Inhibitory Action of a Met-Enkephalin on ACTH Release in Man

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ABSTRACT In order to study the mechanism of action of a Met-enkephalin (FK 33824) on the pituitary-adrenal axis eight normal male volunteers were subjected to an ACTH stimulation test. Lysine-vasopressin (LVP), 5 IU, was injected intramuscularly after pretreatment with 0.5 mg FK 33824 i.m. or a placebo. In six of the subjects the opiate was again administered preceding a single injection of 0.25 mg ACTH β^{1-24} i.m. Blood was collected at regular intervals and ACTH and cortisol concentrations analyzed in all samples. LVP induced significant plasma ACTH ($P < 0.05$) and cortisol ($P < 0.001$) increases. Pretreatment with FK 33824 completely antagonized the effect of LVP. Furthermore, the cortisol elevation after exogenous ACTH was not modified by previous administration of FK 33824.

It is concluded that the Met-enkephalin derivative FK 33824 directly suppresses ACTH release from the pituitary without influencing adrenal synthesis of cortisol.

INTRODUCTION

FK 33824 is a stable sulfoxidecarbinol analog of methionine enkephalin found to have high affinity for opiate receptors in rat brain (1) and in posterior pituitary gland (2). A physiological mode of action of the different enkephalin derivatives as putative neurotransmitters or neuromodulators has been reported (3, 4). These data support a possible role for enkephalins in the regulation of the endocrine system, and hormone profiles after treatment with these agents have been outlined in animals and humans (5–8). As has been demonstrated by Stubbs et al. (7) and del Pozo et al. (8), FK 33824 administered to normal men produces decreased plasma concentrations of ACTH and cortisol. These data, however, do not allow localization of the site of action of the opiate, i.e., whether this takes place at pituitary or suprasellar level. Furthermore, a concomitant effect on the adrenals cannot be excluded. To characterize further the mechanism of action of FK 33824 on the ACTH-adrenal axis a study was undertaken in normal volunteers using lysine-vasopressin (LVP)[1] as the ACTH stimulatory agent. Endogenous arginine-vasopressin possesses corticotropin-release factor-like properties (9, 10) and LVP has yielded reproducible results in the testing of the ACTH-adrenal axis (11).

METHODS

Eight healthy male volunteers (mean age 28 yr) were selected for the study and gave written consent before entering the trial. Krieger and Zimmerman (11) have shown that 5–10 IU of LVP administered intramuscularly or intravenously to normal subjects increase plasma levels of ACTH after 15 min and cortisol after 30 min. Previous studies with 0.5 mg FK 33824 i.m. demonstrated that plasma cortisol concentrations declined steadily from 30 to 180 min after drug administration (8). With this background the schedule for substance application in the present study was devised. After a 12-h fasting period a venous cannula was placed in a forearm vein at 8:00 a.m., and the subjects were kept in resting position. They received in a randomized design at weekly intervals: (*a*) an intramuscular injection of 5 IU LVP (Vasopressin, Sandoz Ltd.) at 60 min, following saline at time 0 (8:30 a.m.); (*b*) the same procedure was repeated but administering 0.5 mg FK 33824 i.m. at time 0; and (*c*) an injection of 0.25 mg ACTH β^{1-24} i.m. (Synacthen, Ciba-Geigy, Ltd., Basel, Switzerland) was given 60 min after 0.5 mg FK 33824 i.m. and compared with the same procedure after administration of placebo. Blood sampling was performed at 0, 30, 60, 75, 90, 120, 180, and 240 min. All specimens were centrifuged at 4°C and stored at −20°C until assayed. Previously described radioimmunoassay procedures were used for plasma ACTH and cortisol determinations (12) in all samples. Statistical evaluation was performed by one-way analysis of variance, and Student's *t* test (for ACTH values).

RESULTS

As shown in Fig. 1(A), injection of 5 IU LVP 60 min after placebo was followed by a significant ($P < 0.05$)

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[1] *Abbreviation used in this paper*: LVP, lysine vasopressin.

