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ANTAGONISM OF THROMBOXANE RECEPTOR INDUCED CONTRACTIONS IN ISOLATED HUMAN GROIN LYMPHATICS

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ABSTRACT

In vitro *studies were performed on lymphatics obtained from the groin in 19 patients undergoing vascular surgery. The lymphatics were mounted in tissue baths, and isometric contractions were induced by increasing concentrations of the thromboxane A_2 (TXA_2) mimetic U-46619. In comparison to K^+ (124mM)-induced contraction, which were used as an internal standard, the response to U-46619 had an E_{max} of 105±5.9%. The pEC_{50}-value was 8.14±0.09. The effects of two thromboxane receptor (TP-receptor) antagonists, L-636,499 and BM-13,505, were investigated. Both antagonists caused concentration-dependent right-ward shifts without depression of E_{max} of the U-46619 concentration-response curves. The slopes of the regression lines in a Schild plot for both antagonists did not differ from one, indicating competitive antagonism. The pA_2-value of BM-13,505 (7.89) was 65 times higher than that of L-636,499 (6.08). The results suggest that the receptor involved in the prostanoid contraction in human groin lymphatics is of the TP-subtype.*

It has been proposed that some prostanoids are important for regulation of the contractile activity of human leg lymphatics (1,2). In a previous work, isometric contractions were measured in isolated lymphatic ring segments, taken from the groin of patients undergoing surgery (2). It was shown that the prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) induced no or only weak contractions, and that the prostaglandin endoperoxide (PGH_2) analogue U-44069, uniformly elicited marked concentration-dependent contractions. The rank order of potency for the prostanoids was U-44069 > $PGF_{2\alpha}$ > PGE_2. According to the classification of prostanoid receptors proposed by Coleman et al (3), this suggests that the main prostanoid receptor regulating contraction in human groin lymphatics is of the TP-type (T stands for thromboxane and P for prostanoid). The most potent natural prostanoid for the TP-receptor is TXA_2 (thromboxane A_2).

This investigation was performed in order to further analyze the prostanoid receptor subtype in isolated human groin lymphatics. The effects of two TP-receptor antagonists on contractions induced by another prostaglandin endoperoxide analogue, U-46619, which in some preparations has been shown to be more potent that U-44069 (4), were recorded. U-46619 closely resembles TXA_2 in its biological activity, and is considered as a selective TXA_2 mimetic (5).

MATERIALS AND METHODS

Preparation and mounting

Superficial groin lymphatics were removed from 19 patients (13 men and 6 women, aged 49 to 80 years, mean age 65 years) during vascular surgery. Using a microscope or magnifying glasses, the tissue around a superficial lymph node

was carefully dissected. After identification, a lymph node was extirpated together with the surrounding tissue and immediately placed in a chilled (4°C) Krebs solution of the following composition (in mM):NaCl 119, $NaHC0_3$ 15, KCL 4.6, $CaCl_2$ 1.5, NaH_2P0_4 1.2, $MgCl_2$ 1.2 and glucose 11. The tissue sample was promptly transported to the laboratory. With the sample immersed in Krebs solution, fat and connective tissue were carefully removed. When a lymphatic was found, it was excised and kept in a refrigerator (4°C) over night.

The next day the lymphatic was divided into ring segments, which were suspended between two L-shaped metal holders (50μm in diameter) arranged in parallel. The equipment used to measure isometric tension in lymphatics has been described earlier (2), and is a modification of that described by Högesätt et al (6) for measurement of contractions in small blood vessels. The vessels were repeatedly stretched until a stable basal tension of about 1.5mN was reached. They were allowed to equilibrate in the baths for about 1 hour before the experiments started.

The microscope used in the laboratory was equipped with a scaler, by which the dimensions of the suspended lymphatics were measured. During all manipulation special care was taken to avoid injury to the lymphatics.

Experimental procedures

When the vessels had reached a steady basal tension, the Krebs solution was placed by a preheated (37°C) and bubbled (95% 0_2+5% $C0_2$) K^+-rich Krebs solution of the following composition (in mM): KCl 124, $NaHC0_3$ 15, $CaCl_2$ 1.5, NaH_2P0_4 1.2, $MgCl_2$ 1.2 and glucose 11. The preparation was submerged in the solution until a stable tension was reached, and subsequently the K^+-rich solution was changed to the Krebs solution again, resulting in a decrease of amplitude below the starting tension. The lymphatics were again stretched to the basal tension and when the tension had stabilized a new K^+-induced contraction was evoked. This was repeated until the predetermined tension level was reached after washing out the K^+-rich solution (3-6 times). It had earlier been shown that optimum responses to K^+ were obtained at basal tensions between 1 and 4mN (2). All segments responding to K^+ with contractions below 0.5mN were not used for further experiments. The antagonists were added 15 min. before the cumulative addition of the agonist started (the fluid was replaced with fresh Krebs and antagonist 5 min. before the agonist was added). Only one concentration of antagonist was used for each segment.

