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Effect of somatostatin on resistance and on capacitance rabbit isolated arteries

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Abstract

The effects of somatostatin, a tetradecapeptide isolated from hypothalamus extracts, were studied on the vascular reactivity of aorta and mesenteric arteries isolated from rabbits. We also investigated whether or not Ca^{2+} movements were implicated in these effects. Rabbit aorta and mesenteric (fifth branch) arteries were isolated, cleaned off, and mounted in an organ bath containing Godfraind solution or physiological saline solution (PSS), respectively. Somatostatin (10^{-8}–10^{-4} M) produced a concentration-dependent inhibition of the contractile responses induced by high K^+ (80 mM) or noradrenaline (10^{-6} M in aorta or 10^{-4} M in mesenteric arteries) in both arteries studied. The inhibitory effect of somatostatin was greater in mesenteric resistance vessels (IC_{50} $3.1 \pm 2.3 \times 10^{-5}$ M, and $5.2 \pm 4.8 \times 10^{-8}$ M with KCl and noradrenaline, respectively). Contractile responses produced by the addition of Ca^{2+} (1–5 mM) to Ca^{2+}-free high K^+ solution were also concentration dependently inhibited by somatostatin in aorta. Furthermore, somatostatin decreased noradrenaline-induced contraction attributed to intracellular Ca^{2+} release in aorta, and inhibited $^{45}Ca^{2+}$ uptake stimulated by high K^+ or by noradrenaline. However, it did not modify $^{45}Ca^{2+}$ uptake in resting mesenteric resistance arteries. Taken together, these results suggest that somatostatin exerts an inhibitory effect on vascular contractions induced by some stimulating agents in different arteries isolated from rabbits, being more potent in mesenteric arteries.

Keywords: Somatostatin; Aorta; Mesenteric artery; Ca^{2+}

1. Introduction

Somatostatin, a tetradecapeptide isolated from hypothalamus extracts in 1973 (Brazeau et al., 1973), is present in many peripheral tissues, including those of the heart (Day et al., 1985). The mechanisms by which somatostatin inhibits hormone release are complex and involve, among other things, reduction of intracellular Ca^{2+} by a pertussis toxin-sensitive G-protein mechanism (Zink and Raue, 1992). There is evidence that, in other tissues, somatostatin reduces Ca^{2+} entry. It has been suggested that somatostatin selectively acts on the Ca^{2+} channel of guinea-pig atrial cells to reduce the Ca^{2+} inward current, which in turn gives rise to the negative inotropic effect (Ohmura et al., 1990).

In recent years, some vascular effects of somatostatin have been described, but the mechanism of these actions is still unclear. Leszczynski et al. (1993) have reported that vascular smooth muscle cells express high and low affinity receptors for somatostatin. Somatostatin has been shown to reduce splanchnic blood flow and portal vein flow in dogs (Jaspan et al., 1979; Samnegard et al., 1979) and in humans. The mechanism by which it exerts these effects has not been elucidated. Tyden et al. (1979) suggested that the circulatory effects of somatostatin were due to a direct action on vascular smooth muscle.

In order to elucidate these mechanisms of action, we studied the effects of somatostatin on the vascular reactivity of aorta and mesenteric arteries isolated from rabbits, and investigated whether or not Ca^{2+} movements across the membrane of vascular smooth muscle cells are implicated in these effects.

2. Materials and methods

2.1. General procedure

Male New Zealand White rabbits weighing 2.5–3 kg were obtained from Biocentre (Barcelona, Spain). The

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animals were anesthetized with ethyl ether and killed by exsanguination from the common carotids. The thoracic aorta and mesenteric arteries (fifth branch) were rapidly removed. The thoracic aorta was placed in Godfraind solution of the following composition (in mM): NaCl, 121; KCl, 5.8; HCO_3Na, 14.9; Cl_2Mg, 1.22; glucose, 11; and $ClCa_2$, 1.25. Adherent fat and surrounding tissue were cleaned off and the arteries were cut into rings approximately 2–3 mm wide. The rings were then suspended between two stainless steel hooks in organ baths containing 10 ml of Godfraind solution. The solution was kept at 36 ± 0.5°C and gassed continuously with a 95% O_2–5% CO_2 gas mixture. The aorta rings were mounted under 2-g tension. Each preparation was allowed to equilibrate for 90–120 min. Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and recorded on a Grass polygraph as previously described (Tejerina et al., 1988).

