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European Journal of Pharmacology 474 (2003) 295–301
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Characterisation of somatostatin actions on knee joint blood vessels of the rat

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Received 14 April 2003; received in revised form 8 July 2003; accepted 15 July 2003

Abstract

The effects of somatostatin on blood flow, plasma extravasation and knee joint sizes in the rat were investigated. Topical bolus administrations of somatostatin (10 pmol–100 nmol) onto the exposed rat knee joint capsules produced dose-dependent increases in knee joint blood flow with an ED_{50} value of 1.7 nmol, and a maximum increase of 109.7%. The peak vasodilator response was observed at 1 min following drug administration, and it subsided at 5 min. Treatment of the rat knee with a somatostatin receptor antagonist cyclo(7-aminoheptanoul-Phe-D-Trp-Lys-Thr[Bzl] (cyclo-somatostatin; 2×20 nmol) significantly suppressed the somatostatin-induced vasodilator response, but treatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 2×50 nmol) or the cyclo-oxygenase inhibitor flurbiprofen (2×10 nmol) had no effect. Unilateral intraarticular injections of somatostatin (10 nmol) produced no change on blood flow and sizes of the rat knee joints, but elicited marked ipsilateral Evans blue extravasation. Cyclo-somatostatin at doses of 2×20 and 2×50 nmol did not affect the plasma extravasation response to somatostatin. The present findings indicate the vasodilator effect of somatostatin is mediated by receptors sensitive to cyclo-somatostatin inhibition, but its plasma extravasation effect might be mediated by somatostatin receptor types that are resistant to inhibition by cyclo-somatostatin. There is no evidence that nitric oxide and prostaglandins are involved in the somatostatin-induced vasodilator response. It is suspected that the vascular effects of somatostatin demonstrated in this study would play a part in the innate response of an inflammatory reaction.

Keywords: Somatostatin; Vasodilatation; Plasma extravasation; Knee joint, rat

1. Introduction

Primary afferent neurones synthesize a variety of neuropeptides that are transported into the peripheral nerve fibres (Brimijoin et al., 1980; Gamse et al., 1982) to play a role in joint physiology and in joint diseases, such as rheumatoid arthritis (Walsh et al., 1992; Gronblad et al., 1988). Homeostasis in normal tissues is suspected to require a balance of proinflammatory peptides such as substance P and anti-inflammatory peptides such as somatostatin (Heppelmann and Pawlak, 1997). In inflamed tissues, this balance is lost and proinflammatory peptides predominate, which, together with other mediators, cause sensitization of sensory fibres (Heppelmann and Pawlak, 1999) and a variety of other inflammatory symptoms (see Ferrell and Lam, 1996).

The proinflammatory roles for substance P has been well documented. These include our previous findings that showed capsaicin, a neurotoxin that releases neuropeptides from sensory neurones, produced plasma extravasation in the rat knee joint that could be attenuated by the substance P antagonist [D-Pro^4, D-Trp^{7,9,10}]substance P-(4-11) (Lam and Ferrell, 1989a). Substance P administered topically or intra-articularly into rat knee joints also elicited marked vasodilatation (Lam and Ferrell, 1993a,b) and plasma extravasation (Lam and Ferrell, 1989a,b, 1990, 1993a; Scott et al., 1991). These inflammatory actions of substance P were mimicked by a tachykinin NK_1 receptor agonist [Sar^9, Met$(O_2)^{11}$] substance P, and blocked by a tachykinin NK_1 receptor antagonist FK888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide) (Lam and Wong, 1996). Furthermore, substance P and related peptides (the tachykinins) have been shown to exert other proinflammatory actions such as stimulating the secretion of prostaglandin E_2 and

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doi:10.1016/S0014-2999(03)02107-1

collagenase from synoviocytes (Lotz et al., 1987), promoting mast cell degranulation (Singh et al., 1999), endothelial cell division, migration and angiogenesis (Ziche et al., 1994; 1996), fibroblasts and keratinocytes proliferation (Nilsson et al., 1985), macrophage maturation and the release of cytokines (Lotz et al., 1988; Laurenzi et al., 1990) and recruiting granulocytes at sites of inflammation (Baluk et al., 1995).

