

Met-Enkephalin Effects on Histamine-Induced Bronchoconstriction in Guinea Pigs

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ABSTRACT

We investigated the effects of the neuropeptide met-enkephalin on histamine-induced bronchoconstriction in an experimental model of asthma. Classic Konzett and Rössler's method of whole body plethysmography modified by Gjuriš¹, was applied in the study. This method represents a standard experimental model of bronchoconstriction, suitable for the evaluation of peptide effects on the histamine-induced bronchoconstriction. The results of the measurements implicate a dose-related modulatory effect of met-enkephalin on the bronchoconstrictor action of histamine. Met-enkephalin doses of 1 mg/kg and 10 mg/kg, respectively, caused statistically significant reduction of the histamine-induced bronchoconstriction. Estimated ED₅₀ dose was 0.235 mg/kg. Further studies are needed to define practical and therapeutic use of the presented observations in respiratory pharmacology.

Key words: met-enkephalin, bronchoconstriction, histamine, asthma, plethysmography

Introduction

We evaluated the effects of the neuropeptide met-enkephalin on experimentally induced bronchoconstriction in guinea pigs. Classic Konzett and Rössler's method of whole body plethysmography modified by Gjuriš^{1,2} was applied in this study, since it represents a standard experimental model of bronchoconstriction, suitable for the evaluation of peptide effects on the histamine induced bronchoconstriction.

Met-enkephalin is an endogenous opioid pentapeptide with the amino acid sequence Tyr-Gly-Gly-Phe-Met (molecular weight, MW=573.7). Molecular precursors of met-enkephalin are pro-opiomelanocortin (POMC) and preproenkephalin hormones³. Preproenkephalin molecule contains six copies of met-enkephalin and pro-opiomelanocortin one copy³. Met-enkephalin binds preferentially to delta opioid receptors^{3,4}. Recent investigations showed that zeta opioid receptors mediate its effects on cellular and tissue growth and development⁵.

Met-enkephalin was investigated because of its presence in the respiratory tract^{6,7} and several previous reports showing that it blocks »in vivo« effects of hista-

mine in the models of anaphylactic shock, hypersensitivity reactions and autoimmune diseases^{4,8,9}. Recent pre-clinical and clinical studies of met-enkephalin indicated that it could be a promising new drug for different autoimmune and chronic inflammatory diseases^{4,5,8-15}.

Materials and Methods

Animals

Experimental animals were male and female (1:1) Hartley guinea pigs bred at the Department of Pharmacology, Zagreb University School of Medicine, weighing 500–700 g. The animals were kept in a room with constant temperature (22±1°C) and dark-light cycle (12h/12h). They were fed by standard laboratory food and given water ad libitum. During the experiment animals were anesthetized with 1.5 g/kg body weight of urethan (25% solution). Two thirds were administered intraperitoneally and the rest subcutaneously. When needed, additional anesthesia was given intravenously. This

type of anesthesia minimally suppresses respiratory reflexes¹. Guinea pigs are suitable for plethysmographic evaluation because of rich smooth muscle network around the bronchioles that is highly sensitive to histamine¹⁶. The protocol of the investigation complied with the European Community guidelines for the use of experimental animals, and was approved by the institutional ethics committee.

Method

Whole body plethysmography with continuous spirogram registration allows monitoring of any change in the respiratory rate, type of respiration or amplitude of respirations. Konzett and Rössler’s method of whole body plethysmography, modified by Gjuriš, was applied in this study^{1,2}.

Bronchoconstriction was induced by intravenous histamine (10 µg/kg). Each animal was its own control, i.e., the histamine responses without pretreatment were compared to histamine responses following pretreatment with the tested substances, measured as changes in the amplitude of respirations. The protective effect of met-enkephalin was defined as the percentage of histamine blockade (the reduction of histamine-induced bronchoconstriction). Met-enkephalin (LUPEX®, Biofactor, Germany) was administered in three doses (0.1 mg/kg, 1 mg/kg, 10 mg/kg). Investigated met-enkephalin doses, presented in Table 1, were given intravenously, 90 seconds prior to histamine. Plethysmography was performed continuously.

