

# Effects of vasoactive neuropeptides on human saphenous vein

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## Abstract

**Objective**—To assess the role of neuropeptides in the control of vascular tone in the human saphenous vein the actions of substance P, vasoactive intestinal peptide, calcitonin gene related peptide, neuropeptide Y, and somatostatin on this blood vessel were examined.

**Methods**—In vitro organ bath techniques were used with preparations of saphenous veins obtained from 29 patients (aged 41–66) who were undergoing coronary bypass surgery.

**Results**—Substance P, vasoactive intestinal peptide, and calcitonin gene related peptide relaxed pre-constricted vessels in a dose dependent manner with a rapid onset of action, taking one to two minutes to reach a plateau at each dose. Substance P ( $10^{-9}$  to  $10^{-6}$  mol/l) induced relaxation with a maximum response (mean (SEM)) 23.0 (6.6)% of the total relaxation induced by glyceryl trinitrate 1 µg/ml and a 50% maximal effective concentration of  $6.8 \times 10^{-9}$  mol/l. Vasoactive intestinal peptide ( $10^{-10}$  to  $10^{-7}$  mol/l) produced a relaxation of 27.0 (5.1)% at  $10^{-7}$  mol/l. The maximum responses induced by substance P and vasoactive intestinal peptide were significantly reduced, to 3.7 (2.8)% and 4.7 (2.0)% respectively, after removal of the endothelium. Calcitonin gene related peptide ( $10^{-10}$  to  $10^{-7}$  mol/l) elicited only 14.3 (2.6)% relaxation at  $10^{-7}$  mol/l, and this was not affected by removal of the endothelium. By contrast, neuropeptide Y and somatostatin exerted concentration dependent constriction on resting vessels. Neuropeptide Y ( $10^{-10}$  to  $10^{-7}$  mol/l) caused prolonged contraction (roughly 20 minutes to reach a maximum plateau at each dose). At  $10^{-7}$  mol/l, the constriction amounted to 28.0 (12.0)% of the response to 90 mM KCl, in ring segments with or without endothelium. Somatostatin ( $10^{-10}$  to  $10^{-6}$  mol/l) quickly caused contraction with a maximum response of 42.7 (15.0)% and a 50% maximal effective concentration of  $6.7 \times 10^{-6}$  mol/l. The constriction was greatly increased when endothelium was removed, with a maximum response of 78.2 (16.8)% and a 50% maximal effective concentration of  $4.3 \times 10^{-7}$  mol/l.

**Conclusions**—Vasoactive peptides have diverse effects on the vascular tone of the human saphenous vein. Some of

these effects are endothelium dependent. The exact physiological role and implication for performance of bypass grafts require further investigation.

The control of vascular smooth muscle tone may partly be mediated by peptidergic mechanisms. Neuropeptides are ubiquitously distributed in human blood vessels,<sup>1</sup> contributing to vasodilatation (through endothelium dependent or direct action on smooth muscle) and vasoconstriction.<sup>2,3</sup> The endothelium has an important role in controlling vascular tone through the secretion of relaxing and contracting factors derived from the endothelium.<sup>4-6</sup> Several peptides induce significant vasomotor effects in human blood vessels through endothelium dependent and endothelium independent mechanisms.<sup>3,7,8</sup>

The human saphenous vein is of particular interest as it is the vessel most commonly used for aortocoronary artery bypass grafting.<sup>9</sup> Understanding the mechanisms that control vascular tone in this vessel will help the understanding of how the saphenous vein behaves as a bypass graft. We therefore examined the effects of five endogenously occurring peptides on isolated human saphenous veins.

## Methods

The study was performed on 111 ring segments taken from 29 patients (aged 41–66) undergoing coronary artery bypass surgery. Human saphenous veins were placed immediately in modified Tyrode's solution composed of 136.8 mM NaCl, 11.9 mM NaHCO<sub>3</sub>, 2.6 mM KCl, 0.4 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM MgCl<sub>2</sub>, 2.5 mM CaCl<sub>2</sub>, 11.1 mM glucose, and 0.04 mM disodium EDTA. The veins were dissected free of adherent tissues and cut into ring segments 3–5 mm long, which were mounted on two L shaped metal hooks in 5 ml organ baths within one hour. The organ bath solution was maintained at 37°C and gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. One hook was fixed and the other attached to a force displacement transducer (Grass FTO-3C). The transducer was connected to a polygraph (Grass 79D) which monitored isometric changes in the tension of the vessel wall.

