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CONTRACTILE PROPERTIES OF LYMPHATICS FROM THE HUMAN LOWER LEG

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ABSTRACT

Lower leg lymphatics, taken from seven volunteers, were investigated in vitro. *Isometric contractions were induced by noradrenaline, 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and the thromboxane A_2 (TXA_2)-mimetic U-44069. Noradrenaline induced phasic contractions in 6 of 7 segments. The amplitude of the contractions were up to 100% of a previous K^+ (124mM)-induced contraction and the frequency at 10^{-6}M of noradrenaline was 5-13 min^{-1}. These contractions were unaffected by propranolol (10^{-6}M), but they were abolished by phentolamine (10^{-6}M). Noradrenaline had an E_{max} (tonic contraction) of 7% of the K^+ (124mM) contraction ($n=7$) and the pEC_{50}-value was 6.9 ($n=3$). The corresponding values for 5-HT were 21% ($n=5$) and 6.4 ($n=3$). PGF_{2a} elicited contractions in all segments investigated ($E_{max}=87\%$, $pEC_{50}=5.8$, $n=5$). U-44069 had high contractile capacity and potency in all investigated segments ($E_{max}=137\%$, $pEC_{50}=8.9$, $n=7$). Phasic contractions were elicited also by 5-HT, $PGF_{2\alpha}$ and U-44069. Compared to earlier studies of human peripheral lymphatics, these results show that there are regional differences in susceptibility to these vasoactive agents.*

Several investigations show that the contractile and pulsatile properties of lymphatics from animals such as sheep (1,2), bovine (3-7), and dog (8,9) are activated after α-adrenoceptor stimulation. Most of these studies have been performed on mesenteric lymphatics. The effects have been described as an increase in lymph flow or lymphatic contraction frequency, or as an increase in lymphatic tension or pressure. These effects are not only blocked by α-adrenoceptor antagonists (5,7) but ß-adrenoceptor stimulation has also been shown to depress lymph flow (2). Furthermore, ß-adrenoceptor blockade facilitates the response to α-adrenoceptor agonists (5,10).

We have previously found that noradrenaline has no contractile effect in isolated human superficial groin lymphatics, and by histochemical examination no nerves could be identified (11). These findings suggest that human groin lymphatics are without contraction-mediating α-adrenoceptors. On the other hand, some prostanoids induce strong contractions in lymphatics from the groin (11,12). Whether these phenomena are representative for other regions in the human body are not known.

This study was performed in order to investigate if specific vasoactive drugs have the same potency and contractile capacity in lymphatics from the human lower leg as in lymphatics from the groin.

MATERIALS AND METHODS

Preparation

Peripheral leg lymphatic segments were excised under local anesthesia (Car-

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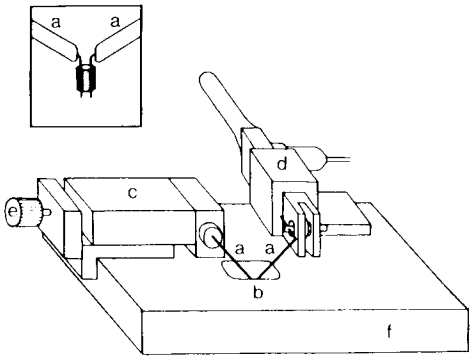

*Fig. 1. Schematic drawing of the myograph used for measuring isometric tension. The myograph block is hollow around the organ bath so that temperature-controlled water can circulate and warm up the bath. (**a**) Metal holders. Inset: lower part of the metal holders with a ring-segment of a vessel mounted over the holders. (**b**) Organ bath containing 2.5ml. The bath is bubbled with a mixture of 95% O_2 and 5% CO_2, giving a pH of approximately 7.4 in the Krebs buffer solution. (**c**) Movable unit used to adjust the distance between the metal holders. (**d**) Force-displacement transducer. (**e**) Micrometer screw to manipulate **c**. (**f**) Myograph block.*

bocain®, Astra, Sweden) from seven volunteers (aged 24-54 years, mean age 32 years). The operations were approved by the Ethics Committee of the University of Lund. A short incision was made 10-15cm above the medial malleolus on one leg. After careful dissection, it was possible to localize and remove a piece of a collecting lymphatic. The lymphatics were immediately placed in a chilled (4°C) Krebs buffer solution of the following composition (in mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, and glucose 11. The extirpated vessels were about 5mm long and had a diameter of about 0.2mm *in situ*. The experimental appliance and procedure used for investigation of isometric tension in human lymphatics has been described earlier (11,12).

