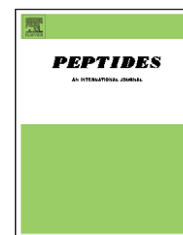


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Serum activity of dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) in breast-fed infants with symptoms of allergy

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ABSTRACT

β-Casomorphins, opioid peptides present in mother's milk, are a good substrate for DPPIV (EC 3.4.14.5) which is a major factor limiting the half-life of biologically active peptides. Serum DPPIV activity of two groups of infants (healthy and atopic dermatitis) and contents of β-casomorphin-5 and -7 in their mothers' milk were determined in the study. We have found correlation between those two parameters in the group of children with atopic dermatitis syndromes, while no such a correlation was found in the control group.

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1. Introduction

β-Casomorphins are opioid-active peptides which may be enzymatically released from milk protein β-casein, the fragments 60–66 and 51–57 for bovine and human β-casein respectively [7,11,26]. Exogenous opioid peptides are the ligands of μ opioid receptor and can exert biological effects similar to those of endogenous opioids [17,36]. The influence of exogenous opioid peptides on the central nervous system, immune system, cardiovascular system and gastrointestinal tract have been widely reviewed [7,16,22,27,29,30,37,38].

It has been demonstrated that human milk except nutrients contains β-casomorphins which are substances with not clear physiological significance [2]. The assessment of effects of oral or intragastric administration of β-casomorphins in human neonates has not been carried out. It has been suggested that casein-derived opioid peptides could play a role in the regulation of infants' gastrointestinal functions and

development [30,33]. The newborns' gastrointestinal wall may be permeable for β-casomorphins immunoreactive materials [4,36] and the permeation of exogenous opioid peptides through the blood–brain barrier seems to be possible [15,34]. Permeability of infant gut to undigested macromolecules is higher than in the adults. This results from immature immune functions, deficiency of proteolytic enzymes, mucosal differentiation and lack of bacterial flora [21,40–42].

Some of researchers suggest that β-casomorphins can evoke the pseudoallergic reactions by inducing histamine release from immune cells [18,19,32,33]. Stepnik and Kurek have shown secretagogue effects on rodents' mast cells after preincubation with synthetic β-casomorphin-7 [33]. Immediate and dose-dependent wheal and flare reactions in the skin in healthy children were observed after β-casomorphin-7 intradermal injection. The skin reactions were similar to test reactions on histamine and codeine and were inhibited by pre-administration of cetirizine, H1-antagonist [19,32]. Moreover it

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has been demonstrated the β -casomorphin-7 induced histamine release from peripheral blood leukocytes in vitro. The reaction has been inhibited by naloxon [19]. Kayser and Meisel have demonstrated the stimulation of the proliferation of the human peripheral lymphocytes by a high concentration of β -casomorphin-7 [14]. These data suggest that casomorphins can be involved in pseudo-allergic hypersensitivity reactions in vivo. It seems to be probable that the β -casomorphins contained in human milk can be specific mediators of pseudo-allergic skin reactions [18,19,32,33]. In this paper we have been demonstrated the presence of β casomorphin-5 and -7 in human milk in different phases of lactation.

Although human breast milk is the most hypoallergenic substance to nourish infants but in the last century the number of a breast-fed children exhibiting atopic dermatitis symptoms has rapidly increased [1,20]. Atopic dermatitis (AD) commonly affects infants and children [5], more than two thirds of the patient demonstrate the beginning of disease within the first year of life [6]. Atopic dermatitis displays a large variety of clinical manifestations. Classic symptoms include erythematous rash with papules, vesicles on the dry skin base and potentially crusting, scaling and lichenification. There is no absolutely known cause of atopic dermatitis [25,28].

An important role in modulating the activity of biologically active peptides in serum play proteases which release dipeptides from the N-terminus of peptides with proline or alanine in penultimate position [9]. The highly specialized aminopeptidase expressed in a variety of cells in many mammalian species is dipeptidyl peptidase IV (CD26; EC 3.4.14.5) [3,35]. DPPIV plays the key role in modification, processing and/or inactivation of peptides, e.g. peptide hormones, various cytokines, chemokines, neuropeptides and growth factors [8,24]. Some of biologically active peptides derived from food contain the sequences with proline in the N- or C-terminal penultimate position that makes them resistant to the attack by most of proteases, even those with broad specificity [23,24]. One of them is β -casomorphin, an opioid peptide which is an excellent substrates for DPPIV [39].

