

Exorphins derived from cow's milk casein elicit pseudo-allergic wheal-and-flare reactions in healthy children

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SUMMARY

Beta-Casomorphin-7 and alpha-casein (90-95) are opioid peptides occurring in cow's milk casein digest products. We investigated the direct histamine releasing properties of these peptides *in vivo*. Intracutaneous skin tests were performed with β -casomorphin-7, alpha-casein (90-95) and cow's milk extract in 25 children without any clinical indication of cow's milk allergy or intolerance. Immediate wheal reactions were measured and compared with skin reactions to histamine and codeine. Tests were repeated after pretreatment with 10 mg cetirizine, a H1-antagonist. The opioid peptides induced wheat-and-flare reactions in all children. These were concentration-dependent and similar to those observed with histamine or codeine. Pretreatment with cetirizine inhibited skin reactivity to both opioid peptides. With one exception, no skin reaction to cow's milk was found. Degranulation of skin mast cells was observed in a biopsy specimen taken from the skin area injected with alpha-casein (90-95). Beta-Casomorphin-7 and alpha-casein (90-95) caused pseudo-allergic immediate type reactions, apparently by direct histamine release. This may be of relevance with regard to clinical reactions to cow's milk.

RÉSUMÉ

Les exorphines provenant de la caséine du lait de vache provoquent des réactions urticariennes pseudo-allergiques chez les enfants sains. – La bêta-casomorphine-7 et l'alpha-caséine (90-95) sont des peptides opioïdes retrouvés dans les produits de digestion de la caséine du lait de vache. Le but de ce travail est d'étudier les propriétés histamino-libératrices directes de ces peptides *in vivo*. Des tests intradermiques ont été pratiqués avec la bêta-casomorphine-7, l'alpha-caséine (90-95) et un extrait de lait de vache chez 25 enfants bien portants n'ayant aucun signe d'allergie ou d'intolérance au lait de vache. Les réactions papuleuses immédiates ont été mesurées et comparées avec les réactions cutanées à l'histamine et à la codéine. Les tests ont été refaits après administration de 10 mg de cetirizine, antihistaminique H1. Chez tous les enfants, les peptides opioïdes ont induit des réactions érythémato-papuleuses. Elles étaient concentration-dépendantes et similaires à celles provoquées par l'histamine ou la codéine. Un prétraitement avec la cetirizine inhibait la réactivité des deux peptides opioïdes. Sauf dans un cas, les tests cutanés au lait de vache restèrent négatifs. Les biopsies de la zone cutanée injectée avec l'alpha-caséine (90-95) mirent en évidence la dégranulation des mastocytes cutanés. La bêta-casomorphine-7 et l'alpha-caséine (90-95) provoquent des réactions cutanées pseudo-allergiques immédiates dues à une histamino-libération directe. Cette constatation peut avoir de l'intérêt pour la compréhension des réactions cliniques au lait de vache.

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TABLE I. – Aminoacids sequences of bovine alpha-casein (90-95), alpha-casein (90-95) and beta-casomorphin-7

<i>alpha-casein:</i>
alpha-casein (90-96): Arg-Tyr-Leu-Gly-Tyr-Leu-Glu
alpha-casein (90-95): Arg-Tyr-Leu-Gly-Tyr-Leu (MW = 783,9 g/M)
<i>beta-casein:</i>
beta-casomorphins: -4, -5, -6, -7, -8, -9, -10, -11
beta-casomorphin-7: Tyr-Pro-Phe-Pro-Gly-Pro-Ile (MW = 789,9 g/M)

