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GENERATION OF A XENOPSIN IMMUNOREACTIVE PEPTIDE BY PEPSINIZATION OF BOVINE MILK

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ABSTRACT

This study was carried out to examine the possible generation of a Xenopsin immunoreactive peptide by pepsinisation of bovine milk. Bovine milk, trypsinised and pepsinised bovine milk, were all assayed for Xenopsin immunoreactivity (XP-IR). XP-IR was present only in pepsinised bovine milk Analysis of pepsinised bovine milk by gel permeation chromatography and high performance liquid chromatography, both resolved a single peak of immunoreactivity with identical chromatographic characteristic to synthetic Xenopsin. These data indicate the generation of a Xenopsin immunoreactive peptide by the action of pepsin on a putative precursor(s), present in bovine milk. The acidic environment of the gastric lumen provides the suitable conditions required for this process. It is possible that this peptide, thus generated, might play a part in the switching off of the gastric induced gastric acid release which occurs in the stomach and also in the release of antral gastrin and pancreatic peptides, glucagon, insulin and pancreatic polypeptide. These two mechanisms are of great significance in the infant whose sole diet consists of milk.

Key Words: Xenopsin,pepsinisation, bovine milk.

INTRODUCTION

The existence of opioid peptides has been reported in partial enzymatic digests of proteins derived from foodstuff (Brantl et al, 1979, Loukas et al, 1983: Zioudrou, et al, 1979). These peptides are called exorphins because of their exogenous origin and morphine-like activit(Gerdes, et al, 2000). Food proteins are therefore potential precursors of bioactive peptides, which are hidden in an inactive state inside the polypeptide chain (FitzGerald and Meisel, 2000., Groziak and Miller, 2000., Meisel and FitzGerald, 2000., Nurminen, 2000). Substances interacting with opioid receptors have been found in the brain (Gintzler et al, 1976) and blood (Pert et al, 1976). The possibility that such opioids can pass from blood to milk brought about the search for these components in milk and its products. The action of several proteolytic enzymes in the digestive tract could give rise to free peptides. Milk protein is also a rich source of biologically active peptides e.g. casomorphins and caseinophosphopeptides (Meisel, 1988) and others (Frank-Peterside, 1999). Such opioid peptides have been discovered in enzymatic digests of whole bovine casein and were designated B-casomorphins because their sequences identified them as fragments of bovine B-casein (Schusdzarra et al, 1983). This group of casomorphins are high in proline content and this confers protection against proteolysis. Therefore, their concentration can rise to a level of physiological significance in the gut. Generation of other peptides from large molecular weight proteins by pepsin-like enzymes has also been reported. One such peptide is Xenopsin. Xenopsin is a biologically active octapeptide originally discovered in skin extracts of the African Amphibian, *Xenopus laeavis* (Araki et al, 1987) Araki et al, 1975). Since then the presence of Xenopsin immunoreactivity has been reported in the brain and GIT of many amphibians (Carraway et al, 1982) and gastric juice from patients with duodenal ulcers (Shaw, et al, 1987b). Generation of Xenopsin-related peptides during acid extraction has been reported in gastric tissues of mammalian and avain origin (Caraway and Feurle, 1985). When injected into the pancreaticoduodenal artery, Xenopsin was shown to release gastrin, insulin and glucagon (Kawanishi, et al, 1973) and to inhibit tetra-gastrin stimulated gastric acid secretion (Zinner, et al, 1982). The aim of this study is to examine the possible generation of Xenopsin-immunoreactivity by pepsinisation of bovine milk.

MATERIALS AND METHODS

Materials

Pepsin and trypsin were purchased from Sigma Chemical Company. Fresh bovine milk was obtained from a local diary farm, Spring Vale

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Enzymatic degradation of bovine milk

To determine the effects of pepsin and trypsin on fresh bovine milk, 10ml of fresh bovine milk was measured into each of two beakers. In one of thetest tubes the sample pH was brought to 2.02 using glacial acetic acid, and the other sample was maintained at pH 6.8. In the former, was added 0.1g of pepsin and into the other 0.1g of trypsin. The samples were incubated at 37 C for 24hrs, extracted with 20ml of acidified ethanol and assayed for Xenopsin immunoureactivity.

