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THE EFFECTS OF OPIATE RECEPTOR AGONISTS AND ANTAGONISTS ON THE STRESS-INDUCED SECRETION OF CORTICOSTERONE IN MICE

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1 Intraperitoneal administration of normorphine, morphine or naloxone or exposure to ether vapour for 1 min, elevated plasma corticosteroid concentrations in mice.

2 Injection of saline or exposure to ether vapour rendered mice less sensitive to a subsequent exposure to ether vapour 15 min later.

3 Treatment with normorphine (50 mg/kg) potentiated the corticosteroid response to ether stress whilst pentazocine (20 mg/kg), naltrexone (10 mg/kg), morphine (24 mg/kg), levorphanol (20 mg/kg) and naloxone (50 mg/kg) prevented the stress-induced elevation of plasma corticosteroids.

4 Both naloxone and morphine inhibited the potentiation by normorphine of the response to ether, the dose of naloxone required being higher than that for inhibition of normorphine analgesia.

5 It is concluded that endogenous opioid peptides may be involved in the control of the response to ether stress in mice.

Introduction

Identification of the enkephalins (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) and endorphins (Cox, Opheim, Teschemacher & Goldstein, 1975) has been followed by experimental evidence which suggests that these endogenous morphine-like compounds may have neutrotransmitter function (Henderson, Hughes & Kosterlitz, 1978; Iversen, Iversen, Bloom, Vargo & Guillemin, 1978). Early interest centred on their roles in pain perception (Belluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976) but the multiplicity of effects of morphine on the endocrine system has focused attention on their possible involvement with anterior pituitary function. Thus, in rats, endogenous opioids or their stable analogues increase plasma concentrations of prolactin (Lien, Fenichel, Garsky, Sarantakis & Grant, 1976) and growth hormone (Dupont, Cusan, Garon, Labrie & Li, 1977) but decrease those of luteinizing hormone and thyrotrophin (Bruni, Van Vugt, Marshall & Meites, 1977).

It is well known that morphine blocks the stress-induced rise of plasma corticosteroid and this, together with the report by Akil, Madden, Patrick & Barchas (1976) that foot-shock in rats increases the amounts of endogenous opioid peptides in brain, suggested that these substances might play a part in the control of the hypothalamus-pituitary-adrenal system (HPA). However, the direction of this possible action of opioids as agonists on the HPA is not clear: the action of morphine itself is not a reliable guide since there is relatively little separation of its agonistic and antagonistic potencies measured on the guinea-pig ileum (Kosterlitz & Watt, 1968) and knowledge is lacking of the effects of selective opioid antagonists in this system. In the present study, therefore, the effects and interactions of opioids of predominantly agonist character, partial agonists and of selective opioid antagonists on plasma corticosteroid levels in stressed and unstressed mice were investigated.

A preliminary account of part of this work has been published (Gibson, Ginsburg, Hall & Hart, 1977).

Methods

Adult male albino mice (LACA strain, 30 to 40 g) were used in all experiments. The animals were housed in a room with a controlled light cycle (12 h). On the day of experimentation the mice were caged in groups of five and were allowed to acclimatize to the conditions of a quiet laboratory for 2 h before the experimental procedures were started. Experiments were performed between 10 h 00 min and 12 h 00 min each day.

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Injections of 0.9% w/v NaCl solution (saline) alone or containing one of the drugs were given intraperitoneally. The doses and timing of the treatments are given in Results. The following drugs were used: etorphine (Reckitt and Colman), levorphanol tartrate (Roche), morphine hydrochloride (May and Baker), naloxone hydrochloride (Endo Laboratories), naltrexone hydrochloride (Endo Laboratories), normorphine (Miles Laboratories and Wellcome Laboratories), pentazocine (Bayer).

Stress was induced by exposing the mice for 1 min to an atmosphere saturated in ether vapour at room temperature (19–21°C). The mice were killed by decapitation and blood collected from the severed neck blood vessels. Plasma corticosteroid was estimated by a modification of the method of Zenker & Bernstein (1958), using corticosterone (Sigma) as the reference compound.

