Vagally mediated release of gastrin and cholecystokinin following sensory stimulation

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The aim of the present study was to investigate whether gastrin, cholecystokinin (CCK) and somatostatin secretion can be influenced by sensory stimulation and if so, whether such effects are mediated via the vagal nerves. Male rats anaesthetized with chloral hydrate were exposed to three different stimuli, i.e. to low frequency (2 Hz) electrical stimulation of muscles via needles (electro-acupuncture), to thermal stimulation at 40 °C or to vibration at 100 Hz. The two former stimuli activate mainly small and medium sized myelinated fibres from muscles and skin respectively, whereas vibration activates large myelinated fibres from skin, subcutaneous tissue and muscles. Experiments were also performed on animals that were vagotomized or exposed to prior treatment with atropine (0.5 mg kg⁻¹). Blood was collected at various time intervals and plasma levels of gastrin, CCK and somatostatin were measured with radioimmunoassay (RIA).

All three stimuli, i.e. electro-acupuncture, vibration and thermal stimulation caused significant elevations of gastrin (103 ± 11-151 ± 16 pm, 105 ± 8-140 ± 12 pm and 105 ± 14-162 ± 4 pm) and cholecystokinin (9 ± 0.8-15 ± 2.8 pm, 8 ± 0.5-10 ± 1.5 pm and 8.0 ± 0.5-10.5 ± 1.5). Somatostatin was raised in response to electro-acupuncture (10 ± 1-14 ± 3 pm). Vagotomy and atropinization abolished the release of gastrin and CCK in response to all three stimuli. CCK levels were significantly reduced following electro-acupuncture in atropinized rats. In conclusion, gastrin and cholecystokinin release is stimulated by activation of sensory afferent, originating in skin, subcutaneous tissue and muscle. Some of these effects are vagally mediated.

Key words: A-β and A-δ afferent, CCK, electro-acupuncture, gastrin, non-noxious stimulation, somatosensory afferents, somatostatin, thermal stimulation, vibration.

The endocrine system of the gut is activated in response to feeding. Part of this effect is mediated by vagal efferents. Thus, in dogs sham feeding causes a rise of gastrin and CCK (Nilsson 1975, Schafmayer et al. 1988). Electrical vagal stimulation in rats also causes a release of gastrin and CCK and a decrease or increase in somatostatin levels (Lindén et al. 1989, Aliño et al. 1983). Recently, we have been able to show that gastro-intestinal hormones such as gastrin and CCK are released in response to suckling in lactating animals of several species including rats (Lindén et al. 1987, 1990). In rats the maternal CCK release triggered by suckling of the pups is vagally mediated via a central reflex possibly involving oxytocinergic transmission (Lindén et al. 1990). Electrical stimulation of the sciatic nerve induced a release of gastro-intestinal hormones in anaesthetized cats, indicating that activation of somatosensory afferent, not only...
from the teats but also from other parts of the skin, may influence the release of gastro-intestinal hormones (Uvnäs-Moberg et al. 1986). Since thin C-fibre afferents are not likely to be activated by such stimulation, the effect is possibly mediated via thicker fibres, normally activated by various kinds of non-noxious stimuli (Uvnäs-Moberg et al. 1986). It has also been shown that different modes of peripheral stimulation activate spinal cord mechanisms that affect pain and peripheral blood flow (Lundeberg et al. 1988a & b, Kjartansson et al. 1988).

The present study investigates in more detail how sensory stimulation influences the release of gastrin, CCK and somatostatin. Male rats were exposed to three types of treatment, known to set up activity in different types of somatosensory nerve fibres, i.e. vibration at 100 Hz which activates mainly thick myelinated fibres from the skin, subcutaneous tissue and muscles. Mild thermal stimulation at 40°C which activates medium sized A-δ and C-fibers, originating in the skin and electro-acupuncture (2 Hz) which activates thin and medium sized myelinated muscular afferents (Chung et al. 1984a & b, Lu et al. 1986). In order to explore the involvement of the vagal nerves and of cholinergic mechanisms in hormonal effects caused by such stimulations, experiments were also performed on vagotomized and atropinized animals.

METHODS

Experiments were performed on male Sprague Dawley rats (300 g) allowed free access to food and water. The animals were anaesthetized with chloral hydrate (500 mg kg⁻¹, given i.p.). Experiments were routinely performed between 9 and 11 a.m. After the experiment, the animals were killed by decapitation and blood (5 ml) was collected. (The study was approved by the local Ethics Committee.)