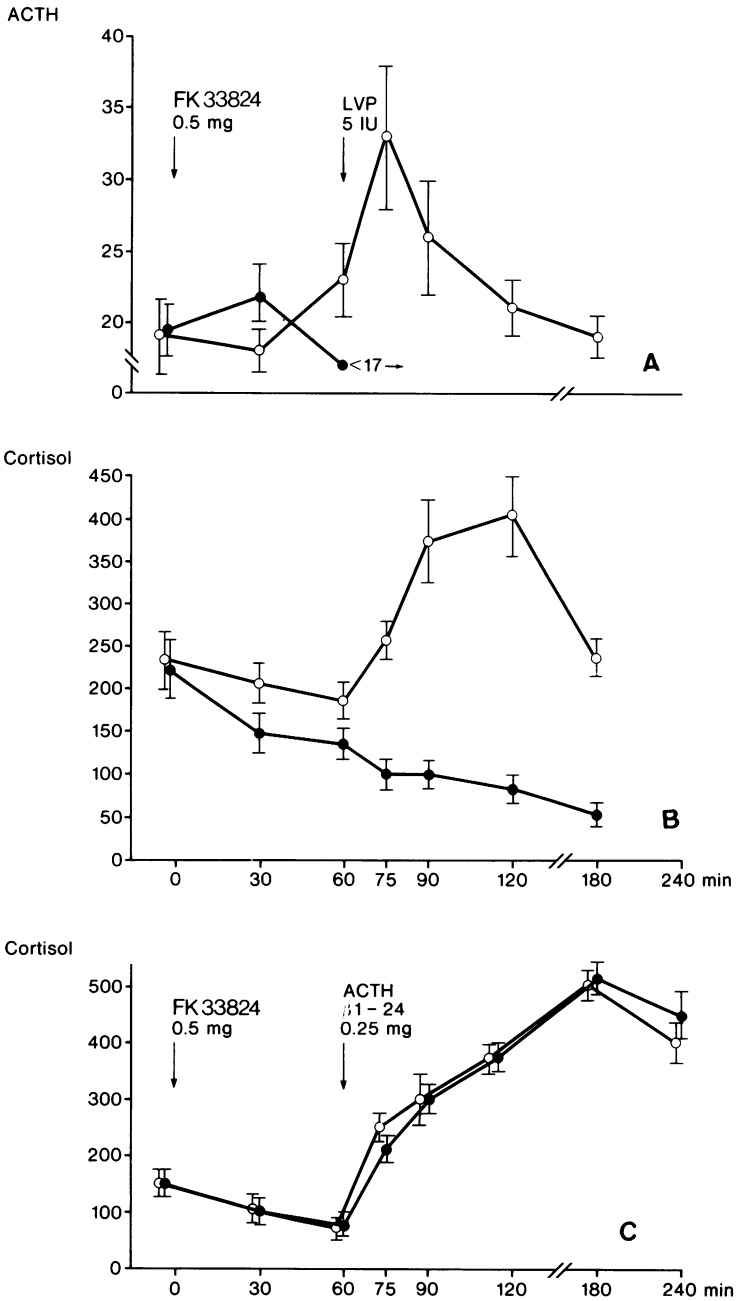

FIGURE 1 As compared with a placebo (○) FK 33824 suppressed the ACTH increment (picograms per milliliter induced by LVP below assay sensitivity (<17 pg/ml) and completely inhibited the cortisol response (nanomoles per liter) (A and B, $n = 8$). The opiate was unable to counteract the effect of ACTHβ^{1-24} on cortisolemia (C, $n = 6$).

ACTH elevation peaking at 15 min, and by substantial plasma cortisol increments ($P < 0.001$ compared with base line 0–60 min, [B]).

FK 33824, injected at time 0, and LVP at 60 min resulted in abolition of the stimulation of both ACTH and cortisol (A and B).

In the last part of the trial in which a possible direct effect of FK 33824 on the adrenals was studied, synthetic ACTH β^{1-24} was administered. Fig. 1(C) shows significantly increased cortisol concentrations ($P < 0.001$, compared with base line 0–60 min) when ACTH was given 60 min after placebo. Cortisol levels

rose from 15 min, reaching peak values after 120 min. Administration of FK 33824 at time 0 did not modify the action of ACTH (C), confirming the central mechanism of action of the opiate.

DISCUSSION

It has been reported that plasma cortisol was significantly suppressed below the physiological morning decline in subjects treated with the Met-enkephalin derivative FK 33824 (7, 8). Data recorded here clearly show an inhibiting effect of the opiate on ACTH and cortisol release after stimulation with LVP. Although the existence of a different hypothalamic excitatory pathway cannot be completely ruled out (13), a direct action of LVP on the pituitary gland can be assumed on the basis of experimental and clinical data (9–11). Appreciable anterior pituitary concentrations of endogenous Met-enkephalin have been detected in man with immunoreactive techniques (14), lending support to an effect of FK 33824 at this level. The central mode of action is further evidenced by the failure of FK 33824 to prevent the release of cortisol that follows the administration of exogenous ACTH, and rules out a dual effect at both the pituitary and adrenal level. Furthermore, in vitro data have shown that FK 33824 neither modified the release of corticosterone by dispersed fasciculata cells of rat adrenals nor interfered with the effect of ACTH on this system when both substances were added simultaneously to the medium.[2]