Somatostatin is stored in a subgroup of the capsaicin-sensitive primary afferent neurones (Gamse et al., 1981; Holzer, 1992). All dorsal root ganglion cells in the rat express somatostatin receptor mRNA (Señaris et al., 1995). Somatostatin receptors are also present in high density in synovia of patients with rheumatoid arthritis (Reubi et al., 1994). Stimulation of the sciatic nerve in the rat causes release of somatostatin into the systemic circulation, which mediates a systemic antiinflammatory effect (Szolcsányi et al., 1998). Somatostatin also reduces inflammation in animal models of arthritis (Matucci-Cerinic et al., 1995) and improves pain and joint function in osteoarthritic patients (Silveri et al., 1994; Fioravanti et al., 1995). The antiinflammatory effects of somatostatin have been associated with suppression of neuropeptide actions. Hence, somatostatin was reported to inhibit the release of substance P as well as the vasodilatation in the cat's dental pulp evoked by stimulation of the inferior alveolar nerve (Gazelius et al., 1981). Another study showed that somatostatin inhibits the vasodilatation and plasma extravasation induced by saphenous nerve stimulation, but has no effect on the vascular responses to intraarterial infusion of substance P, indicating a presynaptic mode of action for somatostatin (Lembeck et al., 1982).

The direct actions of somatostatin on blood vessels are poorly documented and are controversial. Consequently, this peptide has been reported to cause decrease (Cooper et al., 1981), increase (Dézsi et al., 1997; Pofahl et al., 1994) or no change (Tyden et al., 1979) in blood flow to various organs. This is due, in part, to species and regional difference in blood flow responses and to variance in doses and routes of administration of the peptide. Somatostatin is suspected to play a role in physiology and pathophysiology of joints, but its actions on knee joint blood vessels have not been investigated. Therefore, the present study attempts to elucidate the actions of somatostatin on blood vessels of rat knee joints.

2. Materials and methods

2.1. Induction of anaesthesia

Experiments were performed on male Sprague–Dawley rats (250–300 g) bred at the Chinese University of Hong Kong. The animals were deeply anaesthetised with urethane (1.8 g/kg) as judged by the absence of a flexor withdrawal reflex response to a pinch applied to the forelimb. All experiments were conducted in accordance with the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.2. Assessment of changes in articular blood flow

The method of laser Doppler perfusion imaging (LDI) described by Lam and Ferrell (1993b) was used to measure the relative change in rat knee joint blood flow. The skin over the knee joint of the anaesthetised rat was removed to expose the anteromedial aspect of the joint capsule. To prevent tissue dehydration, 100 μl NaCl (0.9%) was added to the exposed surface every 5 min throughout the experiment except during drug administrations. A laser Doppler perfusion imager (Moor Instruments, UK), placed 30 cm above the joint, directs a helium–neon laser (633 nm) to the tissue and scans the surface of the joint in a rectangular pattern of 3 × 4 cm in approximately 30 s. A colour-coded perfusion image can subsequently be generated and displayed on the monitor. The actual perfusion (flux) values at each point in the image are stored on disc and can be utilised for calculation of the mean perfusion within a given area, enabling the determination of flux difference of the same selected area on the LDI image before and after experimental manipulation.

Drugs were administered as a bolus applied to the surface of the joint in a volume of 100 μl. Dose–response curves to somatostatin were constructed with 10-min intervals in between each bolus application to allow for recovery. In experiments where an antagonist was included, the joints were pretreated with the antagonist 5 min prior to the control measurement. This was followed by coadministration of the antagonist with somatostatin. Possible changes in systemic blood pressure induced by drugs applied on the joint surface were monitored via a cannula inserted into the carotid artery of the rat.

