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Regional Differences in Nitric Oxide-Dependent Vascular Responses to Somatostatin

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Summary

The mechanisms of the vascular effects of somatostatin (ST) are not well known. This study compares the direct effects of ST in different vascular regions and species. Isolated perfused segments of the cat superior mesenteric artery *in vitro* did not exhibit a vascular response in the resting state, however, ST-induced vasodilatation was observed with norepinephrine preconstriction. In contrast, ST only slightly dilated superior mesenteric vein segments. In the artery, N^G-nitro-L-arginine inhibited both ST and endothelium-dependent nitric oxide (NO) mediated response. No regular dose-response curves were found when ST was applied on the large mesenteric artery in the cat, but rings of small mesenteric artery from both cats and dogs exhibited dose-dependent relaxations. These effects were also NO-dependent. Local application of ST on the rat saphenous artery *in situ* elicited NO-mediated dose-dependent vasodilatation. However, ST constricted rat saphenous veins in the case of either adventitial or intraluminal application. It is concluded that ST exerts different actions on the arterial and the venous vessel wall. The major response in arteries is endothelium-mediated vasodilatation seen in various species and vascular beds. Large and small arteries respond differently to ST but these differences require further elucidation.

Key words

Mesenteric vessels – Saphenous vessels – Endothelium mediated vasodilatation – Peptide hormone

Introduction

Somatostatin (ST) is a general inhibitory peptide synthesized in both the central nervous system and the gastrointestinal tract. The effects of ST include inhibition of hormone secretion in the pituitary gland (Krulich *et al.* 1968), the pancreas and the intestines (Schally *et al.* 1978), as well as the reduction of intestinal motility and secretion (Konturek *et al.* 1981).

ST appears as a vasoactive agent, but its physiological role and the mechanisms of the vascular effects of ST are not well understood. In different species and vascular regions, variable vasodilatory and vasoconstrictive effects are described. ST causes vasodilatation in human coronary (Franco-Cereceda and Rudehill 1989) and rat pulmonary arteries (Tjen A Looi *et al.* 1992). It increases blood flow in cat mesenteric (Dézsi and Hamar 1988) and dog gastrointestinal arteries (Männl and Farthmann 1981). ST causes vasoconstriction in rat cerebral arteries (Long *et al.* 1992), and in human saphenous (Luu *et al.* 1992) and mesenteric veins (Törnebrandt *et al.* 1987). It reduces splanchnic blood flow in rats (Kravetz *et al.* 1988) and mesenteric blood flow in dogs (Konturek *et al.* 1981). It is difficult to determine the mechanism of vascular actions of ST on the basis of the literature. Direct effects on the vessel wall, and indirect ones, such as interactions with other vascular mechanisms and with the production of neurohumoral agents, could be involved.

In the present study we examined the direct effects of ST on the blood vessel wall by recording

changes in vascular calibre. Experiments were conducted on vessels from different species (cat, dog, rat), different regions (mesenteric, saphenous) and parts of the vascular tree (large artery, small artery, vein) using *in vitro* and *in situ* techniques. On the arterial side, unlike the venous side, ST was found to be a potent vasodilator. The involvement of endothelial nitric oxide (NO) was established in these vascular reactions.

Methods

In vitro studies

Large arteries and veins. Cats were anesthetized with sodium pentobarbital (40 mg/kg), and following heparin administration rapidly exsanguinated. Through midline laparotomy, the mesenteric root was exposed and the main trunk of the superior mesenteric artery (SMA) and vein (SMV) were carefully removed. One to three cm long segments of the SMA or SMV (mean outer diameter 2.5 mm) were mounted in a tissue bath containing Krebs-Ringer (KR) solution as described earlier (Monos *et al.* 1989). The temperature was kept constant (37 °C) and the superfusion buffer was bubbled with 95 % O_2 and 5 % CO_2. Vascular segments were set to their in vivo length, and the perfusion pressure was maintained at 60 mm Hg. The external diameter was measured by a strain-gauge cantilever transducer. The experimental setup allowed continuous superfusion and perfusion of the segment.