Analgesia was assessed from the reaction times of mice placed on a hot-plate (55°C). The reaction time of each mouse was determined four times, at 10 min intervals before treatment and thereafter at regular intervals for 2 h. Contact with the hot-plate, on any single occasion, was limited to 45 s.

Results

Effects of naloxone, normorphine and morphine on plasma corticosteroids in unstressed mice

Plasma corticosteroid concentrations rise swiftly after intraperitoneal injection of naloxone (50 mg/kg), normorphine (50 mg/kg) or morphine (24 mg/kg); at the earliest times of measurement (7.5 min after injection with naloxone and 15 min with morphine and normorphine) they are significantly greater than in the control mice injected with saline (Figure 1). At 30 min after injection, corticosteroid concentrations are still elevated but by 45 min they are approaching or have declined to the values in saline-treated mice. The data in Figure 2 show the dose-dependence of the effects of normorphine and naloxone on plasma corticosteroids in animals killed 30 min after treatment. In the dose ranges used, naloxone appears to be up to ten times more potent than normorphine. This estimate must be treated with reserve because the log dose-response regressions appear not to be parallel and because, while the effect of naloxone levels off at around plasma corticosteroid concentrations of 350 ng/ml, there is no evidence that the effect of normorphine is limited to the same maximum. It is interesting to note that with the lowest doses used of both naloxone and normorphine, the plasma corticosteroid concentrations are somewhat lower than in saline-injected controls (184 ± 26 and 187 ± 38 respectively compared with 229 ± 8 ng/ml) but this difference is not statistically significant ($P > 0.05$).

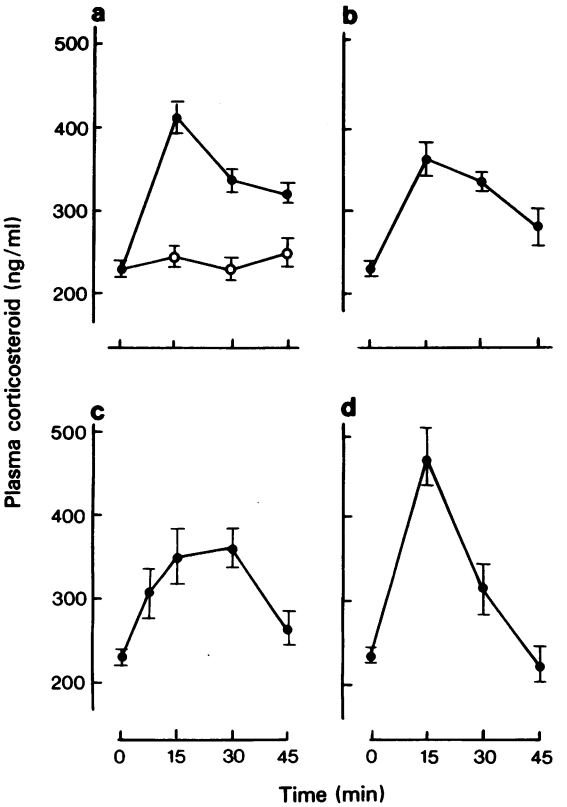

Figure 1 Plasma corticosteroid concentrations (ng/ml) measured at 15, 30 and 45 min after intraperitoneal injection of mice with (a) morphine, 24 mg/kg; (b) normorphine, 50 mg/kg; (c) naloxone, 50 mg/kg; and (d) after exposure to saturated ether vapour for 1 min, all represented by closed circles. In (a) effect of a control injection of saline (i.p.) is represented by (○).

Effect of ether vapour exposure on plasma corticosteroid concentrations

The rise in plasma corticosteroid after 1 min exposure to ether (Figure 1) is considerably greater than the peak effect seen after intraperitoneal injection of morphine, normorphine or naloxone (only naloxone was given here in a dose sufficient to elicit the maximal effect of the drug). The effect of ether also is of shorter duration, there being a significant fall in plasma corticosteroids between 15 min and 30 min after treatment.