INTRODUCTION

In recent years a number of peptides with opioid activity have been described in partial enzymatic digests of proteins derived from alpha-casein, beta-casein, wheat and gluten [23]. These peptides, because of their exogenous origin, have been named exorphins. Peptides responsible for the properties of bovine alpha-casein or beta-casein have been isolated and sequenced [1, 5, 11, 12]. (Table I). Casein derived exorphins appear to be quite resistant to the action of gastrointestinal proteases and may be biologically active [13, 14]. It has been suggested that these "food hormones" may act via gut opiate receptors as exogenous regulators of gastrointestinal motility, permeability and hormone release [16, 17, 21]. We have reported that beta-casomorphin induces histamine release from peritoneal rat mast cells, and anaphylactoid bronchial and jejunal reactions in guinea pigs [7, 9]. Earlier, we extended these studies by investigation of skin reactivity to beta-casomorphin-7 as well as alpha-casein (90-95) in healthy children before and after the administration of the H1-antagonist cetirizine.

MATERIAL AND METHODS

Test substances

Beta-Casomorphin-7 and alpha-casein (90-95) were purchased from Bachem (Budendorf, Switzerland). Histamine dihydrochloride came from Sigma Chemicals (St. Louis, USA). Codeine phosphate solutions were obtained from the pharmacy of the University School of Medical Sciences, Bydgoszcz. For all dilutions as well control tests, physiological saline was used. The cow's milk allergen extract was obtained from Sevac (Prague, Czech Republic). Cetirizine (Zyrtec) came from UCB Pharma (Bruxelles, Belgium).

Subjects

Twenty-five healthy children with no clinical indication of cow's milk allergy or other clinically relevant sensitizations were investigated (13 girls, 12 boys; age 10 to 16 years) with their parents approval. The study protocol was approved by the ethics committee of the University School of Medical Sciences, Bydgoszcz.

Skin tests

Skin test were performed with three concentrations (10^{-5} , 10^{-4} , 10^{-3} mol/l) of beta-casomorphin-7 and alpha-casein (90-95), cow's milk allergen extract, histamine dihydrochloride (10^{-3} mol/l), codeine phosphate (10^{-3} mol/l), and physiological saline. Approximately 0.02 ml of each preparation were injected intracutaneously on the volar surface of the forearm. After 15 min the widest two perpendicular diameters of wheals were measured and the wheal area was calculated as the product of these diameters. These tests were repeated four hours after 10 mg of cetirizine had been ingested.

Total IgE-levels

Total serum IgE-levels were measured with a standard method EIA enzyme immunoassay (Pharmacia, Vienna, Austria). Levels above 100 kU/I were considered as elevated.

STATISTICS

If not mentioned otherwise, mean values and standard deviations are given. For statistical evaluation the student-t-test was used. If the significance level was $p < 0.05$, the null hypothesis was rejected. The correlation between reactions to the opioid peptides and codeine was analyzed by linear regression.

RESULTS

Elevated total IgE levels in the serum were found in 4 children and a positive reaction to the cow's milk test solution was found in only one individual, who had not reported hypersensitivity reactions to milk ingestion; challenge tests were

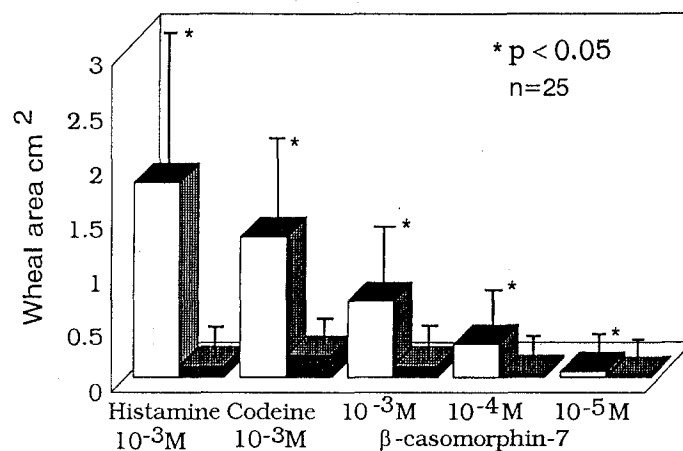


Fig. 1. – Wheal formations after intracutaneous injection of beta-casomorphin-7 before and after pre-treatment with 10 mg of cetirizine.