A vascular reactivity test was performed at the start of the experiment and following treatment for endothelium impairment. Norepinephrine (NE; $4x10^{-6}$ mol/l) was added to the superfusing KR solution in order to constrict the vessel, then perfusion was switched to acetylcholine containing KR solution (ACh; $5x10^{-8}$ mol/l) to induce endothelium-dependent dilatation. After the above tests, the vessels were further studied either in the resting state (without preconstriction) or under NE-precontracted control (C/NE) conditions. NE ($4x10^{-6}$ mol/l) was applied in continuous superfusion, while three doses of somatostatin (Curamed Pharma) were added to the perfusing solution in a concentration range from $3x10^{-12}$ to $3x10^{-7}$ mol/l. Endothelium-derived NO was selectively inhibited by 10^{-4} mol/l N^G-nitro-L-arginine (L-NNA) perfused for 20 min. Intraluminal perfusion of agonists was repeated after endothelial treatment.

Small arteries. Cats and dogs were anaesthetized as described above, and a proximal segment of the jejunum was ligated. The mesentery supplying the intestinal segment was carefully removed and stored in heparinized KR solution. Third order branches of the mesenteric artery (small MA) were dissected into rings (2 mm wide, 300 to 500 μm in diameter). Isolated rings were placed in a thermostated organ bath to record isometric tension. They were mounted on stainless steel wires coupled to a micrometer and a strain-gauge transducer. Further details of the organ bath system is described elsewhere (Szabó *et al.* 1992).

Cumulative NE doses (from 10^{-8} to 10^{-6} mol/l) were used to elicit vasoconstriction. To test endothelial function, ACh doses (10^{-7} and 10^{-6} mol/l) were added to the NE-precontracted segments. Furthermore, various ST doses (from 10^{-9} to $5x10^{-8}$ mol/l) were administered. Small MA rings were incubated with L-NNA ($3x10^{-4}$ mol/l) and the addition of ST doses was repeated.

In situ studies

Large arteries and veins. Sprague-Dawley rats were anaesthetized with sodium pentobarbital (50 mg/kg) and the left saphenous artery (SA; mean diameter 550 μm) and vein (SV; mean diameter 820 μm) were carefully exposed over a length of 2 mm, and superfused with warm (37 °C) KR solution. The diameter of the vessels was measured continuously by a computer-guided video-microangiometer. The microangiometer was connected to a stereomicroscope through a video camera. The microscopic picture was observed on a TV-monitor and using data acquisition software the calibrated diameter data were analyzed by a personal computer (for details see Monos *et al.* 1993).

Somatostatin was applied in a wide concentration range on both arteries and veins. In case of SA, only superfusion of ST (from 10^{-12} to 10^{-7} mol/l) was carried out, whereas in the case of SV an additional local intraluminal infusion (from 10^{-9} to 10^{-6} mol/l) took place. To block NO synthesis, L-NNA (10^{-4} mol/l) was infused locally through a small side branch of the vessel for 10 min. ACh ($5x10^{-8}$ mol/l) was applied to test the presence of functional endothelium before and after L-NNA administration. ACh was superfused into SA under resting conditions. However, in case of SV, superfusion of ACh was ineffective, therefore ACh was given in local infusion together with NE superfusion. NE (10^{-5} mol/l) superfusion was necessary to establish a precontraction level in veins in order to achieve ACh-induced relaxation.

Results

In vitro studies

ST in a wide concentration range (from $3x10^{-12}$ to $3x10^{-7}$ mol/l) did not have any significant vasoactive effects on SMA segments in the resting state (n=3, data not shown), i.e. none of the doses elicited further vasodilatation or vasoconstriction without NE-induced tone.

