced decrease in GH release. However, exogenous administration of an opiate, morphine, can apparently increase opiate receptor activity sufficiently to counteract the suppressive effect of stress on GH. These findings support the view that the stress-induced inhibition and MOR-induced stimulation of GH release are mediated by independent opposing mechanisms.

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