Data analysis

Statistical analysis and data plotting was performed with GraphPad Prism Software (version 4.0). The differences between effects of observed met-enkephalin doses, on the histamine induced bronchoconstriction, were tested by means of Kruskal-Wallis test, and Dunn’s multiple comparison test.

Results

The lowest doses of 0.1 mg/kg met-enkephalin caused no statistically significant change in the histamine-

-induced bronchoconstriction (Table 2). Met-enkephalin doses of 1 mg/kg and 10 mg/kg, respectively, caused statistically significant reduction of the histamine induced bronchoconstriction (Table 2 and Figure 1). The highest dose of 10 mg/kg met-enkephalin had the best pharmacological effect (Table 2, Figures 1–2).

TABLE 2
PERCENTAGE OF MET-ENKEPHALIN INDUCED REDUCTION OF HISTAMINE BRONCHOCONSTRICTION

Animal number	Met-enkephalin		
	0.1 mg/kg (A)	1 mg/kg (B)	10 mg/kg (C)
1	6.7	42.9	66.7
2	0.0	0.0	35.7
3	0.0	25.0	0.0
4	5.0	50.0	62.5
5	0.0	40.0	50.0
6	21.4	46.2	44.5

Kruskal-Wallis test: 6.97, $p < 0.05$, Dunn’s multiple comparison test: A vs. B: rank sum = -5.75, $p > 0.05$, A vs. C: rank sum = -7.75, $p < 0.05$, B vs. C: rank sum = -2.00, $p > 0.05$

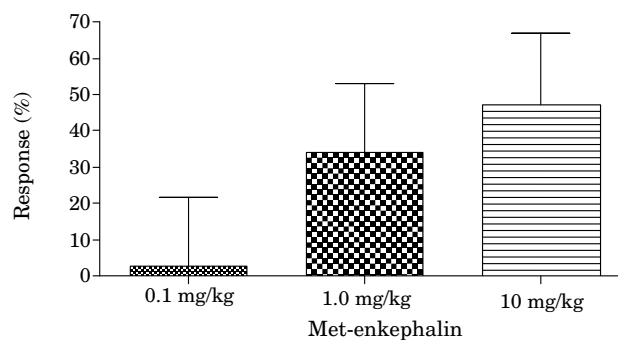


Fig. 1. Blockade of histamine bronchoconstriction by means of different met-enkephalin doses.

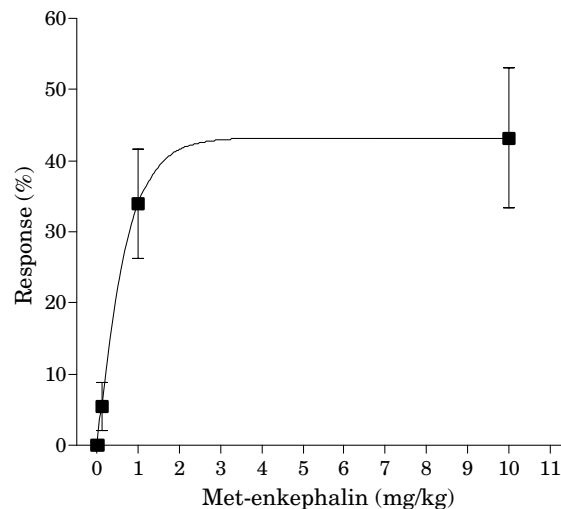


Fig. 2. Dose-response curve of the met-enkephalin induced reduction of histamine bronchoconstriction.

