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# Counteraction by Naltrexone of Stress-Induced Inhibition of TSH Release: Role of Noradrenergic System (41956)

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*Abstract.* The purpose of the present study was to examine the effect of administering an opiate receptor antagonist, naltrexone (NALT) on the decline in pituitary thyrotropin (TSH) release induced by both acute and chronic stress, and to determine whether norepinephrine (NE) is involved in the mechanism by which opiate receptor blockade counteracts inhibition of TSH release during stress. Administration of NALT, a specific opiate receptor antagonist, significantly attenuated the decrease in plasma TSH observed after acute exposure to ether or restraint stress. The ability of NALT to prevent suppression of circulating TSH in ether-stressed rats was blocked by pharmacological suppression of NE activity induced by pretreatment with diethyldithiocarbamate (DDC) or phenoxybenzamine (PB), both NE antagonists. In chronically stressed rats, thrice daily injections of NALT attenuated the sustained decline in circulating TSH, and resulted in a significant elevation in plasma TSH when compared with stressed, saline-treated animals. Pretreatment with DDC prior to NALT injection abolished this stimulatory effect of NALT. These observations indicate that opiate/receptor interaction is prerequisite for the decrease in circulating TSH release during both acute and chronic stress, and support the hypothesis that endogenous opioid peptides (EOPs) mediate the suppressive effect of stress on TSH release. The finding that uninterrupted NE function is necessary for NALT's action on TSH release during stress suggests that the suppressive effect of stress on TSH and its reversal by opiate antagonists involves alterations in hypothalamic NE activity.



There is good evidence that endogenous opiate receptors within central neural pathways are involved in the regulation of pituitary thyrotropin (TSH) secretion. Thus, administration of morphine (MOR) or the endogenous opiate, met-enkephalin, resulted in a decrease in basal plasma TSH levels (1–3), and an attenuation of the increase in TSH release after cold exposure or thyroidectomy (2, 3). Opiate receptor antagonists such as naloxone (NAL) were shown to have no effect on basal circulating TSH levels (1), but to prevent the decrease in TSH release induced by heat or restraint stress (3, 4). These studies suggested that the endogenous opioid peptides (EOPs) do not influence basal pituitary TSH secretion, but they do have a role in mediating the effects of acute stress on TSH release.

The mechanism(s) by which the opiates influence TSH secretion are not entirely resolved, but several studies have provided evidence for a hypothalamic site of action (5, 6). Sharp *et al.* (3) observed that the MOR-induced decrease in TSH in cold-exposed animals is accompanied by an increase in hypothalamic TRH content, suggesting that the EOPs inhibit TSH release by inhibiting TRH release. Opiates apparently do not exert a direct inhibitory effect on the pituitary, since TRH stimulation of TSH release is unaltered by MOR *in vivo* (2) or *in vitro* (3). Other studies indicate that opiates and opiate receptor antagonists alter the activity of several hypothalamic neurotransmitters, including norepinephrine (NE) (7). Since NE is a major hypothalamic promoter of TSH release (8), it is possible that it is involved in the mechanism by which the EOPs and their antagonists influence pituitary TSH release.

Although it has been reported that the EOPs are involved in the decrease in TSH elicited by one type of stress, restraint, it is not clear whether this is a mechanism common to a variety of stress stimuli or if it is unique to this specific stressor. Also, little is known about possible EOP involvement in

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sustained suppression of TSH secretion during chronic stress (9). Information of this nature is important since chronicity is a common characteristic of most stressors encountered in daily life (9). The purpose of the present study was to investigate the role of the EOPs in the decline in TSH release induced by both acute and chronic stress, and to determine whether NE is involved in these processes.

