

## SMALL INTESTINE

## 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 are abundantly present in the gastrointestinal tract. Substance P preferring neurokinin 1 receptors are mainly found in submucosal blood vessels while neurokinin A preferring neurokinin 2 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.

Tachykinins constitute a family of neuropeptides found in both the central and peripheral nervous system. The tachykinins substance P (SP) and neurokinin A (NKA) are found in nerve cells and nerve fibres throughout the gastrointestinal tract.<sup>1</sup> Tachykinins stimulate motility, secretion, and blood flow. In most animal studies, SP causes dilation of vessels although constriction of vessels has been described.<sup>2</sup> Tachykininergic effects on blood flow seem primarily to be mediated by neurokinin 1 (NK1) receptors. SP is thus more potent than NKA and neurokinin B as a vasodilator of the canine mesenteric circulation and isolated rabbit mesenteric arteries.<sup>3,4</sup>

Localisation of NK1 and neurokinin 2 (NK2) receptors in the human intestine has been studied.<sup>5–7</sup> In the antrum and duodenum, NK1 receptors are expressed in the myenteric and submucosal plexuses and on endothelial cells lining blood vessels.<sup>5</sup> In the ileum and colon, expression of the NK1 receptor is localised to the muscular wall of submucosal blood vessels and also to neurones in the myenteric plexus, muscularis mucosa and propria, crypt epithelium, and immune cells.<sup>6,7</sup> Expression of NK2 receptors is found on the muscularis mucosa, propria, and immune cells. Regarding regulation of blood flow, NK2 receptor expression was not found in nerve plexuses or in relation to blood vessels.<sup>6</sup>

In humans, intravenous infusion of SP and NKA increases pulse rate, with SP being 20 times more potent than NKA.<sup>8</sup> Furthermore, infusion of SP and, in higher concentrations, NKA results in a rise in skin temperature and flushing, indicating increased skin blood flow.<sup>8</sup>

In isolated human mesenteric artery preparations, SP induces dilation<sup>9</sup> but the effects of tachykinins on gastrointestinal blood flow in vivo in humans have previously not been described. We hypothesised that tachykinins could increase mucosal blood flow primarily through NK1 receptors,

and therefore decided to study the effects of intravenous administration of SP and NKA on small bowel mucosal blood flow in humans. We used laser Doppler flowmetry, which is a well established method.<sup>10–12</sup> For comparative reasons, laser Doppler flowmetry of the temporal skin was carried out and effects on arterial blood pressure and pulse rate were monitored.

## 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

**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, Järfälla, Stockholm, Sweden) working at a wavelength of 632.8 nm. A latex sheet, 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.<sup>13</sup>

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 recordings from the gut.<sup>10, 13, 14</sup> In these studies we found a stable LDF signal during phase I of the migrating motor complex (MMC), displaying the expected vasomotion of 3–4 cycles/min and a typical dicrotic notch indicative of a true blood flow measurement.<sup>13</sup> In addition, the recording facility with a linear chart recorder was evaluated using a parallel coupled PF3 directly to a PC for digital recordings employing the Perisoft computer software (Perimed AB).<sup>14</sup> The outcome of these investigations indicated no compromise in the accuracy of the registrations by using a chart recorder.

### Skin laser Doppler flowmetry

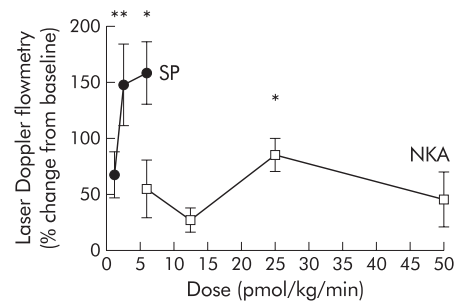
Temporal skin blood flow recordings were carried out using a standard LDF probe (PF408) connected directly to the PF3 (wavelength 632.8 nm) for continuous measurement of perfusion changes during tachykinin challenges. The laser Doppler probe holder for skin measurements was fixed with double adhesive tape. Intestinal (D2 laser probe) and skin laser Doppler readings were performed simultaneously using the same chart recorder.

### Experimental protocol

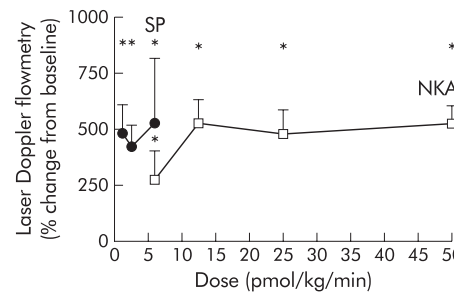
The test subjects were divided into eight groups with 5–7 individuals in each. The control group received saline 0.9% throughout the experiment. Three groups received SP intravenously at doses of 1, 2.5, or 6 pmol/kg/min. Four groups were given NKA intravenously at doses of 6, 12, 25, or 50 pmol/kg/min. Experiments were performed after an overnight fast. Volunteers were in the recumbent position throughout the experiment. Firstly, LDF was studied under control conditions over a period of four hours. Then, immediately after the last MMC, an infusion of either SP or NKA at different doses was started. Measurements of LDF were always carried out during phase I of the MMC within 10 minutes after cessation of phase III of the MMC at the angle of Treitz (D2 laser probe), either under control conditions or during infusion of the tachykinins. LDF readings were then made during steady state conditions and calculated as a mean value over a five minute period. Phase I was defined as part of the MMC with less than two contractions exceeding 10 mm Hg in a 10 minute observation period.<sup>10</sup> We have previously shown small bowel flux measurements to be stable during this sequence of the MMC.<sup>13</sup>

### Statistical evaluation

Values are expressed as mean (SEM). Effects of NKA or SP on blood flow were calculated as per cent increase from basal



**Figure 1** Effects of substance P (SP) and neurokinin A (NKA) on small intestinal blood flow (n=4–7). Values are mean (SEM). Statistically significant difference: \*p<0.05.