When mice are exposed to two periods of ether stress, separated in time by 15 min, the second stress increases corticosteroid secretion but only to an extent sufficient to compensate for the fall in plasma

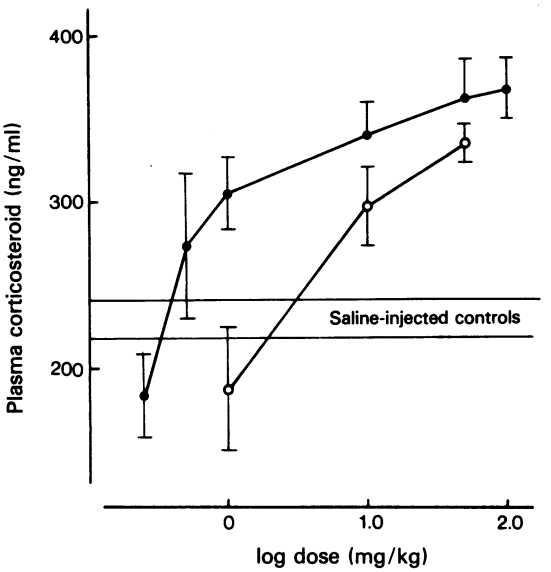

Figure 2 Plasma corticosteroid concentrations (ng/ml) in mice measured 30 min after intraperitoneal injection of naloxone (●) or normorphine (○). Vertical lines show s.e. means. Also shown are the limits of the standard error of the mean from control mice injected 30 min previously with saline (i.p.).