Mesenteric resistance vessels were equilibrated in physiological saline solution (PSS) of the following composition (in mM): NaCl, 139; KCl, 5; $MgCl_2$, 0.98; glucose, 9; $CaCl_2$, 1.15; and HEPES, 4.99. The solution was kept at 36–37°C, gassed continuously with O_2, and maintained at an optimal resting tension of 40 mg (Cauvin et al., 1982). The rings were mounted on a myograph. Two 40-μm tungsten wires were passed through the lumen of an isolated cylindrical segment (2 mm, long) of both arteries (approximately 175 μm inside diameter). One wire was fastened with screws to a fixed, mounted tissue and the other wire was pulled taut by parallel hooks which were attached to a string-gauge force transducer (U-gauge, Shinko). The positions were adjusted with a micromanipulator.

After equilibration the following experiments were carried out: (1) each aorta ring was exposed to single submaximal concentrations of KCl (80 mM) and noradrenaline (10^{-6} M). An initial 10–25-min control contraction was obtained in each experiment with the appropriate stimulating agent. The rings were then washed out and rested for a minimum of 45–60 min. Control contractile responses for each agonist were obtained at the beginning of the experiment until two successive responses were of almost identical height. The rings were then exposed to somatostatin (10^{-6}–10^{-4} M) for 20 min before the addition of KCl or noradrenaline. In all the experiments one of the aorta rings was maintained as time control. (2) To determine whether somatostatin could relax an existing contraction, aorta rings were contracted by a single submaximal concentration of noradrenaline or KCl. When the contractile response to either agent was maximal, somatostatin was added in progressed increasing cumulative concentrations (10^{-8}–10^{-5} M). Rings were allowed to reach a new steady state before each successive concentration of the relaxant agent was added. The results were expressed as a percentage of the maximal control agonist-induced responses. (3) The method used for assessing the inhibitory effects of somatostatin on mesenteric resistance vessels was to contract the vessels with a submaximal concentration of KCl (80 mM) or noradrenaline (10^{-4} M), then wash the activating agent out and repeat the stimulus after 20 min of preincubation of the vessel with PSS containing somatostatin (10^{-8}–10^{-4} M) (Cauvin et al., 1987). Only one agonist was used in each experiment. (4) To determine whether the inhibitory effects of somatostatin were dependent on the Ca^{2+} concentration, aorta rings were incubated in Ca^{2+}-free Godfraind solution for 120 min and then in Ca^{2+}-free high-K^+ (80 mM) depolarizing Godfraind solution for 10 min. Cumulative concentration-response curves for Ca^{2+} were then obtained by increasing the Ca^{2+} concentration in the bath (1–5 mM) stepwise over the next 45 min. Ca^{2+} was then washed out and the rings were re-incubated in Ca^{2+}-free Godfraind solution for 60 min (Barrigon et al., 1984). The high K^+ depolarizing procedure was repeated, but somatostatin was added to the bath 20 min before the first addition of Ca^{2+}. The results were expressed as percentages of the maximal contractile response induced by 5 mM $CaCl_2$. (5) In order to evaluate the possible effects of somatostatin on noradrenaline-induced Ca^{2+} release, a protocol similar to that described previously (Hester et al., 1987) was used. Briefly, somatostatin (10^{-5} M) was added to the aorta rings 20 min prior to noradrenaline to Ca^{2+}-free plus EGTA (10^{-5} M) solution. In another series of experiments, using the above-mentioned solution, after release was complete (approximately 5 min), the tissues were rinsed free of somatostatin and/or noradrenaline with the same Ca^{2+}-free solution for 20 min and then re-stimulated with noradrenaline. (6) $^{45}Ca^{2+}$ uptake was determined as previously described (Meisheri et al., 1981; Leijten and van Breemen, 1984). Mesenteric rings were equilibrated for 30 min in PSS. After equilibration, experimental rings were treated with somatostatin for 20 min and control rings with an equivalent amount of solvent (PSS). The rings were then exposed for 90 s to $^{45}Ca^{2+}$ solution (specific activity 4 μCi/ml). In some experiments noradrenaline (10^{-4} M) or KCl (80 mM) was added simultaneously with $^{45}Ca^{2+}$. At the end of the 90-s exposure to the stimulatory agent, the tissues were washed in ice-cold EGTA-PSS for 45 min to remove extracellular Ca^{2+}. The rings were then removed, blotted, weighed, placed in scintillation vials and 0.5 ml of soluene-350 (Packard) was added, and left overnight in order to digest the tissue. Next, 0.4 ml of an isopropanol/H_2O_2 33% (1:1) solution was added into each vial for 45 min (50°C), and then 20 μl acetic acid was added to neutralize the latter solution. Finally, each vial was filled with 10 ml of a scintillation liquid.