In this paper we have found the correlation between β -casomorphins content in mothers' milk and the DPPIV activity in the serum of their children. We have compared the results of two groups of breast fed children—the group of newborns with syndromes of atopic dermatitis and healthy control group.

2. Materials and methods

2.1. Material

All exclusively breast-fed infants (to 3–5 months age) were the patients of 3rd Department of Paediatric Diseases, Medical University in Białystok, Poland. The mothers gave written informed consent. Blood samples were obtained as a part of routine diagnostic purpose and being analyzed for enzyme activities. Both milk and serum were obtained at the same time. Twenty-three children were diagnosed as having atopic dermatitis symptoms. The control group included 13 breast-fed healthy infants, without allergy symptoms.

The diagnosis of cow's milk allergy was based on clinical symptoms (according to Clein criteria) and positive history of allergy of mothers of those children. There was no correlation between serum DPPIV and specific IgE against cow's milk proteins (casein, alpha- and beta-lactoglobulins) in infants during follow-up period. All biochemical and morphological blood parameters were in normal physiological range.

2.2. Methods

2.2.1. Assay for DPPIV activity

DPPIV activity was determined with the "direct photometric method" [13] adapted to 96-well plates. The reaction mixture contained 50 μ L of 0.3 M Gly/NaOH buffer (pH 8.7), 100 μ L of 3 mM Gly-Pro-*p*-nitroanilide *p*-toluenesulfonate (Sigma G2901), 50 μ L of water and 10 μ L of serum. Instead of serum blank and standard wells contained 10 μ L of water and 10 μ L of 3 mM *p*-nitroanilide (Sigma N2128), respectively. Control wells contained no serum. After 30 min of incubation at 37 °C, the reaction was stopped by adding 50 μ L of chilled (4 °C) 1 M acetate buffer (pH 4.2), 10 μ L of serum was added to control wells. Absorbance was measured at 405 nm with microplate reader (ASYS UVM 340). Calculations were made after adjusting measurements with the blank, enzyme activity was calculated as: $100 \times (E - C)/S$ (where E, C and S are absorbance of experimental, control and standard samples, respectively). One unit of enzyme activity was defined as the amount of enzyme liberating 1 μ mol of product (i.e. *p*-nitroanilide) per minute per liter of serum at 37 °C. All experiments were made in triplicate. All results were statistically analyzed with Dixon test and compared with Students' *t*-test.

2.2.2. Isolation of a peptide fraction

Peptides were extracted from milk according to the method of Halwarker and Elliot. Milk was mixed 2:1 (v/v) with a methanol/chloroform mixture (2:1, v/v), left for 1 h on gentle shaking and centrifuged (5000 $\times$ g for 30 min). Peptide rich water/methanol layer was collected, evaporated and lyophilized.

2.2.3. Purification of a peptide extract

Peptide extracts were purified by SPE with the use of C₁₈ columns (STRATA C-18T, 140 Å, 50 μ m, Phenomenex). Peptide extracts were dissolved in 0.1% TFA and applied on columns previously equilibrated by aspiration of methanol and 0.1% TFA. Loaded column was washed with 10% acetonitrile in 0.1% TFA and peptides were eluted with 50% acetonitrile in 0.1% TFA. The eluate was collected, lyophilized, dissolved to a concentration of 10 mg/mL for HPLC analysis.

2.2.4. HPLC of purified peptide extract

Analysis was performed on the Shimadzu SCL-10Avp HPLC system with SPD-M10Avp diode-array detector and Phenomenex Jupiter Proteo (250 mm $\times$ 4.6 mm, 4 μ m, 90 Å) column. Gradient elution was used with 0.1% TFA as phase A and acetonitrile with 0.1% TFA as phase B. Peaks were detected at a wavelength of λ = 280 nm. Qualitative and quantitative determination of β -casomorphins was performed by comparison with the standards.

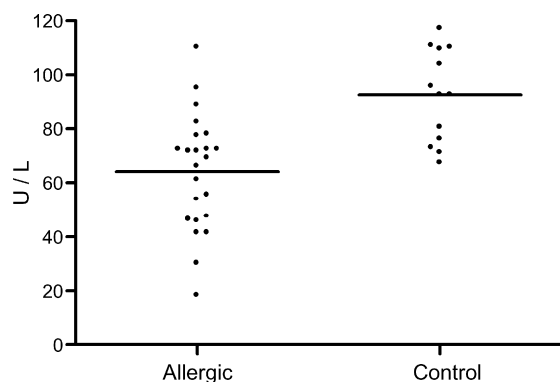


Fig. 1 – Scatter plot of DPPVI activity (horizontal bar indicates mean value).

