Peptides xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Presence of adropin, nesfatin-1, apelin-12, ghrelins and salusins peptides in the milk, cheese whey and plasma of dairy cows

Q1 Suleyman Aydin*

Department of Medical Biochemistry and Clinical Biochemistry (Firat Hormones Research Group), Medical School, Firat University, 23119 Elazig, Turkey

ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 11 February 2013
Accepted 11 February 2013
Available online xxx

Keywords:
Cheese whey
Adropin
Apelin-12
Ghrelins
Nesfatin-1
Salusins

ABSTRACT

Biological fluids (milk and serum/plasma) and cheese whey milk-derived fluid contain numerous molecules, especially amino acids and proteins. Therefore, the purpose of this study was to find out whether cheese whey (n:6), cow milk (n:6) and its blood (n=6) have adropin, nesfatin-1, apelin-12, ghrelins and salusin peptides. Adropin, nesfatin-1, apelin-12 concentrations were measured by ELISA, whereas ghrelin and salusin concentrations were measured by EIA methods. It was found that adropin, nesfatin-1, apelin-12, des-acylated ghrelin and salusins in cheese whey were higher than in the corresponding milk peptides and plasma of dairy cows, with the exception of salusin alpha and acylated ghrelin in milk being the same than that of the corresponding cheese whey concentration and plasma of dairy cows. A correlation was also found between milk peptides and cheese whey, as also with plasma of dairy cows. The data suggest that peptides in cow milk might be an important and nutritious food for (neonatal) calves and human diet due to their biological and physiological properties.

1. Introduction

Cheese whey is an important nutritious liquid produced after milk has been curdled and strained; however, it has not efficently been used in human diet. Most of cheese whey is poured away and pollutes the environment. Cheese whey is a rich and balanced source of amino acids, which act as the precursors of peptides with biological and physiological properties [16]. However, cheese made from milk contains a variety of proteins and peptides with clear biological activity [22]. Several hormones have been found in the milk, including hypothalamic, pituitary, pancreatic (insulin), adrenal, thyroid, gonadal, gut hormones, growth factors (e.g. insulin-like growth factor one-IGF-1), epidermal, transforming, nerve growth factors, and pituitary adenylate cyclase activating polypeptide (PACAP), energy balance regulating hormones (leptin, octanoylated ghrelin [OGR], des-octanoyl ghrelin [DGR) nesfatin-1 [NES-1], adiponectin), arterial pressure regulating peptides (apelins), and copeptin, a derived from the same precursor peptide as arginine vasopressin (AVP) [3]. Some peptides included in this study (ghrelin, nesfatin-1, and apelin-12 [APE-12]) have been previously identifed in human milk [4], but not in animal milk, plasma or cheese whey yet. Presence of salusins and adropin (ADR) have not yet been identified in human milk, animal milk, plasma and cheese whey. Besides the above mentioned identifed hormone in human milk and blood (e.g. apelin-2 and ghrelin) [4], some detail will be given here about these unidentifed substances in human milk and animal milk, plasma and cheese whey before dealing with the major purpose of this study.

Salusins are 2 newly discovered peptides derived from the same precursor, preprosalusin, with 28 and 20 amino acids, designated as salusin-alpha (SAL-α) and salusin-beta (SAL-β), respectively [19]. Administration of both peptides to animals causes hypotension and bradycardia [7]. Salusins increase intracellular Ca^{2+} and induce mitogenesis [19]. Both are expressed and synthesized ubiquitously within human, rat, and mouse tissues, including the central nervous system and kidneys [2,23]; they are also present in human plasma and urine [18]. Salusin production in blood vessels occurs in fibroblast cells in the aorta and smooth muscle cells of the media in the left internal mammary artery (LIMA) and saphena [2].

Adropin (ADR) is a recently discovered peptide implicated in the maintenance of energy homeostasis and insulin resistance [11]; it improves hepatic steatosis, and exerts a direct endothelial protective role via upregulating endothelial nitric oxide (NO) synthase expression through the VEGFR2-phosphatidylinositol 3-kinase-Akt and VEGFR2-extracellular signal-regulated kinase 1/2 pathways [12]. Adropin (molecular weight ~4.5 kDa) is expressed in human umbilical vein and coronary artery endothelial cells (ECs) [12]. This peptide is regulated by fasting and dietary macronutrients. The concentration in mice varies between 4 and 60 ng/mL (1–10 nmol/L) [11,12].

