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Activation of thromboxane receptors and the induction of vasomotion in the hamster cheek pouch microcirculation.

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1 The present study was designed to investigate a possible role of thromboxane A_2 (TXA_2) on arteriolar vasomotion (spontaneous rhythmic variations of the vessel diameter). Therefore the microcirculatory effects of the thromboxane-receptor (TP-receptor) agonist, U 46619, as well as the effects of the TP-receptor antagonists S 17733 and Bay U3405 were evaluated in the hamster cheek pouch microcirculation. For comparison some effects of angiotensin II were also investigated.

2 For microcirculatory measurements, the cheek pouch preparation was placed under an intravital microscope coupled to a closed circuit TV system. The TV monitor display was used to obtain arteriolar internal diameter measurements by means of an image shearing device.

3 Superfusion (0.1 nM to 1 μM) or bolus application (1 pmol to 10 nmol) of U 46619 concentration- or dose-dependently decreased the arteriolar diameter and induced vasomotion in arterioles with a mean initial diameter of 24 ± 2 μm. Both the vasoconstriction and the vasomotion induced by U 46619 were inhibited by the TP-receptor antagonists S 17733 (100 mg kg^{-1}, i.v.) and Bay U3405 (10 mg kg^{-1}, i.v.).

4 Bolus applications of angiotensin II (0.1 pmol to 1 nmol) induced transient vasoconstriction followed by vasodilatation in the cheek pouch arterioles. The dilatation but not the constriction, was sensitive to treatment with the NO-synthase inhibitor N^{ω}-nitro-L-arginine (L-NOARG; 100 μM). Angiotensin II did not induce vasomotion in control conditions or in the presence of L-NOARG.

5 Bolus application of phenylephrine (10 pmol) induced vasoconstriction but no vasomotion in previously quiescent hamster cheek pouch arterioles.

6 These results indicate that activation of TP-receptors causes vasomotion in the hamster cheek pouch arterioles. These spontaneous rhythmic variations in arteriolar diameter are not observed with equipotent doses of angiotensin II and phenylephrine. Thus, the vasoconstriction by itself cannot explain the occurrence of vasomotion observed with the TP-receptor agonist.

Keywords: Hamster cheek pouch; arteriolar diameter; vasomotion; TP-receptor agonist; TP-receptor antagonist; angiotensin II; N^{ω}-nitro-L-arginine

Introduction

Microcirculatory haemodynamics exhibit rhythmic fluctuations of blood flow and haematocrit observed with laser Doppler flowmetry in clinical investigations of the human skin (Bollinger *et al*, 1991) and in precapillary vessels of several tissues in experimental animals, including the hamster cheek pouch (Bouskela & Grampp, 1992; Bouskela & Cyrino, 1994). This phenomen is due to vasomotion, the periodic constriction and relaxation of the arterioles, which manifests itself in related effects termed 'flow motion' and 'flux motion' (Seifert *et al*., 1988). The mechanisms that either cause or control these diameter oscillations are still not well understood but may be related to a delicate balance between membrane currents (Bouskela & Grampp, 1992). Moreover, the possible functional importance of vasomotion in local haemodynamics has not yet been clarified. Some authors have hypothesized that vasomotion may contribute to local regulation of blood flow (Funk *et al*., 1983; Slaaf *et al*., 1987). However, others have focused attention on the possibility that vasomotion may influence fluid exchange at the capillary level, via modulation of pressure and flow values (Mayrovitz *et al*., 1977).

Arachidonic acid (AA) metabolites from either the cyclo-oxygenase or the lipo-oxygenase pathway may alter vascular tone as well as neutrophil and platelet function, which in turn may produce an inflammatory response with concomitant oedema (Vane & Botting, 1992). The cyclo-oxygenase product, thromboxane A_2 (TXA_2) is of particular interest because it is produced by both polymorphonuclear leukocytes and platelets (Hamberg *et al*, 1975; Needleman *et al*., 1986; Cross & Dickinson, 1991; Vane & Botting, 1992); it is a powerful platelet activator and vasoconstrictor which is potentially implicated in thrombotic, bronchopastic and renal diseases (see Misra, 1994). These actions of TXA_2 occur through activation of specific membrane receptors (TP-receptors) present on the target cells; different subtypes of TP-receptors have recently been cloned and were shown to belong to the family of seven transmembrane spanning, G-protein coupled receptors (Hirata *et al*., 1991; Kinsella *et al*., 1994; D'Angelo *et al*., 1994; Raychowdary *et al*., 1994).

Although data, describing the implication of TXA_2 in microcirculatory responses such as adhesion, leakage or constriction are available (see Goldman *et al*., 1991; Mayhan *et al*., 1991; Stücker *et al*., 1996), the precise role of TXA_2 and its possible involvement in vasomotion are not well understood. In one study on the basilar artery of the rat, the TP-receptor agonist U 46619 at 10 μM increased the vasomotion or induced it in previously quiescent tissues; this effect was shared with that of other vasoconstrictors such as 5-hydroxytryptamine and phenylephrine (Fujii *et al*, 1990).

