Tachykinins potently stimulate human small bowel blood flow: a laser Doppler flowmetry study in humans

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**Background:** The two tachykinins substance P and neurokinin A (NKA) are abundantly present in the gastrointestinal tract. Substance P preferring neurokinin 1 (NK1) receptors are mainly found in submucosal blood vessels while neurokinin A preferring neurokinin 2 (NK2) receptors seem to be confined to smooth muscle cells. Tachykinin effects on intestinal mucosal blood flow in humans are not known.

**Aim:** To study the effects of substance P and neurokinin A on small bowel mucosal blood flow in humans.

**Methods:** A manometry tube supplied with single fibre microprobes recorded mucosal blood flow in the proximal small bowel using laser Doppler flowmetry, concomitant with luminal manometry, defining phases I, II, and III of the migrating motor complex. Simultaneously, flowmetry of temporal skin was performed. Under fasting conditions saline was infused intravenously over four hours followed by infusion of substance P, neurokinin A, or saline.

**Results:** During phase I, substance P 1–6 pmol/kg/min increased mucosal blood flow dose dependently by a maximum of 158%. Blood flow of the temporal skin increased in parallel. Neurokinin A 6–50 pmol/kg/min increased mucosal blood flow maximally by 86% at 25 pmol/kg/min while blood flow of temporal skin increased at all doses. Substance P at all doses and neurokinin A at the highest dose only, increased pulse rate. Systolic blood pressure was unchanged by either peptide while substance P at the highest dose decreased diastolic pressure.

**Conclusion:** Tachykinins increase blood flow of the small bowel and temporal skin. With substance P being more potent than neurokinin A, these effects are probably mediated through neurokinin 1 receptors.

**MATERIALS AND METHODS**

Thirty eight healthy volunteers (21 males, 17 females; aged 18–55 years (mean age 28)) participated in the study. Three subjects participated in two different experiments, and two subjects participated in three different experiments, with an interval of at least one month. None reported symptoms or a history of gastrointestinal disease and none was on medication. The experimental protocol was approved by the ethics committee of Karolinska Hospital, and informed consent was obtained from all volunteers.

**Intestinal laser Doppler flowmetry**

A small bowel multichannel polyvinylchloride tube (William Cook Bjaeverskov, Denmark) of length 250 cm and an outer diameter of 4.7 mm was used. The tube has seven channels, each 0.7 mm in width, at different levels. Four side holes, 10 cm apart, were used for motility recordings.

Two single fibre microprobes (PF319: 2L2500), 250 cm in length and 0.5 mm in diameter, were introduced into two channels of the tube and used for intraluminal laser Doppler flowmetry.

**Abbreviations:** MMC, migrating motor complex; NK1, neurokinin 1; NK2, neurokinin 2; NKA, neurokinin A; SP, substance P; LDF, laser Doppler flowmetry; PU, perfusion unit.
Flowmetry (LDF) and connected to Periflux PF3 LDF instruments (Perimed AB, Järfalla, Stockholm, Sweden) working at a wavelength of 632.8 nm. A latex sheet of 8 mm in diameter, located at the angulated tip of each fibre was glued with Histoacryl (Braun Melsungen, Germany) to the manometry tube exactly at the D1 and D2 levels (explained below). Fibres were connected to each PF3 by screw coupling (PF318:2) and a master probe (PF318).

The output signal from each PF3 was linked to a twin channel chart recorder (Servogor; Asea-Brown Boveri, Vienna, Austria) for analogue recordings. Full scale deflection of the chart was 10 V and paper velocity was 3 cm/min. The time constant was set at 0.2 s and the artefact filter was shut off. All LDF values are relative and given in perfusion units (PU); 1 PU corresponds to 10 mV in accordance with the general consensus (European Laser Doppler Users Groups, London 1992). Validity of the LDF recordings required a total backscatter of 3.9–4.7 V of an uninterrupted LDF signal from the intestinal mucosa.7 The tube was passed through a nostril. Fluoroscopy was used to position the side holes in the duodenum with the most distal at the angle of Treitz. The D1 laser probe (spare reading site) was thus positioned in the vertical part of the duodenum and the D2 laser probe (primary reading site) at the angle of Treitz. We have previously evaluated the fidelity of LDF and the D2 laser probe (primary reading site) was thus positioned in the vertical part of the duodenum with the most blood flow measurement.

cycles/min and a typical dicrotic notch indicative of a true blood flow measurement.

Values are expressed as mean (SEM). Effects of NKA or SP on blood flow were calculated as per cent increase from basal level at each dose (fig 1). Infusion of SP and NKA at the same dose, namely 6 pmol/kg/min, SP being more effective. At 25 pmol/kg/min was a significant increase in blood flow seen (86 (14)%, n=6). When comparing the effects of SP and NKA at the same dose, namely 6 pmol/kg/min, SP significantly increased mucosal blood flow (158 (28)%, n=6) while NKA had no significant effect (55 (26)% (fig 1). Hence SP was more efficient than NKA in causing vasodilation (p<0.05) (fig 1). During the measurements, motility remained in phase I of the MMC with less than two contractions exceeding 10 mm Hg during the observation period.

Temporal skin blood flow
Both SP and NKA potently increased skin blood flow at all doses examined (fig 2). SP being more effective. At the comparable dose of 6 pmol/kg/min, SP and NKA increased blood flow (522 (288)% (n=6), 277 (123)% (n=5), respectively) but SP was more efficient than NKA in inducing vasodilation (p<0.05). No clear cut dose-response relationships were found and maximal responses were seen with the doses employed (fig 2).