2.2. Drugs

The following drugs were used: somatostatin was a generous gift from Laboratories Serono, noradrenaline bitartrate (Sigma), potassium chloride and Ca^{2+} chloride

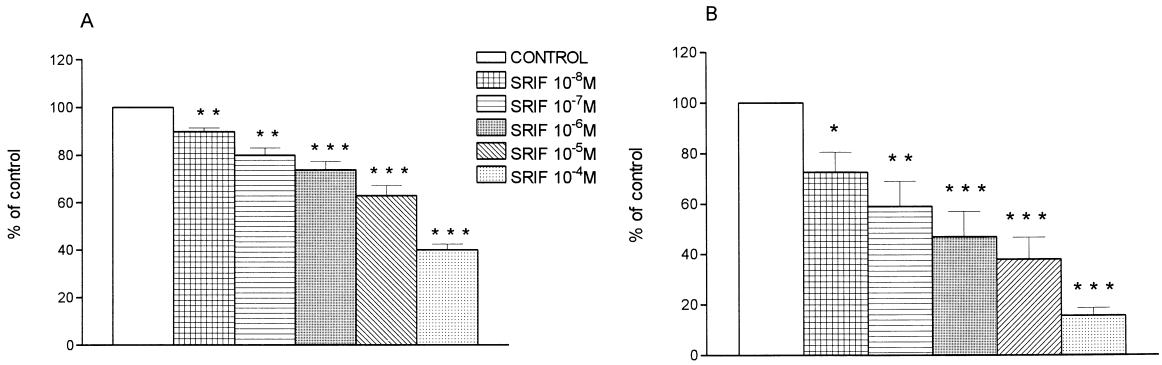

Fig. 1. Inhibitory effect of somatostatin (10^{-8}–10^{-4} M) on the contractile responses induced by high K^+ (80 mM, panel A) and noradrenaline (10^{-6} M, panel B) in rabbit mesenteric artery (fifth branch). Each point represents the mean of 7 experiments and vertical lines indicate S.E.M. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

(Merck) and $^{45}Ca^{2+}$ (specific activity 2 mCi/ml) (Amershan). Stock solutions of somatostatin (10^{-4} M) were prepared by dissolving somatostatin powder in Godfraind solution or PSS; working solutions were made in Godfraind solution or PSS. The concentration for each chemical or drug is expressed as final concentration in the bath in terms of the salt. Ascorbic acid was added to each daily-prepared solution of noradrenaline. The University Complutense of Madrid (EEC official registration 28079-15ABC) approved all protocols concerning animals.

2.3. Statistical analysis

All values used in analyses represent means ± S.E.M. for seven to nine rabbits in each group. Comparisons between the different groups were done with Student's *t*-test. A level of probability $P < 0.05$ was accepted as statistically significant. Concentration–response curves were used to determine the concentration of somatostatin producing 50% inhibition of the maximal contractile response (IC_{50}), using linear regression analysis over the response range of 20–80% of the maximal inhibition obtained.