Surgical procedures. Vagotomy was carried out under anaesthesia. The major branches of the subdiaphragmatic vagus nerve were identified on or near the oesophagus and the anterior and posterior subdiaphragmatic vagi were dissected free and cut. Sham operations were performed according to the same procedure, but the vagi were not cut.

Stimulations. Vibration stimulation (100 Hz) was carried out for a period of 30 min. An electromechanical vibrator (Somedic AB, Stockholm, Sweden) with a probe area of 9 cm² was applied with the constant pressure (3 kPa) to the thorax and abdomen (Th₂-L₁) of the rat. The vibratory movement (peak to peak 1000 µm) was continuously measured throughout the stimulation period (Lundeberg et al. 1984).

Electro-acupuncture was performed for a period of 30 min. Points chosen were UB11 (close to the shoulder joint) and UB54 (close to the hip joint) bilaterally. Stimulation was brought about by manual rotations of the needles (0.3 mm) after insertion to depths of 0.5–0.7 cm, at the points chosen. Thereafter, the rats received electrical stimulation through all the needles (UB11–UB11, UB54–UB54) that were connected to an acupuncture pulse stimulator (B.V. Enraf-Nonius Delft, Delft, the Netherlands) producing bipolar square wave pulses of 0.2 ms duration, and 2 Hz frequency. The current intensity was adjusted so that localized muscle contractions were seen (2–5 V) (Lundeberg et al. 1988c).

Thermal stimulation was applied for 30 min to the skin of the thorax and abdomen (Th₂–L₁) of the rat using a feedback controlled contact thermode. The thermode had a contact surface of 9 cm², was fixed to a glass chamber with a constant temperature of 40°C and applied with a constant pressure of 3 kPa (Lundeberg & Ottoson 1982).

Experimental design. The stimulation was started 10 min after the induction of anaesthesia and was applied for 30 min. The effect of the various stimulations was tested in two different experimental series. In series I blood samples were collected immediately – 0 min (n = 6) or 60 min (n = 6) – after the stimulation was finished. Control samples (n = 6) were collected from anaesthetized non-stimulated animals at time point 0 min. In the group of animals in which the effect of temperature (40°C) was tested, additional controls (n = 6) were performed in which the animals were exposed to the same thermode at body temperature (37°C) for 30 min. In the electro-acupuncture group and controls, samples were also collected 30 min (n = 6) after the end of the stimulation.

To test the effect of anaesthesia alone samples were collected 40 and 100 min after induction of anaesthesia (n = 5).

In series II, stimuli were applied to control animals as well as atropinized, vagotomized or sham-operated animals. Atropine (0.5 mg kg⁻¹ s.c.) was given immediately after induction of anaesthesia (n = 6). Vagotomy or sham operation was performed 1 week before the experiments. Samples were collected 60 min (n = 6) after the end of stimulation in the different experimental groups and controls. Separate controls were performed for each type of treatment.

Treatment of blood samples. Blood samples were collected in tubes containing heparin (10 IU ml⁻¹) and Trasylol (500 IU ml⁻¹). They were centrifuged, the plasma was removed and frozen at −20°C.

RIA's. Gastrin levels were measured in unextracted plasma and by RIA (Nilsson 1975) using antiserum No. 2604, which recognizes gastrin 17 and 34 with
equimolar potency. The detection limit of the assay was 2 pmol l$^{-1}$ and the intra- and inter-assay coefficients of variation were 10 and 13\% respectively.

CCK levels in plasma were measured after SEP-
PAK$_{18}$ extraction using acetonitrile and acetic acid. The RIA method used has been described by Himeno et al. (1983). The antiserum OAL 656 (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) raised against the N-terminal amino acid residue of sulphated CCK-8 detects CCK-8, CCK-33 and CCK-39 but not gastrin. The intra- and inter-assay variation was 10 and 12\% respectively.

Somatostatin in plasma was measured by RIA after SEP-
PAK$_{18}$ extraction of acidified samples using methanol. The antibody R141E was used with the method described by Efendic et al. (1980). The detection limit of the assay was 2 pmol l$^{-1}$ and the intra- and inter-assay coefficients of variation were 9 and 11\% respectively.

Statistical evaluations. The hormone levels in the figures are presented as mean values ± SD. Comparisons between groups were evaluated with the Kruskal–Wallis one-way analysis of variance (ANOVA) and subsequent two-group comparisons were made with the Mann Whitney U-test. Two-tailed tests were used and P-values < 0.05 were considered significant.

