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THE EFFECT OF INTRACEREBROVENTRICULAR ADMINISTRATION OF METHIONINE-ENKEPHALIN ON THE STRESS-INDUCED SECRETION OF CORTICOSTERONE IN MICE

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Ether stress or intracerebroventricular (i.c.v.) administration of saline, met-enkephalin, or naltrexone raised plasma corticosterone levels in mice. The response to ether stress was abolished by a preceding ether stress or by pretreatment with i.c.v. saline or naltrexone. However, following i.c.v. met-enkephalin, plasma corticosterone was significantly elevated by ether stress, the effect being blocked by simultaneous injection of met-enkephalin and naltrexone. Met-enkephalin appears to prevent fast-feedback inhibition of the hypothalamus-pituitary-adrenal system.

Introduction We have reported recently that the plasma corticosterone response to ether stress in mice is enhanced by opioid agonists and reduced by opioid antagonists (Gibson, Ginsburg, Hall & Hart, 1979). Although the effects of the endogenous opioid peptides themselves were not investigated, due to their rapid inactivation following intraperitoneal administration, the results provided indirect evidence that these peptides might be involved in regulating the activity of the hypothalamus-pituitary-adrenal (HPA) system. In the present study therefore we have investigated the direct effect of intracerebroventricular administration of methionine-enkephalin (met-enkephalin) on plasma corticosterone levels in normal and ether-stressed mice.

Methods Adult male albino mice (LACA; 20 to 30 g) were housed in a room with a controlled 12 h light cycle (on at 07 h 00 min). On the day of experimentation the mice were caged in groups of five and were allowed to acclimatise to the conditions of a quiet laboratory for 2 h before experimental procedures were started. Experiments were performed between 10 h 00 min and 12 h 00 min each day. Drugs and 0.9% w/v NaCl solution (saline) were given by intracerebroventricular (i.c.v.) injection into conscious mice by the method of Haley & McCormick (1957). The injected volume was 5 μl in all cases. Met-enkephalin and naltrexone were administered in doses of 50 μg per mouse, which is similar to the median antinociceptive dose for met-enkephalin given by i.c.v. injection to mice (Roemer, Beuscher, Hill, Pless, Bauer, Cardinaux, Closse, Hauser, & Huguemin, 1977).

Stress was induced by exposing the mice for 1 min to an atmosphere saturated in ether vapour at room temperature (19 to 21°C). The mice were killed by decapitation and blood collected from the severed neck blood vessels. Plasma corticosterone levels were estimated by a modification of the method of Zenker & Bernstein (1958), using corticosterone (Sigma) as the reference compound. Statistical analysis was performed by Student's *t* test.

Drugs used were: methionine-enkephalin (Calbiochem) and naltrexone hydrochloride (Endo Laboratories).

Results The effects of the various procedures on plasma corticosterone levels are shown in Figure 1. The rise in plasma corticosterone concentrations in naive mice subjected to ether exposure occurs mainly within 7.5 min of the stress, although there is a further small increase in the succeeding 7.5 min. Increases in plasma corticosterone levels of similar magnitude and time course were seen when mice were given saline by the i.c.v. route. A second exposure to ether, 7.5 min after the first, did not result in a further elevation of corticosterone levels in the succeeding 7.5 min. Similarly in animals given saline (i.c.v.) and exposed to ether vapour 7.5 min later, plasma corticosterone concentrations were not different from those in saline (i.c.v.)-injected controls. That is, there was no apparent adrenocortical response to an ether stress following a previous exposure to ether or a previous injection of saline (i.c.v.).

The increases in plasma corticosteroid levels observed after i.c.v. administration of met-enkephalin or naltrexone can thus be attributed to the stressful effects of the handling and injection, though it should be noted that, in contrast to saline or met-enkephalin, following naltrexone there was no further increase in plasma corticosterone between 7.5 and 15 min after injection. Although the effects of met-enkephalin itself could not be distinguished from those of saline alone, ether exposure 7.5 min after met-enkephalin administration produced a significant elevation of plasma corticosterone levels. However, ether exposure had no effect in naltrexone-treated mice or in mice treated simultaneously with met-enkephalin and naltrexone.