Ring segments were cut from the lymphatics. They were mounted in myographs *(Fig. 1)* and repeatedly stretched to a basal tension of about 1mN. The segments were allowed to equilibrate in Krebs buffer solution 1.5 hour before repeated K^+ (124mM)-induced contractions were obtained. The K-rich solution had the same composition as the Krebs buffer solution apart from exchanging all NaCl with equimolar amounts of KCl. Segments responding to K^+ (124mM) with contractions below 0.5mN were excluded for further experiments.

Drugs

Dilutions of 5-hydroxytryptamine creatinine sulphate (5-HT), (-)-noradrenaline hydrochloride, phentolamine methanesulfonate (Ciba-Geigy), (±)-propranolol hydrochloride (Sigma), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (supplied as an aqueous solution, Amoglandin®, Astra) were formulated in NaCl containing 1mM ascorbic acid. U-44069 [(15S)-hydroxy-9,11-(epoxymethano)prosta-5Z,13 E-dienoic acid] (Upjohn) was mixed in absolute ethanol (5mg/ml) and stored at -20°C. Fresh dilutions of U-44069 were made with phosphate buffer at neutral pH just before use. The concentrations are given as final molar concentrations in the organ baths.

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 (tonic contractions) 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. If the maximum contraction obtained with an agonist was less than 10% of the K^+-induced contraction, the pEC_{50}-value was not calculated.

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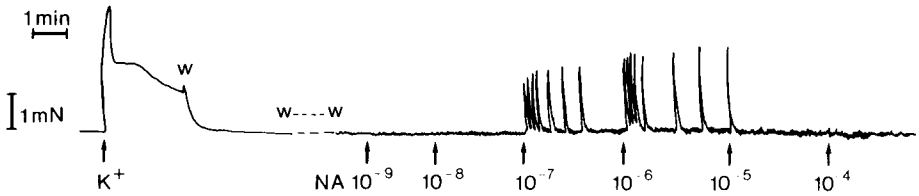

Fig. 2. Noradrenaline (NA)-induced phasic contractions in lymphatics obtained from the human lower leg. K^+ indicates the previous potassium (124mM)-induced contraction. W=wash out with fresh Krebs buffer solution. Concentrations are given in M.

RESULTS

Seventy-five percent (75%) of the examined ring segments (31 of 41) elicited contractions exceeding 0.5mN on K^+ (124mM) exposure and were accordingly used in the investigation. The distance between the metal holders at basal tension was 0.56±0.04mm, and the length of the lymphatic segment was 0.62±0.02mm (mean ± SEM, n=21).

Noradrenaline induced phasic contractions at 10^{-7} and 10^{-6}M in lymphatics from 86% of the subjects (6 of 7) *(Fig. 2)*. The amplitudes of the phasic contractions were up to 100% of the previous K^+-induced contraction. At a noradrenaline concentration of 10^{-6}M, the phasic contractions had a frequency of 5-13 min^{-1}. A decrease in frequency was obtained at noradrenaline 10^{-5}M, and at 10^{-4}M the phasic contractions were either few or had ceased. Together with the phasic contractions a small increase in tonic tension was recorded in 5 of 7 segments *(Figs. 2-4, Table 1)*.

Propranolol $3x10^{-7}$M and 10^{-6}M did not influence the noradrenaline-induced phasic contractions, but phentolamine 10^{-6}M abolished them *(Fig. 4)*. Furthermore, one experiment indicated that prazosin was more effective than rauwolscine in antagonizing the effect of noradrenaline. When a subthreshold concentration of U-44069 ($3x10^{-12}$M) was present in the bath, the susceptibility to noradrenaline increased *(Fig. 5)*.

5-HT 10^{-6}-10^{-4}M induced tonic and phasic contractions in lymphatic segments from 3 of 5 participants. $PGF_{2\alpha}$ and U-44069 induced phasic and tonic contractions in segments from all participants. Concentration-response curves from all agonists are shown in *Fig. 3* and E_{max}- and pEC_{50}-values are given in *Table 1*.