3. Results

3.1. Inhibitory effect of somatostatin on contractions induced by KCl and noradrenaline

The inhibitory effects of somatostatin on the contractile responses induced by high K^+ (80 mM) and noradrenaline (10^{-6} or 10^{-4} M) in rabbit aorta and mesenteric (fifth branch) arteries were studied. As shown in Figs. 1 and 2, preincubation with somatostatin concentrations, ranging from 10^{-8} to 10^{-4} M in the mesenteric artery, and 10^{-6}–10^{-4} M in aorta, produced a concentration-dependent inhibition of the contractile response induced by the stimulating agents (K^+ and noradrenaline) in both arteries. Somatostatin 10^{-4} M almost suppressed the contractile response induced by noradrenaline in the mesenteric artery (fifth branch). Comparing the inhibitory effect in both groups of arteries, we found that somatostatin was more potent in inhibiting the contractions induced by noradrenaline or KCl in the mesenteric artery than in the aorta. Thus, the maximal effect reached in the aorta with somatostatin (10^{-4} M) was 42.7 ± 17.3% ($P < 0.05$, $n = 7$) and 39.7 ± 17.6% ($P < 0.05$, $n = 7$ with respect to the control)

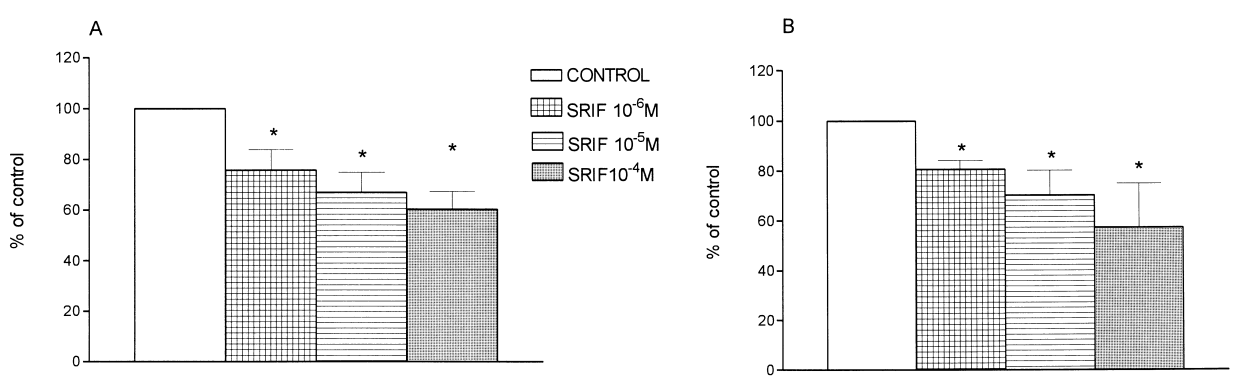

Fig. 2. Inhibitory effect of somatostatin (10^{-6}–10^{-4} M) on the contractile responses induced by high K^+ (80 mM, panel A) and noradrenaline (10^{-6} M, panel B) in rabbit aorta. Each point represents the mean of 7 experiments and vertical lines indicate S.E.M. $^*P < 0.05$.

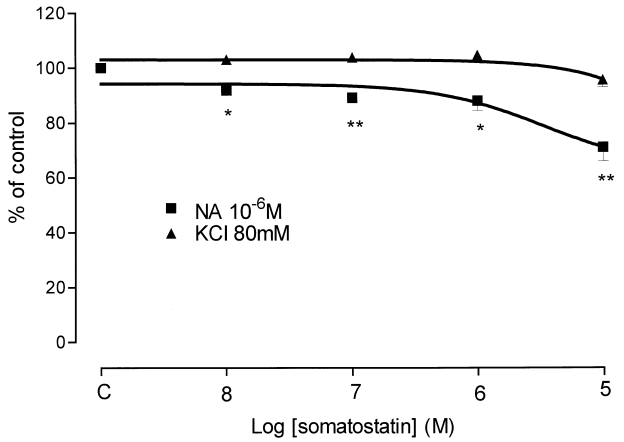

Fig. 3. Relaxing of high K^+ and noradrenaline-induced contractions by somatostatin in aorta. Each point represents the mean of 7 experiments and vertical lines indicate S.E.M. $^*P < 0.05$, $^{**}P < 0.01$.

when the arteries were contracted with noradrenaline and KCl, respectively (Fig. 2).