Xenopsin Radioimmunoassay

Xenopsin immunoreactivity was measured using anti-serum GXP 5 raised in a guinea pig to synthetic Xenopsin. The anti-serum was used at a dilution of 1:120,000. The anti-serum showed <0.01% cossreactivity with neurotensin (Shaw et al, 1987b). All samples were assayed in duplicate and all assays were carried out in assay buffer. Assay buffer is 400ml of Buffer A (buffer A is 23g disoduim hydrogen orthophosphate and 5.97g of sodium dihydrogen orthophosphate dissolved in 2litres of distilled water + 1g of thiomersalate made up to 5litres with distilled water and shaken, pH=4.7) to which 0.8ml of sodium chloride and 0.2g of Bovine serum albumen were added. Total assay volume was 300ul.

Gel permeation chromatography

One (1) ml of pepsinised bovine milk was dried with a stream of air and reconstituted with 2ml of Buffer A. The pep4ic digest was chromatographed on a column (90x1.6cm) of Sephadex G-50 (fine) equilibrated with 2M acetic acid. The column was eluted at 11.2m1hr^{-1} and fractions of 2.8ml were collected. Five hundred (500) ml of each fraction was lyophilized and reconstituted with 100ml of essay buffer for measurement of Xenopsin immunoreactivity.

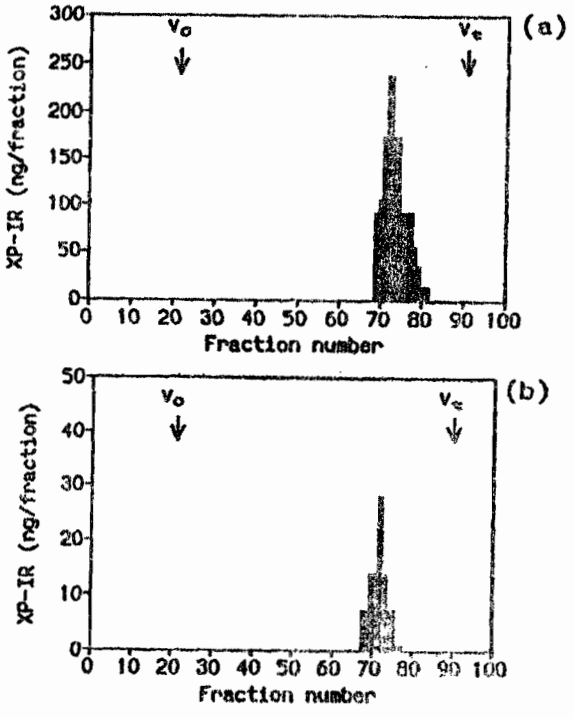

FIGURE 1: Gel permeation chromatogram (Sephadex-G50) of synthetic Xenopsin (a) and Xenopsin immunoreactivity generated by pepsinisation of bovine milk (b). Arrows indicate column void volume (V_o) and total volume (V_t). XP was applied to the column after fractionation of the milk extract.

Elution volume of Blue dextran (Vo), potassium dichromate (V_t) and synthetic Xenopsin were also determined

High performance liquid chromatography

Fractions 68-75 from the gel permeation chromatography were pooled and injected onto a partisil 10, 005-3(60 x1cm) column equilibrated with 0.1% (v/v) trifluoroacetic acid. The column was eluted at a flow rate of 3ml min using a linear gradient of 0-70%. Seventy (70) fractions were collected. 500 of each fraction was dried, reconstituted with 100 assay buffer and assayed for Xenopsin immunoreactivity. The retention time of synthetic Xenopsin was also determined.

RESULTS

Xenopsin Radioimmunoassay

XP-IR was absent in bovine whole milk and trypsinised milk but was present in pepsinised milk.

Gel permeation chromatography

Gel permeation chromatography of pepsinised bovine milk resolved a single peak of XP-IR, which co-eluted with synthetic Xenopsin, fraction 72 (Figure 1).

High performance liquid chromatography

Reverse phase HPLC analysis of gel permeation chromatographic fractions of pepsinised bovine milk, resolved a single peak of XP-IR co-eluting with synthetic Xenopsin, with a retention time of 44min (Figure 2).