2.3. Assessment of plasma extravasation

The method for assessment of plasma extravasation was as described previously (Lam and Ferrell, 1991). Briefly, on the anaesthetised rat, Evans blue (50 mg/kg) was injected into the external jugular vein, then 100 μl of somatostatin was administered into the synovial cavity of one knee, the contralateral knee was injected with the same volume of 0.9% physiological saline to provide an internal control. The animal was maintained anaesthetised for 1, 2 or 4 h and then exsanguinated. The anterior and posterior portions of the knee joint capsule on both sides were dissected free. The amount of tissue obtained from each animal was small, necessitating pooling of samples from four rats. These were weighed and Evans blue extracted by a dye extraction technique described previously (Lam and Ferrell, 1989a). The amount of dye recovered was calculated by comparing the absorbance of the fluid obtained at 620 nm (Biochrom 4060) with that of a standard curve prepared with known concentrations of Evans blue solution. As Evans blue binds

to plasma proteins normally restricted to the vascular compartment, its presence in the capsule provides an index of altered vascular permeability.

In experiments where an antagonist was included, the test knee was injected with 100 μl of the antagonist and the contralateral knee injected with equal volume of saline. After 10 min, the test knee was injected with 100 μl of a solution containing the antagonist and somatostatin, and the contralateral knee, again, received injection of 100 μl saline. Thereafter, the procedure was the same as above.

2.4. Assessment of changes in knee joint sizes and basal knee joint perfusion

At 1, 2 or 4 h after unilateral intraarticular administration of somatostatin, knee joint sizes of each animal were measured by placing a digital micrometer (Mitutoyo, Japan) across the medial aspect of the knee joint. Basal blood flow of each knee joint was than determined using LDI as described above.

2.5. Drug formulation and statistical analysis

Somatostatin, cyclo(7-aminoheptanoul-Phe-D-Trp-Lys-Thr[Bzl] (cyclo-somatostatin), N^G-nitro-L-arginine methyl ester (L-NAME), flurbiprofen and urethane were purchased from Sigma (USA). Evans blue was purchased from BDH (England). All drugs were dissolved in 0.9% NaCl. Results from knee joint blood flow studies are expressed as means ± S.E.M. of basal blood flow (in flux), or represented as means ± S.E.M. of percentage of flux increase from basal values that are normalized to zero. Those from plasma extravasation experiments are expressed as means (in μg/g tissue) ± S.E.M. of the amount of Evans blue extravasated. Knee joint sizes are expressed as means ± S.E.M. of sizes measured in millimeters. Mean values were compared by paired or unpaired Student's *t*-test as appropriate. Differences between dose–response curves were analyzed by repeated measures two-factor analysis of variance (ANOVA). P values <0.05 were considered statistically significant.

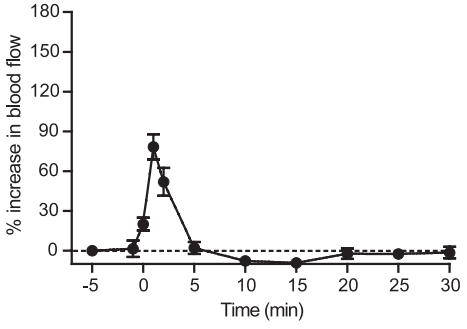

Fig. 1. Time course of somatostatin-induced vasodilatation in the rat knee joint. Topical bolus application of somatostatin (3 nmol) onto the rat knee joint surface produced immediate increase in blood flow that reached a peak of 78.4 ± 9.5% (n = 7) at 1 min. The response subsided at 5 min. Data are shown as means ± S.E.M. (shown by vertical bars) of percentage change of blood flow from basal values that are normalized to zero. Somatostatin was administered at time 0.

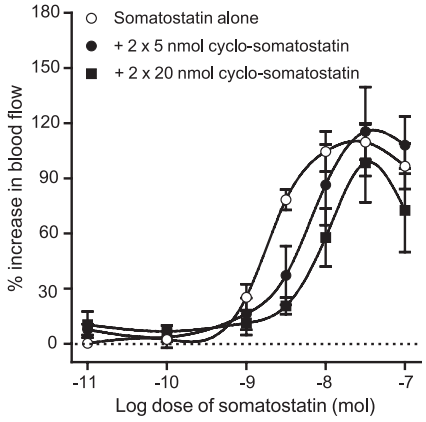

Fig. 2. Effects of the somatostatin receptor antagonist, cyclo-somatostatin, on somatostatin-induced vasodilatation. Topical bolus applications of somatostatin produced dose-dependent vasodilator responses in the rat knee joint (n = 8). Administrations of two 5 nmol cyclo-somatostatin (see Section 2) had no effect on the somatostatin-induced responses, but administrations of two 20 nmol cyclo-somatostatin produced significant inhibition (n = 5 for both; $P > 0.05$ and < 0.05, respectively; two-factor ANOVA).