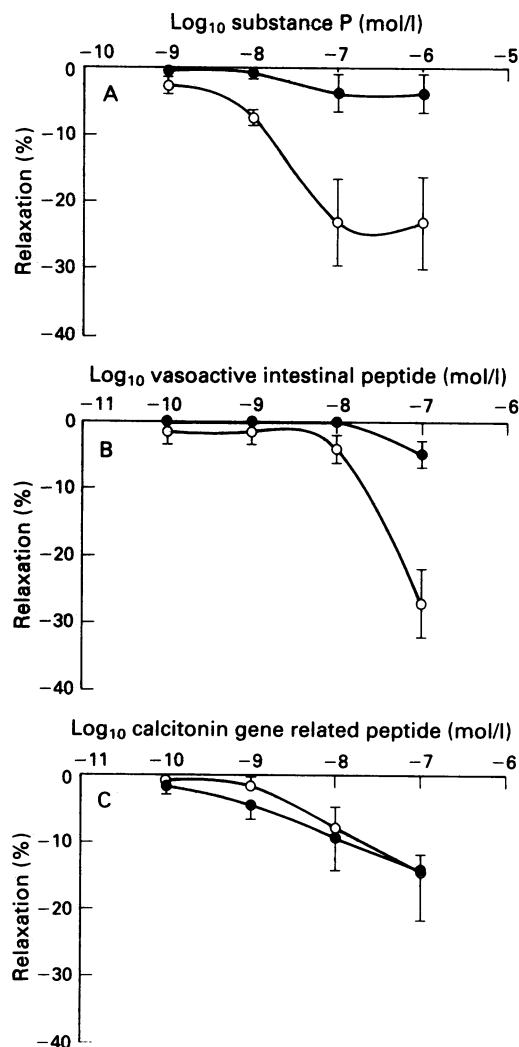
After an initial pre-tension of 60 mN was applied to each vessel the segments were allowed to relax out to a stable baseline. They

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Figure 1 Relaxant effect (measured as percentage of that induced by glyceryl trinitrate 1  $\mu\text{g/ml}$ ) of increasing doses of substance P (A,  $n=7$ ), vasoactive intestinal peptide (B,  $n=4$ ), and calcitonin gene related peptide (C,  $n=6$ ) on the human saphenous vein in the presence ( $\circ$ ) and absence ( $\bullet$ ) of endothelium.



were then challenged twice with 90 mM KCl to assess the viability of each vessel segment. When the maximum response to each dose of KCl had been measured the buffer solution was changed twice and the vessels allowed to return to their resting tension.

Dose-response curves were determined for several different peptides. Each peptide was added to the bath cumulatively in  $\log_{10}$  units until the maximum response of each vessel to each dose of drug had been reached. Each segment was tested with only a single peptide. Some ring segments were pre-constricted with the thromboxane mimetic U46619 to achieve a tension of 60–70% of the contraction seen with KCl before peptides were added.

To assess the endothelium dependent action of peptides some vessel segments were denuded by gently rubbing the intimal surface with a metal spatula. These segments showed absence of endothelium when examined histologically with the specific endothelium marker EN4.

All drugs used (substance P, calcitonin gene related peptide, neuropeptide Y, and somatostatin (Sigma) and vasoactive intestinal peptide (Peninsula)) were reconstituted in distilled water and stored at  $-20^{\circ}\text{C}$ . Aliquots were diluted in modified Tyrode's solution immediately before use.

The contractile effect was expressed as the percentage of the maximum contraction induced by 90 mM KCl, and the relaxation response was calculated as the percentage of the response induced by glyceryl trinitrate (1  $\mu\text{g/ml}$ ). Data are given as means (SEM). In all experiments  $n$  is the number of patients from whom the blood vessels were taken and at least six segments were taken from each patient. The unpaired Student's  $t$  test was used for statistical analysis, and  $p < 0.05$  indicates a statistical difference.

