Potentiation of Histamine-Induced Itch and Flare Responses in Human Skin by the Enkephalin Analogue FK 33-824, \(\beta\)-Endorphin and Morphine*

B. Fjellner and Ö. Hägermark

Department of Dermatology, Karolinska Hospital, S-10401 Stockholm, Sweden

Summary. The effect of various opioid or putative neurotransmitter peptides on histamine-induced itch and flare responses was studied in humans after intradermal injection. Significant enhancement of the histamine responses was induced by the stable methionine-enkephalin analogue FK 33-824, \(\beta\)-endorphin and morphine. The putative neurotransmitters substance P and vasoactive intestinal polypeptide (VIP) — which moreover are potent histamine liberators — had no enhancing effect. The potentiation induced by FK 33-824 was induced neither by local pretreatment with Compound 48/80 to deplete the local stores of mast-cell-bound histamine, nor by oral pretreatment with indomethacin to inhibit prostaglandin formation in the skin. Thus, the enhancement did not seem to be due to histamine release or to prostaglandin formation and the mechanism of the effect remains to be shown. The specific morphine antagonist naloxone did not inhibit the potentiation by FK 33-824, which might indicate that ordinary opiate receptors were not involved. The results support the idea that pain and itch are qualitatively separate processes and suggest possible mechanisms of morphine-induced pruritus. The findings are of particular interest in view of recent reports on the presence of methionine-enkephalin in Merkel cells.

Key words: Pruritus — Histamine — Opioid peptides — Morphine

Introduction

The opioid peptides are endogenous compounds with opiate-like properties. They are assumed to be involved in the endogenous control of pain, but the physiological role of these peptides seems to be far more extensive [1, 26]. As yet, three naturally occurring opioid peptides have been identified, viz leucine-enkephalin, methionine-
enkephalin (met-enkephalin) and \(\beta\)-endorphin. The two enkephalins, widely distributed in the central and peripheral nervous system, are rapidly metabolized. \(\beta\)-Endorphin, which is concentrated in the hypothalamic region and the pituitary, has a distribution quite distinct from that of the enkephalins and is more stable [21]. Since the natural enkephalins are labile and exert only weak and transient effects after systemic administration, analogues have been synthesized which are less susceptible to enzymatic degradation, e.g. the met-enkephalin analogue FK 33-824 [22]. This peptide has a long-lasting and potent analgesic effect in animals [22] and increases tolerance to pain in man [24].

There are close neuro-anatomical and neurophysiological relations between pain and pruritus [17, 23]. In recent years our interest has focused on whether or not peptides with putative neurotransmitter function may be involved as stimulators of the dermal itch receptors [7, 11]. In this context, we therefore considered it of interest to extend these studies and investigate whether the opioid peptides would influence experimental itching in man. Most experiments were performed with the stable met-enkephalin analogue FK 33-824. Intradermal injection of FK 33-824, in doses which were not pruritogenic per se, was found to potentiate the itching evoked by intradermal injection of histamine. After this unexpected finding the investigations were extended to include morphine, the naturally occurring opioid peptides met-enkephalin and \(\beta\)-endorphin, as well as the putative peptide neurotransmitters substance P and vasoactive intestinal polypeptide (VIP). The latter two substances are potent histamine liberators in man [7, 11].

**Materials and Methods**

**Experimental Itch and Flare**

Healthy volunteers, 5 men and 41 women aged 17—59 years, participated in this investigation. Many of them took part in more than one of the experiments. We used the same technique as described previously [7]. After a 0.01 ml intradermal injection of the solution to be studied in the lateral aspect of the upper arms, the duration of the itch response was recorded. The size of the flare reaction was outlined on the skin 5 min after injection, traced on transparent film and measured with a planimeter (model 317 from Gebrüder Haff GmbH, Pf Time, FRG). No internal medication was allowed at least 1 week prior to the experiment. The test solutions were injected in a double-blind fashion.

**Agents Studied**

FK 33-824, [D-Ala\(^2\) MePhe\(^4\), Met(0)\(^3\)-ol]-enkephalin, was synthesized and generously supplied by Sandoz AG, Basle, Switzerland. \(\beta\)-Endorphin was a gift from Prof. L. Terenius, Department of Pharmacology, Uppsala University, Uppsala, Sweden. Prostaglandin E\(_2\) (PGE\(_2\)) was supplied by Upjohn Co., Kalamazoo, MI, USA. VIP was prepared and donated by Prof. V. Mutt, Department of Biochemistry II, Karolinska Institute, Stockholm, Sweden. Compound 48/80 was a gift from Prof. B. Högborg, Leo AB, Helsingborg, Sweden. Met-enkephalin and substance P were obtained from Peninsula Laboratories, San Carlos, CA, USA and naloxone from Endo Laboratories, New York, NY, USA. As a histamine H\(_1\)-receptor antagonist, we used mepyramine from Pharma Rhodia A/S, Copenhagen, Denmark. Morphine hydrochloride and histamine dihydrochloride were obtained from ACO, Solna, Sweden.

Stock solutions of the following compounds were prepared and stored at \(-20^\circ\text{C}\) until use: \(\beta\)-endorphin (30 \(\mu\text{g/ml}\)) dissolved in distilled water slightly acidified with hydrochloric acid; PGE\(_2\) (1 mg/ml) in 95% ethanol; and substance P (10\(^{-4}\) M), VIP (10\(^{-4}\) M) and Compound 48/80 (100 \(\mu\text{g/ml}\)) in sterile, pyrogen-free physiological saline, containing 10% (v/v) Sörensens phosphate buffer (\(\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4\), 67 mM), pH 7.4. Solutions of FK 33-824, met-enkephalin, morphine and