RESULTS

Effect of anaesthesia

Gastrin, CCK and somatostatin levels were not significantly different in samples collected 40 and 100 min after induction of anaesthesia i.e. corresponding to 0 and 60 min after stimulation.

In the five experiments performed, gastrin levels were 115 ± 8 pm and 116 ± 10 pm, CCK 10 ± 4 pm and 12 ± 4 pm and somatostatin 12 ± 4 pm and 14 ± 3 pm.

Effect of vibration

Gastrin and CCK levels were significantly (P < 0.05) elevated when compared to control values 60 min after the stimulation, whereas somatostatin levels were unchanged (Fig. 1).

Effect of atropine and vagotomy

A significant rise (P < 0.05) of gastrin and CCK levels was seen in response to vibration also in this series of experiments. This effect was abolished by vagotomy or following administration of atropine. Somatostatin levels were unchanged in all groups (Table 1). Results obtained from sham operation experiments did not differ from those obtained in unoperated animals and were therefore included in the control group.

**Fig. 1.** Gastrin (a), CCK (b) and somatostatin (c) levels in control rats as well as 0 and 60 min after exposure to vibration at 100 Hz for 30 min.
Effect of thermal stimulation

A clearly significant \((P < 0.001)\) increase of gastrin levels was seen in response to thermal stimulation at \(40^\circ\)C. CCK levels were also raised \((P < 0.05)\) but somatostatin levels were not altered (Fig. 2). No effect was induced by the thermode at body temperature (data not shown).

Effect of atropine and vagotomy

Gastrin levels were elevated \((P < 0.05)\) in response to thermal stimulation – an effect which was abolished by atropine or vagotomy (Table 2). Results obtained in sham-operation experiments did not differ from those obtained in
Table 1. Effect of vibration at 100 Hz on gastrin, CCK and somatostatin levels collected 60 min after end of stimulation in control animals as well as in animals treated with atropine, 0.5 mg kg\(^{-1}\) and vagotomy

<table>
<thead>
<tr>
<th></th>
<th>Gastrin (pmol l(^{-1}))</th>
<th>CCK (pmol l(^{-1}))</th>
<th>Somatostatin (pmol l(^{-1}))</th>
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<tr>
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<tr>
<td>Control</td>
<td>11 104±18</td>
<td>12 8±2</td>
<td>12 11±2</td>
</tr>
<tr>
<td>60 min after stimulation</td>
<td>12 132±25*</td>
<td>12 11±3*</td>
<td>12 9±3</td>
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<tr>
<td>Control – atropine</td>
<td>5 126±21</td>
<td>6 10±3</td>
<td>6 11±3</td>
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<td>60 min after stimulation</td>
<td>5 119±30</td>
<td>6 9±2</td>
<td>6 10±1</td>
</tr>
<tr>
<td>Control – vagotomy</td>
<td>5 190±19</td>
<td>6 7±2</td>
<td>6 11±3</td>
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<tr>
<td>60 min after stimulation</td>
<td>6 186±23</td>
<td>6 7±2</td>
<td>6 10±1</td>
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Table 2. Effect of thermal stimulation at 40 \(^\circ\)C on gastrin, CCK and somatostatin levels collected 60 min after end of stimulation in control animals as well as in animals treated with atropine, 0.5 mg kg\(^{-1}\) and vagotomy

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<thead>
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<th></th>
<th>Gastrin (pmol l(^{-1}))</th>
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<tr>
<td>Control</td>
<td>10 112±22</td>
<td>12 9±2</td>
<td>12 8±2</td>
</tr>
<tr>
<td>60 min after stimulation</td>
<td>11 132±21*</td>
<td>12 10±1</td>
<td>12 10±3</td>
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<tr>
<td>Control – atropine</td>
<td>4 117±29</td>
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<td>5 12±3</td>
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<tr>
<td>60 min after stimulation</td>
<td>6 120±31</td>
<td>6 13±3</td>
<td>6 6±2</td>
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<tr>
<td>Control – vagotomy</td>
<td>6 179±34</td>
<td>6 11±4</td>
<td>6 7±3</td>
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<tr>
<td>60 min after stimulation</td>
<td>6 163±31</td>
<td>6 10±3</td>
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Table 3. Effect of electro-acupuncture at 2 Hz on gastrin, CCK and somatostatin levels collected 60 min after end of stimulation in control animals as well as in animals treated with atropine, 0.5 mg kg\(^{-1}\) and vagotomy