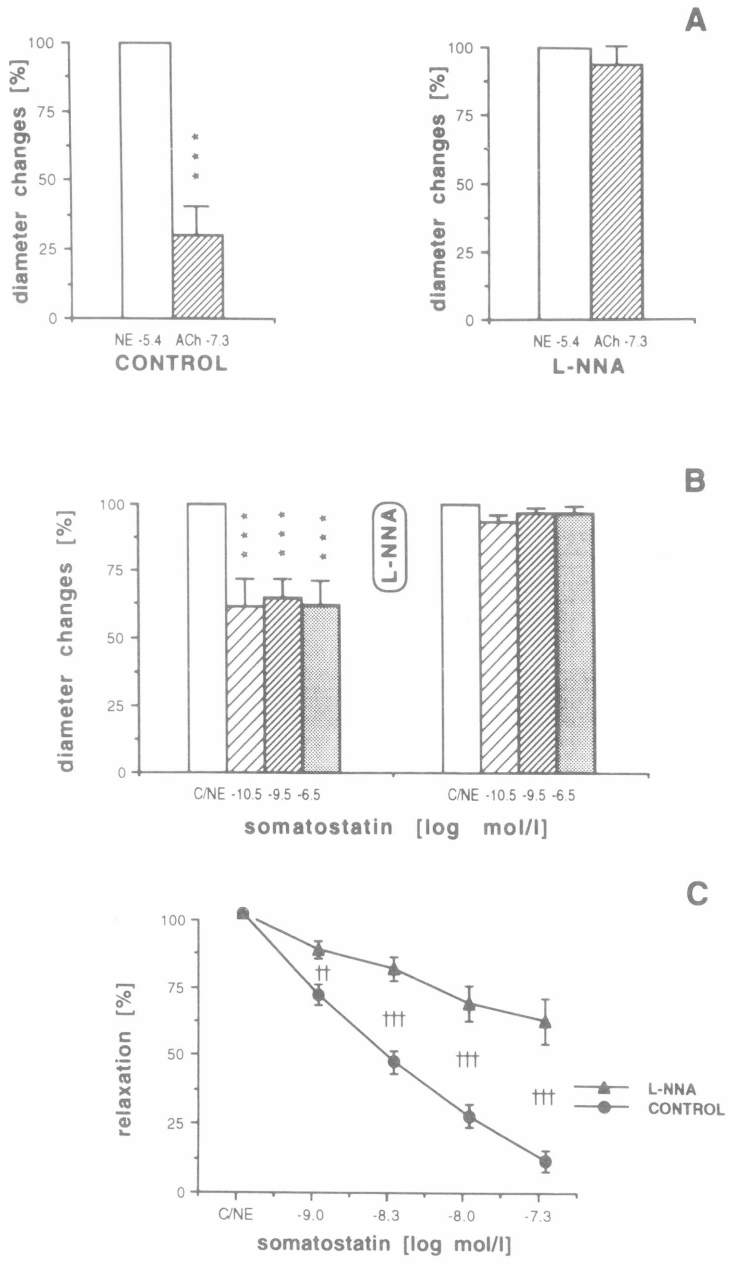

Fig. 1
*Relative diameter changes in response to vasoactive agents in vitro. (A). Percentage changes in diameter of norepinephrine (NE)-preconstricted cat superior mesenteric artery (SMA) segments. Endothelium-dependent acetylcholine (ACh)-induced dilatation in CONTROL conditions and its abolishment by N^G-nitro-L-arginine (L-NNA, 10^{-4} mol/l) treatment. NE and ACh concentrations are given as log mol/l. *** – significant difference vs NE ($p<0.001$). (B) Somatostatin-elicited vasodilatation upon norepinephrine-induced (C/NE) tone in cat SMA segments. Note that the reactions did not follow a sigmoid dose-response curve. L-NNA almost completely abolished ST-induced dilatation. *** – significant difference vs C/NE ($p<0.001$). (C) Percentage relaxation by different doses of somatostatin in cat small mesenteric artery (MA) rings. Dose-dependent relaxation of NE precontracted small MA rings (CONTROL) was significantly suppressed by L-NNA ($3x10^{-4}$ mol/l) treatment. †† – ($p<0.01$) and ††† – ($p<0.001$): significant differences between CONTROL and L-NNA. For statistical analysis ANOVA with Fisher's post hoc test was used. Data are mean values ± S.E.M.*

Continuous NE superfusion ($4x10^{-6}$ mol/l) resulted in a constant preconstriction level. Resting diameter of the SMA segment decreased from 2.5±0.1 mm to 1.7±0.1 mm (n=6, $p<0.001$). ACh ($5x10^{-8}$ mol/l) elicited a large percentage change (75 % dilatation, $p<0.001$) in SMA diameter (Fig. 1A; CONTROL), which was abolished following L-NNA treatment (Fig. 1A; L-NNA).

Various doses of ST elicited significant vasodilatation with NE preconstriction, shown as percentage of the preconstriction level (Fig. 1B). Interestingly, no sigmoid dose-response curve in cat SMA segments was found in the concentration range tested. The maximal diameter change reached 45±9 % ($p<0.001$) of the control level. Treatment with the selective NO synthesis inhibitor L-NNA (10^{-4} mol/l) almost completely suppressed ST-induced dilatation (Fig. 2B). The diameter upon ST administration hardly changed (to 94±3 % of the control preconstriction level, n.s.).

