Mechanism underlying substance P-induced relaxation in dog isolated superficial temporal arteries

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1 In helical strips of dog superficial temporal arteries with intact endothelium, substance P elicited a concentration-related relaxation with an EC_{50} of $2.8 (2.4–3.2) \times 10^{-10}$ M.

2 The relaxant response to the peptide in low concentrations ($1–4 \times 10^{-10}$ M) sufficient to produce approximately half maximal relaxation was not inhibited by indomethacin, but was markedly suppressed by N^G -nitro-L-arginine (L-NOARG), a nitric oxide (NO) synthase inhibitor, and by endothelium denudation.

3 High concentration (10^{-7} M) of substance P produced marked relaxations in endothelium-intact strips. Removal of the endothelium attenuated the relaxation, and indomethacin or tranylcypromine suppressed the endothelium-independent relaxation. In indomethacin-treated strips with intact endothelium, L-NOARG attenuated but did not abolish the relaxation. The residual, L-NOARG-resistant relaxation was not significantly inhibited by ouabain, glibenclamide or tetraethylammonium.

4 Substance P (10^{-7} M) increased the levels of cyclic GMP and cyclic AMP. The increase in cyclic GMP was abolished by endothelium denudation and treatment with L-NOARG, whereas the cyclic AMP increment was abolished by indomethacin.

5 Three different mechanisms may be involved in the substance P-induced relaxation: (1) an endothelium-dependent relaxation mediated by the release of NO from the endothelium, resulting in an increase of cyclic GMP (low and high concentrations of the peptide); (2) an endothelium-independent relaxation in association with cyclic AMP increment caused by prostaglandin I_2 released from sub-endothelial tissues (high concentration), and (3) another endothelium-dependent relaxation possibly mediated by unidentified mediator(s) released from the endothelium (high concentration).

Keywords: Superficial temporal artery; endothelium; nitric oxide; substance P; prostaglandin I_2 ; EDRF

Introduction

Peptides, amines and other endogenous vasoactive substances are implicated in the pathophysiology of various forms of headache, local pain reactions and vasospasms following subarachnoid haemorrhage. It is postulated that one of the main causes of migraine headache is dilatation of extracranial arteries, such as superficial temporal arteries (Drummond & Lance, 1983; Olesen, 1991). Histochemical studies on extracranial arteries have demonstrated that there are abundant nerve fibres containing vasoactive peptides, such as substance P, calcitonin gene-related peptide, and vasoactive intestinal polypeptide (Uddman *et al.*, 1986; 1989; Edvinsson *et al.*, 1988; Uddman & Edvinsson, 1989), mediating vasodilatation of intra- and extra-cranial arteries.

Substance P dilates blood vessels mainly via a release of relaxing substances from the endothelium (Furchgott, 1983; Toda *et al.*, 1991). Goadsby *et al.* (1988) have reported a marked elevation of plasma level of substance P in external jugular venous blood during stimulation of the trigeminal ganglion, which contributes to the innervation of superficial temporal arteries and is involved in the modulation of cranial pain (Moskowitz, 1984). The peptide release may produce vasodilatation in extracranial arteries, including the superficial temporal artery, and lead to the provocation of vascular headache. Nevertheless, effects of substance P in superficial temporal arteries have not been clarified.

The present study was undertaken to analyse the mechanism of action of substance P in the dog superficial temporal arteries, with special reference to the endothelium, nitric oxide (NO) and prostaglandin I_2 (PGI_2). We found that the mechanism of action differ with low and high concentrations of the peptide, and endothelium-derived relaxing factor (EDRF), as distinct from NO, is partially involved in the substance P-induced relaxation.

Methods

Preparation

Mongrel dogs of either sex, weighing 7–15 kg, were anaesthetized with i.v. injections of sodium pentobarbitone (30 mg kg^{-1}) and killed by bleeding from the carotid arteries. Superficial temporal arteries were rapidly isolated. The arteries (0.6–1.5 mm o.d.) were cut helically into strips, approx. 15 mm long. The strips were fixed vertically between hooks in a muscle bath of 20 ml capacity containing the modified Ringer-Locke solution, which was maintained at $37 \pm 0.3^\circ\text{C}$ and aerated with a mixture of 95% O_2 and 5% CO_2 . The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-Koden Kogyo, Tokyo, Japan). The resting tension was adjusted to 1.5 g, which is optimal for inducing the maximal contraction. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.2, $MgCl_2$ 1.0 and dextrose 5.6. The pH of the solution was 7.35–7.41. Before the start of experiments, the arterial strips were allowed to equilibrate for 60–90 min in the control medium, during which time the solution was replaced every 10–15 min.