* Correspondence address: Department of Medical Biochemistry and Clinical Biochemistry (Firat Hormones Research Group), Firat University, School of Medicine, 23119 Elazig, Turkey. Tel.: +90 5334934643; fax: +90 424 2379138.
E-mail addresses: saydin1@hotmail.com, saydin1@firat.edu.tr

0196-9781/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.peptides.2013.02.014

Although a variety of peptides have been identified in milk, little is known regarding the presence and concentration of peptides in cheese whey. (1) the purpose of this research is to determine whether cheese whey, plasma, and milk from cows have adropin, nesfatin-1, apelin-12, ghrelins and salusins peptides and (2) whether the cheese whey peptides show any correlation with plasma and milk peptide levels.

2. Materials and methods

2.1. Sample collection

Cows were housed with free access to the outdoors during the experiment. The protocol has been approved by the Institutional Ethic Committee. Milk (n:6) and blood samples (n:6) per animal were simultaneously collected in the morning between 8 and 10 am from adult lactating 3–4 year Holstein–Friesian cows during the Summer. 10 mL animal blood was taken from the jugular vein into ice-cold glass tubes bearing EDTA (20 mg), trasylol (1500 U) [20], or tubes bearing EDTA (20 mg), trasylol (1500 U and 20 μl Tween-20) [17]. 10 mL samples of milk were similarly collected at the morning milking of the same animals. Tubes containing trasylol (1500 U and 20 μl Tween-20) were used for salusins analysis, while those without Tween-20 were used for the other hormones. Cheese was made from the morning milking of these same animals [9,15]. Briefly, using the legal pasteurized milk cheese control method in Turkey, it was made at 63–65 °C for 30 min the whole raw milk heated to 72–75 °C for 15–20 s before being allowed to cool to 28–32 °C. After cooling to the fermentation temperature before the addition of yeast [Brand name: Super maya-intermak, Konya, contents: H_2O, NaCl%15, microbial protease (*M. miehei*, 1%), sodium benzoate, force: 1:8000 ± 500 mcu], usually for ~30 min, the acidity of the milk was raised up to a pH 6.3–6.5. Liquid yeast was added for milk coagulation, a teaspoonfull being sufficient for 10 l of milk. The container was closed and wrapped with a clean cloth to keep warm for good curdling to continue. The cheese curds were transferred to clean filter-cloth bags and weighed yielding ~3 kg per 10 l of milk. Produced white cheese was made, with the whey dripping from the filter bag containing it. 10 mL cheese whey samples (n:6) were taken and trasylol (1500 U) or plus trasylol (1500 U, and 20 μl Tween-20) added. Samples containing trasylol were used for ghrelin, adropin, and apelin-12 and nesfatin-1 analysis. After collection, milk, cheese whey and plasma of dairy cows samples were stored at 4 °C during transportation and before centrifugation for 5 min (2000 × g) at 4 °C. The lipid supernatant was removed and the samples stored at −80 °C for further analysis.

2.2. Hormone assays

Plasma, cheese whey and milk peptides levels were measured blindly by a technician using commercially available ELISA kits to detect peptides in the biological fluids and were read with using the ELX 800 ELISA reader. Adropin (EK-032-35) and Apelin-12 (EK-057-15) levels were measured using EIA kits (Phoenix Pharmaceuticals, Inc., CA, USA). Nesfatin levels (EIA) were measured using the Ray-Biotech Inc. made (The Protein Array Pioneer Company) EIA kit. Serum acylated ghrelin levels were measured using the human acyl ghrelin ELISA commercial kit (Cat No. A05106, SPI-BIO, Kit, France). Serum des-acylated ghrelin levels were quantified using unacyled ghrelin ELISA commercial kit (Cat. No: A05119, SPI-BIO, Kit, France). Salusin-α(E91892Hu) and salusin-β measurements were conducted using specific commercial ELISA kits (Uscn Life Science Inc., Wuhan, PR China).