Cat SMV segments exhibited a weak endothelial response compared with SMA. This was marked by a slight dilatation to ACh. Accordingly, administration of ST doses induced less than 20 % venodilatation (n=7; data not shown). In other segments loss of the vasoactive response to ST was observed in correlation with histological damage of the endothelium.

In small MA rings of the cat, NE elicited dose-dependent vasoconstriction, characterized by a 2.4±0.3 g (n=19) increase in tension during 10^{-6} mol/l NE administration. Both ACh and ST induced relaxation of NE-precontracted rings. In contrast to the lack of dose-dependence of ST-induced dilatation in cat SMA, ST relaxed cat small MA rings in a concentration-dependent manner (Fig. 1C). This dose-dependent relaxation was significantly ($p<0.001$) suppressed by NO inhibition induced by L-NNA ($3x10^{-4}$ mol/l).

Small MA rings from the dog reacted similarly to those from the cat. Dose-dependent vasoconstriction resulted in 1.6±0.1 g (n=10) increase in tension when 10^{-6} mol/l NE was administered. ST concentrations (from 10^{-9} to $5x10^{-8}$ mol/l) induced dose-dependent relaxation of small MA rings in the dog.

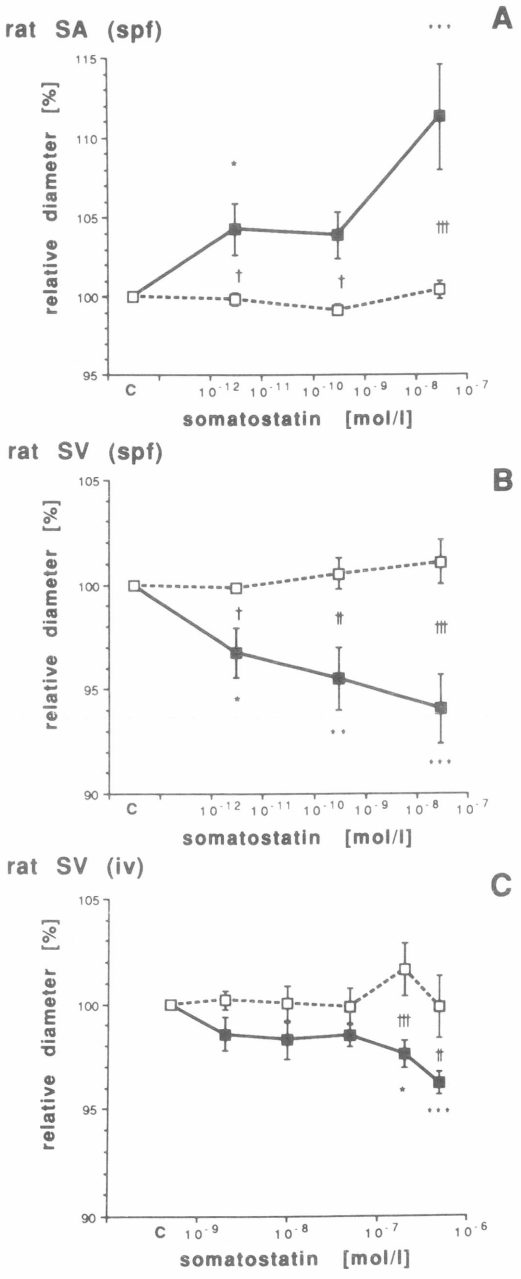

Fig. 2

*Relative diameter changes in response to vasoactive agents in situ. (A). Solid line: Somatostatin (administered in superfusion) induces dose-dependent vasodilatation in rat saphenous artery (SA). Broken line: Local infusion of L-NNA (10^{-4} mol/l) abolished somatostatin-induced vasodilatation in rat SA. (B). Solid line: Somatostatin (administered in superfusion) induces dose-dependent vasoconstriction in rat saphenous vein (SV). Broken line: Local infusion of L-NNA abolished somatostatin-induced vasoconstriction in rat SV. (C). Solid line: Somatostatin (administered intravenously) induces dose-dependent vasoconstriction in rat SV. Broken line: Local infusion of L-NNA abolished somatostatin-induced vasoconstriction in rat SV. * – ($p<0.05$), ** – ($p<0.01$), and *** – ($p<0.001$): significant differences vs. control diameter (C). + – ($p<0.05$), ++ – ($p<0.01$), and +++ – ($p<0.001$): significant differences between intact and L-NNA treated vessels. For statistical analysis ANOVA with Fisher's post hoc test was used. Data are mean values ± S.E.M.*

In situ studies

Rat vessels differ substantially from cat and dog vessels both in their size and vascular reactivity. Functionally large rat vessels hardly exceeded cat and dog small vessels in diameter, and during ST administration the percentage of their diameter also changed to a much lesser extent.