The maximal effect of somatostatin (10^{-4} M) in mesenteric arteries was $84.4 \pm 3.2\%$ ($P < 0.001$, $n = 7$) in arteries contracted with noradrenaline, whereas when the contraction was induced by KCl, this was $60.0 \pm 2.3\%$ ($P < 0.001$, $n = 7$ with respect to the control) (Fig. 1).

The concentrations at which somatostatin inhibited 50% of the maximal contractile response (IC_{50}) induced by high K^+ and noradrenaline in both arteries were $3.1 \pm 2.3 \times 10^{-5}$ and $5.2 \pm 4.8 \times 10^{-8}$ M with KCl and noradrenaline, respectively, in mesenteric arteries, and $> 10^{-4}$ with KCl and noradrenaline in aorta.

3.2. Effect of somatostatin added to aortic rings precontracted with noradrenaline or KCl

In another group of experiments, somatostatin (10^{-8}–10^{-5} M) was cumulatively added to aortic rings maximally contracted with KCl (80 mM) or noradrenaline (10^{-6} M) in order to test whether or not it could relax already established contractions.

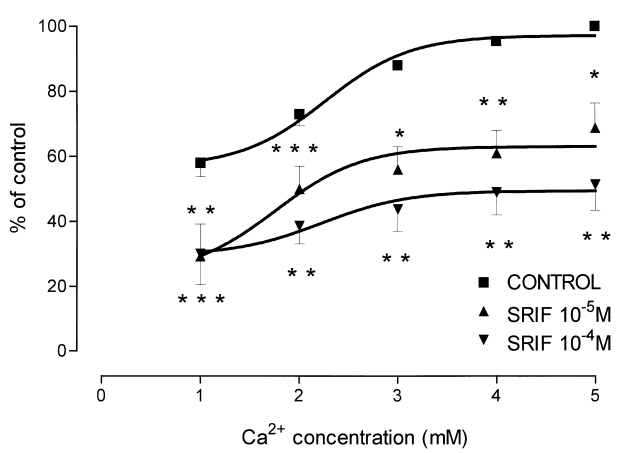

Fig. 4. Effect of somatostatin on restoration of the isometric contraction of aorta rings by addition of Ca^{2+} (1–5 mM) to Ca^{2+}-free high K^+ (80 mM) medium. Ordinate scale: percentage of the maximum control contractions obtained with the highest concentration of Ca^{2+} in each experiment. Each point represent the mean of 5 experiments and vertical lines indicate S.E.M. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

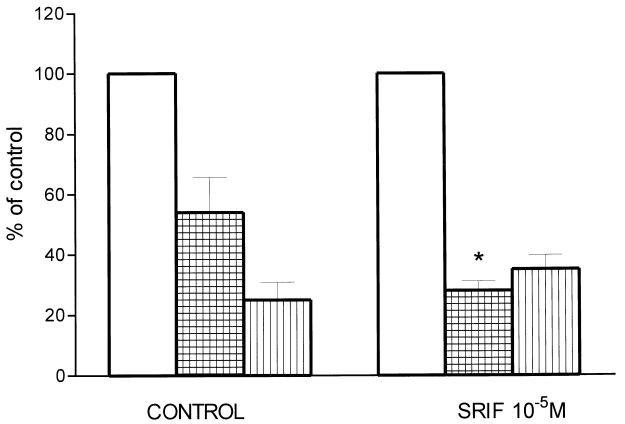

Fig. 5. Effects of somatostatin on the intracellular Ca^{2+} movements. Each point represents the mean of seven experiments and vertical lines indicate S.E.M. Open columns represent the initial-phase response with NA (10^{-6} M); solid columns represent the initial-phase response with NA (10^{-6} M) in a Ca^{2+}-free plus EDTA solution and score columns represent the 2nd-phase response in the same solution. The left panel represents a control and the right panel the presence of somatostatin. $^*P < 0.05$.