Validation of ELISA assay kits

Levels of adropin, nesfatin-1, apelin-12, ghrelins and salusins in the milk, cheese whey and plasma of dairy cows were measured by a previously published method [4], as given briefly below:

Precision assay:

(a) Intra-assay: the intra-assay (within-day) variation [coefficient of variation (CV)] was determined for each peptide (2 samples, 3 replicates per assay).
(b) Inter-assay: the inter-assay (between-days) variation for 4 days was also determined for 2 different samples for each kit using the means of several duplicates for each. For the intra and inter-assay variations, the CVs were is calculated by CV = standard deviation (SD)/mean.

Recovery test: standard peptides were added in 3 concentrations to 2 plasma, milk and cheese whey samples for assay. The recoveries of percentage were calculated as follows: observed value-baseline value/amount added 100×, with the concentrations being given in ng/mL or pg/mL.

Dilution test: Plasma, milk and cheese whey samples were serially diluted and assayed. Data displayed linearity for each peptides.

2.3. Statistical analysis

Data were analyzed using the SPSS 11.0 for Windows™ statistical software (SPSS Inc., Chicago, IL, USA). Statistical analysis to compare peptides in the plasma, cheese whey and and milk of the same animals used the Anova test. Data are expressed as means ± SD for the plasma, milk and cheese whey measurements. Significant differences were considered significant at $P < 0.05$.

3. Results

The performance characteristics ELISA assays are given Table 1, which verify that adropin, nesfatin-1, apelin-12, ghrelins and salusins have been accurately, reliably and quantitatively detected in milk, cheese whey and plasma, with relatively small inter-individual differences. Correlation between plasma and peptide levels of milk in the study was also reported in Table 2. The concentration of adropin in cheese whey (5.13 ± 0.57 ng/mL) was almost twice that in the plasma of the same animals (3.00 ± 0.43 ng/mL), but a little higher than in the milk (5.00 ± 1.11 ng/mL) (Fig. 1). Plasma nesfatin-1 level (4.02 ± 1.51 ng/mL) were also ~1.4 times lower than in whole milk (5.03 ± 1.43 ng/mL) and cheese whey (5.25 ± 1.13 ng/mL) nesfatin-1 level of the same animals (Fig. 2). However, whole milk and cheese whey concentrations of nesfatin-1 were almost identical (Fig. 2). Comparison of whole milk apelin-12 (4.50 ± 1.01 ng/mL) with cheese whey concentrations (5.03 ± 1.25 ng/mL) showed that the former was a little lower, but ~1.3 times higher than in the corresponding plasma samples (4.37 ± 1.61 ng/mL) (Fig. 3). Ghrelins (Fig. 4) and salusins (Fig. 5) concentration (acylated: 105.95 ± 31.61 pg/mL; desacylated: 680.00 ± 100.28 pg/mL) in the milk, cheese whey (acylated: 112.20 ± 20.23 pg/mL; desacylated: 768.27 ± 104.10 pg/mL) and plasma (acylated: 69.92 ± 12.43; desacylated: 700.67 ± 98.44 pg/mL) showed the same trend as apelin-12 concentrations. Acylated ghrelin concentration was ~7–8 times higher than in des-acylated ghrelin in the milk, cheese whey and plasma. SAL-β concentrations (milk: 3987.67 ± 939.86 pg/mL; whey: 4985.33 ± 1634.53 pg/mL; plasma: 3770.50 ± 944.86 pg/mL) was also ~4–5 times greater than SAL-α (milk: 692.38 ± 74.87 pg/mL; whey: 760.00 ± 109.18 pg/mL; plasma: 663.33 ± 165.33 pg/mL) in the all the biological fluids tested (Fig. 5).

Please cite this article in press as: Aydin S. Presence of adropin, nesfatin-1, apelin-12, ghrelins and salusins peptides in the milk, cheese whey and plasma of dairy cows. Peptides (2013), http://dx.doi.org/10.1016/j.peptides.2013.02.014