Raw soya-bean flour increases cholecystokinin release in man

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1. The aim of the present study was to determine whether oral ingestion of raw soya-bean flour, which contains trypsin inhibitors, alters the release of cholecystokinin (CCK) in man.

2. Eleven healthy volunteers ate two mixed meals: one with raw soya-bean flour and the other with soya-bean flour that had been heat-treated. The two flours inhibited 34 and 3 mg trypsin/g flour respectively.

3. CCK was measured in plasma using a bioassay based on the release of amylase (*EC* 3.2.1.1) from dispersed rat pancreatic acini.

4. The peak CCK response was 16·8 (SE 8·1) pmol/l with raw soya-bean flour but 4·9 (SE 2·8) pmol/l with heat-treated flour ($P < 0·05$).

5. We conclude that ingestion of raw soya-bean flour increases CCK release in man and that heat treatment which reduces the trypsin inhibitor content of the flour also diminishes its CCK-releasing effect.

Cholecystokinin (CCK) is released from the jejunum and stimulates pancreatic enzyme secretion (Baile *et al.* 1986). The release of CCK is inhibited by intraduodenal trypsin providing negative feedback control of enzyme secretion (Ihse *et al.* 1977; Slaff *et al.* 1984; Owyang *et al.* 1986). Soya beans contain trypsin inhibitors (Whitaker & Feeney, 1973; Rackis & Gumbmann, 1981; Wilson, 1981) and increased plasma CCK concentrations have been demonstrated in rats fed on raw soya-bean meal (Adrian *et al.* 1982; Liddle *et al.* 1984). CCK also stimulates pancreatic growth (Folsch *et al.* 1978). Prolonged ingestion of such a diet produces pancreatic hypertrophy (Booth *et al.* 1964), adenomas, and adenocarcinomas (McGuinness *et al.* 1980) and an increased susceptibility to carcinogens (Morgan *et al.* 1977; Levison *et al.* 1979; McGuinness *et al.* 1984) in rats. These effects lead to the suggestion (Levison *et al.* 1979; Adrian *et al.* 1982; Liener, 1986) that the recent massive increase in soya-bean ingestion in the Western world (Wolf & Cowan, 1975) has contributed to the concomitant rise in the incidence of carcinoma of the pancreas (Young *et al.* 1978). However, several lines of evidence appear to provide reassurance. Soya bean trypsin-inhibitor activity is diminished by cooking (Rackis & Gumbmann, 1981; Wolf & Cowan, 1975) and by incubation with human gastric juice (Krogdahl & Holm, 1981). Soya-bean trypsin inhibitors have also been reported to have less effect on human trypsin compared with rat trypsin (Temler *et al.* 1983). We therefore examined the effects of raw and heat-treated soya-bean flour on meal-stimulated CCK release in man.

METHODS

The present study was approved by the Hospital Ethics Committee and eleven normal subjects (24–37 years) took part. Subjects fasted overnight, then ate 5 g soya-bean flour mixed with 40 g apple sauce (Rowntree Mackintosh, Hyde, Cheshire) every 30 min. After the second portion of this mixture they also consumed scrambled egg (two eggs, 50 ml milk, 20 g margarine) and coffee (1·65 g Nescafé (Nestlé, Croydon, Surrey), 25 ml milk, 175 ml water). This protocol was used to provide fresh inhibitor throughout digestion of the meal, because of the time-dependent inactivation of soya-bean trypsin-inhibitor by gastric juice

(Krogdahl & Holm, 1981). Each subject was studied twice, using raw soya-bean flour (Bredsoy; Spillers, Ware, Dorset) on one day and heat-treated soya-bean flour (Trusoy; Spillers) on the other, in random order. The trypsin-inhibitor activity of the two flours was estimated by the method of Kakade *et al.* (1969).