3. Results

3.1. Effects of topical administrations of somatostatin

As illustrated in Fig. 1, bolus topical administration of 3 nmol somatostatin onto the rat knee joint produced increase in basal laser flux signal, indicating that it is a vasodilator in the rat knee joint. The peak increase of blood flow at this

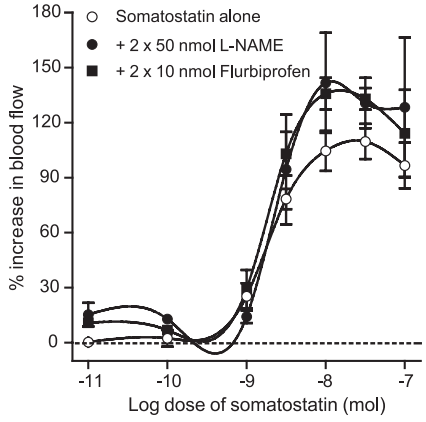

Fig. 3. Effects of the nitric oxide synthase inhibitor, L-NAME, and the cyclo-oxygenase inhibitor, flurbiprofen, on somatostatin-induced vasodilatation. The dose–response curve to topically administered somatostatin (n = 8) was not affected by treatment of the knee joint with two 50 nmol L-NAME or two 10 nmol flurbiprofen (n = 5 and 7, respectively; P>0.05 for both; two-factor ANOVA).

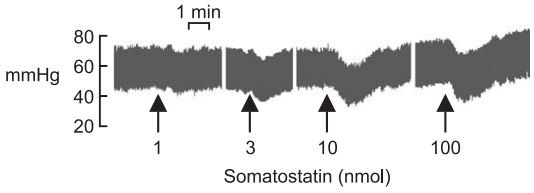

Fig. 4. Recording of blood pressure responses to somatostatin. Topical bolus applications of somatostatin onto the rat knee joint produced marked decrease in systemic blood pressure at doses of 3 nmol and above.

dose was 78.37 ± 9.51% ($n = 7$). This was attained at 1 min and it subsided at 5 min after drug administration.

The full dose–response curve of somatostatin is shown in Fig. 2. It has a mean ED_{50} value of 1.72 ± 0.14 nmol ($n = 8$), and a maximum increase of blood flow of 109.70 ± 9.60% attained at 30 nmol. The dose–response curve of somatostatin was not affected by treatment of the knee joint with 2 × 5 nmol of the somatostatin receptor antagonist cyclo-somatostatin ($P > 0.05$; $n = 5$), but a significant rightward shift of the dose response curve was produced with 2 × 20 nmol cyclo-somatostatin ($P < 0.05$; $n = 5$) (Fig. 2). The same treatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 2 × 50 nmol) or the cyclo-oxygenase inhibitor flurbiprofen (2 × 10 nmol) had no effect on the dose–response curve to somatostatin ($P > 0.05$; $n = 5$ and 7, respectively) (Fig. 3). Somatostatin also produced marked drop in systemic blood pressure at doses of 3 nmol or above (Fig. 4). All doses of cyclo-somatostatin and the enzyme inhibitors used in this study did not alter basal blood flow of the rat knee joint on their own.

3.2. Effects of intraarticular administrations of somatostatin

Unilateral injections of 10 nmol somatostatin into synovial cavities of the rat knees produced insignificant increase in Evans blue extravasation compared with the contralateral saline-injected knees at 1 h following drug administrations ($P > 0.05$; $n = 7$), but at 2 and 4 h, there were significant and comparable ipsilateral increases ($P < 0.01$ compared with their respective contralateral knees; $n = 7$ for both) (Fig. 5A). Basal blood flow and sizes of the rat knee joints were unaffected at all time points ($P > 0.05$ compared with their