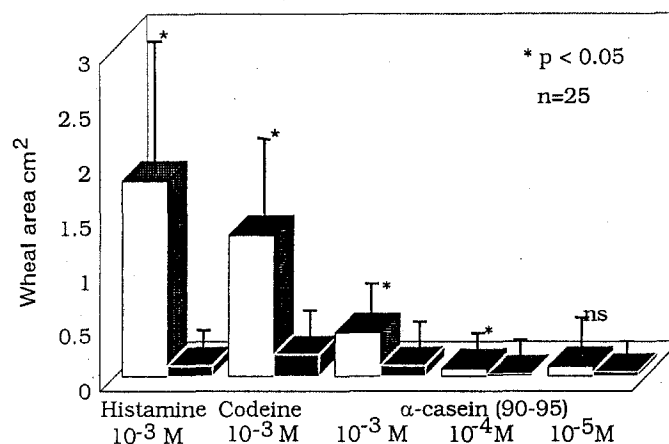


Fig. 2. – Wheal formations after intracutaneous injection of alpha-casein (90-95) before and after pretreatment with 10 mg of cetirizine.

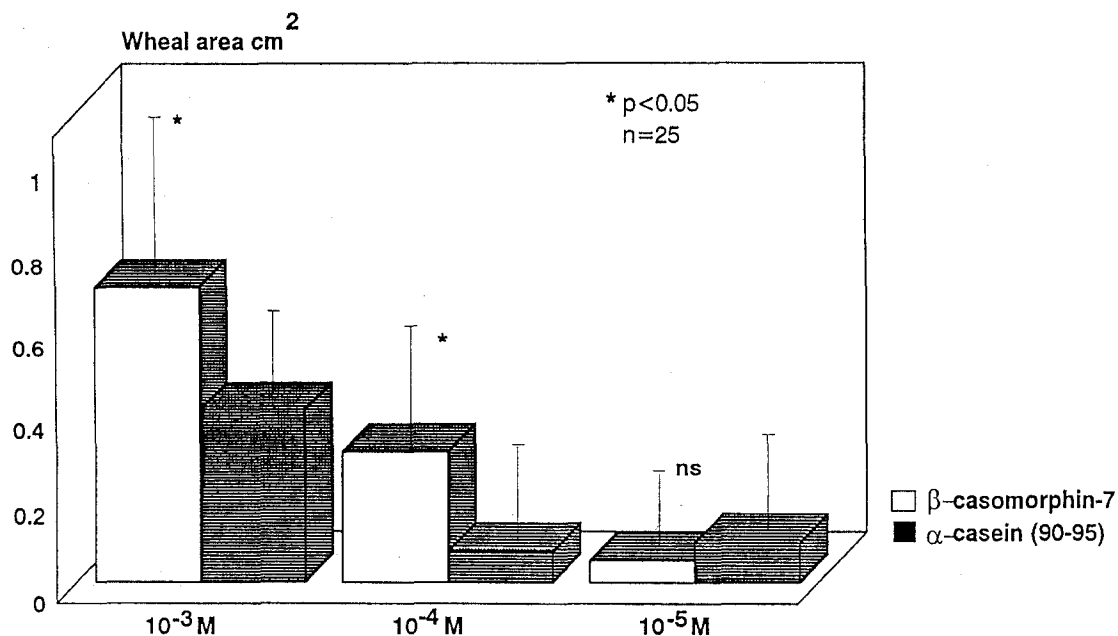


Fig. 3. – Comparison of wheal formations after intracutaneous injection of beta-casomorphin-7 and alpha-casein (90-95).