## Results

Initial studies showed that cumulative addition of substance P, vasoactive intestinal peptide, and calcitonin gene related peptide had no effect on resting vessels. Pre-constricted segments, however, relaxed to these peptides in a dose dependent manner, with a rank order of maximum relaxation: substance P = vasoactive intestinal peptide > calcitonin gene related peptide. They all quickly induced relaxation, which lasted 1–2 minutes for each dose. Substance P ( $10^{-9}$  to  $10^{-6}$  mol/l) produced relaxation with a maximum response of 23.0 (6.6)% and a 50% maximal effective concentration of  $6.8 \times 10^{-9}$  mol/l (fig 1A). Vasoactive intestinal peptide ( $10^{-10}$  to  $10^{-7}$  mol/l) induced relaxation up to 27.0 (5.1)% at the highest dose used (fig 1B). The maximal vasodilatations achieved by substance P and vasoactive intestinal peptide were significantly attenuated to 3.7 (2.8)% and 4.7 (2.0)% respectively after removal of the endothelium ( $p < 0.01$ ). Calcitonin gene related peptide ( $10^{-10}$  to  $10^{-7}$  mol/l) caused only 14.3 (2.6)% relaxation at  $10^{-7}$  mol/l, and this was not affected by removal of endothelium (fig 1C).

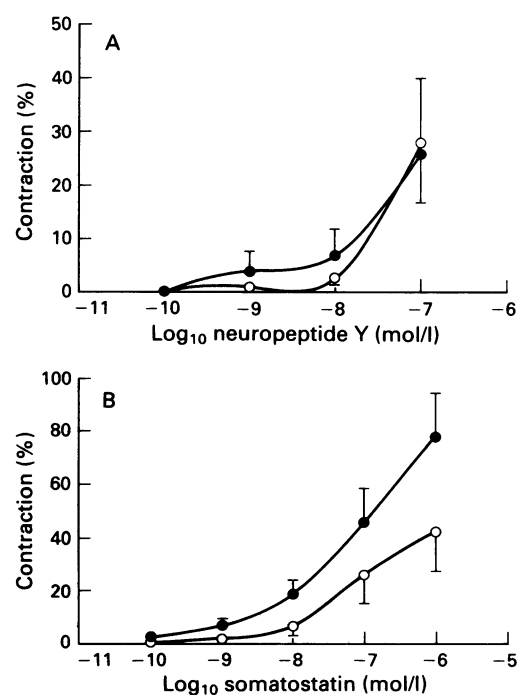


Figure 2 Contractile effect (measured as percentage of maximum concentration induced by 90 mM KCl) of neuropeptide Y (A,  $n=6$ ) and somatostatin (B,  $n=6$ ) on the human saphenous vein in the presence ( $\circ$ ) and absence ( $\bullet$ ) of endothelium.

Glyceryl trinitrate (1  $\mu\text{g/ml}$ ) relaxed pre-constricted blood vessels back to their resting tension but no further. This suggests that glyceryl trinitrate does not reduce resting tension.

Neuropeptide Y and somatostatin caused further constriction of pre-constricted vessels. Cumulative administration of these peptides resulted in contraction of resting vessels that depended on the concentration. Neuropeptide Y ( $10^{-10}$  to  $10^{-7}$  mol/l) induced prolonged contraction (roughly 20 minutes for each dose to reach its maximum plateau), amounting to 28.0 (12.0)% at  $10^{-7}$  mol/l, and this effect was unaltered by removing the endothelium (fig 2A). Somatostatin ( $10^{-10}$  to  $10^{-6}$  mol/l) quickly elicited contraction, each dose taking roughly two minutes to reach its maximum effect. Somatostatin had a maximum response of 42.6 (15)% and a 50% maximal effective concentration of  $6.7 \times 10^{-6}$  mol/l. This constriction was significantly increased when the endothelium was removed, the response being 78.2 (16.8)% and the 50% maximal effective concentration  $4.3 \times 10^{-7}$  mol/l ( $p < 0.01$ ; fig 2B).

### Discussion

Peptidergic mechanisms have been implicated in controlling vascular smooth muscle tone.<sup>10 11</sup> The neuropeptides used in our study (substance P, vasoactive intestinal peptide, calcitonin gene related peptide, neuropeptide Y, and somatostatin) are widely distributed in the body and have vasoactive effects on several human vascular beds.<sup>3 12 13</sup> We have shown that substance P, vasoactive intestinal peptide, and calcitonin gene related peptide are weak vasodilators whereas neuropeptide Y and somatostatin elicit constriction in human saphenous vein.<sup>5</sup>

The mode of action of substance P has been studied in the human coronary and gastroepiploic arteries.<sup>14 15</sup> It induces vasodilatation by interacting with the endothelium to release nitric oxide, which has recently been identified as the substance responsible for the action of endothelium derived relaxing factor.<sup>16</sup> In our studies substance P produced only weak endothelium dependent relaxation, which could be due to a differential density of receptors on the vein compared with other human vascular tissues or the low release of nitric oxide in saphenous veins as previously reported.<sup>17</sup>