TABLE 1
MET-ENKEPHALIN TREATMENT IN EXPERIMENTAL BRONCHOCONSTRICTION

Group	Study design (substance and dose applied pro animal)
A	histamine 10 µg/kg (control for group A)
N = 6	met-enkephalin 0.1 mg/kg, histamine 10 µg/kg, 90 seconds later
B	histamine 10 µg/kg (control for group B)
N = 6	met-enkephalin 1 mg/kg, histamine 10 µg/kg, 90 seconds later
C	histamine 10 µg/kg (control for group C)
N = 6	met-enkephalin 10 mg/kg, histamine 10 µg/kg, 90 seconds later

The results of the measurements implicate a dose-related modulatory effect of met-enkephalin on the bronchoconstrictor action of histamine in the experimental model of bronchoconstriction, as presented in Figure 2. Estimated ED₅₀ dose¹⁷ was 0.235 mg/kg (R²=0.617).

Discussion

Met-enkephalin is a multifunctional neuropeptide that exerts gut cytoprotection, in addition to a number of other antiinflammatory and immunomodulating effects on different organs and tissues^{4,5,8–14}. Consequently, it was recently proposed to be classified as a cytokine⁴.

The beneficial effects of the met-enkephalin were reported for several disease models including arthritis, encephalomyelitis, graft-versus host reaction, ischaemia and inflammatory bowel disease^{4,5,8–14}. The pentapeptide also induces analgesia, and antioxidant action^{4,13,14,18}. According to the present knowledge the administration of met-enkephalin enables pharmacologic effects of both steroidal and non-steroidal antiinflammatory drugs, without the majority of their side-effects^{8,9,13,14}.

Despite of the extensive studies, including pre-clinical pharmacology and several clinical trials of different autoimmune diseases, tumors, AIDS and infertility^{4,5,9,10,13,14,19,20}, met-enkephalin was not (to our knowledge) investigated with respect to asthma therapy. Met-enkephalin and its metabolic activity were detected in the rat and guinea pig respiratory tract^{6,7,21}. However,

no changes in the plasma met-enkephalin were found in acute asthma bronchoconstriction produced by exercise²². The inhibition of non-adrenergic non-cholinergic bronchoconstriction in guinea pig airways was reported to be via mu-opioid receptors²³ that bind met-enkephalin with low affinity⁴. This study showed that met-enkephalin significantly reduces histamine induced bronchoconstriction in guinea pigs regardless of the fact that it is a peptide selective for delta and zeta opioid receptors^{4,5}. Local immunoregulatory role of preproenkephalin and met-enkephalin may be also of interest for the immunotherapy of asthma²⁴.

The results of our study, conducted on the classic model of experimental bronchoconstriction, indicate that further investigations are justified. The localization of the peptide within the respiratory tract^{2,6,7}, seems to be in line with our observation that met-enkephalin doses of 1 mg/kg and 10 mg/kg, respectively, cause statistically significant reduction of the histamine induced bronchoconstriction. Estimated ED₅₀ dose of 0.235 mg/kg is also in the range of pharmacologically active human met-enkephalin doses, applied subcutaneously, in different autoimmune diseases and tumors^{4,9,10,13,14}. Further studies are needed to define possible therapeutic targets, doses and modes of met-enkephalin administration in respiratory pharmacology.

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UČINCI MET-ENCEFALINA U EKSPERIMENTALNOM MODELU BRONHOKONSTRIKCIJE

S A Ž E T A K

U radu su ispitani učinci neuropeptida met-encefalina (LUPEX®) na bronhokonstrikciju izazvanu histaminom u eksperimentalnom modelu astme. Uporabljena je klasična metoda pletizmografije cijelog tijela po Konzettu i Rösleru, modificirana po Gjurišu. Metoda predstavlja standardni eksperimentalni model bronhokonstrikcije, pogodan za procjenu efekta peptida na bronhokonstrikciju izazvanu histaminom. Mjerenja su ukazala da je modulatorni učinak met-encefalina na ovisan o dozi. Doze met-encefalina od 1 mg/kg i 10 mg/kg izazvale su statistički značajnu redukciju histaminom inducirane bronhokonstrikcije. Procjenjena ED₅₀ doza bila je 0.235 mg/kg. Dodatne studije potrebne su kako bi se definirali mogući terapijski učinci met-enkefalina u respiratornoj farmakologiji.