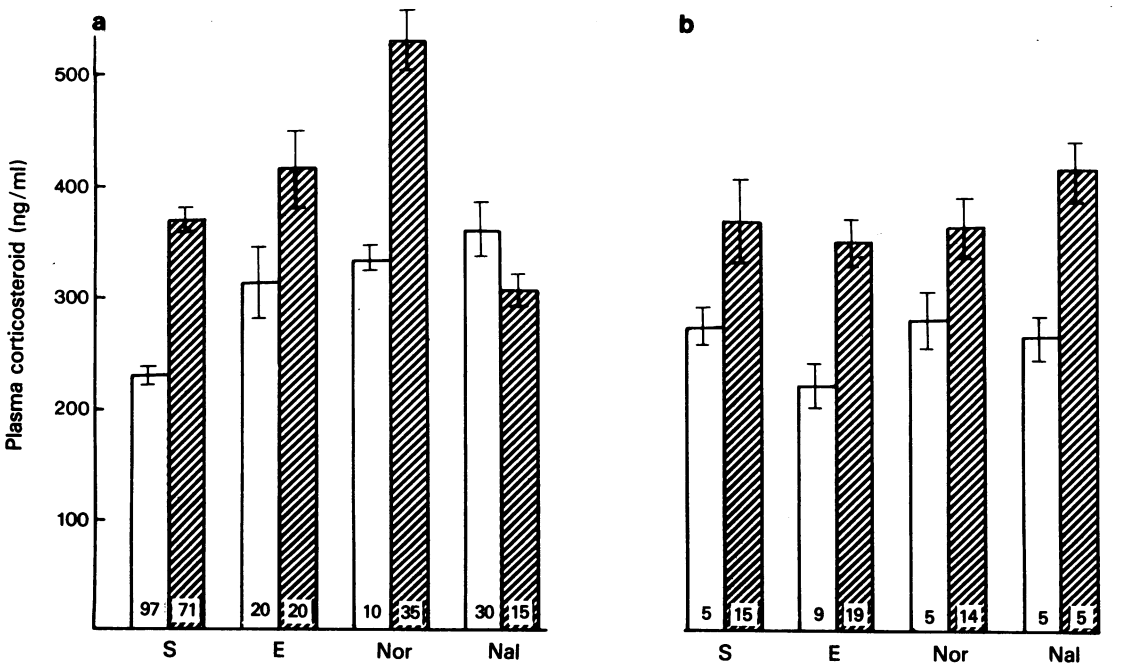

Figure 3 Plasma corticosteroid concentrations (ng/ml) in mice measured at 30 min (a) or 45 min (b) after treatment indicated. Open columns with no additional treatment, hatched columns with 1 min exposure to saturated ether vapour 15 min before measurement of corticosteroids. Numbers within columns refer to number of mice, vertical lines show s.e. means. S = saline; E = ether vapour for 1 min; Nor = 50 mg/kg normorphine; Nal = 50 mg/kg naloxone.

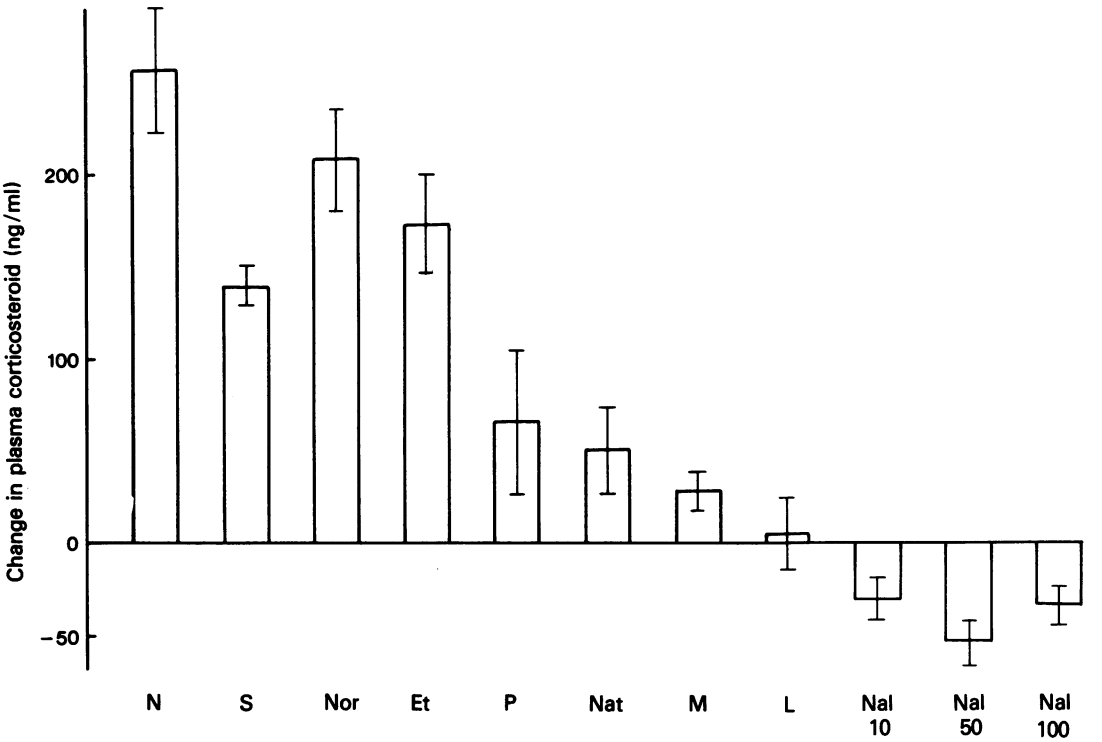

Figure 4 Change in plasma corticosteroid concentrations (ng/ml) in mice produced by exposure to saturated ether vapour for 1 min, 15 min before measurement of corticosteroids. Histogram shows difference in plasma corticosteroid concentration between (1) mice receiving treatment, as indicated, at zero time followed by exposure to ether vapour at +15 min and meusurement at +30 min and (2) mice receiving treatment at zero time and measurement at +30 min. Vertical lines show s.e. means. N = naive; S = saline; Nor = 50 mg/kg normorphine; Et = 2 mg/kg etorphine; P = 20 mg/kg pentazocine; Nat = 10 mg/kg naltrexone; M = 24 mg/kg morphine; L = 20 mg/kg levorphanol; Nal 10 = 10 mg/kg naloxone; Nal 50 = 50 mg/kg naloxone; Nal 100 = 100 mg/kg naloxone.