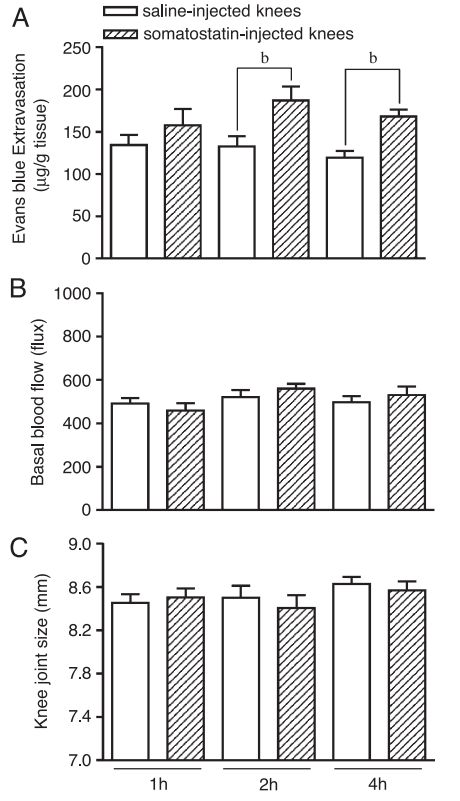

Fig. 5. Time course of actions of unilateral intraarticular administrations of somatostatin (10 nmol) on (A) Evans blue extravasation, (B) basal blood flow and (C) knee joint sizes in the rat. For all three parameters, values for the somatostatin-injected knees were not significantly different to those of their respective contralateral saline-injected knees ($n = 6–8$; $P > 0.05$ for all, Student's paired t-test), except that the amounts of Evans blue extravasated in somatostatin-injected knees were greater than those of their respective contralateral saline-injected knees at 2 and 4 h ($n = 7$ for both; $^bP < 0.01$, Student's paired t-test).

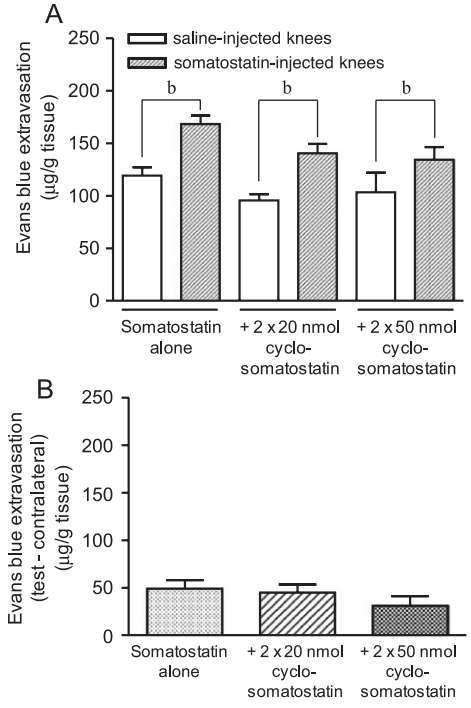

Fig. 6. Effects of the somatostatin receptor antagonist, cyclo-somatostatin, on somatostatin-induced Evans blue extravasation at 4 h. (A) Unilateral intraarticular administrations of somatostatin (10 nmol) alone, in the presence of 2 × 20 nmol cyclo-somatostatin and in the presence of 2 × 50 nmol cyclo-somatostatin; all produced significant increase in Evans blue extravasation compared with their respective contralateral knees ($n = 8$, 5 and 7, respectively; $^bP < 0.01$, Student's paired t-test). (B) The amounts of Evans blue extravasated in the test knees of these three groups of animals (after subtracted the amount of Evans blue extravasated in their respective contralateral knees) were not significantly different to each other ($P > 0.05$, Student's unpaired t-tests).

respective contralateral knees; $n = 6–8$) (Fig. 5B and C, respectively).

As illustrated in Fig. 6A, the Evans blue extravasation response to somatostatin (10 nmol) at 4 h was not significantly affected by treatments of the rat knees with 2×20 or 2×50 nmol cyclo-somatostatin. Thus, the amount of Evans blue extravasated in their test knees (after subtracted from their respective contralateral knees) were 48.94 ± 8.97 ($n = 7$), 44.74 ± 8.712 ($n = 5$) and 31.05 ± 10.11 μg/g tissue ($n = 6$) for somatostatin alone, somatostatin in the presence of 2×20 nmol and somatostatin in the presence of 2×50 nmol, respectively. These values show a trend of decrease in Evans blue extravasation in the presence of increasing doses of cyclo-somatostatin. However, statistical analysis on these values indicates that they are not significantly different to each other ($P > 0.05$ for all) (Fig. 6B).

