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HIGH LEVELS OF VASOACTIVE INTESTINAL PEPTIDE IN HUMAN MILK

Haim Werner, Yitzhak Koch, Mati Fridkin*,
Jan Fahrenkrug** and Illana Gozes

Departments of Hormone Research, *Organic Chemistry,
The Weizmann Institute of Science, Rehovot 76100, Israel
**Department of Clinical Chemistry, Bispebjerg Hospital,
DK-24000 Copenhagen NV, Denmark

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SUMMARY. The presence of immunoreactive vasoactive intestinal peptide (VIP) in human milk has been demonstrated by high performance liquid chromatography and a specific radioimmunoassay. Immunoreactive VIP-like peptide co-eluted with the synthetic marker on a reversed phase C_{18} column. The levels of the neuropeptide ranged between 67 and 161 pg VIP/ml milk.

The vasoactive intestinal peptide, originally isolated from porcine intestinal tissue by Said and Mutt (1), has been shown to be widely distributed through the central and peripheral nervous systems, the gastrointestinal tract and the pancreas (2). In addition to its vasodilatory activity, several other biological effects are attributed to this 28-amino acid peptide: VIP was shown to act on the gastrointestinal, respiratory, cardiovascular and endocrine systems, as well as on central and peripheral nervous structures (for a review see 3).

Hormones, neuropeptides and growth factors are natural constituents of milk of various mammals (4,5). We and others have reported that immunoreactive and bioactive materials resembling luteinizing hormone-releasing hormone (LHRH) and thyrotropin-releasing hormone (TRH) are present in the milk of man, cow and rat (6-8), and recently we have demonstrated the presence of somatostatin-like material in human and sheep milk (9). It was found that the concentrations of these peptides in milk are significantly higher than the corresponding hormone levels in plasma.

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We now report the presence of immunoreactive VIP in human milk. The peptide was comparatively characterized, along with the synthetic octacosapeptide, using high-performance liquid chromatography (HPLC) and a specific radioimmunoassay (RIA).

METHODS

Preparation of milk extract: Milk was collected 2-3 days post-partum from 4 healthy donors and immediately frozen at -20°C. Individual milk samples were processed as described (9). Briefly, milk samples were defatted by centrifugation, acidified to pH 4 and then lyophilized. The residual powders were extracted with 2 N acetic acid in methanol for 24 h, filtered through Whatman No. 1 filter paper, and the filtrates evaporated under reduced pressure. The residues were reconstituted in distilled water (3 ml), clarified by centrifugation and purified by HPLC.

HPLC purification of milk VIP: Chromatography was performed using a reversed-phase C_{18} column (Merck, Darmstadt, 250x4.6 mm, particle size 10 um). The column was equilibrated with a solution of 0.1% Trifluoroacetic acid (TFA) and the milk extracts were eluted using a linear gradient (from 0% to 80%) of acetonitrile in 0.1% TFA over 60 min. Fractions of 2 ml were collected, evaporated in a spin-vacuum concentrator, and the residues reconstituted in 1 ml solution containing 10 mM phosphate buffer, pH 7.4, 0.9% NaCl, 0.4% bovine serum albumin and 0.05 M EDTA, and assayed for their VIP content.

Radioimmunoassay of VIP: The rabbit anti-VIP serum (No. 5006-9) was characterized (10). The synthetic VIP was purchased from Peninsula Lab., Belmont, CA. It was iodinated employing the chloramine T method (11). RIA was performed as previously described (12).

RESULTS AND DISCUSSION

The acid/methanol extraction procedure has been shown to be a very efficient method for the isolation of peptides from tissues and biological fluids (6). In order to avoid false positive results due to carry over of peptides from one HPLC run to another, the column was extensively washed with 80% acetonitrile/0.1% TFA and then with methanol. Before the purification of the milk extracts, a blank injection was performed under the same experimental gradient conditions. The total elution profile of this blank injection was assayed for the presence of immunoreactive VIP, and was found to be free of any contamination.