corticosteroid that would have occurred in the ensuing 15 min (compare Figure 3a with Figure 1d). Similarly, when the second period of ether exposure is 30 min after the first, only a small increase is seen in plasma corticosteroid 15 min later, although at that time in the absence of a second stress, the plasma corticosteroid concentration would have returned to the resting value (compare Figure 3b with Figure 1d). It would seem, therefore, that mice in which plasma corticosteroid is elevated by exposure to ether are at least partially refractory to plasma corticosteroid elevation by subsequent ether stress.

Response to ether vapour in presence of opioids

Administration of normorphine (50 mg/kg), 15 min before exposure of mice to ether vapour, enhances the response to the stress as shown by the difference between plasma corticosteroid concentrations in stressed and non-stressed mice measured 30 min after the normorphine injection (Figure 3a).

In contrast, in mice injected with naloxone (50 mg/kg) 15 min before exposure to ether vapour, plasma corticosteroid is lower than in mice injected with naloxone but not exposed to ether but this difference is not significant ($0.1 > P > 0.05$, Figures 3a and 4). A similar inhibition of the stress-induced rise in plasma corticosteroids is obtained with naloxone at 100 mg/kg (Figure 4). Indeed when the results from the three doses of naloxone are combined, ether stress, 15 min after naloxone treatment, causes a statistically significant reduction in plasma corticosteroids compared with the value in mice receiving naloxone but no stress ($P < 0.04$.)

The effects of both normorphine and naloxone on the corticosteroid response to ether stress are of short duration. When the interval between the intraperitoneal injections of the drugs, and the exposure to

ether, is increased from 15 min to 30 min the increase in plasma corticosteroid is not enhanced in the normorphine-treated animals and is not inhibited in the naloxone-treated animals (Figure 3b).

The contrasting effects of an opioid agonist and antagonist on plasma corticosteroid concentrations after exposure of mice to ether vapour prompted the investigation of the effects of other opioids on this system. Opioids were injected intraperitoneally 15 min before exposure to ether vapour and plasma corticosteroids measured 15 min after the stress. Figure 4 shows that the ether stress produces a greater rise in plasma corticosteroid concentrations in naive mice than in those receiving saline 15 min before the stress. In mice treated with normorphine (50 mg/kg) the ether-induced increase in plasma corticosteroid concentrations was significantly greater ($P < 0.01$) than that in animals given control saline injections. With etorphine (2 mg/kg) the increase due to ether is not significantly different from that seen in saline-treated mice. In contrast, after treatment with pentazocine (20 mg/kg), naltrexone (10 mg/kg), morphine (24 mg/kg), or levorphanol (20 mg/kg) the corticosteroid responses to ether exposure are greatly reduced and, in levorphanol-treated mice, ether stress does not significantly alter plasma corticosteroids. As already noted, with naloxone (10, 50 and 100 mg/kg) the corticosteroid concentration after exposure to ether is lower than that in unstressed animals.

The reduced plasma corticosteroid responses to ether stress after saline injection, or after an initial exposure to ether, may be attributable to the influence of the raised plasma corticosteroid at the time of the final stress. However, it is clear that this does not apply to the effects of the opioid agonist and antagonist because, as results in Figure 1 show, there is no difference in the plasma corticosteroid levels in animals treated with normorphine or naloxone alone.

Effect of naloxone and morphine on the normorphine-induced responses to ether vapour

The results in Figure 4 show that the effect of morphine more closely resembles that of the opioid antagonists, than that of the opioid agonists. If enhancement of the corticosteroid response to ether stress is a manifestation of an agonistic action at opioid receptors it should be possible to block the effect with opioid antagonists.