**Methods and Materials.** *Animals and drugs.* Young adult male Sprague–Dawley rats (2 months of age) 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 14-hr light:10-hr dark schedule and received food and water *ad libitum.* Morphine sulfate (MOR) was a gift from Mallinckrodt, Inc. (St. Louis, Mo.), and naltrexone hydrochloride (NALT) was kindly provided by Dr. Robert Wilette, National Institute on Drug Abuse (Bethesda, Md.). Sodium diethyldithiocarbamate (DDC), an inhibitor of NE synthesis, was purchased from Fisher Scientific Company (Fair Lawn, N.J.), and phenoxybenzamine hydrochloride (PB), an $\alpha$-adrenergic receptor blocker, from Smith, Klein and French Labs (Philadelphia, Pa.). All drugs were weighed and dissolved in sterile NaCl (0.9%) immediately before use.

*Experiment 1: Effect of NALT or NALT and MOR on decreases in plasma TSH induced by restraint stress.* Two days prior to experimentation, rats (280–315 g body wt) were implanted with Silastic intraatrial cannulae under light ether anesthesia and housed in individual cages. On the day of the experiment, rats were divided into three groups (seven animals per group). Three prestress blood samples (0.7 ml each) were obtained via cannula from each animal at 20-min intervals. Throughout the experiment, all blood samples were centrifuged immediately after withdrawal to separate plasma from cells, and cells were resuspended in sterile saline and returned to the animals via cannula. At time zero, all three groups were subjected to continuous immobilization stress according to a previously described method (10). With the onset of stress, animals in group 1 received a subcutaneous injection of NALT (2 mg/kg body wt), while group 2 was injected with the same dose of NALT plus 5 mg MOR/kg body wt. Group 3 received a sc injection of the vehicle, saline (SAL). Subsequent blood samples were withdrawn via cannula at 30, 60, 90, and 180 min after the onset of immobilization.

*Experiment 2: Effect of NALT or NALT and MOR on decrease in plasma TSH induced by ether stress.* Prior to experimentation, animals (300–350 g body wt) were divided into three groups (12 animals per group). A 1-ml blood sample was then obtained by orbital sinus puncture from each animal. Immediately thereafter, animals were injected as follows: group 1 received a sc injection of NALT (2 mg/kg body wt), and group 2 was injected sc with the same dose of NALT plus 5 mg MOR/kg body wt. Group three received a sc injection of SAL. Animals in each group were then exposed to saturated ether vapor for a total of 15 min. Additional blood samples were obtained by orbital puncture at 15, 35, and 60 min after onset of etherization.

*Experiment 3: Effect of DDC pretreatment on ability of NALT to prevent decrease in TSH release induced by ether stress.* Animals (300–355 g body wt) were divided into two groups (group 1, $N = 10$; group 2, $N = 11$), bled by orbital sinus puncture, and injected sc with DDC (500 mg/kg body wt), an NE synthesis inhibitor. At the end of 90 min, each animal was bled for a second time and then injected as follows: group 1 received a sc injection of NALT (2 mg/kg body wt), and group 2 was injected sc with SAL. Immediately thereafter, the rats were exposed to saturated ether vapor for 15 min and blood samples were taken 15 and 40 min later.

*Experiment 4: Effect of pretreatment with PB on ability of NALT to prevent decrease in TSH release after ether stress.* The experimental protocol described in Experiment 3 was repeated, with the exception that 120 min prior to ether exposure, animals (390–450 g body wt) received an ip injection of PB (20 mg/kg body wt), an $\alpha$-receptor antagonist. At time zero, animals were divided into two groups, bled for a second time, and injected as follows: group 1 ($N = 11$) received

NALT (2 mg/kg body wt) sc, while group 2 ($N = 10$) was injected with SAL. Both groups were then exposed to ether vapor for 15 min and bled 15 and 40 min later.

*Experiment 5: Effect of 3× daily NALT injection on inhibition of TSH release induced by a chronic stress.* On Day 1 of the experiment, rats (310–360 g body wt) were divided into three groups. On the same day, groups 1 ($N = 9$) and 2 ($N = 8$) were subjected to chronic stress induced by implantation of a 2-in.-square gauze pad under the skin of the abdomen (11). Rats in group 3 ($N = 8$) served as unoperated controls. On Day 2, rats in group 1 received three sc injections of NALT (2 mg/kg body wt) at 4-hr intervals, while groups 2 and 3 received three sc injections of SAL at the same time intervals. Animals were bled by orbital sinus puncture 20 min after the last injection of NALT or SAL. Injections were repeated on Days 3 and 4, and blood samples were obtained after the last injection of NALT or SAL on both days.