Tension recording

Isometric contractions and relaxations were displayed on an ink-writing oscillograph. Contractions induced by 30 mM K^+ were obtained first and then the strips were washed three times with fresh media. Relaxant responses to the agents were obtained in the strips partially contracted with $PGF_{2\alpha}$, the contraction being in a range between 20 and 35% of the contractions induced by 30 mM K^+ . At the end of each series of experiment, 10^{-4} M papaverine was added to attain the maximal relaxation. Since tachyphylaxis to substance P rapidly developed, the peptide (10^{-7} M) was applied

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repeatedly until the response was stabilized. Then, substance P in a single concentration (10^{-10} – 10^{-7} M) was applied to obtain the concentration-response relationship. In some experiments, substance P, sodium nitroprusside and NO in single concentrations were applied successively. The relaxant response to the drug was expressed as the relative value to that induced by papaverine. After the responses to the agents were stabilized, the effects of blocking agents were examined by treatment of strips for 20 min or longer. In other experiments, one to three pairs of strips with and without the endothelium were obtained from each dog, and paired comparisons were made in the responses of endothelium-intact and -denuded strips. The endothelium was removed by gently rubbing the intimal surface with a cotton ball. The endothelium denudation was confirmed by the abolition or marked suppression of the relaxation produced by 10^{-6} M acetylcholine, and also by silver staining procedure (Abrol *et al.*, 1984).

Measurement of cyclic AMP and cyclic GMP

Contents of adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP) in dog superficial temporal artery strips with and without the endothelium were measured. After 20 min of equilibration in the control bathing medium, and that containing indomethacin or N^G -nitro-L-arginine (L-NOARG), the strips were exposed to 10^{-7} M substance P, then plunged into liquid nitrogen. The tissues were homogenized in 1.5 ml of 6% trichloroacetic acid at 0°C with a glass homogenizer. After centrifugation at 800 g for 10 min, an ether extraction procedure was carried out on the supernatant. An aliquot of the extract was then used for determination of cyclic AMP and cyclic GMP by the use of commercial radioimmunoassay kit (Yamasa Co., Chiba, Japan).

Statistics and drugs used

The results shown in the text and figures are expressed as mean values $\pm$ s.e.mean. EC_{50} is expressed as geometric means with 95% confidence limits. Statistical analyses were made with Student's unpaired *t* test and Tukey's method after one-way analysis of variance. Drugs used were substance P, N^G -nitro-L-arginine (L-NOARG), N^G -nitro-D-arginine (D-NOARG) (Peptide Institute Inc., Minoh, Japan), L- and D-Arginine (L- and D-Arg), tetraethylammonium bromide, tranilcypromine hydrochloride (Nacalai Tesque, Kyoto, Japan), indomethacin, glibenclamide (Sigma, St. Louis, MO., U.S.A.), sodium nitroprusside, ouabain octahydrate (Merck, Darmstadt, Germany), beraprost sodium (Toray-Kaken Pharmaceutical Co., Tokyo), prostaglandin $F_{2\alpha}$ (Ono Co., Osaka, Japan), papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka), and acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo). Responses to nitric oxide (NO) were obtained by adding the $NaNO_2$ solution adjusted to pH 2 (Furchgott, 1988).

Results

Mechanical responses to substance P

The addition of substance P (10^{-10} – 10^{-7} M) produced a concentration-related relaxation in dog superficial temporal artery strips partially contracted with $PGF_{2\alpha}$; the EC_{50} was 2.8 (2.4 – 3.2) $\times 10^{-10}$ M ($n = 6$) (Figure 1, left panel). Removal of the endothelium markedly inhibited the relaxant response to the peptide in concentrations from 1 to 4×10^{-10} M, and moderately attenuated the response to the higher concentrations. The EC_{50} of the substance P-induced relaxations in endothelium-denuded strips was 1.3 (1.0 – 1.7) $\times 10^{-9}$ M ($n = 6$). Substance P in low concentra-

