

The influence of casein-derived exorphins on mast cells in rodents

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INTRODUCTION

A number of peptides with opioid activity have been found in partial enzymatic digests of food proteins [1]. These peptides, because of their exogenous origin, have been named exorphins. Casein-derived exorphins appear to be quite resistant to the action of gastrointestinal proteases and may be biologically active. It has been suggested that the exorphins play the role of food hormones as exogenous regulators of gastrointestinal functions [2]. A number of opioid peptides from bovine casein, human casein and gluten have been sequenced and resynthesised. In their communication, Barantl et Teschemacher found increased opioid activities in milk baby food (Milupa) and in enzymatically digested casein as compared with fresh bovine milk [3]. Svedberg et al. have demonstrated nanomolar concentrations of β -casomorphin-7, 4 and -6 immunoreactive materials in small intestine contents in human volunteers after bovine milk ingestion [4]. These results indicate that casein-derived exorphins appear in the human gut and justify the hypothesis that these peptides might play a role in some gastrointestinal diseases. In the late 80-ties, Kurek speculated that β -casomorphin-7 may be the starting point of pseudoallergic reactions and described the ability of this peptide to induce

histamine release from rat peritoneal mast cells and isolated human leukocytes [5, 6]. We have reported that β -casomorphin-7 induces anaphylactoid bronchial and jejunum reactions in guinea pigs as well as pseudoallergic wheal-and-flare skin reactions in healthy volunteers [6, 7]. Similarly, wheal-and-flare formations were found in healthy children, when α -casein (90-95) was injected intradermally [8].

THE AIM OF THE STUDY AND THE TECHNIQUES

Here, we investigated the secretagogue effect of synthetic α -casein (90-95) and β -casomorphin-7 on two different populations of mast cells in rodents. Mast cells of "connective tissue type" were obtained from rat peritoneal cavity (PMC) and purified using density gradient centrifugation on Percoll. The "mucosal-like type" mast cells (BMMC) were cultured from mouse bone marrow cells in using the medium prepared from 48 h co-culture of concanavaline A activated spleen cells from C57Bl/6J and C3H mice or in the presence of purified murine recombinant IL-3 (60 U/ml). The increase in released β -hexosaminidase activity was considered to be a marker of mast cell secretory activity.

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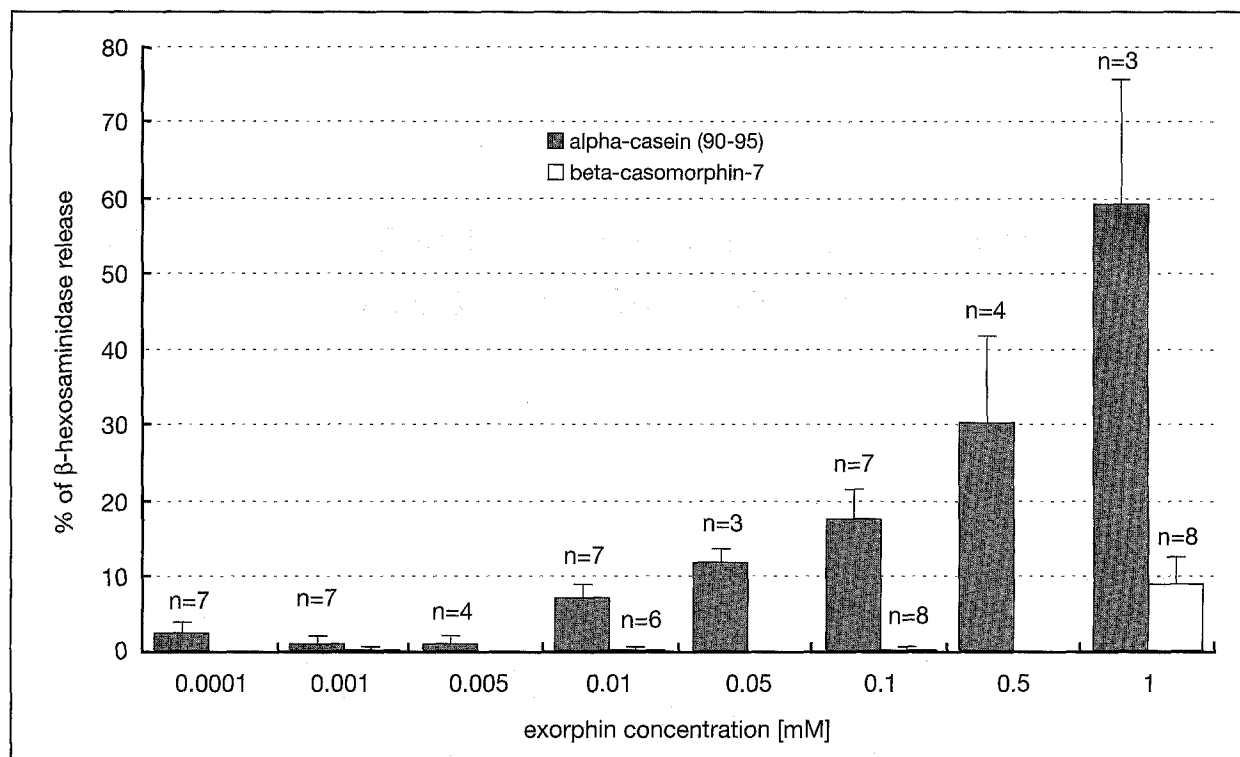


Fig. 1. – Influence of casein-derived exorphins on PMC.

CONCLUSIONS

Both synthetic casein-exorphins were able to exert a secretagogue effect on PMC by mili- and micromolar concentrations of β -casomorphin-7 and α -casein (90-95), respectively (fig. 1). For this reason, α -casein (90-95) was selected for further experiments. No reaction was observed after stimulation of BMMC with exorphins at concentrations of up to 10^{-3} M. The PMC were activated in non-cytotoxic and dose-dependent manner. The reaction of PMC was temperature (4°C , 37°C , 47°C), energy (2-deoxy D-glucose 2×10^{-3} M and antimycin A 10^{-3} M) dependent. However, PMC were responsive in a calcium and

magnesium free buffer, the reaction was abolished when preincubation with EDTA was performed. Preincubation of PMC with opiate antagonist was effective only when high 10^{-3} M concentration of naloxone was used. The effect of naloxone in this concentration may be due to mechanisms other than receptor blockage. Interestingly, after preincubation with benzalkonium chloride up to $6 \mu\text{g/ml}$, PMC become unresponsive. The significant decrease in PMC reactivity to α -casein (90-95) after preincubation with benzalkonium chloride strongly suggests a peptidergic pathway which involves a membrane-assisted but receptor-independent stimulation of G_i -like proteins [9].

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