The goals of the present study were to investigate the effects of the TP-receptor agonist, U 46619, on vasoreactivity in the hamster cheek pouch microcirculation. By use of techniques described previously (Bouskela & Grampp, 1992), the effects of U 46619 were studied with special attention to the development of vasomotion. The ability of TP-receptor antagonists (S 17733 and Bay U3405) to interfere with the action of U 46619 was also analysed. In order to distinguish between vasomotion and vasoconstriction, some comparative studies with angiotensin II and phenylephrine were also performed.

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Methods

Cheek pouch preparation

Experiments were performed on the cheek pouch preparation of male hamsters (Mesocricetus auratus, Harlan CPB, Austerlitz, Holland), weight between 90 and 130 g. Anaesthesia was induced by an intraperitoneal injection of 0.1–0.2 ml of sodium pentobarbitone (Pentobarbital sodium, Sanofi, France, 60 mg ml^{-1}) and maintained with α-chloralose (1,2-O-(2,2,2-(trichloroethylidene) α-D-glucofuranose; Merck, Darmstadt, Germany, 100 mg kg^{-1}) administered through the femoral vein. Throughout the surgery and the subsequent experiment, the temperature of the animals was kept at 37.0°C with a heating pad controlled by a rectal thermistor (LB 750 Uppsala Process Data AB, Sweden). A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a microscope stage similar to that described by Duling (1973) with minor modifications (Bouskela & Grampp, 1992). The cheek pouch was gently everted and pinned with 4–5 needles into a circular well filled with silicone rubber to provide a flat bottom layer, thus avoiding stretching of the tissue, but preventing shrinkage. In this position, the pouch was submerged in a superfusion solution that continuously flushed the pool of the microscope stage. Before the pouch was pinned, large arterioles and venules were located with the aid of a Zeiss binocular stereomicroscope. In order to produce a single-layer preparation, an incision was made in the upper layer so that a triangular flap could be displaced to one side. The exposed area was dissected at 10–16X magnification under the stereomicroscope, and the fibrous, almost avascular, connective tissue converging the vessels was removed by using ophthalmic instruments. The dissected part of the pouch was 125 to 150 μm thick. Dissected pouches with petechial haemorrhages and those without blood flow in all vessels were discarded.

The superfusion solution was a HEPES-supported HCO^{-}_{3}-buffered saline solution (composition in mM: NaCl 110.0, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18.0, HEPES 15.39 and HEPES Na^{+}-salt 14.61) whose temperature was maintained at 37.0°C, and the superfusion rate was 4 ml min^{-1}. The pH was set to 7.40 by bubbling the solutions continuously with 5% CO_2 in 95% N_2.

For measurements of microvascular variables, the preparation was placed under an intravital microscope (Ergolux, Leitz) coupled to a closed circuit TV system, where it was allowed to rest for 30 min (stabilization period). A Sony CCD video camera sends the image to a video recorder (Sony Umatic VO 5800 PS) and to a video monitor (Sony). After the stabilization period, internal diameter measurements of selected arterioles were performed by use of an image shearing monitor (IPM model 907). The diameter changes as well as eventual vasomotion were recorded on to a chronolog recorder. Vasomotion was analysed as previously described by Bouskela & Grampp (1992) and it was defined both in terms of frequency, expressed in cycles min^{-1} and amplitude, expressed in μm. From the videotape recordings, continuous measurements of the internal diameter of selected arterioles were obtained. Time averages of the vessel diameter and, if vasomotion was present, of its frequency and amplitude could then be inferred from the whole length of each diameter recorded (3 min in each case). If present, vasomotion normally causes the vessel diameter to oscillate quite symmetrically around a stable average value. The latter could therefore be assessed by simply computing the means of maximum and minimum diameter readings. In all experiments, the vasomotion amplitude was defined between average maximal and minimal vessel diameter and the vasomotion frequency as the inverse of the average cycle length, as could be determined from each diameter recording.

Pharmacological agents

For the experiments, the following drugs were used: U 46619 (9-11-dideoxy-11α, 9α epoxy methanoprostaglandin $F_{2\alpha}$; Sigma), phenylephrine (Sigma), angiotensin II (Sigma), N^{ω}-nitro-L-arginine (L-NOARG; Sigma), Bay U3405 ((3**R**)-3-(4-fluorophenyl-sulfonamido)-1, 2, 3, 4-tetrahydro-9-carbazolepropanoic acid) and S 17733 (prepared by Dr G. Lavielle, Servier Research Institute). S 17733 is the sodium salt of 5Z-7-[2-(4-chlorophenyl sulphonylamimoethyl)-indane-2-yl]heptane-4-oic.

The TP-receptor agonist U46619 was either applied topically, added to the superfusion solution (0.1 ml min^{-1}) to reach a final concentration of 0.1 nM to 1 μM or injected as a bolus into the perfusion circuit (final doses: 1 pmol to 10 nmol). Dose-response curves to U 46619, given by topical perfusion, were constructed by increasing the concentration of the agonist stepwise every 15 min (cumulative dose-response curve).

The TP-receptor antagonists (Bay U3405, S 17733) were injected into the femoral vein (0.5 ml 100 g^{-1} body weight) 15 min before the application of the agonist to reach the final concentrations indicated in the results.

