 Plasma cholecystokinin concentrations after breast feeding in healthy 4 day old infants

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Abstract
The aim of the present study was to characterise plasma concentrations of cholecystokinin (CCK) after breast feeding in newborn infants. Fifty eight healthy full term exclusively breast fed infants were investigated at 4 (1) (2-6) days of age. Each infant contributed one blood sample collected just before, immediately after, or 10, 30, and 60 minutes after breast feeding. Plasma concentrations of CCK were measured with a technique consisting of high pressure liquid chromatography separation of gastrins and CCKs and consequent analysis with radioimmunoassay. Mean (SD) preprandial plasma concentrations of CCK (CCK8+CCK-33,39) were 68 (17) pmol/l. A significant increase was seen immediately after breast feeding, which was followed by a decline at 10 minutes and a secondary rise was seen at 30 and 60 minutes. The first peak is likely to be due to a suckling related activation of the vagal nerve and the second to a stimulatory effect of food on CCK-producing cells. An inverse relationship between basal concentrations of CCK and age of the infant was found. In rats peripheral injections of CCK reduce food intake and cause postprandial sedation and sleepiness via activation of an afferent vagal mechanism. CCK release in response to breast feeding may therefore in addition to exerting stimulatory effects on digestion and metabolism contribute to relaxation and sleepiness seen after breast feeding. The high CCK concentrations seen in younger infants may help the infant to remain satiated and calm despite receiving very little food during the first days of life.

Subjects and methods
Fifty eight healthy infants, 30 boys and 28 girls, were studied with the approval of the local medical ethics committee and with consent from the parents. They were all born after uncomplicated pregnancies and at term. Their mean (SD) gestational age and birth weight were 40 (1) weeks and 3490 (405) g, respectively. All infants were exclusively breast fed. Blood samples were collected at 4 (1) days of age (range 2-7 days) in connection with breast feeding. The feed took place 3 (1) hours after the preceding feed and was demanded by the infant according to the mother’s evaluation. The infants were allowed to suck to satiety and the feed duration was 27 (10) minutes. The amount of milk ingested, determined by weighing the infants (n=50) before and after a meal, was 50 (20) ml

Each infant contributed one blood sample, which was taken just before breast feeding (n=12), immediately after breast feeding was ended (n=12) or 10, (n=13), 30 (n=9), 60 (n=12) minutes later. Samples of approximately 2 ml venous blood were taken from the back of the hand with an open needle technique in connection with routine metabolic screening. Blood samples were collected into ice chilled plastic tubes containing 10 IU heparin and 500 KIE aprotinin per ml blood and centrifuged at 4°C within 30 minutes of collection. The plasma samples were stored at -20°C until analysed for CCK.

Cholecystokinin (CCK) is produced in endocrine cells of the proximal intestine. Plasma concentrations have been shown to rise in response to feeding in several species including rats, dogs, and man. However, vagal nerve activity may contribute to the feeding related response, as in dogs sham feeding stimulates the release of CCK, and in rats electrical vagal stimulation causes a release of CCK.

We have recently shown that sucking a pacifier in infants causes an increase of plasma concentrations of insulin and a decrease of the intraluminally secreted somatostatin. These results indicate that sucking triggers an activation of the vagal nerves which in turn influence the secretion rate of vagally controlled hormones.

CCK is known to stimulate gall bladder contraction and also pancreatic growth and enzyme secretion. In addition, it has been shown that CCK released from the intestine, via an afferent vagal mechanism, induces satiety and feeding associated sedation and sleepiness.

The aim of the present study was to measure plasma concentrations of CCK during the first days of life and to characterise the response during breast feeding. Samples were collected at different time points during and after a breast meal in order to obtain evidence of separate vagal and food related regulatory effects on CCK release.

*Arch Dis Child* 1993;68:46–8}
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CCK DETERMINATIONS
Blood samples were thawed, centrifuged, and the plasma loaded onto C18 SEP-PAK cartridges for purification. Each cartridge was activated with 10 ml of 100% acetonitrile and then washed with 10 ml of 0.1% acetic acid before plasma samples in 1 ml portions were applied onto the cartridge. After washing with 5 ml of 0.1% acetic acid, the samples were eluted by 6 ml of a mixture containing 50% acetonitrile and 50% of 0.1% acetic acid. The eluted samples were lyophilised and stored before further processing.

HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)
HPLC was used for separation of CCK from gastrin and also for separation of the different molecular forms of CCK (CCK-8, -33,39). Lyophilised samples were dissolved in a mixture of 50% distilled water and 50% of 0.1% trifluoroacetic acid (500 µl). Samples were injected on a TSK ODS-120 T column (4.6 × 250 mm, 5 µm particle size), using an isocratic system of 34% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. With this HPLC system, good separation was obtained between non-sulphated and sulphated gastrin-17 and non-sulphated gastrin-34 and the different molecular forms of CCK, CCK-8 was clearly separated from CCK-33,39 which eluted closely. Fractions containing CCK-8 and CCK-33,39, respectively, were pooled and frozen and then lyophilised before later analysis by radioimmunoassay. The standards used were human gastrin-17 (non-sulphated, sulphated), human gastrin-34 (non-sulphated), CCK-8 (sulphated), from Universal Biological Ltd and porcine CCK-33 and CCK-39 (sulphated) were gifts from Professor V Mutt's laboratory.