**Figure 2** Effects of substance P (SP) and neurokinin A (NKA) on temporal skin blood flow (n=4–7). Values are mean (SEM). Statistically significant difference: \*p<0.05.

flowmetry signal during phase I of the MMC. The statistical significance of effects induced by NKA or SP was evaluated using Wilcoxon's test for paired observations (two sided test). For comparison of the efficiency of the peptides, the Mann-Whitney U test was employed (one sided test). Differences resulting in p values less than 0.05 were considered significant.

## RESULTS

### Intestinal mucosal blood flow

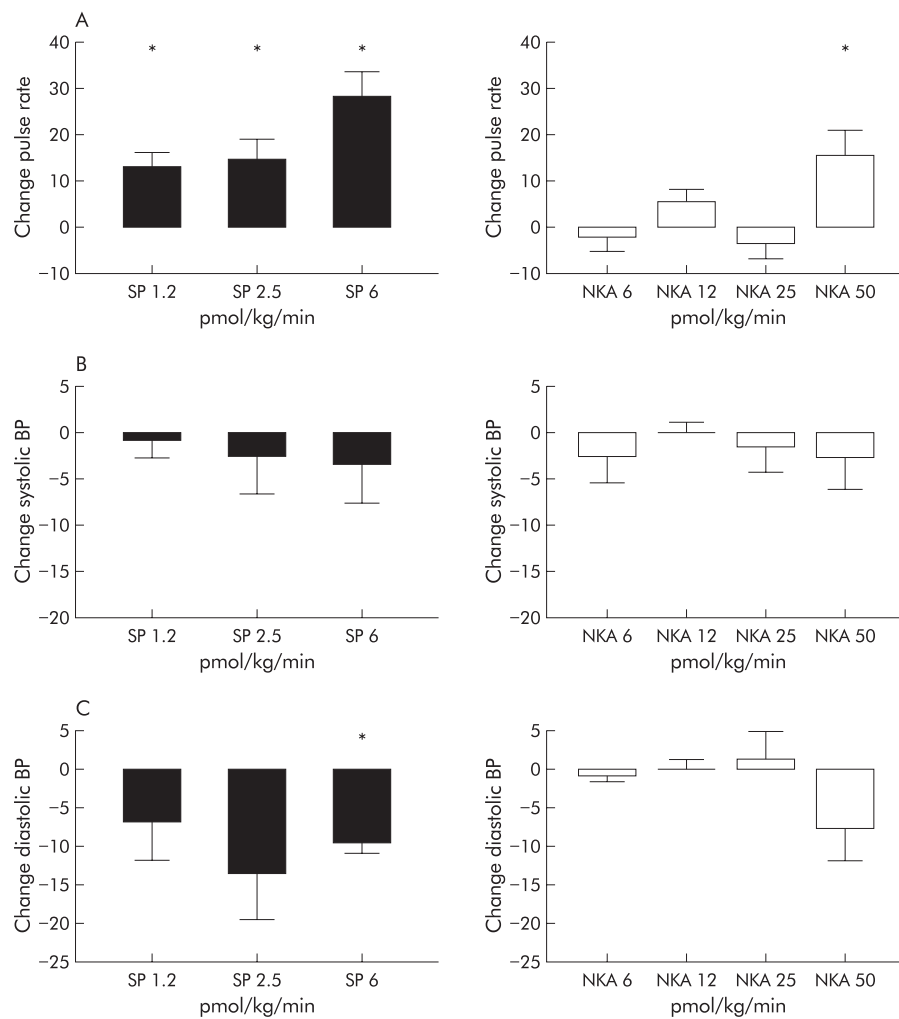
The effect of infusion of SP and NKA on mucosal blood flow is illustrated in fig 1. Infusion of SP at doses of 1.2, 2.5, and 6 pmol/kg/min increased small bowel blood flow dose dependently to a stable level within 10 minutes. NKA 6, 12, 25, and 50 pmol/kg/min achieved a stable but less pronounced effect. Only 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)%, n=5) (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 only 1–2 contractions above 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



**Figure 3** Effects of substance P (SP) and neurokinin A (NKA) on pulse rate (A), systolic blood pressure (B), and diastolic blood pressure (C) (n=4–7). Values are mean (SEM). Statistically significant difference: \*p<0.05.

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 ( $r^2=0.058$ ,  $p=0.26$ ), diastolic blood pressure ( $r^2=0.058$ ,  $p=0.26$ ), or pulse rate ( $r^2=0.011$ ,  $p=0.63$ ).

## 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,<sup>8</sup> 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.<sup>10 15 16</sup> 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<sup>17</sup> 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.<sup>18</sup> 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.<sup>5–7</sup> 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<sup>19</sup> 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.<sup>19</sup>

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.<sup>19</sup> 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, Romerio and colleagues<sup>20</sup> 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.<sup>8, 21</sup> 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.<sup>22, 23</sup> 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 and compared with healthy controls.<sup>24</sup> No difference was found between the two groups. Although neurokinin antagonists may have a therapeutic potential in irritable bowel syndrome, the beneficial effects of such compounds are more likely to be due to effects on smooth muscle layers, intrinsic excitatory neurones, and visceral afferents,<sup>25</sup> rather than on mucosal blood flow.

We conclude that SP and NKA stimulate small bowel blood flow in humans, probably through NK1 receptors. This finding may constitute a basic mechanism for the tissue reactions that occur in inflammatory states of the gut or generalised symptoms after massive release of peptides from neuroendocrine tumours.

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