The NO synthase inhibitor, L-NOARG, was added to the superfusion solution to a final concentration of 100 μM.

Angiotensin II was added to the superfusion solution as a bolus of 20 μl in doses of 0.1 pmol to 1 nmol.

Phenylephrine was added to the superfusion solution as a bolus of 20 μl at a dose of 10 pmol.

Agonists, when added as a bolus, elicited responses that terminated within 10 min after their administration. A minimum of 15 min was allowed between two bolus injections.

Study design

In the first part of the study, 22 arterioles (a larger and a smaller one from each animal) obtained from 11 hamsters were studied for the occurrence of spontaneous vasomotion and for the effects of topical application of increasing concentrations of U 46619. The remainder of the study was performed on previously quiescent arterioles only.

The effects of bolus injections with U 46619 were studied in further groups of animals: control studies (9 arterioles from 5 hamsters); in the presence of S 17733 (one group of 7 arterioles from 4 hamsters and a second group of 6 arterioles from 3 hamsters); in the presence of Bay U3405 (8 arterioles from 4 hamsters).

The effects of angiotensin II were studied in two groups of animals (8 arterioles from 4 hamsters each). The effect of phenylephrine was studied in a further group of 6 hamsters (12 arterioles).

Statistical analysis

Results are expressed as mean ± s.e.mean, unless otherwise stated. To verify the significance of the changes observed, the data presented in the tables were analysed by use of the repeated measures ANOVA followed by the Dunnett's multiple comparison test, except for the comparison with zero in Table 2, for which Student's *t* test for paired observations was used. The data with the S 17733 were analysed with Student's *t* test for unpaired observations. *P* values smaller than 0.05 were considered to be significant.

Results

Spontaneous arteriolar vasomotion

Twenty two arterioles from 11 hamsters (107 ± 3 g) were studied for the occurrence of spontaneous vasomotion. In 13 of these arterioles (obtained from 7 hamsters), mean internal diameter 25 ± 3 μm, no spontaneous vasomotion could be recorded. In 9 arterioles (obtained from 5 hamsters), mean internal diameter 24 ± 2 μm, spontaneous rhythmic contractions and dilatations could be observed with a mean frequency of 3.5 ± 1.1 cycles min^{-1} and mean amplitude of

2.0 ± 0.4 μm. In these preparations, spontaneous vasomotion could be observed in small arterioles (mean internal diameter 17 ± 2 μm) and larger ones (mean internal diameter 31 ± 2 μm).

Arteriolar vasomotion induced by the TP-receptor agonist U 46619

Topical application of increasing concentrations of U 46619 In all 22 arterioles described above, topical application of U46619 in increasing concentrations (0.1 nM to 1 μM) caused concentration-dependent decreases in diameter (Table 1). The TP-receptor agonist induced or augmented vasomotion in small and in large arterioles (Table 1). In previously quiescent arterioles U 46619 induced vasomotion whereas it augmented the amplitude of the oscillations in arterioles which already showed spontaneous vasomotion (Table 2). A typical example of the induction of vasomotion by U 46619 is shown in Figure 1. The induction of vasomotion by U 46619 seemed independent of the degree of constriction caused by the agent (Tables 1 and 2).

Vasomotion induced by bolus injection of U 46619 These studies were only performed in previously quiescent arterioles.

Control studies In 9 quiescent arterioles from 5 hamsters (90 ± 8 g) with a mean internal initial diameter of 24 ± 3 μm, bolus injections of U46619 in increasing concentrations (10 pmol to 10 nmol) into the superfusion solution caused dose-dependent decreases in mean internal arteriolar diameter and induced vasomotion (Figure 2). At 1 nmol, a concentration subsequently used for pharmacological evaluation, the mean internal diameter decreased by $47 \pm 4\%$ of its initial value and vasomotion occurred with a mean frequency of 3.8 ± 0.4 cycles min^{-1} and a mean amplitude of 3.5 ± 0.3 μm (Figure 2).

Effect of TP-receptor antagonists In 7 quiescent arterioles from 4 hamsters (96 ± 2 g) with a mean initial internal diameter of 27 ± 3 μm, the i.v. injection of 10 mg kg^{-1} of the TP-receptor antagonist S 17733, 15 min before the U 46619 injection, did not significantly change the mean internal diameter (26 ± 4 μm) and did not induce vasomotion. S 17733 partially inhibited the U 46619-induced constriction but only modestly reduced the rhythmic oscillations (Figure 3a). In 6 quiescent arterioles from 3 hamsters (109 ± 9 g) with a mean initial internal diameter of 28 ± 4 μm, the i.v. injection of 100 mg kg^{-1} S 17733, 15 min before the bolus of U 46619 (1 nmol) did not change the mean internal diameter (29 ± 3 μm) or induce vasomotion. At this dose, S 17733 completely prevented the vasoconstriction and the induction of vasomotion by the TP-receptor agonist, U 46619 (Figure 3b).