TABLE II. – Patients with high IgE levels and positive skin test reaction to cow's milk extract

Patient No.	IgE (IU/ml)	wheal area mm ² (milk)
13	122	56.5
14	100	0
23	260	0
24	1 000	0
25	127	0

not performed. (Table II). Intradermal injection of beta-casomorphin-7 caused immediate wheal-and-flare reactions, which at 10^{-3} , were somewhat smaller than those to histamine dihydrochloride or codeine phosphate. The size of wheals was dose-dependent. Wheal reactions occurred in all children after injection of 10^{-3} mol/l beta-casomorphin-7, while 88 per cent and 36 per cent reacted to 10^{-4} mol/l and 10^{-5} mol/l concentrations respectively. Pre-treatment with cetirizine significantly decreased the whealing due to beta-casomorphin-7 in all concentrations. Detailed results are shown in figure 1. A significant correlation was found between skin reactions to codeine phosphate and each of the three concentrations of beta-casomorphin-7 (10^{-5} mol/l: $r = 0.60$, $p < 0.01$; 10^{-4} mol/l: $r = 0.53$, $p < 0.01$; 10^{-3} mol/l: $r = 0.69$, $p < 0.001$). Injection of alpha-casein (90-95) induced wheal reactions in 96 per cent, 40 per cent to the 10^{-3} , 10^{-4} , and 10^{-5} mol/l. After administration of cetirizine whealing reactions to 10^{-3} and 10^{-4} mol/l concentrations of alpha-casein (90-95) were significantly decreased. (fig. 2).

Compared with reactions to beta-casomorphin-7, wheal areas to alpha-casein (90-95) were significantly smaller. (fig. 3). All wheal reactions to both peptides were accompanied by flare reactions (not quantified). A significant correlation between reactions to codeine phosphate and alpha-casein (90-95) was found only between reactions to codeine phosphate and alpha-casein (90-95) with the 10^{-5} mol/l concentration of the peptide ($r = 0.50$, $p < 0.02$).

DISCUSSION

Both cow's milk casein-derived exorphins, beta-casomorphin-7 and alpha-casein (90-95), elicit immediate wheal-and-flare reactions in the skin of children without indications of cow's milk allergy. In addition, a skin test reaction to cow's milk allergen was found in only one and elevated serum total IgE levels in four out of 25 individuals, indicating that skin test reactions to the exorphins are pseudo-allergic. Skin reactions to both opioid peptides occurred in a dose dependent fashion and could be inhibited significantly by pre-administration of cetirizine.

In a previous study, we found that beta-casomorphin-7 induces histamine release from peripheral blood leukocytes in vitro and provokes immediate wheal-and-flare skin reactions in healthy adult volunteers without cow's milk allergy [8]. The present study extends this finding to children, and shows that also alpha-casein (90-

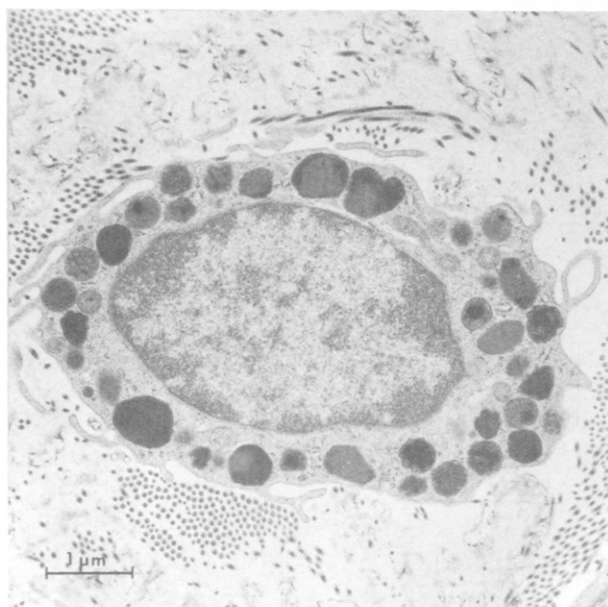


Fig. 4. – Electron micrograph of typical skin mast cell in biopsy specimen before injection of alpha-casein (90-95). (Magnification x 17000).

