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Is caseinomacropeptide from milk proteins, an inhibitor of gastric secretion?

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ABSTRACT

The aim of this work was to study, *in vivo*, the effect of the ingestion of not glycosylated caseinomacropeptide (CMP) on gastric secretion. In Experiments #1 and #2, 7 calves fitted with a gastric pouch received either a diet without CMP (C diet) or C diet in which CMP was introduced (equal to and 5 folds that of CMP quantity contained in cow milk, diets CMP1 and CMP5, respectively). In Experiment #3, 2 calves (with gastric pouch) were fed C diet followed by an "iv perfusion" of CMP. In Experiment #4, 25 calves fed either C, CMP1 or CMP5 diets were fitted with a blood catheter for sample collections. The quantities of daily gastric secretions seemed few modified by CMP ingestion but the profile of these secretions was changed along the day. The most important result is that CMP can inhibit gastric secretions (mainly hydrochloric acid) stimulated by the meal, but there was no dose-dependent response. No similar observations were obtained after perfusion of CMP in jugular vein. CMP was not detected in blood. Results obtained in our experiments are not in favor of its significant intestinal absorption. Gastrin, somatostatin and VIP could be implicated in the mechanisms of regulation.

1. Introduction

The protein fraction of milk contains many valuable components and biologically active substances. Moreover, milk proteins are precursors of many various biologically active peptides which are inactive within the sequence of the precursor protein but can be released by enzymatic proteolysis during gastrointestinal (GI) digestion or food processing. In this context, from the beginning of the 1980s, many authors have described peptides isolated from caseins which have a biological activity [1].

Among these biologically active molecules, phosphorylated peptides or phosphopeptides (PP) form a particular group that could be active on digestive absorption. Phosphopeptides are known to exert an effect on calcium metabolism but also on other minerals in animal and human species [2–9]. Among PP group, a peptide produced *in vivo*, the caseinomacropeptide (CMP) has received a particular attention concerning its bioactive action on gastrointestinal (GIT) functions [10]. Thus, it was suggested that CMP participates to the defense in the gut (ability to bind cholera toxin and *E. coli* enterotoxins, inhibition of bacterial and viral adhesion), to the development of favorable microflora in the gut (bifidobacterial) and to the modulation of immune system responses [10,11]. Caseinomacropeptide could act on the regulation of digestive secretions from pancreas [12–14] and stomach [15–19] as well as on gut regulatory peptide (GRP) production as demonstrated for cholecystokinin (CCK) [20] and gastrin [19]. But there are few data concerning gastric secretions and they were obtained in different species following intra venous (iv), parenteral or intestinal perfusion of CMP and most often the results are contradictory. To our knowledge, no study reports data obtained in this scientific field, after CMP ingestion in entire and vigil animal or human.

When a liquid diet is offered to a young calf, the meal is ingested during 2 to 3 min and by-pass the rumen to enter directly the abomasum via the reticular groove. The rumen does not work and the digestion processes are similar to those observed in monogastric animals fed milk since abomasum corresponds to the chemical stomach of the GIT in monogastric species. Naturally, the calf fed milk or milk substitute based on skim-milk proteins, produces pepsin and chymosin in stomach [21,22] which cleave rapidly the Phe_{105}–Ala_{106} bond of κ-casein and this single splitting is responsible for milk clotting in the abomasum, resulting in CMP release. The CMP primary structure is relatively well preserved in the different species and CMP contains all the post-translational modifications of κ-casein (phosphorylation and glycosylation) and the mutations of the genetic variants. Thereby, CMP is a very

Abbreviations: BPP, bovine pancreatic polypeptide; BW, body weight; CCK, cholecystokinin; CMP, caseinomacropeptide; DM, dry matter; GI, gastrointestinal; GIT, gastrointestinal tract; GMP, glycosylated CMP; GRP, gut regulatory peptides; iv, intra venous; PP, phosphopeptides; RIA, radioimmunoassay; VIP, vasointestinal polypeptide.

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heterogeneous fraction including glycosylated molecules (GMP) which were isolated [23] and shown to have a specific action on GIT functions [12]. Moreover, the presence of intact CMP in the jejunum of human after ingestion of diet containing casein, indicates that CMP resists hydrolysis by pancreas enzymes in the GIT [24] whereas *in vitro*, CMP is hydrolysed in 5 min by trypsin and chymotrypsin [25].

We have used this interesting mammal model fitted with a gastric (or abomasal) pouch, to study *in vivo* the effect of the ingestion of this biological peptide (CMP) incorporated into the meal, on gastric secretion. The aim of this work was also to elucidate the possible mechanisms implicated, particularly by the way of GRPs. Finally, since it was hypothesized that CMP could be absorbed across the intestinal wall we have measured the gastric secretion after iv perfusion of this molecule.

2. Materials and methods

2.1. Animal preparation and diet

Treatments and experiments were carried out according to the regulation of the European Community regarding the experimental animals' protection. Experiments were carried out on 32 Holstein-Friesian calves aged from 58 to 167 days (from 62 to 157 kg body weight (BW)), coming from the cow flock of the UMR-PL (INRA-Saint Gilles) or from the Coopérative des Agriculteurs de Bretagne. Until experimentation, they were fed a milk-substitute diet based on spray-dried skim-milk powder and tallow which contained 250 g protein and 190 g fat/kg of dry matter (DM).