In 8 quiescent arterioles from 4 hamsters (121 ± 1 g) with a mean initial internal diameter of 28 ± 4 μm, i.v. injection of the TP-receptor antagonist Bay U3405 10 mg kg^{-1}, 15 min before the bolus of U 46619 (1 nmol) did not change the mean internal diameter (25 ± 5 μm) or induce vasomotion. As shown in Table 3, Bay U3405 completely prevented the vasoconstriction (from $42 \pm 5\%$ to $6 \pm 3\%$; $P < 0.05$) and the induction of vasomotion (frequency from 2.3 ± 0.8 to 0.4 ± 0.4 cycles min^{-1}, $P < 0.05$; amplitude from 1.6 ± 0.5 to 0.2 ± 0.2 μm, $P < 0.05$) caused by the TP-receptor agonist. U 46619-induced responses slightly returned toward control values 45 min after the i.v. injection of Bay U3405 (Table 3).

Table 1 Effect of U 46619 on diameter and on vasomotion in arterioles of the hamster cheek pouch: comparison between small and large arteries

	Concentration U 46619 (nM)					
	0	*0.1*	*1*	*10*	*100*	*1000*
All arterioles ($n = 22$)						
Diameter (μm)	24 ± 2	22 ± 2	21 ± 2*	20 ± 2*	19 ± 1*	19 ± 1*
Vasomotion: Amplitude (μm)	0.8 ± 0.3	1.7 ± 0.4	2.1 ± 0.4*	2.3 ± 0.4*	3.3 ± 0.4*	3.3 ± 0.5*
Frequency (cycles min^{-1})	1.4 ± 0.6	3.4 ± 0.9	3.9 ± 0.8*	4.0 ± 0.6*	5.6 ± 0.6*	5.2 ± 0.5*
Small arterioles ($n = 11$)						
Diameter (μm)	17 ± 2	16 ± 2	16 ± 2	15 ± 1*	15 ± 1*	15 ± 1*
Vasomotion: Amplitude (μm)	0.8 ± 0.4	1.5 ± 0.6	2.1 ± 0.6	2.3 ± 0.6	3.4 ± 0.6*	3.2 ± 0.6*
Frequency (cycles min^{-1})	1.8 ± 1.0	4.0 ± 1.4	4.6 ± 1.2*	4.4 ± 1.0	5.6 ± 0.9*	5.5 ± 0.8*
Large arterioles ($n = 11$)						
Diameter (μm)	31 ± 2	28 ± 3	26 ± 2*	25 ± 2*	23 ± 1*	23 ± 1*
Vasomotion: Amplitude (μm)	0.8 ± 0.3	1.8 ± 0.6	2.1 ± 0.5	2.3 ± 0.4	3.4 ± 0.6	3.5 ± 0.8*
Frequency (cycles min^{-1})	1.1 ± 0.6	2.8 ± 1.0	3.2 ± 0.9	3.7 ± 0.9*	5.6 ± 0.7*	4.9 ± 0.7*

Values are shown as means $\pm$ s.e.mean. *Value significantly different from that obtained in the absence of U 46619 ($P < 0.05$; ANOVA + Dunnett's test)

Table 2 Effect of U 46619 on diameter and vasomotion in arterioles of the hamster cheek pouch with or without spontaneous vasomotion

	Concentration U 46619 (nM)					
	0	*0.1*	*1*	*10*	*100*	*1000*
Arterioles without spontaneous vasomotion ($n = 13$)						
Diameter (μm)	25 ± 3	22 ± 3	21 ± 3	20 ± 3**	19 ± 2**	20 ± 2**
Vasomotion: Amplitude (μm)	0	1.1 ± 0.6	1.0 ± 0.3*	1.8 ± 0.4*	3.3 ± 0.7*	3.5 ± 0.8*
Frequency (cycles min^{-1})	0	2.0 ± 1.0	2.4 ± 0.9*	3.5 ± 0.9*	5.5 ± 0.8*	4.0 ± 0.5*
Arterioles with spontaneous vasomotion ($n = 9$)						
Diameter (μm)	24 ± 2	23 ± 1	21 ± 2	19 ± 2**	18 ± 1**	18 ± 1**
Vasomotion: Amplitude (μm)	2.0 ± 0.4	2.5 ± 0.5	3.7 ± 0.4**	3.0 ± 0.6	3.5 ± 0.4	3.1 ± 0.5
Frequency (cycles min^{-1})	3.5 ± 1.1	5.6 ± 1.3	6.1 ± 1.0	4.8 ± 0.8	5.7 ± 0.8	7.0 ± 0.8**

Values are shown as means $\pm$ s.e.mean. *Value significantly different from that in the absence of U 46619 ($P < 0.05$; Student's t test for paired observations) **Value significantly different from that in the absence of U 46619 ($P < 0.05$; ANOVA + Dunnett's test)

Effects of angiotensin II and the NO-synthase inhibitor L-NOARG

In 8 quiescent arterioles from 4 hamsters (116±6 g) with a mean initial internal diameter of 23±4 μm, a bolus injection of angiotensin II (0.01 nmol) caused a significant vasoconstriction (decrease in mean diameter by 36±4%) followed, after 2 min, by a vasodilatation (increase in mean diameter by 28±7%). No vasomotion was induced in any of the arterioles tested. (Typical example: Figure 4).