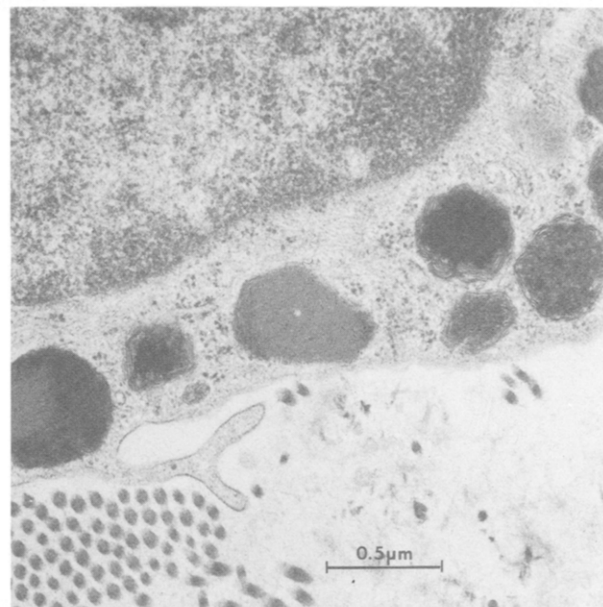


Fig. 5. – Higher magnification of figure 5. Mast cell with crystal and granular scroll patterns. (Magnification x 44000).

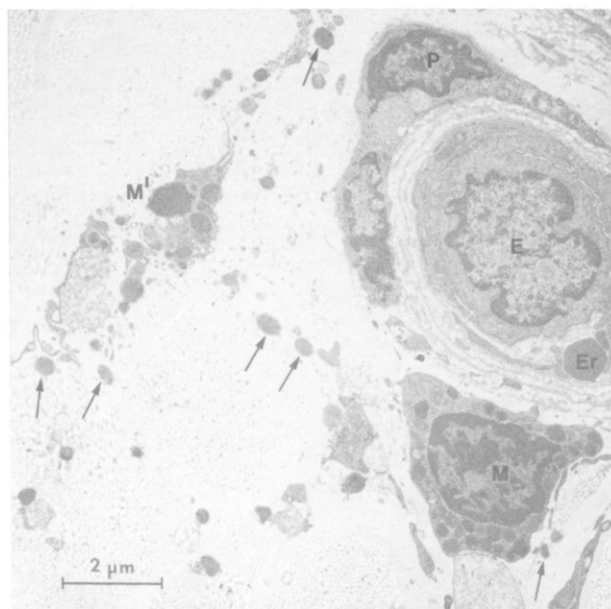


Fig. 6. – Electron micrograph of biopsy specimen 20 min after i.c. injection of 10^{-3} mol/l concentration of alpha-casein (90-95). Perivascular mast cell (M.). Degranulated mast cell (M') and mast cell granules between collagen fibres. Endothelial cell (E). Pericyte (P). Erythrocyte (Er). (Magnification x 9500).

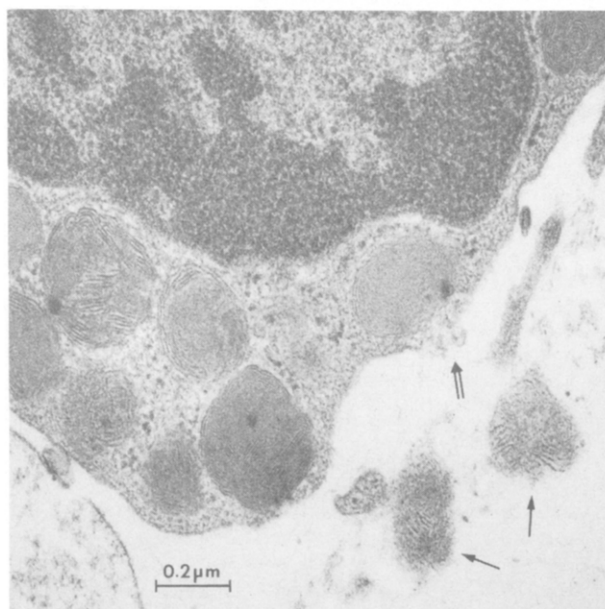


Fig. 7. – Higher magnification of perivascular mast cell (M) from figure 6 demonstrating that perigranular membranes of adjacent cell granules are fused to cell membrane. (Magnification x 68000).