*Experiment 6: Effect of DDC pretreatment on ability of NALT to counteract depressing effect of chronic stress on TSH release.* Rats were divided into five groups (seven animals per group) as follows: groups 1 through 4 were implanted with 2-in.-square gauze pads as described above, whereas group 5 served as unoperated controls. At 48 hr after implantation, groups 1 and 2 were injected sc with DDC (500 mg/kg body wt), whereas groups 3–5 were given SAL. By 90 min later, animals in groups 2 and 4 received sc injections of NALT (2 mg/kg body wt), whereas groups 1, 3, and 5 received SAL. Animals were killed by decapitation and trunk blood was collected 20 min after injection of either NALT or SAL.

*Hormone analysis and statistical procedure.* Plasma TSH concentrations were determined by radioimmunoassay, using a NIAMDD TSH kit. Plasma TSH levels were expressed as nanograms per milliliter, in terms of NIAMDD-TSH-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.** Figure 1 shows that continuous immobilization of SAL-injected animals resulted in a significant reduction in plasma TSH from prestress levels at all time points

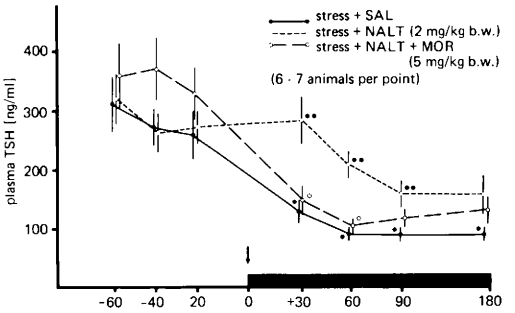


FIG. 1. Effects of NALT and NALT together with MOR on restraint stress-induced depression of plasma TSH levels. Duration of restraint is indicated by the solid bar on the abscissa. At the onset of stress, animals were injected sc with SAL, NALT, or NALT together with MOR (indicated by arrow). Solid asterisk: $P < 0.05$, compared to prestress values. Two solid asterisks: $P < 0.05$, compared to SAL-treated group. Empty asterisk: $P < 0.05$, compared to NALT-treated group.

examined. NALT administration at the onset of stress reversed this decline. In this group, TSH plasma levels were significantly elevated above the vehicle-treated control group at 30, 60, and 90 min of restraint, and did not differ significantly from prestress values for at least 30 min after onset of stress. Administration of MOR together with NALT prevented the NALT-induced elevation of TSH during stress. At both 30 and 60 min after onset of stress, TSH levels in rats given NALT together with MOR were significantly lower than corresponding values in the group given NALT alone, and remained significantly depressed compared to prestress levels throughout the entire restraint period.

The effect of NALT on ether stress-induced changes in circulating TSH levels was also investigated (Table I). Plasma TSH was significantly reduced at both 30 and 60 min after onset of a 15-min period of etherization. Rats injected with NALT prior to stress showed significantly higher TSH levels when compared to the vehicle-treated group at both 30 and 60 min poststress. Administration of MOR together with NALT reversed the effect induced by NALT alone, resulting in a significant decrease in TSH as compared to the NALT-injected group at both 30 and 60 min. In order to investigate the role of NE in the stimulatory effect of NALT on TSH release in stressed rats, an NE synthesis