<table>
<thead>
<tr>
<th></th>
<th>Gastrin (pmol l(^{-1}))</th>
<th>CCK (pmol l(^{-1}))</th>
<th>Somatostatin (pmol l(^{-1}))</th>
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<tr>
<td>Control</td>
<td>12 115±23</td>
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<td>12 9±3</td>
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<tr>
<td>60 min after stimulation</td>
<td>11 152±26**</td>
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<td>12 11±1</td>
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<tr>
<td>Control – atropine</td>
<td>8 120±25</td>
<td>6 15±2</td>
<td>8 11±2</td>
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<tr>
<td>60 min after stimulation</td>
<td>6 121±18</td>
<td>6 10±2*</td>
<td>6 10±3</td>
</tr>
<tr>
<td>Control – vagotomy</td>
<td>5 186±32</td>
<td>6 8±1</td>
<td>6 9±2</td>
</tr>
<tr>
<td>60 min after stimulation</td>
<td>5 179±19</td>
<td>6 9±2</td>
<td>5 7±1</td>
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Table 4. Basal levels of gastrin, CCK and somatostatin levels in controls, vagotomized and atropine-treated (0.5 mg kg\(^{-1}\)) animals

<table>
<thead>
<tr>
<th></th>
<th>Gastrin (pmol l(^{-1}))</th>
<th>CCK (pmol l(^{-1}))</th>
<th>Somatostatin (pmol l(^{-1}))</th>
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<td>Basal levels</td>
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<td>(n = 36) 9±2</td>
<td>(n = 36) 9±2</td>
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<tr>
<td>Atropine</td>
<td>(n = 17) 120±25</td>
<td>(n = 18) 13±2**</td>
<td>(n = 19) 11±2</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>(n = 16) 185±34**</td>
<td>(n = 18) 9±2</td>
<td>(n = 18) 9±2</td>
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</table>
unoperated animals and were therefore included in the control group.

**Effect of acupuncture**

CCK levels were significantly elevated 60 (P < 0.01) and 330 (P < 0.05) min after stimulation was ended. Somatostatin levels were significantly elevated at 0 and 330 min (P < 0.05). Gastrin levels were significantly increased (P < 0.05) at 60 min (Fig. 3). The results of control experiments performed at 0 and 330 min did not differ and were therefore combined.

**Effect of atropine and vagotomy**

Gastrin and CCK levels were again influenced by acupuncture (P < 0.01). This effect was abolished by vagotomy and by administration of atropine. In the case of CCK, a significant decrease was seen after atropinization. Somatostatin levels were not influenced (Table 3). Results obtained in sham-operation experiments did not differ from those obtained in unoperated animals and were therefore included in the control group.

**Effect of atropine and vagotomy on basal levels of gastrin, CCK and somatostatin**

When data from the three separate experimental groups were analysed together, atropine (0.5 mg kg⁻¹) caused a significant (P < 0.01) increase in CCK and vagotomy produced an increase of gastrin levels in comparison to untreated animals. Somatostatin levels were not influenced (Table 4).

**DISCUSSION**

The main findings of the present study were that vibration at 100 Hz and thermal stimulation at 40°C as well as electro-acupuncture (2 Hz) significantly increased gastrin and CCK levels, effects which were abolished by vagotomy or atropine pretreatment. The results show that different modes of sensory stimulation may induce a vagally mediated cholinergic release of some gastro-intestinal hormones.

Basal gastrin and CCK levels were high under the present experimental conditions. Both the anaesthesia and the fact that animals were not fasted contributed to the high gastrin and CCK levels. The animals had to be anaesthetized in order to receive the standardized simulations. Furthermore, we chose to work with freely fed animals in which intragastric pH is high due to the buffering effect of food in the stomach to be able to demonstrate increases of gastrin levels. At low antral pH, gastrin release is inhibited and previous attempts to evoke a vagally induced release of gastrin in fasted rats had proved unsuccessful (Lindén et al. 1990).