4. Discussion

The present study demonstrates that somatostatin has vasodilator and plasma extravasation effects in the rat knee joint. The vasodilator effect is immediate and transient (< 5 min), requiring activation of somatostatin receptors that can be blocked by cyclo-somatostatin, but it does not involve nitric oxide or prostaglandins. Plasma extravasation occurs after 1 h and peaks at 2 h, which unlike the vasodilator response, is mediated by somatostatin receptor types that are not susceptible to inhibition by cyclo-somatostatin. The present findings indicate that somatostatin, although labeled as an "antiinflammatory" neuropeptide, may act in concert with proinflammatory neuropeptides to promote the innate reactions (vasodilatation and increase vascular permeability) that contribute to inflammatory processes.

A large proportion of primary afferent neurones produce neuropeptides that are transported to their sensory endings in the periphery and to their terminations in the spinal cord. The releases of some of these neuropeptides in the periphery produce plasma extravasation and/or vasodilatation as components of the innate reactions that support inflammatory processes in the tissue. Most attention on sensory neuropeptides has focused on substance P. The accumulated evidence suggests substance P serves both the afferent and efferent functions of sensory neurones: it mediates pain transmission when released from central nerve terminals, and it mediates neurogenic inflammation when released from the peripheral nerve terminals (see Ferrell and Lam, 1996).

Less is known about the functions of the neuropeptide somatostatin in primary afferent neurones. The matter is further confounded by inconsistent reports on spinal somatostatin levels in inflamed animals, some of which have demonstrated increased somatostatin levels (Ohno et al., 1990; Zhang et al., 1994), while others have reported no change (Smith et al., 1992; Hanesch et al., 1995). Nonetheless, it is known that noxious mechanical and thermal stimuli of the skin could evoke release of somatostatin into spinal cord of the cat (Duggan et al., 1988, Morton et al., 1989). Ionophoretic application of somatostatin onto dorsal horn neurones in spinal cord slices produces hyperpolarisation and reduces spontaneous firing of these neurones (Murase et al., 1982). Intrathecal application of somatostatin in high doses is antinociceptive (Gaumann and Yaksh, 1988). Furthermore, somatostatin reduces inflammation in animal models of arthritis (Matucci-Cerinic et al., 1995). This is echoed by clinical findings that showed somatostatin reduces pain and improves joint function in patients with osteoarthritis (Silveri et al., 1994; Fioravanti et al., 1995). Somatostatin is also reported to play a systemic antiinflammatory role when released from activated vagal capsaicin-sensitive nerve terminals of the rat and somatic nerves of guinea pig (Thán et al., 2000).

Therefore, the accumulated evidence indicates somatostatin has an antiinflammatory role. There is speculation that the antiinflammatory actions of somatostatin are important for balancing the effects of proinflammatory peptides such as substance P in normal tissues (Heppelmann and Pawlak, 1997). Such hypothesis is supported by the findings that showed somatostatin reduced the release of substance P from sensory afferent neurones and attenuated the vasodilatation and plasma protein leakage evoked by antidromic nerve stimulation (Gazelius et al., 1981; Holzer, 1992). Thus, presynaptic mechanisms are thought to be responsible for mediating the antiinflammatory effects and also the antinociceptive effects of somatostatin (Lembeck et al., 1982; Heppelmann and Pawlak, 1997; Carlton et al., 2001).