Blood was withdrawn from an arm vein into chilled tubes containing aprotinin (2000 kIU/ml blood) and EDTA (10 nmol/l blood) and plasma was stored at −20°. Plasmas were prepared for bioassay as described by Liddle *et al.* (1984). Briefly, CCK peptides were extracted by passing 6 ml plasma through Waters Sep-pak C18 cartridges (Millipore, Harrow, Middlesex) and eluted in a mixture of 800 μl ethanol, 198 μl water and 2 μl trifluoroacetic acid. The eluates were dried in siliconized tubes under nitrogen at 45°. CCK activity was measured by incubation for 30 min with dispersed rat acini, prepared by collagenase (*EC* 3.4.24.3) digestion, as described by Gardner & Jensen (1985). The amylase (*EC* 3.2.1.1) released from the unknown samples was measured using the Phadebas kit (Pharmacia, Uppsala, Sweden) and compared with the release obtained with CCK8 standards.

The assay has a detection limit of 3 pmol/l and the coefficient of variation of triplicates within the assay was 11%. The coefficient of variation for basal amylase release was 29%, determined on eight different days, and the coefficient of variation for amylase release from acini incubated with CCK8, 30 nmol/l, was 21%, measured on eight different days. In view of this interassay variability all samples from each subject were processed in the same bioassay.

Gastrin was measured by radioimmunoassay using antibody 1611 (Rosenquist & Walsh, 1980) and neurotensin by radioimmunoassay using C-terminal antibody NT58 (Lee *et al.* 1984).

Statistical analysis employed Wilcoxon's matched-pairs test, and $P < 0.05$ was taken to be significant.

RESULTS

The rise in plasma CCK after the meal was greater and more sustained when the meal was given with raw soya-bean flour (Bredsoy) than when the soya-bean flour had been cooked (Trusoy) (Fig. 1). There were no statistically significant differences between the postprandial rises in plasma gastrin (mean peak gastrin rise (pmol/l): 19 (SE 7) with raw flour and 24 (SE 8) with cooked flour) or neurotensin (raw flour 24 (SE 7), cooked flour 30 (SE 10)) on the two study days. Bredsoy and Trusoy inhibited 34 and 3 mg of bovine trypsin/g flour respectively.

DISCUSSION

In the present study normal subjects ingested soya-bean flour which was either raw or had received heat-treatment which destroyed about 90% of trypsin-inhibitor activity. The plasma CCK response to a mixed meal was substantially greater during ingestion of raw flour than with heat-treated flour. Although we cannot be certain, it seems likely that the heat-labile factor responsible was soya-bean trypsin-inhibitor. The magnitude of the CCK response in the presence of heat-treated flour was similar to human postprandial responses reported by others using the same bioassay system (Liddle *et al.* 1985; Owyang *et al.* 1986). The postprandial rise in plasma CCK concentration was followed by a second smaller rise, as recorded by others (Byrnes *et al.* 1981; Himeno *et al.* 1983). We believe this to be the first demonstration of increased CCK release associated with administration of raw soya-bean flour in man. Gastrin and neurotensin release were unaltered, suggesting that there was no generalized change in gut hormone release.

In the present study, raw soya-bean flour had no significant effect on CCK release until the egg meal was also given. This contrasts with the marked effects of soya-bean trypsin-

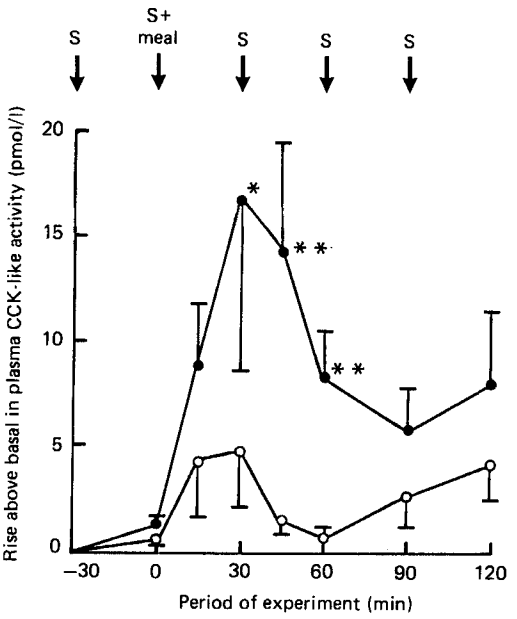

Fig. 1. The effect of eating raw (●) or heat-treated (○) soya-bean flour at the times indicated (S) on plasma cholecystokinin (CCK)-like activity following a scrambled-egg meal. Values are means, with their standard errors represented by vertical bars, for eleven subjects. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