DISCUSSION

This study has demonstrated the generation of a Xenopsin immunoreactive peptide by pepsinisation of bovine milk. Whole milk and trypsinised milk were also assayed for XP-IR but were negative. This generation of a XP-IR peptide from pepsinised milk is similar to the generation of Xenopsin-related peptides during

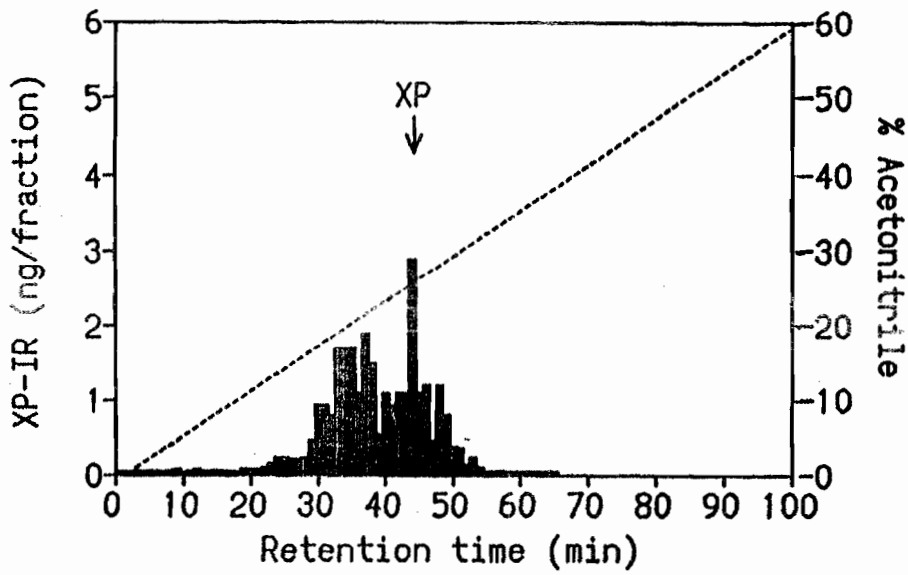

FIGURE 2: RPHPLC elution profile of Xenopsin immunoreactivity generated by pepsinisation of bovine milk. XP indicates elution position of synthetic Xenopsin. XP standard was applied to column after factionation of milk XP-IR.

acid extraction of gastric tissues as reported by Carraway and Feurle, (1985). In that study it was reported that the generation of this peptide involved the action of a pepsin related enzyme(s) on tissue substrate of Mr.>70,000. The absence of XP-IR in bovine milk is a further indication that this peptide is most likely present as part of an inactive part of a larger molecule with a pepsin sensitive bond. The physiological significance of the generation of Xenopsin from gastrin protein(s) is not clear. Synthetic Xenopsin given intravenously to dogs potently inhibit gastrin-driven gastric acid secretion (Zinner et al, 1982,) stimulates exocrine pancreatic secretion (Feurle et al, 1982), and stimulated the release of pancreatic polypeptides insulin and glucagon (Zinner, et al, 1982). Xenopsin has also been shown to release insulin and glucose (Kawanisho, et al, 1973). The significance of this generation mechanism in bovine milk could be important in the newborn where it might be necessary to switch off the gastrin stimulated gastric acid release and in the increase in the level of peptides which occur after feeding (Lucas et al, 1980b: Lucas et al, 1987, Frank-Peterside, 1999).

These increases in gut peptide levels could be the triggering mechanism for the physiological and structural changes that occur after birth in the GIT of the newborn. These changes are necessary for the adjustment in the infant, in changing from intravenous nutrition via the placenta to intermittent enteral feeding. Such peptides, which have been reported to increase after milk feeding in infants, include pancreatic polypeptide, insulin enteroglucagon, motilin and plasmagastrin (Lucas et al, 1980b: Lucas et al, 1980a). Gastrin and enteroglucagon being of particular interest in the neonatal period since in addition to their other functions, they are proven trophic factors for the GIT e.g the trophic effect of gastrin on the fundic mucosa was shown by increases in deoxyribonucleic and ribonucleic acid synthesis (Johnson, 1976; Johnson, 1977). The increase in peptide levels after milk consumption was attributed to the presence of a bombesin immunoreactiv peptide in milk (Jahnke and Lazarus, 1984). Bombesin has been reported to increase the secretion of gut peptides upon infusion. A Xenopsin/pepsin system could be another factor in this peptide release mechanism upon milk ingestion in newborns. If pepsin is the enzyme involved in the generation of immunoreactive XP, this reaction might be possible within the acidic environment of the gastric lumen. The highest concentration of XP-IR in the amphibian is reported to be in the stomach (Carraway et al, 1982). In this case, immunoreactive Xenopsin could be functioning as a signal within the lumen, a lumone.

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