The haemodynamic actions of somatostatin are poorly documented and are conflicting. This is due, in part, to species and regional difference in blood flow responses and to variance in doses and in routes of administration of the peptide. Consequently, somatostatin has been reported to cause decrease (Cooper et al., 1981), increase (Dézsi et al., 1997, Pofahl et al., 1994) or no change (Tyden et al., 1979) in blood flow to various organs. Evidence from the present study indicates that somatostatin is an effective vasodilator in the rat knee joint. It produced vasodilatation with an ED_{50} value of 1.75 ± 0.14 nmol. At doses above 3 nmol, it also lowered the systemic blood pressure of the rat, indicating it is a depressor agent on the systemic circulation in this species. The vasodilator effect of somatostatin is immediate and short. Hence, it produced maximum vasodilatation ($109.72 \pm 9.58\%$) within the first minute after drug administration, and the response was subsided at 5 min. Similarly, a previous study showed that somatostatin produced decrease in portal blood pressure that lasted no more than 5 min in cirrhotic patients with portal hypertension (Cirera et al., 1995). Substance P also produced transient (< 5 min) vasodilator response in the rat knee joint with a mean maximum increase of $89.40 \pm 8.55\%$, and an ED_{50} of 1.73 ± 0.28 pmol (results not shown). Therefore, the patterns of the vasodilator response to substance P and somatostatin are similar, except that substance P is a much more potent vasodilator in the rat knee joint.

Mechanisms of action of somatostatin on blood vessels have been reported to be direct and indirect. In the latter instance, it has been proposed that somatostatin inhibits release of vasodilatory substance and, thus, indirectly causes vasoconstriction and decrease in blood flow (Szentivanyi et al., 1996). Direct action of somatostatin on blood vessels has been shown to involve endothelium-derived nitric oxide and prostaglandins (Dézsi et al., 1997; Tjen-A-Looi et al., 1992; Ancha et al., 2003). The present results indicate that somatostatin-induced vasodilatation in the rat knee joint is mediated by somatostatin receptors that are susceptible to inhibition by cyclo-somatostatin. However, a role for nitric oxide or prostaglandins in mediating the vasodilator response to somatostatin is not substantiated since the nitric oxide synthase inhibitor L-NAME and the cyclo-oxygenase inhibitor flurbiprofen, at doses that are known to inhibit their respective target enzymes (Lam and Yip, 2000), had no effect on somatostatin-induced vasodilatation.

Topical application of somatostatin onto exposed blood vessels of parietal cortex of rats has been shown to produce an Evans blue extravasation response, indicating a change in the vascular permeability (Long et al., 1992). The present study also demonstrated an increase in Evans blue extravasation (48.94 ± 8.97 μg/g tissue) following unilateral intraarticular injection of 10 nmol somatostatin into the rat knee joint, thus confirming somatostatin has the capacity to increase vascular permeability. Nevertheless, somatostatin produced no change in blood flow and in sizes of the rat knees at 1–4 h following intraarticular administration of the drug. This supports the notion that the vascular effects of somatostatin are short-lived. Similarly, intraarticular injection of 1 nmol substance P produced no change in blood flow or sizes of the rat knee joint, but increased Evans blue extravasation by 50.68 ± 28.74 μg/g tissue (results not shown). This indicates substance P is ten times more effective than somatostatin in increasing vascular permeability.

Heppelmann and Pawlak (1999) have reported inhibition of somatostatin-induced responses in sensory neurones by 20 nmol cyclo-somatostatin. However, the present study showed that cyclo-somatostatin at doses as high as 2×50 nmol had no effect on somatostatin-induced Evans blue extravasation. This does not necessarily imply that somatostatin receptors are not involved in mediating the plasma extravasation effect of somatostatin. Five types of somatostatin receptors have been identified (Patel et al., 1995), but the selectivity of cyclo-somatostatin on these receptors is unknown. Therefore, at present, it cannot be ruled out that a somatostatin receptor type(s) resistant to inhibition by cyclo-somatostatin could mediate the plasma extravasation effect of somatostatin.

In conclusion, somatostatin was found to elicit vasodilator and plasma extravasation effects in the rat knee joint similar to those produced by substance P. Somatostatin is much less potent than substance P in producing these vascular effects. Nevertheless, if sufficient amount of somatostatin were released from the articular sensory neurones, it is expected to mediate neurogenic inflammation along with substance P. In the long run, the proinflammatory effects of somatostatin would subside and its well-known antiinflammatory effects would prevail to limit the extent of the inflammatory reaction.

Acknowledgements

This research was supported by the Research Grants Council (RGC) of Hong Kong.

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