3. Results

3.1. DPPIV activity in serum

DPPIV activity was found to be significantly higher ($p < 0.001$) in sera from healthy control group (92.6 ± 4.8 U/L; mean $\pm$ S.E.) than in sera from allergic group (64.2 ± 13.2 U/L). Results ranged from 18.3 to 110.7 U/L in sera from allergic group and from 67.5 to 117.3 U/L in sera from control group (Table 1). The mean value in allergic group (64.2 U/L) was lower than the lowest value in control group (67.5) which gives 48% of allergic group results below lower range for control group. On the other hand only 15% of control group results were above the upper range of the allergic group (Fig. 1).

3.2. β -Casomorphins content of mothers' milk

Both casomorphins were positively identified in most of peptide extracts. β -Casomorphin-5 was present in all extracts, whereas β -casomorphin-7 was absent in 25% and 12% of extracts in control and allergic group, respectively. A typical HPLC chromatogram is shown in Fig. 2.

In the mothers' milk of the allergic group, the content of β -casomorphin-5 was observed to be significantly higher than that of β -casomorphin-7 ($p < 0.001$). The content of both β -casomorphins (i.e. -5 and -7) in mothers' milk in the allergic group was found to be significantly lower ($p < 0.005$ and $p < 0.05$, respectively) than in the control group (Fig. 3).

3.3. Correlation between enzyme activity and β -casomorphins content

Statistically significant Pearson correlations were found between enzyme activity in infants' serum and β -casomor-

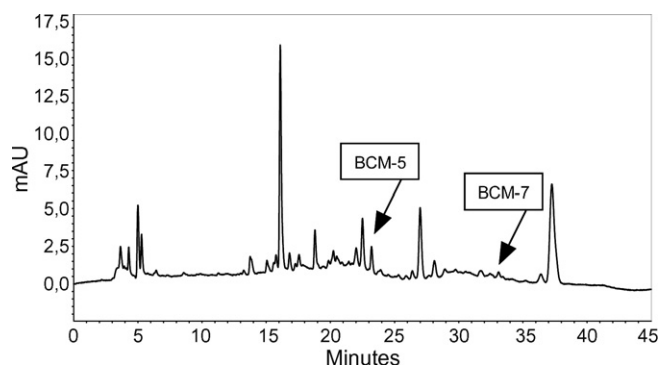


Fig. 2 – Fractionation of peptide extracts from human milk by HPLC. The arrows indicate β -casomorphin-5 (BCM-5) and β -casomorphin-7 (BCM-7).

phins content of their mothers' milk in the allergic group (Fig. 4). Pearson r values were -0.6927 for β -casomorphin-5 ($p < 0.05$) and -0.8335 for β -casomorphin-7 ($p < 0.005$). No statistically significant correlation was found in the control group.

4. Discussion

Our research has shown the negative correlation between DPPIV activity in infant's serum and casomorphin contents of mothers' milk in the AD group. It means that in allergic group the high level of BCM in mother's milk corresponds the low DPPIV activity in infant's sera. We have not found such a dependence in the control group. Moreover DPPIV activity in the AD group was significantly lower in comparison with the control group, 91% of the results in the AD group were lower than an average for the control group (Fig. 1).

Despite lower contents of β -casomorphins in mothers' milk of the AD group it seems possible that β -casomorphins can pass from the intestine to blood and may have prolonged half-life due to a lower DPPIV activity. The β -casomorphins, before they get cleaved, can induce a physiological effect (e.g. pseudoallergic reactions [18,19,32,33]) which can intensify skin symptoms of atopic dermatitis.

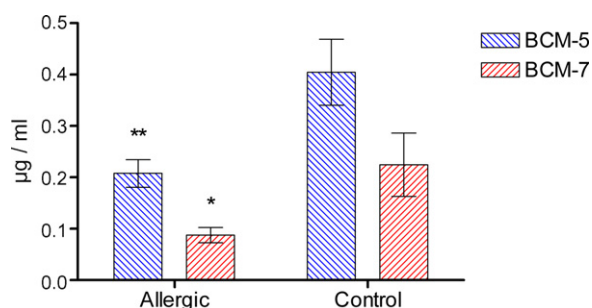
In the control group both casomorphins content in milk and DPPIV activity in infants' sera were higher. Thus the opioid peptides could be cleaved before or after they reached the intestine–blood barrier. Surprisingly we have not found any dependence between the content of β -casomorphins in mothers' milk and DPPIV activity in infant's serum in the control group. At current stage of our research we are not able to explain that observation. The presence of casomorphins in