95) induces such reactions. Also, an inhibitory action of an H1-antagonist was demonstrable. Naloxone was found to inhibit the histamine release from peripheral blood leukocytes induced by beta-casomorphin-7 (00). The inhibitory effect of naloxone on immediate skin reactions due to beta-casomorphin-7 was smaller than on codeine-induced whealing. These findings give evidence that beta casomorphin-7 is a histamine releasing agent, whose action is via opioid receptors and probably also mechanisms not related to opioid receptors [2]. Presumably skin reactions to alpha-casein (90-95) are also due to direct mediator release, as a biopsy taken from the skin injected with this peptide showed mast cell degranulation. This was additionally demonstrated in one adult healthy volunteer (male, 40 years old, no cow's milk allergy or intolerance). (fig. 4 and fig. 5). Inhibition by cetirizine, an H1-antagonist, of wheal-and-flare reactions due to the opioid peptides indicates that histamine is evidently the most important mediator of these actions. The significant correlation between skin reactions elicited by the opioid peptides on the one hand and codeine phosphate on the other gives additional evidence of an involvement of opioid receptors.

Whealing induced by alpha-casein (90-95) was significantly smaller than that elicited by beta-casomorphin-7 at equimolar concentrations. This may be due to differences in the affinities of the molecules to opiate receptors or other membrane

structures involved in the mast cell activation [2, 3, 10, 19].

The treatment of children with hypersensitivity reactions to cow's milk has greatly improved since hydrolysate formulas were introduced. According

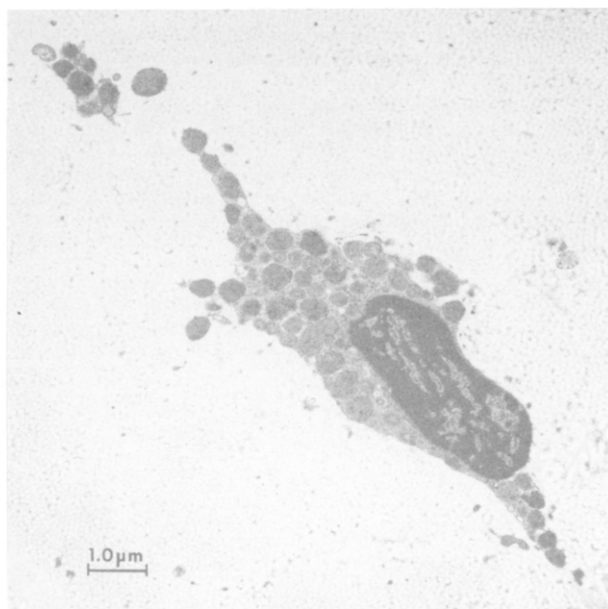


Fig. 8. – Degranulated mast cell. (Magnification x 11500).

to the US pharmacopoeia, the transition from an immunogenic to a non-immunogenic state of casein hydrolysate is related to the break-down of protein to peptides with molecular weight no greater than 1.500 daltons [6]. Furthermore, it has been reported that some children still react to hydrolysate cow's milk formula [18]. Also, cow's milk hydrolysates have been found to induce reac-

tions in the skin prick test [15], and the possibility of residual allergenic properties of hydrolysate formulas has to be considered [22]. However, the demonstration of cow's milk derived exorphins [4, 20] suggests that such peptides could cause immediate-type pseudo-allergic hypersensitivity reactions in vivo. Further work will address this issue.

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