In 8 quiescent arterioles from 4 hamsters (111±4 g) with a mean initial internal diameter of 23±4 μm, the addition of the NO-synthase inhibitor, L-NOARG (100 μM) to the superfusion solution caused a significant vasoconstriction (mean internal diameter: 20±2 μm) but did not induce vasomotion. Fifteen minutes later, a bolus injection of angiotensin II (0.01 nmol) further enhanced the vasocontriction by 40±6% but no longer caused vasodilatation; two min after injection of angiotensin II the mean internal diameter returned to control values (1±8%). Again, no vasomotion was induced in any of the arterioles tested. A typical example is shown in Figure 4.

Effect of phenylephrine

In 12 quiescent arterioles from 6 hamsters (92±2 g) with a mean initial internal diameter of 21±2 μm, bolus injections of phenylephrine (10 nmol) caused significant vasoconstrictions (decrease in mean diameter by 31±4%; $P<0.05$) but did not induce significant vasomotion (only in 1 out of 12 arterioles a brief period of 3 cycles of spontaneous contraction with a mean amplitude of 0.8±0.5 μm was observed). A typical example of a phenylephrine response is shown in Figure 4.

Discussion

The major findings of the present study can be summarized as follows: The TP-receptor agonist U 46619 produced concentration-dependent vasoconstriction and induced vasomotion in hamster cheek pouch arterioles. These effects could be demonstrated during continuous superfusion of the preparation with U 46619 or during a topical bolus application of the compound. Both the constrictor responses and the vasomotion induced with U 46619 could be prevented by the TP-receptor antagonists S 17733 and Bay U3405, indicating that TP-receptors are involved in these effects. The vasoconstriction as such does not explain the occurrence of vasomotion since angiotensin II, at doses causing comparable constriction in the cheek pouch arterioles, did not induce vasomotion either under control conditions or in the presence of L-NOARG. Also phenylephrine at a dose which evoked comparable constriction to that obtained with U 46619, did not induce vasomotion in the cheek pouch arterioles. Angiotensin II, under control conditions caused constriction immediately followed by dilatation in hamster cheek pouch arterioles. The NO-synthase inhibitor L-NOARG selectively prevented the dilator responses to angiotensin II, suggesting that they are due to NO-release.

Rhythmic changes of microvascular diameter, termed vasomotion, are a typical feature of arteriolar networks (Intaglietta, 1981; 1991; Slaaf *et al*., 1987; Bertuglia *et al*., 1994). Several observations suggest that a complex relationship exists between vessel wall tension and vasomotion frequency. Thus,

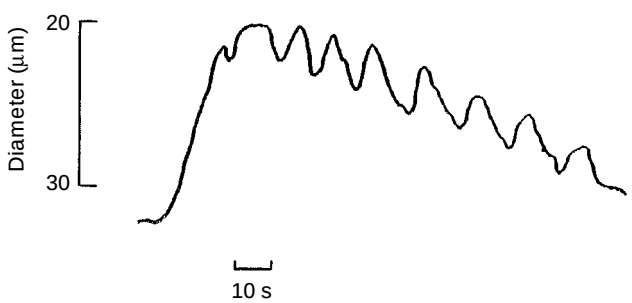

Figure 1 Typical example of the constrictor response to a bolus application of 1 nmol of U 46619 in an arteriole of the hamster cheek pouch. This previously quiescent arteriole developed vasomotion as a result of TP-receptor activation.