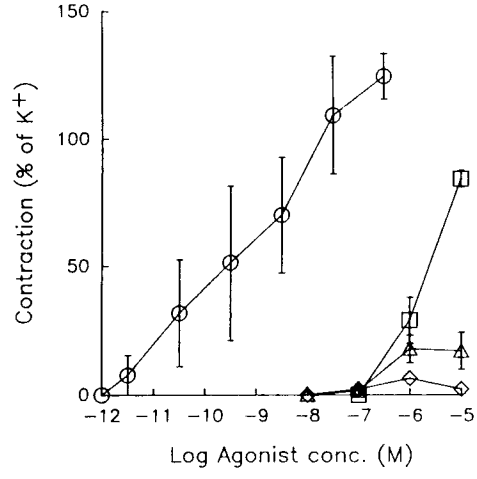

Fig. 3. Concentration-response curves for U-44069 (○), $PGF_{2\alpha}$ (□), 5-HT (△), and noradrenaline (◇) in isolated lymphatics taken from the human lower leg. The contractile responses are expressed as percentage of the previous K^+ (124mM)-induced contraction.

DISCUSSION

In the present study, we investigated the contractile effects of noradrenaline, 5-HT, $PGF_{2\alpha}$, and U-44069 in human lymphatics taken from the lower leg. By using an identical experimental technique for this investigation, as in a previous study on human groin lymphatics (11), we attempted to compare the effect of these drugs on lymphatics in a different region.

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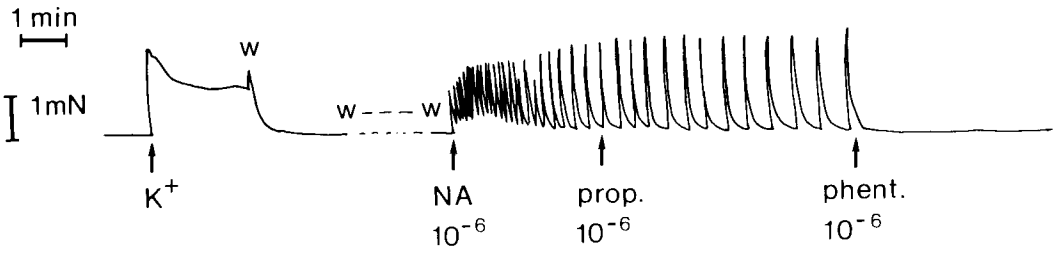

Fig. 4. Tracing from an experiment with a lymphatic segment taken from the human lower leg. Noradrenaline (NA) 10^{-6}M induced phasic contractions, which were unaffected by propranolol (prop.) 10^{-6}M, but abolished by phentolamine (phent.) 10^{-6}M. K^+ indicates the previous potassium (124mM)-induced contraction, and W indicates wash out with fresh Krebs buffer solution.

Table 1
Effect of Different Vasoactive Substances on Tonic Contractility of Lymphatics Obtained from the Human Lower Leg

The pEC_{50}-value was calculated only in experiments where E_{max} exceeded 10% of the K^+-induced contraction. Values represent mean and standard error of mean (SEM). n denotes the number of different preparations (participants) examined and on which the E_{max}-value is based. z denotes the number of preparations on which the pEC_{50}-value is based.

	n	E_{max}	pEC_{50}	z
Noradrenaline	7	7±2	6.9±0.3	3
5-HT	5	21±6	6.4±0.1	3
$PGF_{2\alpha}$	5	87±2	5.8±0.1	5
U-44069	7	137±8	8.9±0.5	7

In contrast to lymphatics from the groin where noradrenaline had no effect on contraction (11), noradrenaline elicited phasic contractions on lower leg lymphatics. The ability to block this noradrenaline-induced phasic contraction with phentolamine (an unselective α-adrenoceptor antagonist) corroborates that noradrenaline exerts its contractile effect through α-adrenoceptors. The main α-adrenoceptor population is presumably of the α_1-subtype, as the α_1-adrenoceptor antagonist prazosin antagonized the noradrenaline-induced phasic contractions more notably than rauwolscine, an α_2-adrenoceptor antagonist. The increased potency of noradrenaline when a subthreshold concentration of U-44069 was present in the bath *(Fig. 5)* suggests a close association between α-adrenoceptors and thromboxane A_2-receptors (12) in human lymphatics. Based on animal experiments, it has been suggested that the sympathetic nervous system influences the contraction frequency of the lymphatics through a balance between α- and ß-adrenoceptors (excitatory and inhibitory effects, respectively), whereas arachidonate metabolites (e.g., TXA_2) act on the intrinsic mechanism regulating spontaneous and agonist-induced contractile events (13, for review see 14). The thromboxane A_2-mimetic U-44069 was significantly (6 times) more potent on lymphatics taken from the lower leg (pEC_{50}=8.9±0.5) when compared to lymphatics taken from the groin (pEC_{50}=8.1±0.1). The contractile capacity of U-44069 was also greater in the lower leg lymphatic; E_{max} was 102% in the groin and 137% in the lower leg. This difference in response from lymphatics in the lower leg compared to those from the groin may reflect the greater requirement for propulsion of surplus interstitial fluid from the distal portion of the leg when standing and particularly when exercising (15,16).

