Br. J. Pharmac. (1980), **70**, 509–511

LEUCINE-ENKEPHALIN AND METHIONINE-ENKEPHALIN PRODUCE OPPOSING EFFECTS ON PLASMA CORTICOSTERONE LEVELS IN ETHER-STRESSED MICE

ALAN GIBSON, STEPHEN L. HART, & AMEL SHABIB

Department of Pharmacology, Chelsea College, University of London, Manresa Road, London SW3 6LX

The inhibitory effects of intracerebroventricular administration of saline on the plasma corticosterone response to ether stress in mice was reduced by Met-enkephalin and enhanced by Leu-enkephalin. When administered simultaneously the effects of the two peptides opposed each other. Met and Leu-enkephalin may subserve different physiological functions in the response of the hypothalamus-pituitary-adrenal system to stress.

Introduction The plasma corticosterone response to ether stress in mice is enhanced by some opioid drugs and reduced by others (Gibson, Ginsburg, Hall & Hart, 1979a), suggesting a role for the endogenous opioid peptides in the control of the hypothalamus-pituitary-adrenal (HPA) system. In addition, the effect of intracerebroventricular (i.c.v.) administration of methionine-enkephalin (Met-enkephalin) on ether stress suggested that, within the hypothalamus, the enkephalins might act to remove central inhibitory influences on HPA activity (Gibson, Ginsburg, Hall & Hart, 1979b). In the present study we confirm this effect of Met-enkephalin but report that i.c.v. administration of leucine-enkephalin (Leu-enkephalin) produces the opposite effect and appears to enhance central restraints on the activation of the HPA system by stress.

Methods Male albino mice (20 to 30 g; LACA) were housed in a room with a controlled 12 h light cycle (on at 07 h 00 min). On the day of the experiment the mice were caged in groups of five and were allowed to acclimatize 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 saline (0.9% w/v NaCl solution) were given by i.c.v. injection into conscious mice (Haley & MacCormack, 1957), the injected volume being 5 μl in all cases. Drugs were administered in doses of 50 μg per mouse which is close to the median antinociceptive dose for Met-enkephalin given by i.c.v. injection to mice (Roemer, Buescher, Hill, Pless, Bauer, Cardinaux, Closse, Hausser & 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). Mice were killed by decapitation and blood collected from the severed neck blood vessels. Plasma corticosterone levels were estimated by a slight 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: leucine-enkephalin (Wellcome Research Laboratories); methionine-enkephalin (Calbiochem); naltrexone hydrochloride (Endo Laboratories).

Results The main results of this study are shown in Figure 1.

In naive animals following ether exposure plasma corticosterone levels were increased by 97% after 5 min and by 140% after 10 min. All of the pretreatments involving i.c.v. injections by themselves raised plasma corticosterone concentrations, presumably due to the handling and injection. However, the pretreatments differed in their effects on a subsequent ether stress. In animals given saline and exposed to ether 5 min later the plasma corticosterone levels were similar to those in saline injected controls. Thus, i.c.v. administration of saline appeared to attenuate the activation of the HPA system by stress. However, following pretreatment with Met-enkephalin, ether exposure did produce a significant elevation of plasma corticosterone levels in the succeeding 5 min. Conversely, in mice pretreated with Leu-enkephalin, plasma corticosterone levels were significantly reduced by ether exposure.

In other experiments, not shown in Figure 1, simultaneous administration of the opioid antagonist, naltrexone (50 μg i.c.v.), prevented the enhancing effect of Met-enkephalin and the inhibitory effect of Leu-enkephalin on ether-induced changes in plasma corticosterone levels suggesting that both effects were mediated by opioid receptor activation.

Since Met-enkephalin and Leu-enkephalin by themselves had produced opposite effects on ether stress, simultaneous i.c.v. administration of the two