Table 1. Immunoreactive vasoactive intestinal peptide in four samples of human milk

Sample	Volume of milk sample (ml)	VIP content (pg)	VIP concentration (pg/ml milk)
1	18	2511	139
2	37	2490	67
3	43	6560	152
4	13	2092	161

After initial purification on HPLC, the fractions (2 ml) were evaporated, reconstituted in 1 ml buffer, and tested for the presence of VIP by using a specific RIA system.

The levels of VIP detected in four samples of human milk were established by evaluating the VIP content of the HPLC fractions and were found to range between 67 pg/ml and 161 pg/ml (Table 1). The elution profile of one sample of milk (No. 2) is presented in Fig. 1A. Fractions 21 to 29 were pooled, evaporated and repurified by HPLC under the same conditions. RIA of the second HPLC run shows that the peak of immunoreactive VIP from the first run was sharpened to some extent and it coelutes with the synthetic VIP marker (Fig. 1B). Two additional immunoreactive peaks were eluted at higher concentrations of acetonitrile and may represent partial degradation products of the VIP. The synthetic marker (15 ug) was applied to the column only after purification of the milk extracts. The marker was eluted at fraction 23 with a recovery of 90%.

The level of immunoreactive VIP reported with this assay in extracted plasma from normal subjects is below 20 pmol/l or 66 pg/ml (10). These values are several fold lower than the concentrations of VIP that were detected in milk. Higher concentrations of neuropeptides in milk as compared to plasma have previously been reported

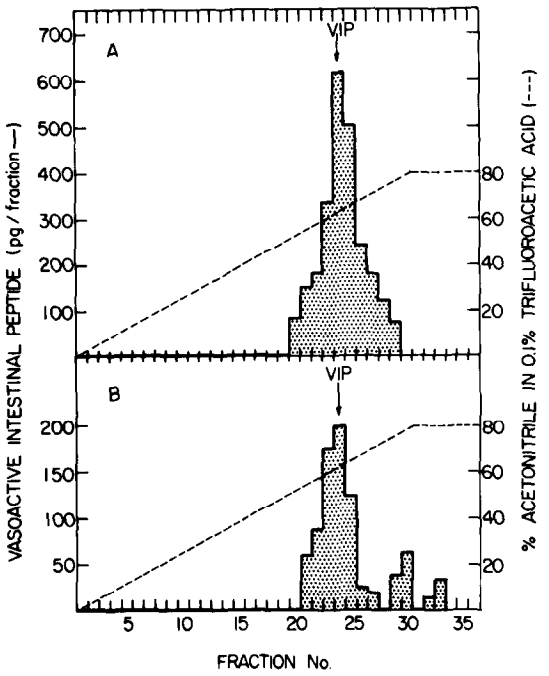

Figure 1. Reversed-phase C_{18} HPLC purification of milk VIP. (A) An equivalent of 37 ml milk (sample No. 2) was injected onto a RP-C_{18} column and eluted by increasing linearly the concentration of acetonitrile (dashed line). Fractions (2 ml) were assayed for their VIP content by RIA. (B) The remainder of the immunoreactive fractions (21 to 29) were pooled and repurified on HPLC at the same conditions. The arrow indicates the elution position of synthetic VIP. Flow rate was 1 ml/min.

for LHRH (6-8), for TRH (6,8), for somatostatin (9), and for bombesin, neurotensin and pro-γ-melanotropin (13). It is not yet clear whether milk VIP is concentrated from the plasma at the mammary gland by an active concentration mechanism, or whether this organ is an additional source for the synthesis and secretion of the peptide.

It is tempting to speculate about the possible physiological role of VIP in the neonate. At least for the neuropeptide somatostatin, which is also transmitted from mother to progeny via the milk, we have been able to demonstrate that the milk peptide can be absorbed by the gastrointestinal tract of the suckling rat in an intact molecular form and transferred via the blood to various tissues, where it is apparently able to interact with specific receptors (14).

Likewise, we suggest that the high levels of VIP present in milk may have some physiological regulatory function in the suckling newborn, most probably at the digestive system level.

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