Vasoactive intestinal peptide is a 28 amino acid peptide originally isolated from porcine intestine,<sup>18</sup> and subsequently found in the central nervous system and in peripheral nerves supplying many organs.<sup>19</sup> Its main physiological role has been postulated to be that of a local neurotransmitter.<sup>20</sup> It produces endothelium dependent dilatation of the rat aorta,<sup>21 22</sup> rabbit mesenteric artery,<sup>23</sup> and human coeliac artery.<sup>24</sup> By contrast, endothelium independent relaxation due to vasoactive intestinal peptide has been seen in cat cerebral arteries,<sup>25</sup> dog coronary arteries,<sup>26</sup> and human pulmonary arteries.<sup>27</sup> Our study showed endothelium dependent relaxation of isolated

human saphenous vein in response to vasoactive intestinal peptide.

Calcitonin gene related peptide elicits only weak relaxation in the saphenous vein, apparently through a direct action on the vascular smooth muscle. This peptide is claimed to be the most potent endogenous vasodilator in human blood vessels.<sup>28 29</sup> It causes both endothelium dependent and endothelium independent relaxation.<sup>7 30 31</sup> Minor dilatation caused by calcitonin gene related peptide in the saphenous vein might be due to the absence of the endothelium-dependent relaxation mechanism.<sup>7 31</sup>

Neuropeptide Y is a 36 amino acid peptide that is found with noradrenaline in sympathetic neurones.<sup>32</sup> It has a direct vasoconstrictive action and may influence the action of noradrenaline as well as other transmitters in the control of vascular tone in many blood vessels.<sup>33-35</sup> Compared with other reported vasoconstrictors, neuropeptide Y and somatostatin induce only moderate contraction in the saphenous vein.<sup>36</sup> Furthermore, the constriction induced by somatostatin was greatly increased in the absence of endothelium, whereas that of neuropeptide Y was not affected. The difference between the responses to neuropeptide Y and somatostatin after removal of the endothelium may be explained by lack of basal secretion of endothelium derived relaxing factor by the saphenous vein<sup>37</sup> coupled with the ability of somatostatin but not neuropeptide Y to stimulate the release of the relaxing factor.

The concentrations of peptide used in this study are comparable with those used in *in vitro* studies of human mesenteric arteries and veins.<sup>3</sup> We avoided using high concentrations to enable better comparison with the concentrations of peptides circulating in the body to be made. Circulating concentrations of various peptides have been assayed in healthy patients: substance P and calcitonin gene related peptide concentrations have been measured at 1.2 (0.1) and 35.0 (4.0) pmol/l respectively,<sup>38</sup> and plasma vasoactive intestinal peptide concentration at 0.5–21 pmol/l.<sup>39</sup> It is difficult, however, to predict the local concentrations of these peptides at their receptors.

The sensitivity of the saphenous vein to these five neuropeptides differs from that of other human vascular tissues.<sup>37</sup> This further highlights the heterogeneity of the response of human blood vessels to vasoactive compounds. A similar heterogeneity between human arteries and veins has been reported for both endothelial function and the action of vasoconstrictors.<sup>37 40 41</sup> This difference in response could have important physiological and pathological implications.

Neuropeptides have been shown to be localised with neurotransmitters—for example, neuropeptide Y is stored with noradrenaline,<sup>32</sup> and acetylcholine and vasoactive intestinal peptide are present together in parasympathetic nerves.<sup>42</sup> As the saphenous vein is supplied *in situ* with both sympathetic and parasympathetic nerves, neuropeptides may act as neuromodulators<sup>42</sup> and play an integral part in the neuronal control of vascular smooth muscle

tone before the vein is removed during surgery. Harvesting the saphenous vein for bypass grafting results in total denervation of the vessel. Hence the sensitivities of the endothelium and smooth muscle become the determinant factors in the action of neuropeptides when the vein is used as a bypass graft.

Evidence is increasing that saphenous vein grafts cannot be regarded as rigid tubes with little tendency for vasomotion.<sup>43,44</sup> Their dynamic properties could modulate blood flow and hence affect myocardial perfusion. We have characterised the action of certain vasoactive peptides on the isolated human saphenous vein and their possible dependence on endothelium. More studies are required to determine the exact role of neuropeptides in controlling flow in the saphenous vein before and after its use as a bypass graft and their possible effects on long term patency.

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