Figure 1 Modification by endothelium denudation (left panel), indomethacin (10^{-6} M) and indomethacin plus N^G -nitro-L-arginine (L-NOARG, 3×10^{-4} M) (right panel) of relaxant responses to substance P in dog superficial temporal artery strips partially contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3×10^{-7} M). Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values at 10^{-10} M substance P in the strips with (●) and without the endothelium (○) were 240 ± 11 and 196 ± 21 mg ($n = 6$), respectively (left panel) and those in control arterial strips (●), treated with indomethacin (○) and indomethacin plus L-NOARG (Δ) were 230 ± 26 , 200 ± 19 and 248 ± 38 mg ($n = 5$), respectively (right panel). There were no significant differences in the size of the relaxant response to 10^{-4} M papaverine in each concentration of substance P (10^{-10} , 2×10^{-10} , 5×10^{-10} , 2×10^{-9} , 10^{-8} and 10^{-7} M). Left panel; significantly different from the values in endothelium-intact arteries. ^a $P < 0.01$; ^b $P < 0.05$. Right panel; significantly different from control, ^a $P < 0.01$; ^b $P < 0.05$. Significantly different from the value treated with indomethacin, ^c $P < 0.01$; ^d $P < 0.05$ (Tukey's method). Values are means $\pm$ s.e.mean.

tions produced only endothelium-dependent relaxation, whereas the peptide in concentrations higher than 4×10^{-10} M produced both endothelium-dependent and -independent relaxations. Modifications by indomethacin (10^{-6} M) and L-NOARG (3×10^{-4} M) of the concentration-related relaxant responses to substance P in the endothelium-intact strips are shown in right panel of Figure 1. In endothelium-intact artery strips, indomethacin did not influence the relaxant responses, but additional treatment with 3×10^{-4} M L-NOARG markedly suppressed the relaxant responses to the peptide in low concentrations, and moderately attenuated the response to the higher concentrations. Indomethacin (10^{-6} M) or L-NOARG (3×10^{-4} M) slightly increased the basal tone and augmented the contractile response to $PGF_{2\alpha}$. However, the preconstriction levels were adjusted to a magnitude similar to that in control media by decreasing the amount of $PGF_{2\alpha}$ applied. In the remainder of this paper, mechanisms of the substance P-induced relaxation were analysed separately by using low (1 – 4×10^{-10} M, producing approximately half maximal relaxation) and high (10^{-7} M) concentrations.

In endothelium-intact artery strips partially contracted with $PGF_{2\alpha}$, substance P (1 – 4×10^{-10} M) and sodium nitroprusside (SNP, 3×10^{-8} M) produced moderate relaxations (Figure 2). Indomethacin (10^{-6} M) did not alter the relaxation, but additional treatment with L-NOARG (10^{-4} M) abolished the peptide-induced relaxation, whereas the response to SNP was not inhibited. L-NOARG inhibited the peptide-induced relaxation in a concentration-dependent manner; mean values of the relaxation relative to that caused by 10^{-4} M papaverine in control media and those containing 10^{-6} M indomethacin and indomethacin plus 10^{-5} or 10^{-4} M L-NOARG were $47.3 \pm 2.6\%$ ($n = 17$), $44.9 \pm 2.5\%$ ($n = 23$), $10.4 \pm 1.9\%$ ($P < 0.01$ vs. control and indomethacin, $n = 18$) and $1.8 \pm 0.9\%$ ($P < 0.01$ vs. control, indomethacin and indomethacin plus 10^{-5} M L-NOARG, $n = 9$), respectively.

Figure 2 Typical recordings of the response to substance P (SP, 2×10^{-10} M) and sodium nitroprusside (SNP, 3×10^{-8} M) of dog superficial temporal artery strips with and without the endothelium obtained from the same dog. Responses of the endothelium-intact strip were obtained before (control) and after treatment with 10^{-6} M indomethacin or indomethacin plus 10^{-4} M N^G-nitro-L-arginine (L-NOARG). The strips were partially contracted with prostaglandin F_{2α} (PGF_{2α}, $2-5 \times 10^{-7}$ M); the level before the addition of PGF_{2α} is shown as a horizontal line just left of each tracing. Pap represents 10^{-4} M papaverine that produced maximal relaxation.