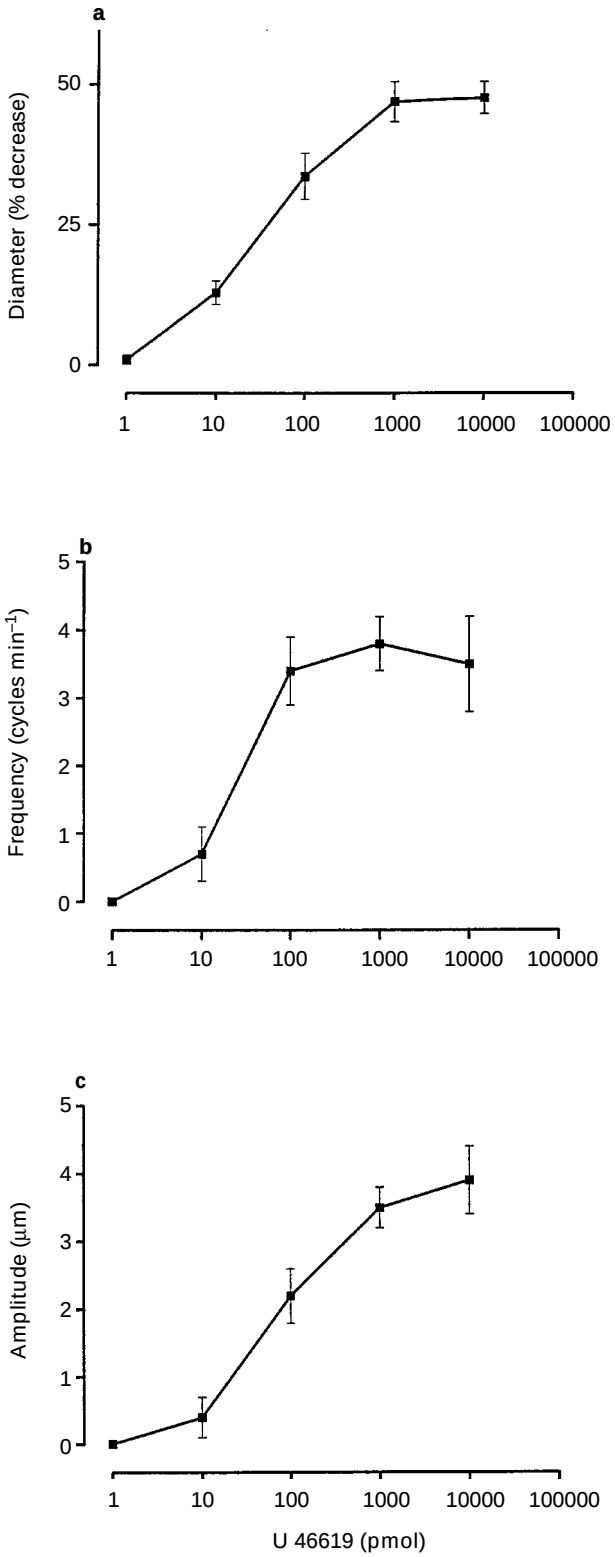

Figure 2 The TP-receptor agonist U 46619 causes dose-dependent constriction (a) and vasomotion (frequency-b and amplitude-c) in hamster cheek pouch arterioles. Data shown are means and vertical lines indicate s.e.mean.

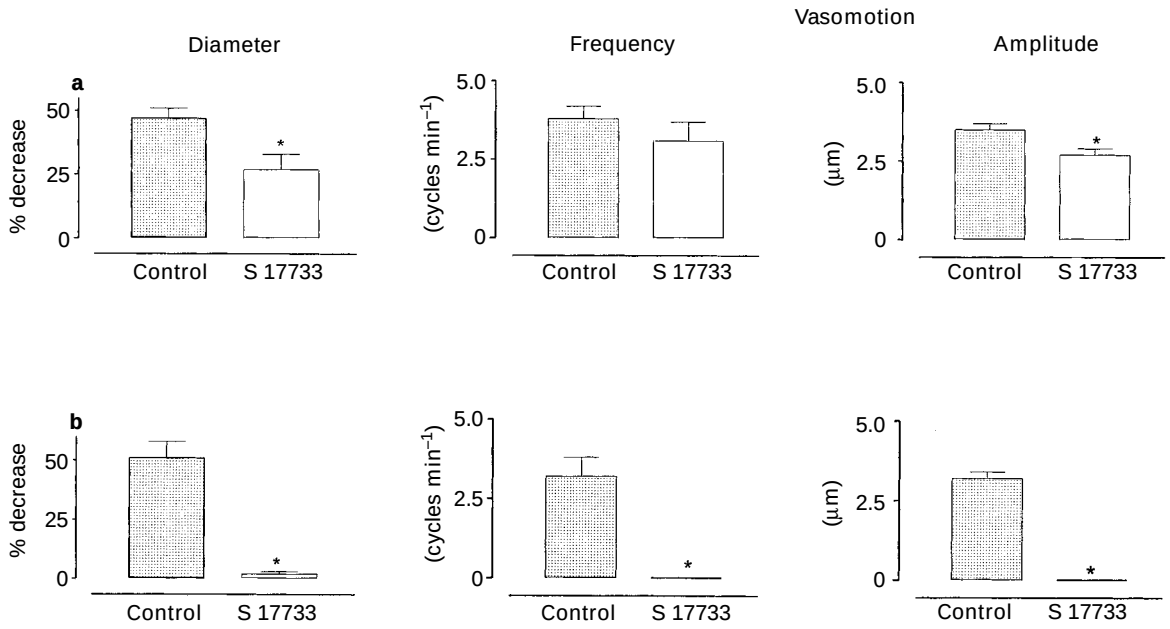

Figure 3 Antagonism by the TP-receptor antagonist S 17733 of the constrictor responses and of the vasomotion caused by U 46619 (1 nmol) in hamster cheek pouch arterioles. At (a) 10 mg kg^{-1}, the antagonist partially and at (b) 100 mg kg^{-1} completely inhibited the responses. *Effect of S 17733 was significant ($P<0.05$; Student's t test for unpaired observations).

Table 3 Effect of i.v. treatment with the TP-receptor antagonist Bay U3405 (10 mg kg^{-1}) on U 46619 (nmol)-induced responses in the hamster cheek pouch arterioles

		Vasomotion	
	Diameter (% decrease)	*Frequency* (cycles min^{-1})	*Amplitude* (μm)
Control	42±5	2.3±0.8	1.6±0.5
15 min after Bay U3405	6±3*	0.4±0.4*	0.2±0.2*
45 min after Bay U3405	26±4	0.7±0.4	0.5±0.3*

Values are shown as means±s.e.mean. *Value significantly lower than control ($P<0.05$; ANOVA + Dunnett's test).