Table 1 shows the results of experiments on the interaction of naloxone and normorphine. The antagonist was given 15 min before the agonist, followed after 15 min by the ether challenge and 15 min later the animal was killed. Thus the interval, 30 min, between naloxone injection and stress was such that even at the highest dose of naloxone (50 mg/kg) there would be little or no impairment of the corticosteroid response to ether (see Figure 3b). Enhancement of the corticosteroid response by normorphine is blocked by the pretreatment with naloxone in doses of 2 mg/kg or greater. With the same protocol, if

Table 1 Effects of naloxone (Nal) and morphine (M) on the normorphine (Nor) enhancement of the corticosteroid response to ether vapour in mice

Treatment				*Corticosteroid (ng/ml)*	*Change in CS due*
0 min	*15 min*	*30 min*	n	*mean ± s.e.*	*to stress (ng/ml)*
	Saline		97	229 ± 8	
	Saline	Ether	71	369 ± 11	140 ± 11
	Nor (50)		10	335 ± 11	
	Nor (50)	Ether	35	531 ± 27	209 ± 28
Nal (50)			5	265 ± 20	
Nal (50)		Ether	5	413 ± 27	148 ± 27
Nal	Nor				
0.5	50	Ether	5	536 ± 81	201 ± 80
1	50	Ether	5	587 ± 84	252 ± 84
2	50	Ether	10	423 ± 24	88 ± 24
4	50	Ether	10	401 ± 28	66 ± 28
8	50	Ether	11	345 ± 36	10 ± 36
50	50	Ether	20	333 ± 35	−2 ± 35
M (24)			5	320 ± 12	
M (24)	Nor (50)		5	347 ± 25	
M (24)	Nor (50)	Ether	9	428 ± 38	81 ± 38

Treatment columns show time of drug administration (intraperitoneal) and dose (mg/kg); all animals were killed, and plasma corticosteroids (CS) measured, 15 min after the ether stress.

in the pretreatment, naloxone is replaced with morphine, a block of the enhancement of the corticosteroid response to stress by normorphine is again observed.

Analgesia produced by normorphine and its antagonism by naloxone

Within 20 min of injection, normorphine (100 mg/kg) produces analgesia of similar intensity, but longer duration, to that obtained with morphine (20 mg/kg). Treatment with naloxone (0.05 mg/kg), 15 min before administration of normorphine, delays the onset (90 min) but does not decrease the intensity of normorphine analgesia. The onset is delayed further with 0.1 mg/kg naloxone (130 min) and is blocked completely with 0.5 mg/kg naloxone. In the presence of normorphine plus the higher doses of naloxone, or with 0.1 mg/kg naloxone alone, the reaction times of the mice are shorter than during the pretreatment control testing suggesting that naloxone is hyperalgesic.

Discussion

Changes in plasma corticosteroid concentrations induced by opioids could involve an action at one or more of several sites in the HPA system. In the case of morphine it is considered that the site of action is the hypothalamus because injection of morphine into the medial, but not the rostral or caudal, hypothalamus raises plasma corticosteroid concentrations (Lotti, Kokka & George, 1969), and morphine has no action in rats with lesions of the median eminence (George & Way, 1959).

In the present study, exposure of mice to ether vapour produced a sharp rise in plasma corticosteroid concentrations but when the stress was repeated 15 or 30 min later the elevation of plasma corticosteroids was markedly diminished. The mechanism of this tolerance to repeated ether stress is unclear. It does not appear to be due to the fast, rate-sensitive feedback inhibition by circulating corticosteroids of corticotrophin releasing factor (CRF) from the hypothalamus and of corticotrophin (ACTH) from the pituitary (Dallman & Yates, 1969; Jones, Brush & Neame, 1972; Jones, Hillhouse & Burden, 1977), since 30 min after exposure to ether vapour plasma corticosteroid levels were falling rapidly.