As shown in Fig. 3, when the arteries were precontracted with KCl (80 mM), somatostatin evoked a non-significant relaxing effect, whereas when the arteries were precontracted with noradrenaline (10^{-6} M) somatostatin relaxed these contractions, reaching significant values ($P < 0.05$) at concentrations equal to or higher than 10^{-8} M. The maximal relaxation was $29.0 \pm 5.3\%$ ($P < 0.01$, $n = 7$) reached with somatostatin 10^{-5} M.

3.3. Effects on Ca^{2+}-induced contractions

In aorta rings previously depolarized by high K^+, somatostatin produced a concentration-dependent decrease of

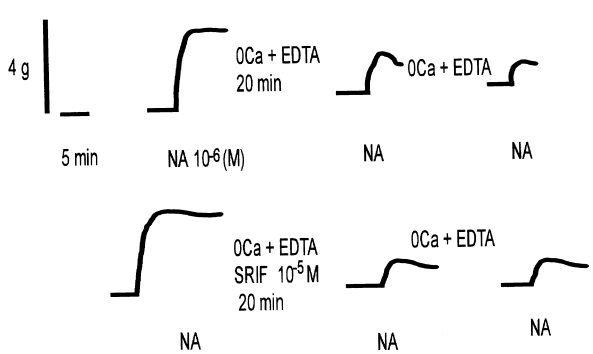

Fig. 6. Effects of somatostatin on initial-phase responses of aorta to noradrenaline in a Ca^{2+}-free plus EDTA solution (middle tracing, bottom) and 2nd-phase responses in the same solution (right tracings) 20 min after rinsing somatostatin and/or noradrenaline from the tissues and bath with a similar Ca^{2+}-free plus EDTA solution. Control responses to noradrenaline in Godfraind solution are also included (left tracings). Top series represents control responses to noradrenaline in Godfraind solution and in Ca^{2+}-free plus EDTA (initial- and 2nd-phase responses) in the absence of somatostatin.

the contraction induced by Ca^{2+} and shifted the concentration–response curve downwards and to the right. Somatostatin 10^{-5} M reduced the maximal response of aorta rings to 5 mM Ca^{2+} by 68.0 ± 7.0 ($P < 0.05$, $n = 5$) and at 10^{-4} M by 51.1 ± 8.1 ($P < 0.01$, $n = 5$) (Fig. 4).

3.4. Effect on intracellular Ca^{2+} release

In order to determine whether somatostatin could have an inhibitory effect on noradrenaline-induced contractions attributed to Ca^{2+} release from intracellular stores, we tested the effects of the drug on phasic contractions resulting from the exposure to noradrenaline in the presence of Ca^{2+}-free plus EGTA (10^{-5} M) solution. As shown in Fig. 5 (middle panel) somatostatin (10^{-5} M) depressed the transient contractions induced by noradrenaline (10^{-6} M) in the aorta ($54.0 \pm 1.1\%$ vs. $28.2 \pm 3.4\%$ with respect to each control). When tissues were rinsed free of somatostatin and/or noradrenaline with the Ca^{2+}-free solution and were re-exposed to the same concentration of noradrenaline, there was only a residual response both from the control aorta and from the aorta previously exposed to somatostatin ($25.2 \pm 5.9\%$ vs. $35.1 \pm 4.6\%$ with respect to each control) (Fig. 5, right panels). Fig. 6 shows a typical recording.