D-NOARG was without effect; mean values of the relaxation in control media and those containing 10^{-5} and 10^{-4} M D-NOARG were $44.5 \pm 5.2\%$, $40.3 \pm 6.6\%$, and $37.3 \pm 8.3\%$ ($n=6$), respectively. The inhibitory effect of 10^{-5} M L-NOARG was reversed by the addition of 3×10^{-3} M L-arginine (L-Arg) but not by 3×10^{-3} M D-Arg; mean values of the relaxation in control media and those containing 10^{-5} M L-NOARG, 10^{-5} M L-NOARG plus 3×10^{-3} M D-Arg and 10^{-5} M L-NOARG plus 3×10^{-3} M L-Arg were $42.3 \pm 4.4\%$, $4.2 \pm 1.4\%$ ($P < 0.01$ vs. control), $0.8 \pm 0.4\%$ ($P < 0.01$ vs. control) and $29.5 \pm 4.5\%$ ($P < 0.05$ vs. control, $P < 0.01$ vs. 10^{-5} M L-NOARG) ($n=4$), respectively. Removal of the endothelium abolished the relaxation caused by substance P in low concentrations (Figure 2, lower); mean values of the relaxation in endothelium-intact and -denuded strips were $45.1 \pm 3.8\%$ and $1.9 \pm 1.4\%$ ($P < 0.01$, $n=9$), respectively.

The addition of substance P (10^{-7} M) produced marked relaxations in the endothelium-intact artery strips. Typical responses to substance P and NO are illustrated in the upper tracing of Figure 3. Treatment with indomethacin (10^{-6} M) did not affect the magnitude of the relaxation, but accelerated the recovery. Additional treatment with 10^{-4} M L-NOARG significantly suppressed but did not abolish the relaxation. Relaxations caused by exogenous NO were not influenced by indomethacin and L-NOARG. Increase in the concentration of L-NOARG to 3×10^{-4} M did not produce the additional inhibition; mean values of the relaxation in control media and those containing 10^{-6} M indomethacin and indomethacin plus 10^{-5} M, 10^{-4} M or 3×10^{-4} M L-NOARG were $75.3 \pm 2.0\%$ ($n=29$), $74.9 \pm 2.6\%$ ($n=31$), $44.1 \pm 7.4\%$ ($P < 0.01$ vs. control and indomethacin, $n=15$), $43.6 \pm 4.3\%$ ($P < 0.01$ vs. control and indomethacin, $n=31$)

Figure 3 Typical tracings of the response to substance P (SP, 10^{-7} M) and nitric oxide (NO, 10^{-6} M) of dog superficial temporal artery strips with and without the endothelium. Responses of the endothelium-intact strips were obtained before (control) and after treatment with 10^{-6} M indomethacin or indomethacin plus 10^{-4} M N^G-nitro-L-arginine (L-NOARG) (upper tracing). Responses of the endothelium-denuded strips were obtained before (control) and after treatment with indomethacin (lower tracing). The strips were partially contracted with prostaglandin F_{2α} (PGF_{2α}, $2-5 \times 10^{-7}$ M); the level before the addition of PGF_{2α} is shown as a horizontal line just to the left of each tracing. Pap represents 10^{-4} M papaverine that produced maximal relaxation.

and $40.7 \pm 4.2\%$ ($P < 0.01$ vs. control and indomethacin, $n=23$), respectively. The inhibitory effect of 10^{-5} M L-NOARG was reversed by the addition of 3×10^{-3} M L-Arg but not by D-Arg ($n=3$, data not shown). The residual relaxation under combined treatment with indomethacin and 3×10^{-4} M L-NOARG was not significantly influenced by 10^{-6} M ouabain (from $47.2 \pm 5.4\%$ to $43.3 \pm 5.9\%$, $n=8$), 10^{-6} M glibenclamide (from $55.0 \pm 8.7\%$ to $42.7 \pm 11.5\%$, $n=5$), 10^{-3} M (from $39.8 \pm 9.5\%$ to $49.0 \pm 7.4\%$, $n=5$) or 10^{-2} M tetraethylammonium (from $40.1 \pm 4.8\%$ to $29.5 \pm 6.2\%$, $n=8$). Although, ouabain and tetraethylammonium *per se* evoked a contraction and augmented the response to PGF_{2α}, the precontraction level was matched to that in the absence of the inhibitors. On the other hand, glibenclamide had no effect on the basal tone nor the contractile response to PGF_{2α}. In the endothelium-denuded artery strips, substance P produced moderate relaxation, which was significantly less than the relaxation in endothelium-intact strips (Figure 1, left). The relaxation in the strips without endothelium was abolished by treatment with 10^{-6} M indomethacin (Figure 3, lower) and suppressed by 3×10^{-4} M tranylcypromine; mean values of the relaxation in control media and those containing 10^{-6} M indomethacin and 3×10^{-4} M tranylcypromine were $48.7 \pm 2.4\%$ ($n=28$), $3.9 \pm 0.7\%$ ($P < 0.01$ vs. control, $n=24$) and $24.4 \pm 6.2\%$ ($P < 0.01$ vs. control, $n=12$), respectively. Tranylcypromine in the same concentration attenuated the relaxant response to beraprost, a stable analogue of PGI₂ (Toda, 1988), in concentrations of $1-3 \times 10^{-8}$ M, sufficient to produce similar magnitude of relaxation to those elicited by substance P; however, the inhibition ($30.9 \pm 10.2\%$, $n=12$) was significantly less than that induced by substance P ($53.8 \pm 8.6\%$ inhibition, $n=12$; $P < 0.05$).