Table 1 – Activity of DPPIV in infants' sera

Group	No. of subjects	Age (years)	Activity (U/L)	
			Mean $\pm$ S.E.	Range
Allergic	23	<1	64.2 ± 13.2	18.3–110.7
Control (healthy)	13	<1	92.6 ± 4.8	67.5–117.3

Table 2 – Activity of DPPIV in blood samples of infants with symptoms of food allergy and healthy controls in comparison with literature data

Group	No. of subjects	Age (years)	Enzyme activity mean $\pm$ S.D. or S.E. (as indicated)	Note
Allergic	23	<1	64.2 $\pm$ 21.6 (S.D.); 64.2 $\pm$ 4.5 (S.E.)	
Control (healthy)	13	<1	92.6 $\pm$ 17.2 (S.D.); 92.6 $\pm$ 4.8 (S.E.)	
Healthy [13]	88	15–81	54.9 $\pm$ 1.5 (S.E.)	Japanese population
Hepatitis [13]	3	n/a	143.0 $\pm$ n/a (n/a)	–
Gastric cancer [13]	3	n/a	25.3 $\pm$ n/a (n/a)	–
Osteoporosis [10]	30	52–91	70.7 $\pm$ 1.8 (S.E.)	Females (Japanese population)
Healthy [12]	20	n/a	80.3 $\pm$ 17.1 (S.D.)	Females (German population)
Anorexia nervosa [12]	11	n/a	108.4 $\pm$ 28.4 (S.D.)	–
Bulimia [12]	23	n/a	91.1 $\pm$ 18.7 (S.D.)	–

**Fig. 3 – Content of β -casomorphins-5 (blue bars in both groups) and -7 (red bar in both groups) in mothers' milk (mean $\pm$ S.E.). The asterisk labels show statistical significance: $p < 0.05$, $^{**}p < 0.005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)**

human milk has already been shown [2] but establishing physiological values in milk and influence of those peptides on infants' requires further research.

The activity of DPPIV in human serum was found to be a promising diagnostic index in such diseases like hepatitis, gastric cancer, autoimmune diseases, depression and osteoporosis [10,13]. It was also shown that DPPIV activity of small intestine enterocytes is significantly lower in celiac disease comparing with healthy children [31]. Our results show the

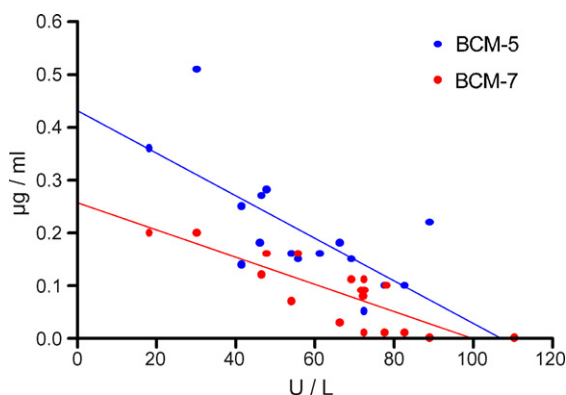
higher activity of infants' serum DPPIV in the control group than in the allergy group. We were unable to locate any publication describing neither normal nor pathological values for DPPIV activity in infants' serum. Most of published data on DPPIV activity in serum concerns adults—healthy and with diseases like hepatitis and gastric cancer [13], anorexia nervosa and bulimia [12] or osteoporosis [10] (data shown in Table 2 in comparison with the obtained results). DPPIV activity in the allergic group (64.2 $\pm$ 4.5 U/L; mean $\pm$ S.E.) was found to be higher than values reported for gastric cancer (25.3 U/L; mean, S.E. or S.D. not available) and for healthy Japanese population (54.9 $\pm$ 1.5 U/L; mean $\pm$ S.E.) but latter difference was not statistically significant. All the other tabulated values (including value for the control group, as described in Section 3.1) were significantly higher than DPPIV activity in the allergic group. DPPVI activity in the control group (92.6 $\pm$ 4.8 U/L; mean $\pm$ S.E.) was found to be significantly lower than values reported for hepatitis (143.6 U/L; mean, S.E. or S.D. not available) and anorexia nervosa (108.4 $\pm$ 28.4 U/L; mean $\pm$ S.D.). All the other tabulated values were lower, of which only difference with the value reported for bulimia [12] was not statistically significant.