Gastrin and cholecystokinin secretion is in part under control of the autonomic nervous system. Vagal activation induced electrically or by sham feeding raises the plasma levels of these hormones in dogs, cats and rats (Nilsson 1975, Åliño et al. 1983, Schaafmayer et al. 1988, Lindén et al. 1989). In contrast, activation of the sympathetic-adrenal system inhibits vagally-induced hormone responses. Thus gastrin release caused by electrical vagal stimulation in cats is abolished during concomitant stimulation of the splanchnic nerve (Uvnäs-Wallensten & Järhult 1982). Furthermore, in rats, administration of x₂-adrenergic agonists such as clonidine inhibits the vagally-induced release of somatostatin into the gastric lumen of anaesthetized rats (Uvnäs-Moberg & Åliño 1991). Activation of the unmyelinated C-fibre afferents is related to pain transmission and activation of the sympathetic-adrenal system and consequently to an inhibition of vagally induced effects on the release of GI hormones.

In a previous study performed on anaesthetized cats, the levels of gastrin and some other gastro-intestinal hormones were raised in response to afferent electrical stimulation of the sciatic nerve at low stimulatory intensity (Uvnäs-Moberg et al. 1986). At such stimulation, thick and medium sized afferents, but not C-fibres, are likely to be activated.

In order to further explore the effect of non-noxious somatosensory stimulation on the release of gastro-intestinal hormones, three different kinds of afferent stimulations known to activate specific populations of sensory nerve fibres (i.e. vibration at 100 Hz, thermal stimulation at 40°C and deep muscle stimulation at 2 Hz) were applied. More specifically vibratory stimulation activates all types of low threshold mechanoreceptors in the stimulated area and the activity is transmitted in A-β fibres (Merzenich & Harrington 1969). Thermal stimulation is com-
plex and the types of fibres that are activated depend on the temperature.

Above 43 °C heat receptors are activated and the sensation of heat is conducted in afferent C-fibres. In the present studies in which a thermode of 40 °C was used, A-δ and C-fibres are likely to have been activated (Kenshalo 1970). Finally, electro-acupuncture (i.e. low frequency electrical stimulation of muscles) activates several types of mechanoreceptors within the muscle. Among these are the so-called ergoreceptors. The activity set up in these receptors is transmitted in A-δ fibres (Kniffki et al. 1981).

The present data show that stimulation of sensory afferents from skin and muscle caused a release of gastrin and CCK (and sometimes of somatostatin). Whether the sensory stimuli used really are equipotent as to their hormone-stimulating potential is difficult to say. It seemed as if thermal stimulation was particularly efficient in stimulating gastrin release and electro-acupuncture in CCK release. However, the intensities of the stimulations are difficult to compare and also basal levels which are of importance for the outcome of the stimulations varied somewhat between the experimental groups and series.

The finding that vagotomy abolished the effects on gastrin and CCK release caused by vibration, thermal stimulation and acupuncture indicates that the effects were vagally mediated. It is also possible that it is difficult to further enhance the high basal values of gastrin seen after vagotomy. Pretreatment with atropine also abolished the responses, indicating that the vagal effect of gastrin and CCK secretion is mediated by cholinergic mechanisms.

However, administration of atropine alone also caused a rise of basal CCK levels, indicating the presence of a cholinergic inhibitory tone on CCK release. Indeed, an enhanced secretion of CCK following feeding in rat has been demonstrated to occur following treatment of atropine in rats. This increase was suggested to be a consequence of a reduced secretion of pancreatic juice and hence to lower levels of inhibitors of CCK secretion in the duodenum (Nakano et al. 1990).

Electro-acupuncture caused a significant decrease of CCK levels when they were previously raised by treatment with atropine. This finding indicates that electro-acupuncture may not only increase CCK levels but also in case of elevated basal CCK levels lead to an inhibition of CCK release. By which mechanism this effect is mediated is not known.

Acupuncture stimulation has previously been shown to reduce sympathetic nerve activity and in addition Sato et al. have shown that noxious stimulation of the skin leads to a reflex inhibition of both sympathetic nerve activity and catecholamine secretion (Sato 1987). The present data show that not only is sympathetic transmission inhibited by these kinds of somatosensory stimulation, in addition some parasympathetic vagally mediated effects are induced (Sato & Schmidt 1987).

In lactating rats the maternal levels of gastrin, and particularly of CCK, rise in response to suckling of the pups. This effect is abolished by vagotomy. Since lesions of the afferent nervous pathways within the CNS, leading to oxytocin secretion inhibit suckling related CCK release and since oxytocinergic neurons from the paraventricular nucleus innervate the dorsal vagal motor nucleus, the suckling related release may involve a central oxytocinergic pathway (Linden et al. 1990). Whether central mechanisms are also involved in the gastrin and CCK release caused by vibration, thermal stimulation at 40 °C and electro-acupuncture remains to be established.

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