Drugs

A stock solution of the prostaglandin endoperoxide analogue U-46619 (15S)-hydroxy-11α-(epoxymethano)prosta-5Z,13E-dienoic acid; Upjohn, USA) was made up in absolute ethanol (5mg/ml) and stored at 20°C. Fresh dilutions of U-46619 were made with phosphate buffer at neutral pH and L-636,499 (Merck-Frosst, Canada) was dissolved in sodium bicarbonate (1mM) both just before use. BM-13,505 (Boehringer-Mannheim, FRG), was dissolved in NaOH (1mM), and stored at -80°C until used. The final dilutions of the antagonists were made in saline.

Analysis of data

The contractions produced by each concentration of the agonist were related to the maximal amplitude of the previous K^+-induced contraction. All concentration-response curves were plotted graphically. E_{max}, i.e., the maximum contraction, obtained with the agonist, was established. The contraction was regarded as maximum when two subsequent contractions gave a response of the same amplitude or when the subsequent concentration produced a decreased contraction amplitude. The pEC_{50}-value, i.e., the negative logarithm of the EC_{50}-value, was determined from the graph as the concentration giving half maximal contraction.

The concentration ratio (CR) was calculated as the ratio between the EC_{50}-values in the presence and absence of the antagonist. The pA_2-value, i.e., the negative logarithm of the antagonist concentration that displaced the agonist concentration-response curves toward higher concentrations with a factor of 2, was determined according to the method of Arunlakshana and Schild (7).

RESULTS

At basal tension, before the K^+-rich Krebs solution was added to the baths, the measured length of the lymphatic segments was 0.59±0.03mm, and the distance between the metal holders was 0.67± 0.04mm (mean ± S.E.M., n=79). No spontaneous contractions were observed in the vessels. The K^+ (124mM)-induced contraction, to which the agonist induced contractions were related, was 2.26± 0.17mN (mean ± S.E.M., n=79).

Contractile effect of U-46619 without antagonists

U-46619 induced concentration-dependent contractions in all lymphatic segments. In approximately 50% of the experiments low agonist concentrations ($3x10^{-10}$-10^{-8}M) induced phasic contractions, while at higher concentrations the contractions were stable. In the rest of the experiments, the contractions were stable at all agonist concentrations. Maximum contraction was reached in all experiments. E_{max} was found to be 105±5.9% of the preceding K^+ (124mM)-induced contraction, and the pEC_{50}-value was 8.14±0.09 (mean ± S.E.M., n=19).

Effects of the TP-receptor antagonists L-636,499 and BM-13,505

L-636,499 ($3x10^{-6}$-$3x10^{-5}$M) and BM-13,505 (10^{-8}-10^{-7}M) caused concentration-dependent right-ward shift of the U-46619 concentration-response curves *(Fig. 1 and 2, respectively)*. The two highest concentrations of L-636,499 showed a tendency to depress the E_{max}. However, the depressions were not significant (Wilcoxon signed ranks test for paired data). BM-13,505 had no depressing effects on the E_{max} of U-46619. The interactions between U-46619 and the antagonists are shown by Schild plots in *Fig. 3*, and data on Schild regressions are given in *Table 1.*

DISCUSSION

Previously, we have shown that noradrenaline, adrenaline, serotonin, dopamine and acetylcholine had little or no contractile effects on isolated human groin lymphatics, whereas some prostanoids showed strong contractions in this preparation (2). Sinzinger et al suggested key roles for PGH_2 and TXA_2 in human lymphatic contractility (1), although the origin of the TXA_2 was probably extralymphatic, since TXA_2 formation was undetectable in the vessel itself (8). To our knowledge, no further *in vitro* studies on TXA_2-induced contractions in human lymphatics have been published.

Recently, Elias and Johnston (9) showed that in isolated bovine mesenteric lymphatics with transmural pressure above 8cm water, U-46619 at a concentration of 10^{-8}M increased lymph flow, whereas higher or lower concentrations of U-46619 decreased lymph flow independ-

Table 1

Data on Schild regressions for L-636,499 and BM-13,505. U-46619 was used as agonist. The values represent mean and the 95% confidence intervals. n denotes number of values on which the regression lines are based.