Normorphine and naloxone pretreatment modified, in opposite directions, the corticosteroid response to ether stress although at the time that the stress was applied there was no difference between the pretreatments in their 'direct' effects on corticosteroid concentration. In normorphine-treated mice, ether stress resulted in a further marked rise in plasma corticosteroids whereas in animals pretreated with naloxone, ether vapour actually lowered corticosteroid concentrations. It appears therefore that in the presence of normorphine there is an impairment of the mechanism through which the corticosteroid response to ether exposure is diminished by a preceding stress. In contrast, with the opioid antagonist, naloxone, restraints on the activation of the HPA system appear to be reinforced. This suggests that endogenous opioid peptides may have a role within the HPA system of reducing a restraint on ACTH secretion and that naloxone blocks and normorphine mimics this action. The site of action of the opioids could be the hypothalamus or pituitary but the high content of opioid receptors (Kuhar, Pert & Snyder, 1973) and enkephalin-like immunoreactive material (Elde, Hökfelt, Johansson & Terenius, 1976) in the hypothalamus makes this the more likely site. In addition, Simantov & Snyder (1977) have reported that opioid receptor binding is much greater (20 to 200 times) in whole rat brain than in the pituitary and that within the pituitary, opioid receptor density is greatest in the neural lobe.

The effect of other opioids on the stress response in mice is of interest. The opioid agonist, etorphine, allowed the full expression of the corticosteroid response to ether stress, whereas morphine behaved more like the opioid antagonist, naloxone, in reducing the stress-induced rise in plasma corticosteroids. There is no paradox here since it is well known that the concentrations at which morphine produces opioid antagonistic effects in guinea-pig ileum are only slightly greater than those required for agonistic action. The present observations reinforce the point that, even in the whole animal, effects of morphine in high doses can be an expression of antagonism at opioid receptors. The nature of this action of the opioids on this system is likely to be dose-dependent, and it is probable that with the opioids normally classified as partial agonists the concentration is critical. The opioids were not studied over a range of concentrations and thus comment on the characteristics of the receptor involved in the interaction of opioids with the HPA system would not be valid. Several workers have shown that opioid neuropeptides elevate plasma levels of prolactin and growth hormone and Shaar, Frederickson, Dininger & Jackson (1977) have reported that this action of met-enkephalinamide in rats is sensitive to 0.2 mg/kg naloxone. In the present study, although normorphine analgesia was sensitive to this dose of naloxone the inhibition of the normorphine-ether interaction required doses greater than 1 mg/kg.

It has been known for many years that opioid analgesia can be affected by activity of the HPA system. Recent reports show that with a rise in the concentration of plasma corticosteroids, animals become less

sensitive to opioid agonists but more sensitive to opioid antagonists (Gebhart & Mitchell, 1972; Gispen, Van Wimersma Greidanus, Waters-Ezrin, Zimmermann, Krivoy & De Wied, 1975; Harris, Loh & Way, 1976). This ability of corticosteroids to affect the activity of opioids, and of opioids to affect the release of anterior pituitary hormones, suggests that the degree of analgesia produced by opioids is a balance of two opposing actions. During stress, exogenous opioid agonists may raise plasma corticosteroid concentrations which would tend to impair the analgesia whereas partial agonists may lower corticosteroids which would tend to enhance analgesia. Thus in untreated mice the level of nociception during stress may depend upon the balance of the interactions between corticosteroids and endogenous opioids. It has been reported that ACTH and β-endorphin are released concurrently from the pituitary following stress (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale & Bloom, 1977). Thus, during stress, substances other than ACTH and corticosterone may be released which affect the control of the HPA system and the present results suggest that the endogenous opioids may be of importance in this respect. Further understanding of this complex system requires knowledge of the turnover of the opioid-like neuropeptides under different physiological conditions.

The results described in this paper suggest that endogenous opioid peptides may be involved in the control of the stress-induced secretion of corticosteroids in mice and support the general contention that these neuropeptides are important in the regulation of the secretion of anterior pituitary hormones.

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