FK 33824 has been found to elevate plasma growth hormone and prolactin (6) and reduce ACTH (7) and vasopressin (15) in normal volunteers. The effect on growth hormone and prolactin is readily antagonized by naloxone (7, 8). However, the actions on the pituitary-adrenal axis and on vasopressin have been found to be resistant to this agent (7, 15). The foregoing data implicate the existence of several types of opiate receptors with different preferences for agonists-antagonists. Thus, results obtained by Lord et al. (16) and confirmed by Kosterlitz (17) have demonstrated the presence of several subclasses of opiate receptors. Essentially, pain and morphine addiction potential are localized in the μ receptor and this can be antagonized by naloxone, whereas enkephalin actions are mediated primarily through binding to δ receptors. However, recent studies of Kream and Zukin (1) show that in rat brain homogenates FK 33824 interacts equally well with μ and δ opiate receptors. Therefore, it is tempting to speculate that the action of FK 33824 not antagonized by naloxone may be mediated by δ receptors. Moreover, Met-enkephalin can exhibit endocrine properties partly different from those of the prototype opiate morphine. Thus, Tolis et al. (18) reported that morphine administered to humans resulted in a significant increase in serum prolactin without altering the levels of growth hormone and cortisol, whereas FK 33824 elevates prolactin and growth hormone while decreasing cortisol (7, 8). It also would be tempting to attribute to Met-enkephalin properties unrelated to opioid mechanisms because in this particular case a direct effect on the pituitary gland speaks against the role of opiates as intermediary neurotransmitters.

[2] Neher and Podesta. Unpublished observations.

REFERENCES

1. Kream, R. M., and S. Zukin. 1979. Binding characteristics of a potent enkephalin analogue. *Biochem. Biophys. Res. Commun.* **90:** 99–109.
2. Simantov, R., and S. H. Snyder. 1977. Opiate receptor binding in the pituitary gland. *Brain Res.* **124:** 178–184.
3. Yang, H-Y., J. S. Hong, W. Fratta, and E. Costa. 1978. Rat brain enkephalins: distribution and biosynthesis. *Adv. Biochem. Psychopharmacol.* **18:** 149–159.
4. Zieglgansberger, W., J. P. Fry, A. Herz, L. Moroder, and E. Wunsch. 1976. Enkephalin-induced inhibition of cortical neurones and the lack of this effect in morphine tolerant/dependent rats. *Brain Res.* **115:** 160–164.
5. Roemer, D., H. H. Büscher, R. C. Hill, J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser, and R. Huguenin. 1977. A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature (Lond.)* **268:** 547–549.
6. von Graffenried, B., E. del Pozo, J. Roubicek, E. Krebs, W. Pöldinger, P. Burmeister, and L. Kerp. 1978. Effects of the synthetic analogue FK 33-824 in man. *Nature (Lond.)* **272:** 729–730.
7. Stubbs, W. A., A. Jones, C. R. W. Edwards, G. Delitala, W. J. Jeffcoate, S. J. Ratter, G. M. Besser, S. R. Bloom, and K. G. M. M. Alberti. 1978. Hormonal and metabolic responses to an enkephalin analogue in normal man. *Lancet.* **II:** 1225–1227.
8. del Pozo, E., B. von Graffenried, J. Brownell, F. Derrer, and P. Marbach. 1980. Endocrine effect of a Met-enkephalin derivative (FK 33-824) in the humans. *Hormone Res.* In press.
9. Gillies, G., T. B. van Wimersma Greidanus, and P. J. Lowry. 1978. Characterization of rat stalk median eminence vasopressin and its involvement in adrenocorticotropin release. *Endocrinology.* **103:** 528–534.
10. Gillies, G., and P. J. Lowry. 1979. Corticotrophin releasing factor may be modulated by vasopressin. *Nature (Lond.).* **278:** 463–464.
11. Krieger, D. T., and E. H. Zimmerman. 1977. The nature of CRF and its relationship to vasopressin. *In* Clinical Neuroendocrinology. L. Martini, and G. M. Besser, editors. Academic Press, Inc., New York. 363–391.
12. Girard, J., J. B. Baumann, U. Bühler, K. Zuppinger, H. G. Hass, J. J. Staub, and H. I. Wyss. 1978. Cyproterone acetate and ACTH adrenal function. *J. Clin. Endocrinol. Metab.* **47:** 581–586.
13. Gillies, G. and P. J. Lowry. 1980. Corticotrophin releasing activity in extracts of the stalk median eminence of brattleboro rats. *J. Endocrinol.* **84:** 65–73.
14. Gramsch, Ch., V. Höllt, P. Mehraein, A. Pasi, and A.

Herz. 1978. Regional distribution of endorphins in humans brain and pituitary. *In* Characteristics and Function of Opioids. J. M. van Ree, and L. Terenius, editors. Elsevier/North-Holland Biomedical Press, Amsterdam. 277–278.
15. Brownell, J., E. del Pozo, and P. Donatsch. 1980. Inhibition of vasopressin secretion by a Met-enkephalin (FK 33-824) in humans. *Acta Endocrinol.* **95:** In press.
16. Lord, J. A. H., A. A. Waterfield, J. Hughes, and H. W. Kosterlitz. 1977. Endogenous opioid peptides: multiple agonists and receptors. *Nature (Lond.).* **267:** 495–499.
17. Kosterlitz, H. W. 1978. Opioid peptides and their receptors. *In* Endorphins '78. L. Graf, M. Palkovitz, and A. Z. Ronai, editors. Excerpta Medica, Amsterdam. 205–216.
18. Tolis, G., J. Hickey, and H. Guyda. 1975. Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. *J. Clin. Endocrinol. Metab.* **41:** 797–800.