Cyclic AMP and cyclic GMP levels

The addition of 10^{-7} M substance P significantly increased the content of cyclic AMP and cyclic GMP in the endothelium-intact artery strips. The peak effect on cyclic GMP was attained 1 min later ($94.3 \pm 28.1\%$ increase, $n=6$; $P < 0.01$) and the plateau of cyclic AMP increment was obtained from 30 s to 2 min ($80.0 \pm 22.6\%$, $n=5$, $P < 0.01$).

Therefore, contents of cyclic AMP and cyclic GMP were measured 1 min after the peptide application in the same samples from different individuals.

In endothelium-intact and denuded artery strips, basal levels of cyclic AMP averaged 166.6 ± 26.3 ($n = 7$) and 112.5 ± 20.5 fmol mg^{-1} frozen tissue ($n = 5$), respectively. Substance P (10^{-7} M) significantly increased the cyclic AMP levels in both strips (Figure 4). The stimulating effect was not influenced by treatment with 5×10^{-6} M L-NOARG, but was abolished by 10^{-6} M indomethacin. Indomethacin alone decreased the basal level of cyclic AMP in endothelium-intact artery strips ($11.4 \pm 3.8\%$ decrease $n = 7$; $P < 0.05$).

The level of cyclic GMP in endothelium-intact artery strips (255.8 ± 43.8 fmol mg^{-1} frozen tissue, $n = 7$) was increased by $149.7 \pm 14.6\%$ following treatment with 10^{-7} M substance P. L-NOARG (3×10^{-4} M) significantly decreased the basal level of cyclic GMP ($89.2 \pm 2.5\%$ decrease, $n = 7$; $P < 0.01$)

Figure 4 Effect of substance P (SP, 10^{-7} M) in the absence and presence of N^G-nitro-L-arginine (L-NOARG, 5×10^{-6} M) or indomethacin (IM, 10^{-6} M) on the cyclic AMP formation in dog superficial temporal artery strips with the endothelium (E) (left panel) and without the endothelium (E) (right panel). Basal levels (C) of the nucleotide in endothelium-intact and -denuded strip were 166.6 ± 26.3 ($n = 7$) and 112.5 ± 20.5 ($n = 5$) fmol mg^{-1} frozen tissue, respectively. Significantly different from control, ^a $P < 0.01$; ^b $P < 0.05$ (Tukey's method). Columns show mean values with s.e.mean.

Figure 5 Effect of substance P (SP, 10^{-7} M) in the absence and presence of N^G-nitro-L-arginine (L-NOARG, 3×10^{-4} M) on the cyclic GMP formation in dog superficial temporal artery strips with the endothelium (E) (left panel); and effect of the peptide in the absence and presence of L-NOARG (5×10^{-6} M) or indomethacin (IM, 10^{-6} M) on the cyclic GMP formation in the strips without the endothelium (right panel). Basal levels (C) of the nucleotide in the endothelium-intact and -denuded strips were 255.8 ± 43.8 ($n = 7$) and 24.2 ± 9.9 ($n = 4$) fmol mg^{-1} frozen tissue, respectively. Significantly different from control, ^a $P < 0.01$; and from the value treated with substance P, ^b $P < 0.01$ (Tukey's method). Columns show mean values with s.e.mean.

and abolished the stimulating effect of substance P on the cyclic GMP level. Removal of the endothelium decreased the cyclic GMP level to 24.2 ± 9.9 fmol mg^{-1} frozen tissue ($90.5 \pm 2.7\%$ decrease, $n = 4$; $P < 0.01$) and abolished the effect of substance P (Figure 5).