Discussion In our previous paper we suggested that endogenous opioid peptides might have a role within the HPA system of reducing a restraint on ACTH

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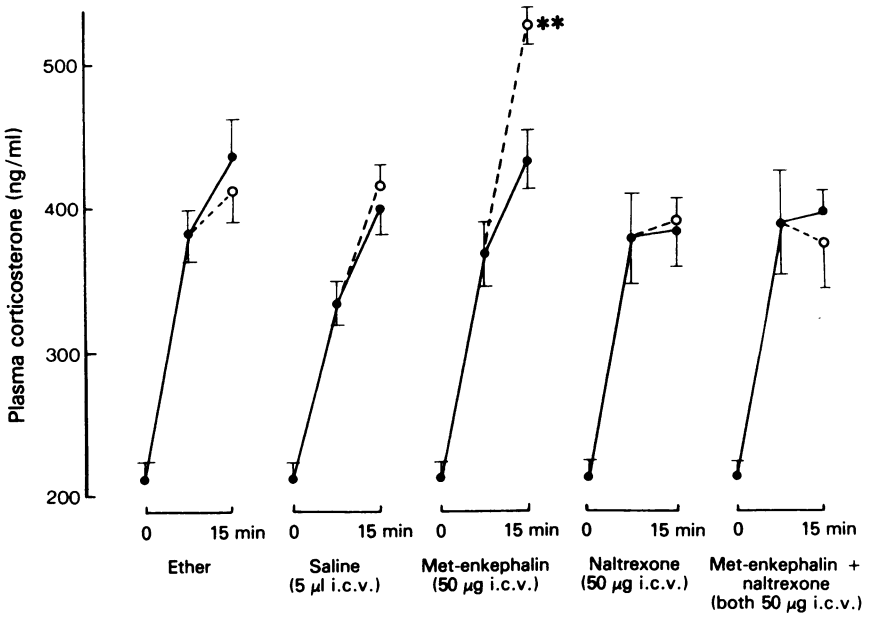

Figure 1 Plasma corticosterone levels (ng/ml) in mice measured at 7.5 min and 15 min after the pretreatment indicated below each graph. The continuous line and (●) represent corticosterone levels in mice receiving only the pretreatment. The dotted line and (○) represent corticosterone levels in mice receiving the pretreatment and exposed to ether stress at 7.5 min. Each point is the mean of at least 5 observations; vertical lines show s.e. mean. **$0.01 > P > 0.001$ Significantly different from 15 min met-enkephalin-treated mice.

secretion following stress (Gibson *et al.*, 1979). The present results support this suggestion since the inhibitory effects of i.c.v. administration of saline were reversed by met-enkephalin, this effect being blocked by the opioid antagonist, naltrexone. The mechanism by which the plasma corticosteroid response to ether stress is reduced by a preceding stress is unclear. It is possible that it is due to the fast, rate-sensitive feedback inhibition of HPA activity mediated by circulating corticosteroids (Dallman & Yates, 1969; Jones, Hillhouse, & Burden, 1977), since all the pretreatments produced increasing plasma corticosterone levels. Recently, Kaneko & Hiroshige (1978) have shown that this fast feedback mechanism is related to central catecholamine neurones since it is absent in animals pretreated with 6-hydroxydopamine. It seems possible therefore that met-enkephalin might act by reducing the release of catecholamines from these nerves as it has been shown to do in other peripheral (Hughes, 1975) and central (Taube, Borowski, Endo, & Starke, 1976) noradrenergic neurones. On the other hand in our previous study the response to ether stress was also reduced by intraperitoneal injections of saline which had no effect on plasma corticosterone levels after 15 min (Gibson *et al.*, 1979). Further, Buckingham (1979) has recently shown that, at least in rats, the feedback mechanism operated by corticosteroids is delayed and is not apparent until 1 h later. Thus the feedback mechanism prevented by met-enkephalin may not be due to circulating corticosteroids but may still be due to interactions with inhibitory noradrenergic neurones in the HPA system (Scapagnini, Van Loon, Moberg, & Ganong, 1970; Buckingham & Hodges, 1978).

The most likely site of action of the endogenous opioid peptides in the HPA system is the hypothalamus. Hypothalamic enkephalin content is reduced by footshock stress (Rossier, Guillemin & Bloom, 1978) and by laparotomy (Gibson, Ginsburg, Hall, Hart, & Kitchen, 1978). Rossier *et al.* (1978) have suggested that the enkephalin containing neurones may be activated by stress and the present results suggest that enkephalin may act to remove central inhibitory influences on HPA activity.

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