Antagonist	Slope of regression line	pA_z-value	n
L-636,499	0.88 (0.50 - 1.26)	6.08 (5.72 - 6.93)	21
BM-13,505	1.02 (0.36 - 1.68)	7.89 (7.61 - 8.75)	27

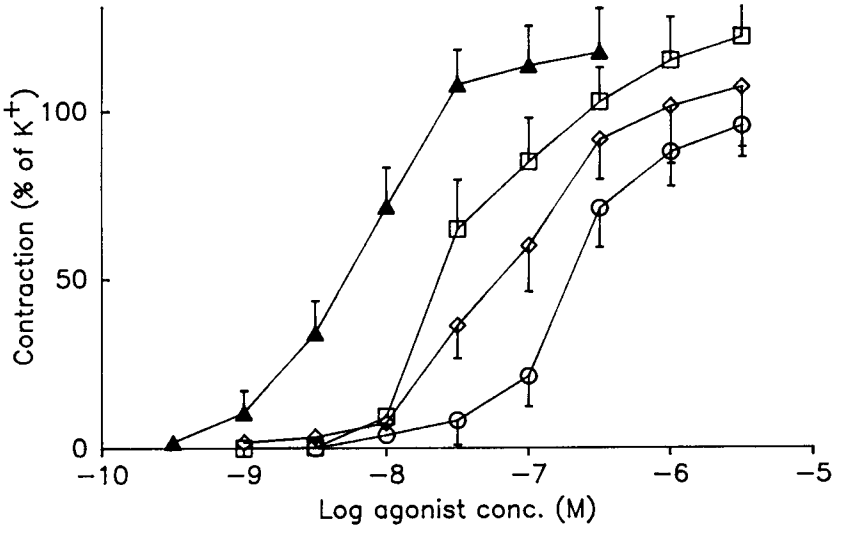

Fig. 1. Concentration-response curves for U-46619 in human groin lymphatics in absence (▲) and presence of increasing concentrations of L-636,499, (□ $3x10^{-6}$M, ◇ 10^{-5}M and ○ $3x10^{-5}$M). The contractile responses are expressed as percentage of the K^+ (124mM)-induced contraction. Each point in the curves indicates mean ± SEM obtained from 7-9 lymphatics (patients).

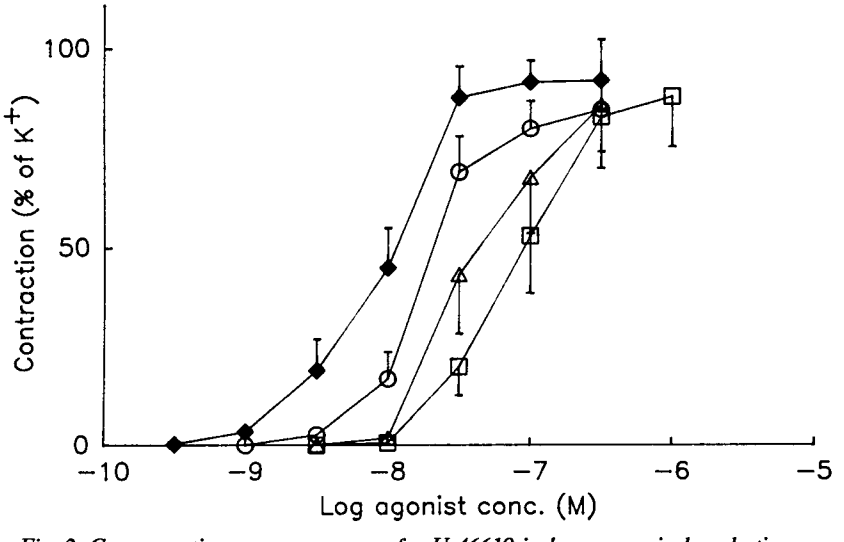

Fig. 2. Concentration-response curves for U-46619 in human groin lymphatics in absence (♦) and presence of increasing concentrations of BM-13,505 (○ 10^{-8}M, △ $3x10^{-8}$M and □ 10^{-7}M). The contractile responses are expressed as percentage of the K^+ (124mM)-induced contraction. Each point in the curves indicates mean ± SEM obtained from 8-10 lymphatics (patients).