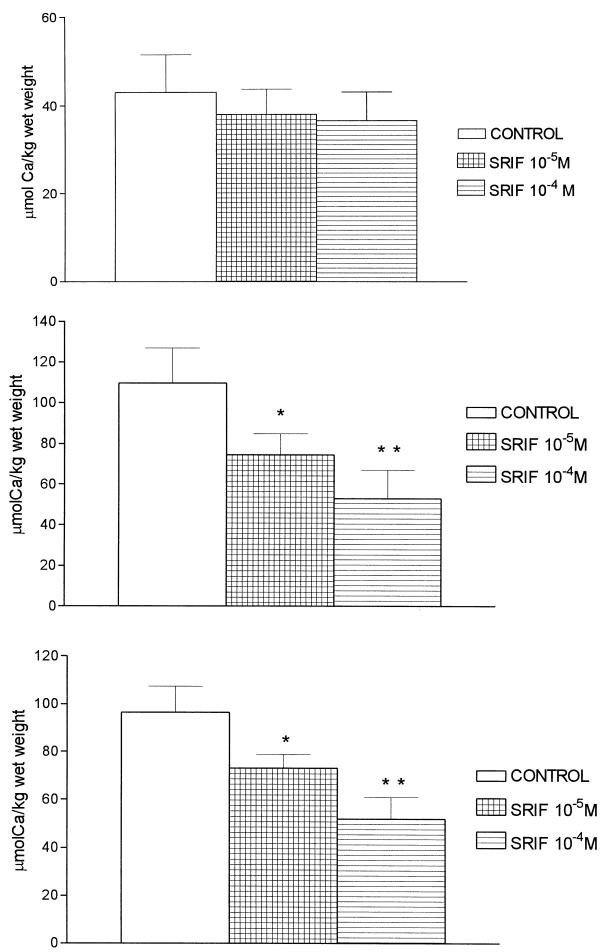

Fig. 7. Effects of somatostatin on $^{45}Ca^{2+}$ influx in resting mesenteric arteries (fifth) (upper panel) and in vessels stimulated with 80 mM KCl (middle panel) or 10^{-4} M noradrenaline (lower panel). The values are the mean of 6 experiments and vertical lines represent the S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$.

3.5. Effects on $^{45}Ca^{2+}$ influx

The effects of somatostatin on $^{45}Ca^{2+}$ influx were studied in resting, non-stimulated mesenteric rings as well as in rings stimulated either by high-K^+ depolarization or by agonist activation noradrenaline (10^{-4} M) (Fig. 7). In resting rings, somatostatin at 10^{-5} M reduced, but not significantly, the $^{45}Ca^{2+}$ content from the control values (43.1 ± 8.5 μmol/kg vs. 31.2 ± 5.8 μmol/kg tissue, $P > 0.05$ $n = 6$). Addition of noradrenaline (10^{-4} M) or high-K^+ (80 mM) increased the $^{45}Ca^{2+}$ influx to 96.5 ± 11.0 μmol, and to 109.8 ± 17.2 μmol of Ca^{2+}/kg of wet weight, respectively. Somatostatin, 10^{-5} M, reduced the uptake induced by noradrenaline from 96.5 ± 11.0 to 73.1 ± 5.7 μmol ($P < 0.05$, $n = 6$), and to 52.4 ± 9.3 μmol of Ca^{2+}/kg of wet weight at 10^{-4} M ($P < 0.01$, $n = 6$). When the $^{45}Ca^{2+}$ influx was induced by high K^+, the reduction was from 109.8 ± 17.2 to 74.5 ± 10.4 μmol ($P < 0.05$, $n = 6$), and to 53.0 ± 14.2 μmol of Ca^{2+}/kg of wet weight ($P < 0.01$, $n = 6$) with somatostatin 10^{-5} and 10^{-4} M, respectively.

4. Discussion

Somatostatin has been used therapeutically in the treatment of esophagic haemorrhages due to portal hypertension. Portal hypertension is a syndrome characterised by an increase in portal vein pressure, which induces the opening of collateral branches in order to conduct the blood towards the systemic circulation. The advantage of the use of somatostatin in the treatment of portal hypertension is that somatostatin exerts its action without changing the systemic circulation. However, there are controversies about the vascular effects of somatostatin. It has been reported that intravenous somatostatin injection in humans is followed by a direct vasoconstricting effect on the splanchnic vascular bed with a consequent decrease in portal venous inflow and pressure (Tyden et al., 1979; Boch et al., 1981). Sieber et al., (1992) suggested that somatostatin as well as octeotride induce their vasoconstricting effect by blocking the secretion of vasodilator agents, as no direct vasoactive effect had been observed for either of the peptides tested.

In this work, we tested whether somatostatin had any direct effect on rabbit mesenteric arteries and aorta under resting tension. We did not find any action of somatostatin on either artery under resting tension. These data are in agreement with results of previous work which showed

that somatostatin did not show any direct effect on rat mesenteric blood flow in arteries perfused in vitro (Sieber et al., 1992).