0007-1188/80/120509-03 $01.00

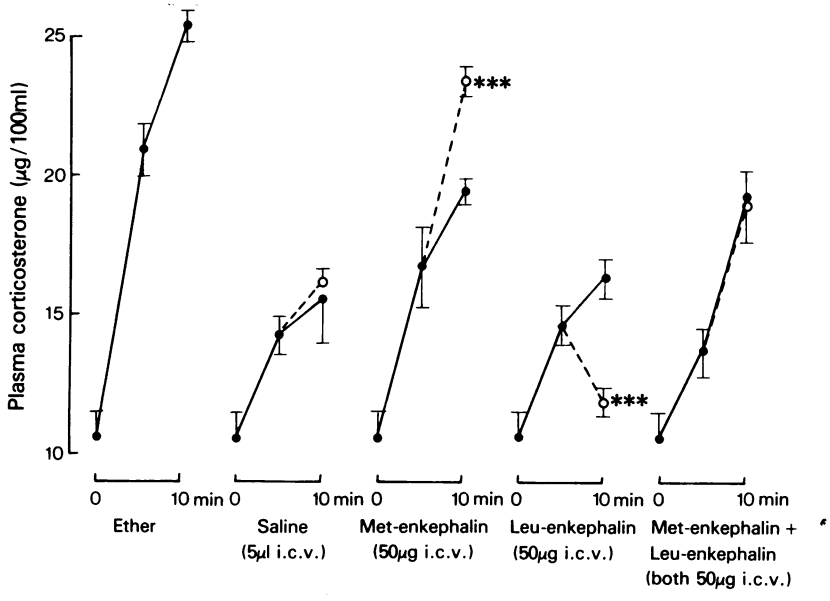

Figure 1. Plasma corticosterone levels in mice measured at 5 min and 10 min after the pretreatment indicated below each graph. The continuous line and (●) represent corticosterone levels in mice receiving only the pretreatment. The dotted line and (○) represent corticosterone levels in mice receiving the pretreatment and exposed to ether stress at 5 min. Each point is the mean of at least 5 plasma samples, each from different mice; vertical lines show s.e. mean. *** $P<0.001$ Significantly different from value of plasma 10 min after pretreatment only.

peptides was studied (Figure 1). In this case the results were similar to those of a saline injection, since following such pretreatment ether exposure failed to produce a significant alteration in plasma corticosterone levels in the succeeding 5 min.

Discussion Although in the present study a slightly different time cycle was employed, the results confirm our previous finding that i.c.v. administration of saline reduces the effect of a subsequent ether stress on plasma corticosterone levels, and that this inhibition can be overcome by Met-enkephalin (Gibson *et al.*, 1979b). However, the general statement that enkephalins may act in the hypothalamus to reduce central inhibitory influences on HPA activity must now be modified in the light of the results obtained with Leu-enkephalin. Thus Leu-enkephalin seemed to reinforce the negative feedback mechanisms within the HPA system which are activated by stress (Jones, Hillhouse & Burden, 1976), and produced an effect which opposed that of Met-enkephalin. It seems possible therefore that the two peptides might subserve different physiological functions in the HPA system. It is of interest that Buckingham & Hodges (1979) have recently found that endogenous opioid peptides can produce mutually antagonistic effects on the output of corticotrophin releasing hormone *in vitro*, and Larssen, Childers & Snyder (1979) have identified distinct Met-enkephalin containing and Leu-enkephalin containing nerve tracts in brain. It is known that various types of stress cause a reduction in hypothalamic enkephalin content, and this has been interpreted as being a result of stress-induced activation of central enkephalin containing nerves (Rossier, Guillemin & Bloom, 1978; Gibson, Ginsburg, Hart & Kitchen, 1980). The present results would suggest that activation of Met-enkephalin containing nerves and Leu-enkephalin containing nerves would produce opposing effects on the HPA system and therefore that the degree of activation of the system by stress may be dependent upon the balance of activity within the brain of the endogenous opioid peptides.

The authors thank Dr T. W. Smith for the gift of leu-enkephalin.

References

BUCKINGHAM, J.C. & HODGES, J.R. (1979) Opioids and hypothalamo-pituitary-adrenocorticotrophic activity in the rat. *J. Physiol.* **295**, 70P.

GIBSON, A., GINSBURG, M., HALL, M., & HART, S.L. (1979a) The effects of opiate receptor agonists and antagonists on the stress-induced secretion of corticosterone in mice. *Br. J. Pharmac.* **65**, 139–146.

GIBSON, A., GINSBURG, M., HALL, M., & HART, S.L. (1979b). The effect of intracerebroventricular administration of methionine-inkephalin on the stress-induced secretion of corticosterone in mice. *Br. J. Pharmac.* **66**, 164–166.

GIBSON, A., GINSBURG, M., HART, S.L., & KITCHEN, I. (1980) Effects of adrenalectomy and hypophysectomy on enkephalin content of rat hypothalamus. *Br. J. Pharmac.* (in press).

HALEY, T.J. & MACCORMACK, W. G. (1957) Pharmacological effects produced by interacerebral injection of drugs in the conscious mouse. *Br. J. Pharmac. Chemother.* **12**, 12–15.

JONES, M.T., HILLHOUSE, E., & BURDEN, J. (1976). Secretion of corticotrophin-releasing hormone in vitro. In *Frontiers in Neuroendocrinology*. ed. Martini, L. & Ganong, W.F. Vol. 4, pp. 195–226. New York: Raven Press.

LARSSEN, L.-I., CHILDERS, S., & SNYDER, S.H. (1979). Met- and leu-enkephalin immunoreactivity in separate neurones. *Nature*, **282**, 407–410.

ROEMER, D., BUESCHER, H.H., HOLL, R.C., PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSSER, D. & HUGUEMIN, R. (1977). A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature*, **268**, 547–549.

ROSSIER, J., GUILLEMIN, R., & BLOOM, F. (1978). Foot-shock induced stress decreases leu^5-enkephalin immunoreactivity in rat hypothalamus. *Eur. J. Pharmac.* **48**, 465–466.

ZENKER, N., & BERNSTEIN, D.E., (1958). The estimation of small amounts of corticosterone in rat plasma. *J. biol. Chem.* **231**, 695–701.

(Received April 30, 1978.)