ST had different effects in the rat SA and SV. In the artery, ST superfusion had a significant vasodilator effect, which was dose-dependent (Fig. 2A; solid line). The maximal dose of ST ($3x10^{-7}$ mol/l) elicited a 11±3 % (n=7, $p<0.001$) increase in relative

diameter. In the rat SV, significant dose-dependent venoconstriction was found when ST was given either in superfusion (Fig. 2B; solid line) or in infusion (Fig. 2C; solid line). In superfusion and in infusion the maximal reductions of diameter were by 6±2 % (n=5, $p<0.001$) and 4±1 % (n=5, $p<0.001$), respectively.

When NO synthesis was inhibited with L-NNA (10^{-4} mol/l), the vasodilator effect of ST in rat SA was totally abolished (Fig. 2A; dashed line). The venoconstrictor effect of ST administered both in superfusion and in infusion was also abolished (Fig. 2B and C; dashed lines). ACh-induced dilatation in each case was also significantly suppressed (to less than 50 % of control reaction) following L-NNA treatment.

Discussion

In the present experiments, direct vascular effects of ST were compared in different species and vascular regions. In isolated arteries from the cat and dog mesenteric region, as well as *in situ* in the rat saphenous region, ST appears to be a vasodilator. However, this vasodilatory effect could only be seen in isolated arteries when NE-induced constrictor tone was established. No vasoconstrictive effect of ST was found either in the resting state of cat SMA, or with NE-induced tone.

The mechanism of the vasodilatory action of ST was further characterized by using a selective inhibitor of NO synthesis, and the mediator of this effect seems to be the release of endothelium-derived nitric oxide. This NO-dependent, ST-induced vasodilatation is similar to the known endothelium-mediated effects of other peptides. Substance P (Bolton and Clapp 1986), the vasoactive intestinal peptide (Davies and Williams 1984), bradykinin (Cherry *et al.* 1982), and partly the calcitonin gene-related peptide (Samuelson and Jernbeck 1992) induce dilatation through the release of endothelial NO.

Large and small mesenteric arteries responded differently to ST. The dilatory effect of ST on large SMA segments did not follow a regular dose-response relationship, since low doses of ST already elicited maximal vasodilatation. This was not the case in small MA of the cat and the dog, where we found dose-dependent endothelium-mediated vasodilatation by ST. An irregular dose-dependence of the ST-induced dilatation is also described in human mesenteric artery segments (Törnebrandt *et al.* 1987). The physiological role of such peculiar kinetics is unclear. Relaxation of large conduit mesenteric arteries by the lowest normal ST concentrations might facilitate the operation of regional circulatory control mechanisms (e.g. functional hyperaemia), which could involve endothelium-mediated vasodilatation by ST in the mesenteric resistance vessels.

We have also found differences between the action of ST on the arterial and venous vessel wall. In contrast to the vasodilatation on arteries from different species and regions and to weak dilatation of SMV segments constriction was elicited by ST in SV. The latter was independent of adventitial (superfusion) or intravenous administration of ST. Regional differences in responses suggest that the vascular action of ST might be the result of complex interaction of various vasoactive mechanisms. The available data on human isolated mesenteric vessels have also shown that ST dilates the arteries and contracts the veins (Törnebrandt *et al.* 1987). The resting human saphenous vein was contracted by ST in a concentration-dependent manner, while the precontracted segment contracted further. The constriction was greatly increased, when the endothelium was removed (Luu *et al.* 1992), showing that a ST-induced dilatory mechanism was also present in the background. This is in agreement with our finding on cat SMV, however, the loss of ST-induced contraction after NO-inhibition in the rat SV is contradicting. The reason for this discrepancy could be due to species differences, but the exact mechanism is still not clear.

The regional and species-dependent distribution of direct effects of ST on the vessel wall, among which NO-dependent mechanisms play an important role, and its interaction with indirect vasoregulatory factors require further elucidation.

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