TABLE I. EFFECT OF NALT OR NALT AND MOR ON ETHER STRESS-INDUCED DECREASES IN PLASMA TSH

| Treatment at time 0 | Plasma TSH (ng/ml) | | | |
|---|---|---|---|---|
| | Time 0 | +15 min | +35 min | +60 min |
| SAL + 15 min ether stress ($N$ = 8) | 371.3 ± 52.6 | 263.4 ± 30.0[a] | 216.2 ± 19.9[a] | 244.7 ± 19.9[a] |
| NALT[d] + 15 min ether stress ($N$ = 8) | 349.1 ± 38.4 | 287.0 ± 17.1 | 382.0 ± 64.9[b] | 362.8 ± 26.6[b] |
| NALT + MOR[e] + 15 min ether stress ($N$ = 8) | 361.1 ± 69.8 | 258.4 ± 36.6 | 277.8 ± 45.8[c] | 234.8 ± 34.7[c] |

[a] $P < 0.05$, compared to time 0.
[b] $P < 0.05$, compared to the SAL-treated group.
[c] $P < 0.05$, compared to the NALT-treated group.
[d] 2 mg/kg body wt, sc.
[e] 5 mg/kg body wt, sc.

inhibitor, DDC, was administered prior to etherization and injection with either SAL or NALT (Fig. 2). By 90 min after DDC treatment, circulating TSH levels were dramatically decreased from initial values. In the SAL-treated group, subsequent ether exposure resulted in a further reduction in plasma TSH levels. However, this decrease was not statistically significant. Animals injected with NALT prior to etherization showed a similar trend toward a further decline in TSH levels after ether exposure. Similar results were observed in animals pretreated with PB (Fig. 3).

Additional experiments were carried out to examine the effect of NALT on the prolonged decline in plasma TSH levels during chronic stress. As shown in Fig. 4, chronic stress produced by gauze pad implantation resulted in a significant reduction in circulating TSH for 3 consecutive days after surgery. Thrice daily NALT treatment blocked this decrease, resulting in a significant increase in plasma TSH compared to the stressed, SAL-treated group on all 3 days of treatment. Pretreatment of chronically stressed rats with DDC (Fig. 5) blocked NALT's ability to elevate the suppressed levels of circulating TSH in these animals.

**Discussion.** The results of the present study show that NALT, a specific endogenous opiate receptor antagonist, can reverse the decline in plasma TSH observed after exposure to either acute or chronic stress. These studies also demonstrate that the ability of NALT to counteract the inhibitory effect of stress on TSH release is mediated by the hypotha-

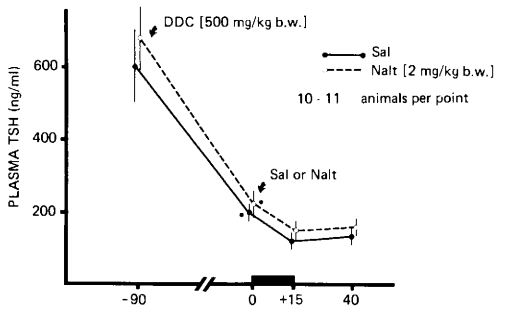


FIG. 2. Effect of DDC on TSH response to NALT after 15 min of ether stress. At 90 min prior to etherization, animals were injected with 500 mg DDC/kg body wt. Immediately before ether stress (indicated by solid bar on abscissa), rats received SAL or NALT sc. Solid asterisk: $P < 0.05$, compared to initial, pre-DDC values.

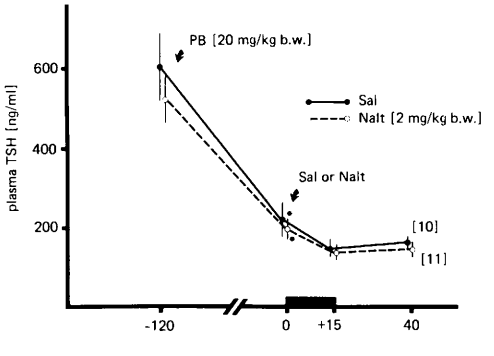


FIG. 3. Effect of PB on TSH response to NALT after 15 min ether stress. At 120 min before etherization, animals were injected with 20 mg PB/kg body wt. Immediately prior to ether stress (indicated by solid bar on abscissa), rats received SAL or NALT sc. Solid asterisk: $P < 0.05$, compared to initial, pre-PB values.