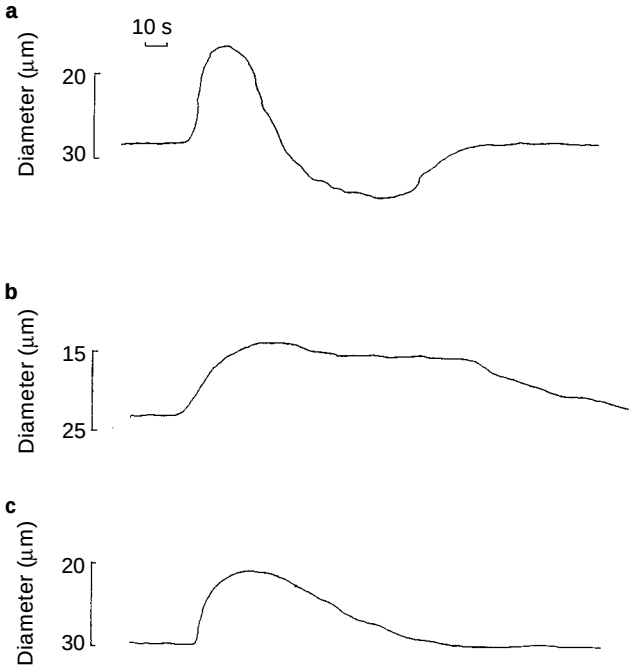

Figure 4 Typical example of the arteriolar responses to bolus applications of angiotensin II (0.01 nmol; a), angiotensin II (0.01 nmol) in presence of L-NOARG (100 μM; b) and of phenylephrine (10 nmol; c) in the hamster cheek pouch. Note the absence of vasomotion in the three preparations.

during vasodilatation, vasomotion was found both to disappear e.g. in pial vessels under hypercapnia (Auer & Galhofer, 1981) and in the hamster skinfold during exposure to adenosine (Colantuoni *et al.*, 1984) and to remain unchanged e.g. in pial vessels under hypercapnia and during exposure to adenosine (Hundley *et al.*, 1988) and in the rabbit tenuissimus muscle during exposure to adenosine (Oude Vrielink *et al.*, 1990). Also during vasoconstriction, vasomotion was enhanced e.g. in the hamster skinfold during exposure to vasopressin (Gertsberger *et al.*, 1987) or unaffected e.g. in the hamster skinfold during exposure to angiotensin (Gertsberger *et al.*, 1987), and in cat pial arterioles during hyperoxygenation (Auer & Galhofer, 1981). Moreover, in the bat wing, warming increases vasomotion frequency without changing either mean arteriolar diameter or vasomotion amplitude (Bouskela, 1989). These findings suggest that a dissociation may exist between vessel wall tonus and vasomotor behaviour.

Thromboxane A_2 (TXA_2) is a powerful vasoconstrictor and platelet activator, produced through the cyclo-oxygenase pathway from arachidonic acid (Hamberg *et al.*, 1975; Vane & Botting, 1992).

TXA_2 exerts effects at the level of the microcirculation. Microvascular permeability in the hamster cheek pouch venules, studied with fluorescein-labelled dextran, can be induced with leukotrienes D_4 and B_4 and this induction implicates an arachidonic acid metabolite that activates TP-receptors; this substance may be TXA_2 itself (Dunham *et al.*, 1984). The TP-receptor agonist U 46619 causes endothelial gaps in pulmonary microvessel endothelial layers (Mineau-Hanscke *et al.*, 1990).

Ischaemia-reperfusion induces TXA_2 and neutrophil-dependent injury (Klausner *et al.*, 1988; 1989). The local injury is characterized by the accumulation of polymorphonuclear leukocytes (PMN) in the microcirculation and by increased permeability. Inhibition of TXA_2-synthesis prevents neutrophil accumulation and protects the vascular barrier. Recent evidence illustrates that TXA_2 mediates diapedesis after ischaemia

through regulation of neutrophil, but not endothelial, cell adhesion receptors (Goldman *et al*., 1991). On the other hand, ICAM-1 expression on the surface of human vein endothelial cells is increased by substances that evoke TXA_2 production, such as IL-1β, TNFα, thrombin and PAF and this expression can be blocked by the TXA_2-synthase inhibitor DP-1904 (Ishizuka *et al*., 1994). Increased platelet TXA_2 production has been observed in the presence of activated polymorphonuclear leukocytes (PMNs): platelets use the arachidonic acid from PMNs to produce TXA_2 (Maugeri *et al*., 1992). All these data illustrate important interactions between leukocytes, platelets and endothelial cells that implicate a role for TXA_2 at the level of the microvasculature.

Since TXA_2 is a powerful vasoconstrictor, it will, once produced affect microvascular contractility. By use of the technique of intravital microscopy, it was shown that TXA_2, or its mimetic U 46619, causes powerful constrictions of arterioles of the rat cremaster muscle (Stücker *et al*., 1996) and of rat cerebral arterioles (Mayhan *et al*., 1991). Moreover, the latter constrictions were observed in both normal and diabetic animals (Mayhan *et al*., 1991). Pial arteries are capable of producing significant amounts of TXA_2 (Rosenblum & Bryan, 1987). The decreased endothelium-dependent relaxations to acetylcholine and ADP in diabetes can be restored by the TP-receptor antagonist SQ 29548 and by indomethacin, suggesting the involvement of TXA_2 or a substance capable of activating TP-receptor in cerebral arterioles (Mayhan *et al*., 1991); such results have also been described for large arteries (Tesfamarian *et al*., 1989). In rabbit small pulmonary arteries, the vasoconstrictions induced by vagal nerve stimulation and acetylcholine are blocked by the TP-receptor antagonists AA-2414 and ONO 3708, indicating that they are produced through TXA_2 or a substance that activates TP-receptors (Shirai *et al*., 1992).