We also studied the effects of somatostatin on contractile responses induced by KCl and by noradrenaline in both resistance and capacitance rabbit-isolated arteries. Somatostatin inhibited the contraction induced by depolarization of the vascular smooth muscle cell membrane with KCl (or with increasing concentrations of extracellular Ca^{2+} in a high-K^+ solution) or that induced by the adrenergic agonist, noradrenaline. These responses have been attributed to Ca^{2+} entry through voltage-operated channels (Hudgins and Weiss, 1964; Sigurdsson et al., 1975; Bolton, 1979; Cauvin et al., 1983). Moreover, depolarization of smooth muscle cells through L-type Ca^{2+} channels has been implicated in the mechanism by which noradrenaline induces vascular smooth muscle contraction.

It is well known that somatostatin has an inhibitory effect on this kind of Ca^{2+} channel. Two types of action have been classically postulated to be involved in the transduction of the somatostatin message: the inhibition of adenylyl cyclase (Cronin et al., 1983) and of Ca^{2+} fluxes (Tsunoo et al., 1986). However, it is not clear whether somatostatin acts directly on the Ca^{2+} channel (Ohmura et al., 1990; Zink and Raue, 1992) or indirectly, acting on a K^+ channel, leading to hyperpolarization and finally shutting down of the Ca^{2+} channel (Koch and Schonbrunn, 1987; Koch et al., 1987; Yatani et al., 1987; Pennefather et al., 1988; White et al., 1991).

Taking into account the present data, we postulated that the inhibitory effect of somatostatin on the contractile response in rabbit arteries was due to inhibition of Ca^{2+} fluxes. This hypothesis is supported by the results of experiments carried out with $^{45}Ca^{2+}$ and in Ca^{2+}-induced contraction. Both sets of conditions are strongly extracellular Ca^{2+}-dependent. The hypothesis is also supported by the differences in potency of the inhibitory effect of somatostatin on contractions induced by KCl or by noradrenaline in aorta or mesenteric artery. The inhibitory effects of somatostatin on vascular reactivity induced by both KCl and noradrenaline in mesenteric resistance vessels were greater than those in aorta. Thus, the IC_{50} value of somatostatin for this effect was lower in the mesenteric artery (order of magnitude of [about] two) than in the aorta artery. The precise explanation for the tissue specificity is not yet known, but it may be due to differences in the structure of the channels themselves from vessel to vessel (Quins et al., 1981; Cauvin et al., 1988) and/or to variations in the membrane potentials of the different arteries (Shibata et al., 1991; Tejerina et al., 1992). However, in the case of agonist-induced responses the different efficacy of the drugs appears to be more related to the source of the Ca^{2+} activator of the contractile process.

Rabbit mesenteric resistance vessels have been found to be extremely sensitive to the inhibitory effects of organic Ca^{2+} channels antagonists (Godfraind et al., 1986; Cauvin et al., 1988; Tejerina et al., 1992). It has been reported that, in these vessels, contractions resulting from receptor activation by noradrenaline are more sensitive to Ca^{2+} channel antagonistic inhibition than are contractions induced by K^+ depolarization (Cauvin et al., 1984, 1988). In the present study, the IC_{50} value for somatostatin inhibition of noradrenaline-induced contraction was lower (about three order of magnitude) than the IC_{50} value for the inhibition of high K^+-induced contraction. A possible explanation for the greater sensitivity of noradrenaline-induced contraction in response to somatostatin in resistance vessels is that noradrenaline modulation of voltage-sensitive Ca^{2+} channels may be particularly important for promoting spike activity and Ca^{2+} influx in small resistance vessels where Ca^{2+} stores are relatively poorly developed (Benham and Tsien, 1988).

In addition, we cannot rule out an action of somatostatin on intracellular Ca^{2+} movements, as demonstrated by the effect of somatostatin on noradrenaline-induced contraction in a Ca^{2+}-free medium (Fig. 5).

In conclusion, these results suggest that somatostatin decreases both KCl- and noradrenaline-induced contractions in aorta and mesenteric arteries, being more potent in mesenteric artery, and this effect is due to an action on both extracellular and intracellular Ca^{2+} movements.

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