The potencies of 5-HT and $PGF_{2\alpha}$ are slightly less in lymphatics from the lower leg (pEC_{50}-values of 6.4±0.1 and 5.8±0.1, respectively) compared to lymphatics from the groin (6.7±0.1 and 6.1±0.4, respectively). However, the con-

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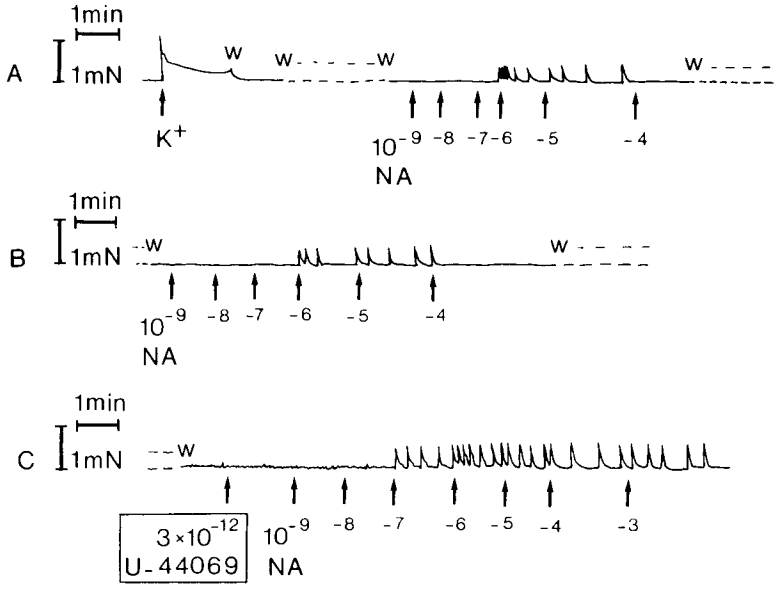

Fig. 5. Tracings obtained from the same lymphatic segment presented in a chronological order (A-C). In A and B, noradrenaline (NA) 10^{-6}M induced phasic contractions. In C, when U-44069 $3x10^{-12}$M was added before noradrenaline, the phasic contractions occurred at a lower concentration of noradrenaline (10^{-7}M). Between the different cumulative additions of noradrenaline several wash out (W) with fresh Krebs buffer solution were made. K^+ indicates the previous potassium (124mM)-induced contraction. The concentrations of noradrenaline above 10^{-9}M are given as log noradrenaline concentration (in mM).

tractile capacity of $PGF_{2\alpha}$ is more than 4 times that in lower leg lymphatics (E_{max}=87±2%) compared to those from the groin (E_{max}=20%). As shown in *Fig. 3*, a high concentration (10^{-5}M) of $PGF_{2\alpha}$ elicits a strong contraction (87%) and the curve between the two highest concentrations (10^{-6} and 10^{-5}M) is steep. Perhaps the strong contraction induced by 10^{-5}M of $PGF_{2\alpha}$ is unselective. The contractile capacity for 5-HT is similar in the two regions.

In conclusion, regional differences exist in susceptibility and response to noradrenaline, U-44069, $PGF_{2\alpha}$, and 5-HT in human lymphatics. In the human lower leg, these drugs induce both phasic and tonic contractions. In contrast, noradrenaline induces no phasic and almost no tonic contractions in lymphatics from the groin (11). The thromboxane A_2-mimetic U-44069 is about 6 times more potent and has greater contractile capacity in lymphatics from the lower leg compared to lymphatics from the groin. The U-44069-induced potentiation of noradrenaline suggests a close connection between α-adrenoceptors and thromboxane-receptors in human lymphatics.

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