5. Conclusions

1. Serum DPPIV activity of infants with atopic dermatitis was significantly lower than in the allergy group.
2. Both casomorphins i.e. -5 and -7 were identified in mothers' milk of both groups and the level of casomorphin-5 was higher than that of casomorphin-7. In the same time the level of both casomorphins in milk of AD group was significantly lower than the control group, which was also accompanied by lower serum DPPIV activity.
3. A correlation was found between serum DPPIV activity and casomorphin contents of mothers' milk in the group of infants with atopic dermatitis. No such a correlation was found in the control group.

REFERENCES

- [1] Arvola T, Moilanen E, Vuento R, Isolauri E. Weaning to hypoallergenic formula improves gut barrier function in breast-fed infants with atopic eczema. *J Pediatric Gastroenterol Nutr* 2004;38(1):92–6.

**Fig. 4 – Correlation (Pearson r) between enzyme activity in infant serum (x-axis) and β -casomorphins content in mothers' milk (y-axis) from allergic group.**

- [2] Assargård U, Larsson C, Nörby U, Lindström L, Nyberg F. Human β -casomorphin-5 containing peptides in human body fluids. In: Brantl V, Teschemacher H, editors. β -Casomorphins and related peptides: recent developments. Weinheim, Germany: VCH; 1994. p. 247–54.
- [3] Boonacker E, Van Noorden CJF. The multifunctional or moonlighting protein CD26/DPPIV. *Eur J Cell Biol* 2003;82:35–73.
- [4] Caporale C, Fontanela A, Petrilli P, Pucci P, Molinaro MF, Picone D, et al. Isolation and characterisation of dipeptidyl peptidase IV from human meconium. Functional role of β -casomorphins. *FEBS* 1985;2:273–7.
- [5] Cheigh NH. Managing a common disorder in children: atopic dermatitis. *J Pediatr Health Care* 2003;17:84–8.
- [6] Christophers E, Fölster-Holst R. Atopic dermatitis versus infantile eczema. *J Am Acad Dermatol* 2001;45:2–3.
- [7] Clare DA, Swaisgood HE. Bioactive milk peptides: a prospectus. *J Dairy Sci* 2000;83:1187–95.
- [8] Cohen M, Fruitier-Arnadin I, Piot JM. Hemorhins: substrates and/or inhibitors of dipeptidyl peptidase IV. Hemorhins N-terminus sequence influence on the interaction between hemorhins and DPPIV. *Biochimie* 2004;86:31–7.
- [9] Durinx Ch, Lambeir AM, Bosmans E, Flamagne JB, Berghmans R, Haemers A, et al. Molecular characterization of dipeptidyl peptidase activity in serum. Soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* 2000;267:5608–13.
- [10] Gotoh H, Hagihara M, Nagatsu T, Iwata H, Miura T. Activity of dipeptidyl peptidase IV and post-proline cleaving enzyme in sera of osteoporotic patients. *Clin Chem* 1988;34(12):2499–501.
- [11] Greenberg R, Groves ML, Dower HJ. Human β -casein. *J Biol Chem* 1984;259:5132–8.
- [12] Hildebrandt M, Rose M, Mayr C, Schüler C, Reutter W, Salama A, et al. Alternations in expression and in serum activity of dipeptidyl peptidase IV (DPPIV, CD26) in patients with hyporetic eating disorders. *Scand J Immunol* 1999;50:536–40.
- [13] Hino M, Fuyamada H, Hayakawa T, Nagatsu T, Oya H, Nakagawa Y, et al. X-prolyl dipeptidyl-aminopeptidase activity, with X-proline *p*-nitroanilides as substrates, in normal and pathological human sera. *Clin Chem* 1976;22:1256–61.
- [14] Kayser H, Meisel H. Stimulation of human peripheral blood lymphocytes by bioactive peptides derived from bovine milk proteins. *FEBS Lett* 1996;383:18–20.
- [15] Koch G, Wiedemann K, Drebes E, Zimmermann W, Link G, Teschemacher H. Human β -casomorphin-8 immunoreactive material in the plasma of women during pregnancy and after delivery. *Regul Pept* 1988;20:107–17.
- [16] Kostyra E, Sienkiewicz-Szłapka E, Jarmołowska B, Krawczuk S, Kostyra H. Opioid peptides derived from milk proteins. *Pol J Food Nutr Sci* 2004;13(54):25–35.