Seven calves were fitted with an abomasal (gastric) innervated pouch prepared from the fundic area on the lesser curvature [26], this part being the most representative of the whole abomasum for digestive enzymes [27]. The gastric juice was permanently collected via a catheter inserted in the gastric pouch. Twenty-five other animals were fitted with a disposal catheter introduced into an external jugular vein to collect blood samples.

During experimentation, all the animals received a milk substitute in which the proteins (about 25% of DM) were exclusively provided by a CMP-free whey protein concentrate coming from hydrochloric casein preparation. This diet was used as Control diet (C diet). The CMP was prepared from cow milk caseins [28]. Briefly, κ-casein was solubilized at pH 6.7–7.0 thanks to the addition of NaOH or KOH and treated with rennet (which contains mainly chymosin) continuously added during acidification at pH 5.0. The protein was hydrolysed into para-κ-casein (residues 1–105) which remains in the curd and CMP (residues 106–169) which is soluble in the whey (which contained exclusively CMP). Then, the curd was separated from the whey, by decantation or centrifugation and CMP was extracted by ultrafiltration. The analysis of the CMP preparation [13] showed that the two genetic variants non-glycosylated, A and B (6783 and 6750 Da respectively), were mainly present and the quantities of glycasylated forms were negligible. Moreover, the preparation was slightly contaminated by the 1–23 fragment of α-1 casein and no other proteic contaminants were present.

Caseinomacropeptide was introduced into the C diet as a partial lactose substitute, at 2 different concentrations, i.e. equal to and 5 folds that of CMP in cow milk (diets CMP1 and CMP5, respectively) (Table 1). Each calf received each experimental diet in different orders. The calves were fed twice daily at 08:30 h and 16:00 h. The DM intake was 44–55 g/kg $BW^{0.75}$ per day and the DM concentration increased with age, from 130 to 170 g/kg milk substitute. The transition between the diet given until experimentation and experimental diets was progressive during 2 weeks.

2.2. Experimental procedure

Four experiments were carried out (Table 2). In Experiment 1 (effects of CMP ingestion on daily gastric secretions) and in Experiment 2 (effects of CMP ingestion on the kinetic of gastric secretions along the day), seven calves fitted with an abomasal pouch were used. Total quantities of gastric juice secreted in 24 h-period were collected and a representative sample was conserved for electrolyte or enzyme assays until analysis. Moreover, in Experiment 2, the quantities of gastric juice secreted during each day of experiment, were collected during 6 periods corresponding to 0–1, 1–2, 2–3, 3–7, 7–23 and 23–24 h after the morning meal; the last period was chosen as the basal period.

In Experiment 3 (effects of iv perfusion of CMP on the kinetic of gastric secretions), 2 calves were used among the 7 animals precedently used. There were fed C diet followed by an "iv perfusion" of either 20 ml of distilled water (control treatment, C) or 200 mg of CMP diluted in 20 ml of distilled water (CMP treatment, CMP), during 10 min, just after morning meal ingestion. Distilled water was used rather than saline solution since, in other studies we have not observed effects or differences between the two vehicles for the same parameters measured. For evening meal, the calves were only fed C diet for the two treatments. The gastric juice was collected following the same experimental design used in Experiment 2. But, the 1st hour before the morning meal was chosen as the basal period (thus, it was possible to perform one repetition each 2 days with the same animal).

Table 1
Chemical composition of the diets (% of dry matter).

Diet	Control (C)	CMP1	CMP5
–Proteins (N × 6.38)	25.00	25.90	29.30
–Fat	15.30	15.30	15.30
–Free N extract	52.90	51.90	48.20
–Ashes	6.80	6.90	7.20
–P	0.53	0.53	0.55
–Ca	0.67	0.68	0.70
–K	1.15	1.16	1.17
–Na	0.34	0.36	0.44
–Mg	0.09	0.09	0.10
–Peptide CMP (a)	0	1.06	5.32

(a) CMP is added (as a mixture with lactose) in the milk substitute, just before meal ingestion by the calves, in replacement of a part of lactose.

Table 2
Experiments, animals, and number of days of collection per animal, per treatment and/or per experiment.

Experiment N°	Calves[a]	Parameter studied	Treatment				
			Ingestion (diet)			Ingestion: C diet	
			C	CMP1	CMP5	Intra venous perfusion	
						C[b]	CMP[b]
1	7-GP	Juice	83	45	38	–	–
		Enzymes	54	23	24	–	–
		Electrolytes	5	5	4	–	–
2	3-GP[c]	Juice	9	7	8	–	–
		Enzymes	5	3	4	–	–
		Electrolytes	5	4	4	–	–
3	2-GP[c] + BC	Juice	–	–	–	6	6
		Enzymes	–	–	–	3	3
		Electrolytes	–	–	–	3	3
4	24-BC	Plasma gut regulatory peptides	17	12	10	–	–

[a] Number of calves used with the chirurgical preparations: gastric pouch (GP) and/or jugular blood catheter (BC);
[b] "Intra veinous perfusion" of either 20 ml of distillated water (Control treatment, C) or 200 mg of CMP diluted in 20 ml of distillated water (CMP treatment, CMP), during 10 min, just after morning meal ingestion;
[c] Among the 7-GP calves used for Experiment 1.