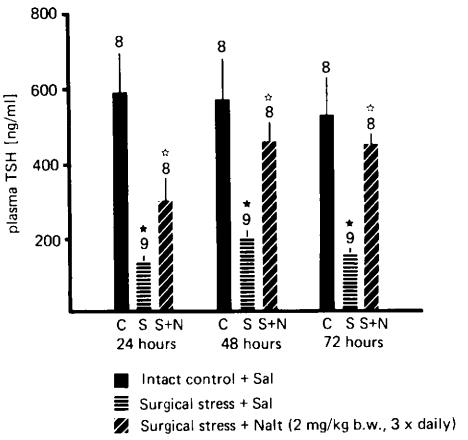


FIG. 4. Effect of daily NALT on chronic stress-induced changes in plasma TSH. Bars represent mean plasma TSH levels, and vertical lines show SEM. Numerals above bars indicate number of animals per group. On Days 2, 3, and 4 of gauze pad implantation, rats received 3 daily sc injections of NALT (2 mg/kg body wt) or SAL. Blood samples were taken 20 min after the last injection on each day of treatment. Solid star: $P < 0.05$, compared to the unstressed, SAL-injected group. Open star: $P < 0.05$, compared to the stressed, SAL-treated group.

lamic noradrenergic system. At present, it is not clearly understood how the EOPs exert an inhibitory influence on TSH in response to specific stimuli such as heat or stress, in the absence of an effect under basal conditions. Since central EOP activity is increased during stress (11, 12), it is possible that stimulation of endogenous receptors is enhanced to an extent necessary to permit an effect. On the other hand, stress may increase opiate receptor responsiveness to EOP stimulation. It was reported previously that NAL, another opiate receptor antagonist, can reverse the decline in circulating TSH levels induced by acute restraint stress (4). The present studies confirm and extend these findings by providing evidence that the EOPs are involved in the decline in TSH release induced by acute exposure to other stressors, both acute and chronic.

Ether exposure and immobilization are two of the most common stressors used experimentally to determine the effects of stress on various endocrine parameters, but the mechanism(s) by which either of these stressors alters hypothalamic–pituitary–thyroid function remains to be clarified. The present results indicate that central opioid neurons are involved in the inhibitory action of both kinds of stress on pituitary TSH release. Previous work has shown that long-term daily immobilization resulted in a sustained decline in plasma TSH levels (9). In the present study, comparable results were observed after prolonged stress induced by gauze pad implantation. Thrice daily NALT injections effectively reversed this inhibition, suggesting that the suppression of circulating TSH levels during chronic stress is mediated, at least in part, via the EOP system.

Administration of either DDC, an NE synthesis inhibitor, or PB, an NE $\alpha$-receptor blocker, prior to acute ether exposure, prevented NALT from counteracting the inhibitory action of ether stress on TSH release. Similarly, DDC pretreatment of chronically stressed rats was observed to effectively block the ability of NALT to counteract the inhibitory action of the chronic stress on TSH

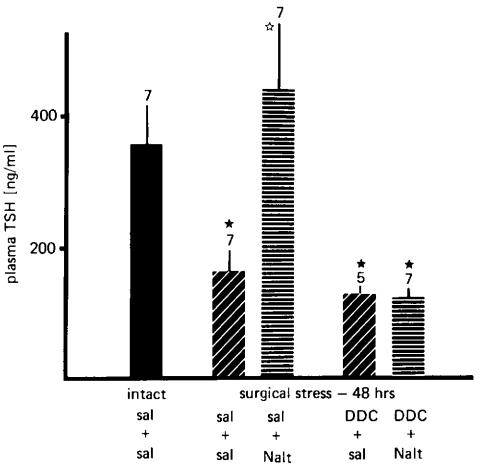


FIG. 5. Effect of DDC pretreatment on plasma TSH response to NALT during chronic stress. Bars represent mean plasma TSH values, and vertical lines show SEM. Numerals above bars indicate number of animals per group. At 48 hr after gauze pad implantation, stressed rats were divided into two groups and received injections of either DDC (500 mg/kg body wt) or SAL prior to stress; the unstressed control group received SAL. By 90 min later, half of the stressed animals in each group were injected sc with NALT (2 mg/kg body wt), and the remainder received SAL. Solid star: $P < 0.05$, compared to the unstressed, control group. Empty star: $P < 0.05$, compared to the stressed, SAL (×2)-treated group.