Arterial blood pressure and pulse rate
No significant effects of SP or NKA on systolic blood pressure were seen (fig 3). Diastolic blood pressure decreased during this sequence of the MMC.
significantly (9.2 (1.5) mm Hg) with SP at 6 pmol/kg/min whereas lower doses had no effect. NKA at all doses had no significant effect on diastolic blood pressure (fig 3).

SP potently increased pulse rate at all doses. At 6 pmol/kg/min, pulse rate increased by 28.5 (5.4) beats/min (n=6). NKA had a significant effect only at a dose of 50 pmol/kg/min (n=6) (fig 3).

There was no correlation between intestinal mucosal blood flow and systolic blood (r²=0.058, p=0.26), diastolic blood pressure (r²=0.058, p=0.26), or pulse rate (r²=0.011, p=0.63).

**DISCUSSION**

Using laser Doppler flowmetry in the small intestine of humans, we found that SP potently increased mucosal blood flow while NKA was less potent. The effects are likely to be mediated by NK1 receptors as this receptor has higher affinity for SP than NKA and SP preferring receptors are expressed in vascular tissue whereas NKA preferring NK2 receptors are not. NK1 receptors generally seem to mediate the vascular effects of tachykinins. SP has thus been found to have more pronounced effects on systemic blood pressure and pulse rate in humans, as we also found.

It has previously been shown that small intestinal blood flow correlates with the MMC so that mucosal blood flow increases when motility is increased. Phase II and particularly phase III of the MMC, where motor activity is highest, is thus accompanied by increased blood flow while phase I (no motor activity) is not. We therefore timed the administration of SP and NKA with the MMC pattern and started infusions of SP or NKA in phase I of the MMC, within 10 minutes of the preceding phase III. The increases in mucosal blood flow in response to SP and NKA infusion seen in this study are therefore not due to concurrent MMC.

Furthermore, NKA has a more potent stimulatory effect than SP on human small bowel motility while the opposite was the case regarding mucosal blood flow, as seen in these experiments. If the increase in blood flow during infusion of SP and NKA was solely secondary to increased motility, NKA should be more potent than SP in stimulating blood flow.

The effects of SP and NKA on mucosal blood flow did not correlate with systemic arterial blood pressure or pulse rate. The effect of SP and NKA on mucosal blood flow does not therefore seem to be secondary (reflex reactions) to effects on the systemic circulation but rather is exerted via specific receptors (that is, NK1 receptors) in the gut.

A structural basis for involvement of tachykinins in the regulation of mucosal blood flow in humans is at hand. Thus SP immunoreactivity is found in nerve fibres in the human intestinal mucosa and nerve cell bodies in the submucosal plexus. Furthermore, immunoreactivity against the NK1 receptor has been found in nerve plexuses of the human intestine and on both the muscular wall of submucosal blood vessels and endothelial cells lining the blood vessels. SP can thus be released from nerve fibres in the intestinal wall to act on NK1 receptors and cause dilatation of blood vessels.
Whether tachykinins actually participate in the physiological regulation of mucosal blood flow in the gastrointestinal tract is still not known. Newby and colleagues studied the vasodilator effect of SP and found that although SP has effects on the systemic circulation, the peptide does not seem to be involved in maintenance of peripheral vascular tone or systemic blood pressure in humans as administration of a NK1 receptor antagonist had no effect on arterial blood pressure or pulse rate.

The same may hold true regarding tachykininergic involvement in the regulation of mucosal blood flow, in the sense that although SP has a potent effect it may not be involved in the physiological regulation of mucosal blood flow. Receptor antagonist studies are needed to clarify this.

In our study, skin blood flow was markedly increased at all doses tested. Tachykinins thus seem to increase skin blood flow more potently than mucosal blood flow of the small intestine. It has previously been shown in humans that the vasodilatory effect of SP on blood flow in the forearm is mediated by NK1 receptors. One could speculate on mechanisms of the more potent effects of tachykinins on skin blood flow compared with intestinal mucosal blood flow. It could be that the tachykinins dilate both arteries and veins in the skin while tachykinins preferentially dilate arteries in the gastrointestinal tract. In line with this assumption, Romero and colleagues found that SP induced dilation of the human hand veins in vivo mediated through NK1 receptors.

Increased knowledge of the effects of tachykinins on mucosal blood flow in the human gastrointestinal tract could be relevant for diseases such as carcinoid tumours and inflammatory bowel disease. Patients with carcinoid syndrome experience episodes of flushing of the skin and also diarrhoea. Tachykinins are known to be released during carcinoid syndrome and administration of SP to healthy individuals results in flushing. The mechanism of diarrhoea in carcinoid syndrome is not known but could involve increased mucosal blood flow and secretion mediated by SP.

In inflammatory bowel disease a 1000-fold increase in the number of NK1 receptors on small bowel blood vessels has been described. SP may therefore have a pathophysiological role in the increased blood flow during gut inflammation and inflammatory bowel disease. In addition, splanchnic venous blood flow has been measured in patients with irritable bowel syndrome. The incremental postprandial portal vein flow response is partly caused by a relationship between fasting flow rate and phase activity of the migrating motor complex. It is also known that the incretin effect in humans is caused by a relationship between fasting flow rate and phase activity of the migrating motor complex.

REFERENCES