Discussion

The present study revealed that low concentrations of substance P elicited only an endothelium-dependent relaxation in dog isolated superficial temporal arteries, which was not influenced by treatment with indomethacin but was abolished by L-NOARG, an NO synthase inhibitor (Moore *et al.*, 1990; Toda *et al.*, 1990). These findings suggest that the peptide releases NO from the endothelium that acts on smooth muscle to increase the production of cyclic GMP, responsible for a muscle relaxation. Similar results were also obtained in monkey temporal arteries (Toda *et al.*, 1991), dog cerebral arteries (Onoue *et al.*, 1988; Toda & Okamura, 1991) and various arteries from different species (Zawadzki *et al.*, 1981; Furchgott, 1983).

High concentrations of substance P produced endothelium-dependent and -independent relaxations. The addition of 10^{-7} M substance P elicited a marked relaxation in endothelium-intact artery strips. Indomethacin did not influence the relaxation, and additional treatment with L-NOARG suppressed the relaxation. In endothelium-denuded artery strips, substance P (10^{-7} M) produced a moderate relaxation, which was abolished by treatment with indomethacin or markedly suppressed by tranylcypromine, a PGI₂ synthase inhibitor (Gryglewski *et al.*, 1970). Substance P increased the cyclic AMP level in endothelium-intact and denuded strips, the increase being abolished by indomethacin. Therefore, endothelium-independent relaxations observed in the arteries stimulated by the peptide may be produced by vasodilator prostaglandins, possibly PGI₂, that are released from subendothelial tissues and are responsible for cyclic AMP formation.

On the other hand, endothelium-dependent relaxation caused by a high concentration of substance P in endothelium-intact strips treated with indomethacin was partially inhibited but not abolished by L-NOARG even when the arteries were treated with high concentrations (up to 3×10^{-4} M). However, the stimulating effect of the peptide on the production of cyclic GMP was completely abolished by 3×10^{-4} M L-NOARG. Therefore, the relaxation appears to be associated partly with endothelium-derived NO, as observed in the arteries stimulated by low concentrations of substance P, and also with a mechanism other than that via NO and cyclic GMP. Similar responses, resistant to NO synthase inhibitors, have been reported in porcine coronary (Richard *et al.*, 1990; Cowan & Cohen, 1991; Matsumoto *et al.*, 1993), human gastropiploic (O'Neil *et al.*, 1991) and coronary arteries (Chester *et al.*, 1990) and canine renal (Pawloski & Chapnick, 1991) and femoral veins (Miller, 1991) in response to substance P, bradykinin, leukotriene D₄, acetylcholine, ADP or thrombin. In the present study, the peptide-induced relaxation resistant to L-NOARG, was not influenced by ouabain, glibenclamide and tetraethylammonium, suggesting that the electrogenic sodium pump, ATP-activated K⁺ channel and voltage-dependent K⁺ channel are not involved. The inability of substance P to liberate endothelium-derived hyperpolarizing factors from guinea-pig small mesenteric arteries has been demonstrated by Bolton & Clapp (1986). On the other hand, Vanhoutte and coworkers have suggested the involvement of sodium pump activation and K⁺ channel opening, because relaxations of canine coronary arteries induced by arachidonic acid and acetylcholine are susceptible to ouabain and glibenclamide (Rubanyi & Vanhoutte, 1985; Feletou & Vanhoutte, 1988; Hoeffner *et al.*, 1989).

Figure 6 Schematic presentation of possible mechanisms of action of substance P in dog superficial temporal artery strips. SP, substance P; NO, nitric oxide; PGI₂, prostaglandin I₂; X, unknown mediator(s); Squares in the endothelium and smooth muscle represent receptors.

Figure 6 is a hypothetical diagram on the mechanism of action of substance P in dog superficial temporal arteries. The peptide acts (1) on receptors in the endothelium to promote the synthesis and release of NO, which activates soluble guanylate cyclase in smooth muscle, resulting in the increase of cyclic GMP responsible for an endothelium-dependent relaxation; (2) on smooth muscle to synthesize and release PGI₂, which generates cyclic AMP in smooth muscle

and produces an endothelium-independent relaxation; and (3) on the endothelium to produce another endothelium-dependent relaxation by releasing relaxing factor(s) distinct from NO ('X' in Figure 6), which does not appear to be mediated by cyclic nucleotides nor associated with sodium pump activation or K⁺ channel opening. Substance P is one of the important vasodilator substances which are thought to be involved in the modulation of cranial vessel tone.

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