- [17] Kostyra K, Kostyra E. Biologically active peptides derived from food proteins. *Pol J Food Nutr Sci* 1992;1(42):5–17.
- [18] Kurek M, Malaczynska T. Nutritive casein formula elicits pseudoallergic skin reactions by trick testing. *Int Arch Allergy Immunol* 1999;118:228–9.
- [19] Kurek M, Ruëff M, Czerwionka-Szaflarska M, Doroszewska G, Przybiela B. Exorphins derived from cow's milk casein elicit pseudo-allergic wheal-and-flare reactions in healthy children. *Rev fr Allergol* 1996;36(2):191–6.
- [20] Lahov E, Regelson W. Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. *Food Chem Toxicol* 1996;34:131–45.
- [21] Lucas A, Bloom SR, Aynsley-Green A. Gastrointestinal peptides and adaptation to extrauterine nutrition. *Can J Physiol Pharmacol* 1985;63:527–37.
- [22] Meisel H. Overview on milk protein-derived peptides. *Int Dairy J* 1998;8:363–73.
- [23] Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and it responsible for their degradation in human serum. *Eur J Biochem* 1993;214:829–35.
- [24] Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul Pept* 1999;85:2–24.
- [25] Eczema PJ. *Nursing Standard* 2000;14(16):49–55.
- [26] Pihlanto-Leppälä A, Antila P, Mäntsälä P, Hellman J. Opioid peptides produced by in vitro proteolysis of bovine caseins. *Int Dairy J* 1994;4:291–301.
- [27] Ramabadran K, Bansinath M. Pharmacology of β -casomorphins, opioid peptides derived from milk protein. *Asia Pacific J Pharmacol* 1989;4:45–58.
- [28] Saini B. Managing eczema in children. *Pharmacist* 2005;24:360–5.
- [29] Schanbacher FL, Talhouk RS, Murray FA. Biology and origin of bioactive peptides in milk. *Livest Prod Sci* 1997;50:105–23.
- [30] Silva SV, Malcata FX. Casein as source of bioactive peptides. *Int Dairy J* 2005;15:1–15.
- [31] Smith MW, Phillips AD. Abnormal expression of dipeptidylpeptidase IV activity in enterocyte brush-border membranes of children suffering from celiac disease. *Exp Physiol* 1990;75:613–6.
- [32] Stepnik M, Kurek M. The influence of bovine casein-derived exorphins on mast cells in rodents. *Rev fr Allergol* 2002;42:447–53.
- [33] Stepnik M, Kurek M. The influence of casein-derived exorphins on mast cells in rodents. *Rev fr Allergol* 1999;57–9 [Numéro spécial].
- [34] Sun Z, Zhang Z, Wang X, Cade R, Elmir Z, Fregly M. Relation of β -casomorphin to apnea in sudden infant death syndrome. *Peptides* 2003;24:937–43.
- [35] Tanaka T, Kameoka J, Yaron A, Schlossman SF, Morimoto Ch. The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity. *Proc Natl Acad Sci USA* 1993;90:5686–90.
- [36] Teschemacher H, Koch G, Brantl V. Milk protein-derived atypical peptides and related compounds with opioid antagonist activity. In: Brantl V, Teschemacher H, editors. β -Casomorphins and related peptides: recent developments. Weinheim, Germany: VCH; 1994. p. 3–17 [review].
- [37] Teschemacher H, Koch G. Opioids in the milk. *Endocr Regul* 1991;25:147–50.
- [38] Teschemacher H. Opioid receptor ligands derived from food proteins. *Curr Pharmaceut Des* 2003;9:1331–44.
- [39] Tiruppathi Ch, Miyamoto Y, Ganapathy V, Roesel RA, Withford GM, Leibach FH. Hydrolysis and transport of proline-containing peptides in renal brush-border membrane vesicles from dipeptidyl peptidase IV-positive and dipeptidyl peptidase IV-negative rat strains. *J Biol Chem* 1990;265:1476–83.
- [40] Weaver LT. Breast and gut: the relationship between lactating mammary function and neonatal gastrointestinal function. *Proc Nutr Soc* 1992;51:155–63.
- [41] Weaver LT. Development of the gastrointestinal tract: anatomy and embryology. In: Walker WA, Durie JR, Hamilton JR, Walter-Smith JA, Watkins JB, editors. *Pediatric gastrointestinal disease*. Toronto: Decker; 1991. p. 195–216.
- [42] Weaver LT. Significance of bioactive substances in milk to the human neonate. *Livest Prod Sci* 1997;50:139–46.