RADIOIMMUNOASSAY
The lyophilised samples were reconstituted in the assay buffer (0.02 M veronal buffer) and CCK concentrations were determined by gastrin radioimmunoassay. The antiserum used was 2717, a gift from Professor J Rehfeld, Copenhagen, which reacts with the carboxy terminal pentapeptide common to both gastrin and CCK. This antiserum detects CCK-8 and CCK-33 with the same potency but showed 50% less affinity for CCK-39. CCK-8 was used as standard. The intra-assay and interassay coefficients of variation were 5% and 11%, respectively. The sensitivity of the assay is 5 pmol/l. CCK-8 and CCK-33 in amounts ranging from 0.05 to 0.4 pmol/l were added to 1 ml dog plasma and submitted to SEP-PAK extraction and HPLC, about 65% of the material was recovered. For a more detailed description of the HPLC and radioimmunoassay methods see Lindén and Uvnäs-Moberg.

PRESENTATION OF DATA AND STATISTICAL ANALYSIS
CCK concentrations are given as CCK-33,39 and CCK-8 separately as well as together (total CCK). Data are given as mean (SD).

RESULTS
The preprandial total CCK concentrations (n=12) was 68 (17) pmol/l. (CCK-33,39 and CCK-8 were 29 (11) pmol/l and 39 (10) pmol/l, respectively). The total CCK concentrations as well as CCK-33,39 were significantly raised immediately after the infant had stopped sucking (p<0.01). A secondary rise was observed after 30 minutes (p<0.05 and p<0.01) and 60 minutes later (figure). The postprandial pattern of CCK-8 was similar to that observed for CCK-33,39 but a significant rise was seen only at 60 minutes after breast feeding (p<0.01). The ratio between large (CCK-33,39) and small (CCK-8) CCK molecules was 1:1 before and 3:1 after breast feeding.

CORRELATIONS
Basal CCK concentrations (total as well as CCK-33,39) were inversely correlated to postnatal age (days 3–7): r=-0.5, p=0.05 and r=-0.7, p=0.005.

DISCUSSION
Only a few reports on plasma concentrations of CCK in man have been published, the reason being methodological difficulties. Due to the chemical similarity between gastrin and CCK, most antisera cross react with gastrin and CCK and specific CCK antibodies have not been commercially available. In the present study we solved the problem by running each sample on HPLC thus separating gastrins from CCKs. The concentrations of CCK-8 and CCK-33,39 were then measured separately with radioimmunoassay.

The basal concentrations recorded in newborns in the present study were about 10 times higher than those recorded with specific CCK radioimmunoassays in adults or in 9 month old children. With our method we have also recorded lower concentrations in...
adult subjects than in the newborns.14 Thus, we think that the CCK concentrations really are high in infancy—an assumption that is supported by inverse correlation between CCK concentrations and postnatal age during the first week of life.

The larger forms of CCK, that is CCK-33,39, dominate CCK-8 after stimulation. This difference is not seen in adult women.14 Interestingly, the same preponderance for larger molecular forms is seen in infants also in the case of gastrin.15

From a physiological point of view, the most interesting finding is the biphasic release of CCK in response to breast feeding. This has not been shown earlier in children or in adults.

It is not likely that nutrients are responsible for the immediate postprandial CCK peak as plasma concentrations returned to basal 10 minutes later. We have shown that insulin concentrations increased2 and that the intragastric secretion of somatostatin decreases when infants suck a pacifier.9 As the release of both these hormones is vagally controlled, we concluded that the sucking stimulus triggers an activation of the vagal nerve.6 As the secretion of CCK is also under vagal influence,6 the initial CCK response is more likely to have been caused by vagal mechanisms included by the sucking stimulus than by food itself.

The second more protracted increase of CCK concentrations, however, appearing 30–60 minutes after breast feeding was expected and likely to be a consequence of the presence of milk in the intestine. It is not yet known which factors in milk are responsible for the releasing effect. Fat, however, has been shown to be the most potent CCK releaser in adults.8

A very rapid growth and maturation of the gastrointestinal tract occur postnatally. The high basal and stimulated CCK concentrations may contribute to the postnatal development of the gastrointestinal tract as CCK exerts trophic effects, for example on the pancreas.10

It is well known that a newborn infant becomes not only satiated after feeding but also sedated and sleepy. Indeed, similar effects can be triggered by sucking of a pacifier alone.10,17 In this context it is of interest that the infants often were asleep postprandially and did not react to the venepuncture. It is known from rat experiments that CCK causes satiety and a postprandial behaviour including sedation and sleep if injected intraperitoneally. The effect is mediated viaafferent vagal fibres as it is abolished by vagotomy.11,12 It is therefore possible that the CCK released in the infant during breast feeding may via afferent vagal nerve activation influence central nervous system function in such a way that satiety, sedation, and sleepiness are induced. Other factors such as bombesin and gastric distention may of course also contribute to this effect.

Another interesting finding was that basal CCK concentrations were inversely related to age, that is the younger the infant the higher was its basal CCK concentration. Hypothetically this difference could be related to shorter feed intervals in the younger infants and therefore to persisting postprandial increases of CCK concentrations. However, no relationship between time for last feed and CCK concentrations could be demonstrated. Considering that CCK may as described above induce satiety and postprandial sedation, it is tempting to suggest that infants have high CCK concentrations in the first days of life in order to stay sedated and calm despite receiving very little food from their mothers.

We wish to thank the Swedish Medical Research Council, the First of May Flower Annual Campaign, Stiftelsen Clas Groschinsky Minnesfond, Stiftelsen Samariten, and Ahlén-Stiftelsen for financial assistance.

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Arch Dis Child 1993 68: 46-48
doi: 10.1136/adc.68.1_Spec_No.46

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