inhibitor and trypsin on basal pancreatic secretion in the rat (Green & Lyman, 1972). It is possible that the buffering capacity of our egg meal preserved the flour from pH-dependent degradation in the stomach. However, there may be a more fundamental difference between responses in rat and man because Owyang *et al.* (1986) found that soya-bean trypsin-inhibitor and trypsin do not affect basal CCK release in man, even when infused directly into the duodenum.

CCK has a number of physiological and pharmacological effects (Baile *et al.* 1986) and raw soya-bean products could be used to increase CCK release for therapeutic purposes. For example, CCK induces satiety resulting in diminished food intake in man (Baile *et al.* 1986) so that diets containing raw soya-bean might be used to stimulate CCK release in obesity. Animals fed on raw soya-bean meal fail to gain weight although impaired digestion of protein may also contribute to this effect (Booth *et al.* 1964; Whitaker & Feeney, 1973; Rackis & Gumbmann, 1981; Struthers *et al.* 1983).

However, before considering such applications there is a need to assess the potential hazards. Chronic ingestion of raw soya-bean meal produces pancreatic hypertrophy in chicks (Garlich & Nesheim, 1966), pigs (Yen *et al.* 1977) and rats (Booth *et al.* 1964), plus benign and malignant tumours (McGuinness *et al.* 1980) and an increased susceptibility to carcinogens (Morgan *et al.* 1977; Levison *et al.* 1979; McGuinness *et al.* 1984) in the rat. These changes in animals have raised the possibility of a link between the recent increases in human soya-bean ingestion and pancreatic cancer in the Western world (Levison *et al.* 1979; Adrian *et al.* 1982; Liener, 1986).

Soya-bean products are indeed widely used in the Western diet. Soya-bean-based sauces and meat substitute are used in many products and soya-bean flour is used in bread and cakes. The soya-bean in these foods is either heat-treated by the manufacturer or cooked later to inactivate most but not all the trypsin-inhibitor present (Liener, 1986). It is reassuring to note that the process used by the manufacturer (Spillers) was sufficient to

diminish CCK release as well as trypsin-inhibitor activity. Some 'health foods' such as soya-bean bran contain raw soya-bean but are not eaten extensively.

At present there is no direct evidence that raw soya-bean, trypsin inhibitors or CCK produce pancreatic hypertrophy or neoplasia in man. Folsch *et al.* (1984) found no change in the maximal pancreatic secretion rate of patients with chronic pancreatitis fed on 30 g raw soya-bean flour three times daily for 6 weeks. However, chronic pancreatitis might limit the organ's capacity to grow so that such a study does not exclude the possibility of a trophic effect on the normal human pancreas.

Trypsin inhibitors are widespread in nature and present in some traditional foods including apple, potato and egg (Whitaker & Feeney, 1973; Rackis & Gumbmann, 1981; Wilson, 1981). They include the Kunitz inhibitor which is inactivated by heat and by gastric juice and the Bowman-Birk inhibitor which survives in both situations (Wolf & Cowan, 1975; Krogdahl & Holm, 1981; Liener, 1986). The former predominates in soya-beans but the Bowman-Birk inhibitor is the major inhibitor present in ground nuts (*Arachis hypogaea*), chick peas (*Cicer arietinum*), lima beans (*Phaseolus lunatus*), adzuki beans (*P. angularis*) and mung beans (*P. aureus*) (Wilson, 1981).

CCK-releasing factors such as those present in raw soya-bean may play a physiological role in meal-stimulated CCK release. They provide some interesting therapeutic possibilities but possible harmful effects of long-term ingestion have not been excluded.

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