release in these animals. These findings indicate that uninterrupted NE activity is prerequisite for NALT to reverse the inhibitory effect of stress on plasma TSH release. Hypothalamic NE has been shown to be essential for promoting TSH release (8), and is believed to act by inducing increased hypothalamic release of TRH. MOR has been reported to decrease NE content in the hypothalamus (13), whereas NAL was demonstrated to increase hypothalamic turnover of this neurotransmitter (14). In conclusion, the results of the present study indicate that the EOPs mediate the suppressive effect of both acute and chronic stress on TSH release, and that this inhibitory effect and its reversal by opiate antagonists involve the hypothalamic NE system.

1. Bruni JF, Van Vugt DA, Marshall S, Meites J. Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. Life Sci **21**:461–466, 1977.
2. Muraki T, Nakadate T, Tokunaga Y, Kato R. Effect of morphine on the release of thyroid-stimulating hormone stimulated by exposure to cold, thyroidectomy and the administration of thyrotrophin releasing hormone in male rats. J Endocrinol **86**:357–362, 1980.
3. Sharp B, Morley JE, Carlson HE, Gordon J, Briggs J, Melmed S, Hershman JM. The role of opiates and endogenous opioid peptides in the regulation of rat TSH secretion. Brain Res **219**:335–344, 1981.
4. Judd AM, Hedge G. The roles of the opioid peptides in controlling thyroid stimulating hormone release. Life Sci **31**:2529–2536, 1982.
5. Lomax P, George R. Thyroid activity following administration of morphine in rats with hypothalamic lesions. Brain Res **2**:361–367, 1966.
6. Lomax R, Kokka N, George R. Thyroid activity following intercerebral injection of morphine in the rat. Neuroendocrinology **6**:146–152, 1970.
7. Meites J, Bruni JF, Van Vugt DA, Smith AF. Relation of endogenous opioid peptides and morphine to neuroendocrine functions. Life Sci **24**:325–336, 1979.
8. Weiner RI, Ganong WF. Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol Rev **58**:905–975, 1978.
9. Du Ruisseau P, Tache Y, Brazeau P, Collu R. Effects of chronic immobilization stress on pituitary hormone secretion, on hypothalamic factor levels, and on pituitary responsiveness to LHRH and TRH in female rats. Neuroendocrinology **29**:90–99, 1979.
10. Mueller GP, Twohy CP, Chen HT, Advis JP, Meites J. Effects of L-tryptophan and restraint stress on hypothalamic and brain serotonin turnover, and pituitary TSH and prolactin release in rats. Life Sci **18**:715–724, 1976.
11. Madden J, Akil H, Patrick RL, Barchas JD. Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. Nature (London) **255**:358–360, 1977.
12. Rossier J, Guillemin R, Bloom F. Foot-shock induced stress decreases leu$^5$-enkephalin immunoreactivity in rat hypothalamus. Eur J Pharmacol **48**:465–466, 1978.
13. De Wied D, Van Ree JM, De Jong W. Narcotic analgesics and the neuroendocrine control of anterior pituitary function. In: Zimmerman F, George R, eds. Narcotics and the Hypothalamus. New York, Raven Press, p251–264, 1974.
14. Van Vugt DA, Aylsworth CF, Sylvester PW, Leung FC, Meites J. Evidence for hypothalamic noradrenergic involvement in naloxone-induced stimulation of luteinizing hormone release. Neuroendocrinology **33**:261–264, 1981.

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