ently of transmural pressure. In isolated ring preparations of bovine mesenteric lymphatics $PGF_{2\alpha}$, U-46619 and the leukotrienes B_4, C_4, and D_4 induced rhythmical contractions (10), and it has been shown that this activity, and spontaneously phasic contractions can be inhibited by cyclooxygenase and lipoxygenase suppression (11). Furthermore, in conscious sheep, the infusion of aspirin and imidazole into popliteal efferent lymphatics reduced the amplitude of the pressure pulses (12). These animal studies indicate that arachidonate metabolites are impor-

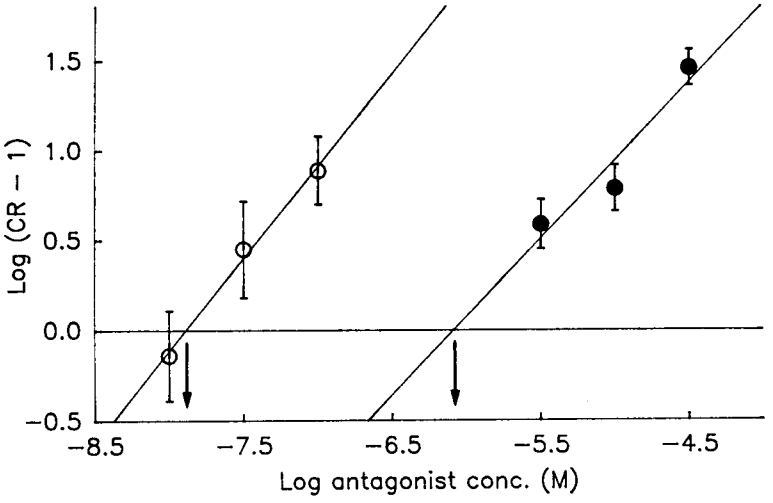

Fig. 3. The effects of BM-13,505 (○) and L-636,499 (•) on U-46619-induced contractions of human groin lymphatics illustrated by Schild plots. The pA_2-values (where the regression lines intersects with the abscissa when log (CR-1) = 0) for CM-13,505 and L-636,499 are indicated by arrows. Each regression is calculated from vessel of 7-10 lymphatics (patients), and each point denotes mean ± SEM.

tant for lymphatic contractility. However, when characterizing prostanoid receptors, the most reliable method is to use selective receptor antagonists. By the use of cyclooxygenase or thromboxane synthetase inhibitors the metabolites of arachidonic acid may be reoriented into other pathways and these can exert effects which influence the results (13-15). A competitive TXA_2-receptor antagonist may have an affinity for the receptor, but without exerting an effect, and perhaps without influencing the arachidonic cascade. The agonist and the antagonist may compete for the receptor in a concentration-dependent way. The slope of the regression line in the Schild plot would not differ from 1 if the antagonism is competitive, and the pA_2-value would then be a measure of the potency of the antagonist.

The prostaglandin-endoperoxide analogue U-46619 has been shown to be both a potent and selective TXA_2 mimetic in different vascular preparations (3). In the present study, U-46619 induced maximal contractions of the same magnitude as the K^+-induced contractions, which previously (2) have been shown to be unmodified by blockade of noradrenaline uptake, β-adrenoceptor blockade and α-adrenoceptor blockage, indicating that TP-receptors are present in the smooth muscle cells. L-636,499 has been shown to inhibit human platelet aggregation induced by arachidonic acid and U-44069 (16). However, its selectivity for TP-receptors has been questioned (17). BM-13,505 is a novel compound, that has been shown to attenuate significantly vasoconstrictor effects of carbocyclic thromboxane A_2 and U-46619 in cat coronary arteries (18). In the latter study, the main factor thought responsible for the antagonism of thromboxane induced vasoconstriction was that BM-13,505 replaced thromboxane A_2 on the vascular receptor.

In the present study, parallel shifts to the right were induced by L-636,499 and BM-13,505. As the antagonists did not significantly depress E_{max}, and the Schild plots yielded slope indexes near unity, competitive antagonism are indicated for both antagonists. The pA_2-value for L-636,499 (6.08) are in the same range as those found in tracheal smooth muscle (19; pA_2 6.0), and in feline basilar artery

(10; pA_2 5.99). However, the potency for BM-13,505 was 65 times higher than for L-636,499 (pA_2-values of 7.89 and 6.08, respectively).

The present study supports the proposals of a functional importance of prostanoids for contractile regulation of human groin lymphatics. The results suggest that the receptor subtype involved is the TP-receptor, and further that clinical use of thromboxane receptor antagonists may interfere with normal lymphatic contractility.

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