The present study confirms and extends these findings for arterioles of the hamster cheek pouch microcirculation. Our data illustrate the potent arteriolar constrictor properties of the TXA_2-mimetic and TP-receptor agonist U 46619; indeed the vasoconstriction observed with increasing doses of U 46619 was eliminated by two selective TP-receptor antagonists, S 17733 (Verbeuren *et al*., 1994) and Bay U3405 (Perzborn *et al*., 1989).

An important new finding of our study is that TXA_2 induces vasomotion, rhythmic constriction and dilatation, in arterioles of different sizes of the hamster cheek pouch. This vasomotion was also sensitive to the TP-receptor antagonists and thus appears to involve receptor activation by U 46619. An enhancement by U 46619 of spontaneous vasomotion has previously been observed in the rat basilar artery (Fujii *et al*., 1990). Future studies will have to determine whether endogenously produced TXA_2 plays a role in vasomotion and whether attenuation of this response is beneficial or deleterious in health and disease. In this respect, it is interesting to note that spontaneous rhythmic contractions caused by the lipoxygenase metabolites 15-HETE and 15-HPETE in canine isolated saphenous veins and splenic and renal arteries, are sensitive to indomethacin treatment; moreover, the vascular contractions caused by these metabolites in these arteries and veins are due to TP-receptor activation (Van Diest *et al*., 1986; 1991). However, in the conscious or anaesthetized hamster, indomethacin did not affect vasomotion or induce vasomotion in the skeletal muscle microcirculation (Bertuglia *et al*., 1994) which may point to species and/or tissue differences.

One fundamental issue raised previously is the possible interaction between increased arteriolar tone and the occurrence of vasomotion (see e.g. Oude Vrielink *et al*., 1990). In order to gather some information about this interaction in the cheek pouch microcirculation, we have compared the arteriolar activity of TXA_2 with that of angiotensin II and phenylephrine. It is generally accepted that angiotensin II is a potent constrictor of arterioles (see e.g. Messina *et al*., 1975; Fleming *et al*., 1987; Vicaut *et al*., 1989) and this has also been illustrated in the hamster cheek pouch (Click *et al*., 1979; Morita-Tsuzuki *et al*., 1993). However, in some arterioles angiotensin II can also evoke endothelium-dependent vasodilator responses, such as in rat cerebral arterioles (Haberl *et al*., 1990). In our present study, a bolus injection of angiotensin II evoked a biphasic response: a powerful vasoconstriction followed by a dilatation. Neither phase of this response was accompanied by vasomotion. In order to separate between the two phases of the response to angiotensin II, NO-synthase activity was inhibited with L-NOARG (Verbeuren *et al*., 1993). It was interesting to note that superfusion of the cheek pouch preparation with L-NOARG caused a significant vasoconstrictor response which was not accompanied by vasomotion. This latter observation is in agreement with the findings obtained in the skeletal muscle microcirculation of conscious hamsters, in which vasomotion was shown not to be related to NO, although NO-inhibition could stimulate vasomotion in smaller arterioles of the anaesthetized hamster (Bertuglia *et al*., 1994). Moreover, in the rat cerebral microcirculation, inhibition of NO-synthase reduced vasomotion, suggesting that NO might be implicated in its control (Morita-Tsuzuki *et al*., 1993). In the presence of L-NOARG, angiotensin II only caused arteriolar constriction, the vasodilator component of its action was completely prevented by the NO-synthase inhibitor. Despite this absence of NO, angiotensin II did not induce vasomotion in the cheek pouch arterioles. These findings are in agreement with studies on rat cerebral arterioles where angiotensin II failed to alter spontaneous vasomotion (Morita-Tsuzuki *et al*., 1993).

Also another vasoconstrictor agent, the α_1-adrenoceptor agonist phenylephrine, used at a dose which causes a comparable constriction to that obtained with U 46619, failed to induce vasomotion in the cheek pouch arterioles. This observation is in agreement with previous studies showing that noradrenaline, in spite of its constrictor properties, in fact decreased spontaneous vasomotion in the hamster cheek pouch (Bouskela & Cyrino, 1994).

Our data thus suggest that vasoconstriction *per se* cannot explain the occurrence of vasomotion: indeed, when TXA_2, angiotensin II or phenylephrine were administered to the hamster cheek pouch preparation, they all cause vasoconstriction, but only TXA_2 induced vasomotion.

In conclusion, the present study illustrates that TP-receptor activation in arterioles of the hamster cheek pouch microcirculation caused not only vasoconstriction, but also the development of spontaneous rhythmic constrictions and dilatations, that is, vasomotion. Whether TXA_2 or a substance that activates its receptor, is implicated in the physiological regulation of vasomotion at the level of the microcirculation, remains to be evaluated.

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