

IN VITRO EFFECTS OF A THROMBOXANE A₂-ANALOGUE U-46619 AND NORADRENALINE ON CONTRACTIONS OF THE HUMAN THORACIC DUCT

T. Sjöberg, S. Steen

Department of Cardiothoracic Surgery, University Hospital, Lund, Sweden

ABSTRACT

A piece of a human thoracic duct removed at operation was investigated in organ baths. The duct was cut in 8 ring segments each about 1mm long and isometric tension recorded. The segments were exposed to a potassium rich (124mM) Krebs buffer solution. In only two of the segments were contractions induced (7.2 and 1.0mN, respectively). Noradrenaline and the thromboxane mimetic U-46619 induced tonic and phasic contractions. At a noradrenaline concentration of $10^{-5}M$ the phasic contractions had a frequency of 5 min^{-1} . The highest frequency, 9 min^{-1} , was recorded with $3 \times 10^{-10}M$ of U-46619 present in the bath. Noradrenaline had a mean E_{max} of 50% of the previous K^+ (124mM)-induced contraction, and the mean pEC_{50} -value was 6.7. The E_{max} and pEC_{50} -values of U-46619 were 142% and 9.5, respectively. Postjunctional α -adrenoceptors and thromboxane A₂-receptors may play a role in the contractility of the human thoracic duct.

Previously we showed that lymphatics obtained from the human groin have a diverse susceptibility to certain vasoactive drugs compared to lymphatics taken from the human lower leg (1,2). Such regional differences are well known from experiments on human arteries and veins (3). However, investigations on isolated human lymphatics have been few and more regions need to be studied and compared. With greater insight into the different receptor types incorporated in the smooth muscle cells of lymphatics obtained from vari-

ous regions of the body, a pharmacological approach to some types of lymphedema may be possible.

We had the opportunity to examine a piece of the mediastinal thoracic duct, which had to be ligated in the right chest for control of a cervical fistula of the thoracic duct injured during a radical neck dissection for treatment of a papillary thyroid carcinoma. The thoracic duct segment was investigated similarly to other lymph vessels previously studied by us to allow a comparison of drug parameters.

MATERIAL AND METHODS

About 1cm of the thoracic duct was extirpated just above the diaphragm through the 9th intercostal space. The patient was a 66-year-old woman who 3 weeks earlier had undergone a hemithyroidectomy and radical neck dissection for treatment of a papillary carcinoma of the right lobe of the thyroid gland. During the operation, the thoracic duct was injured resulting in persistent cervical lymphatic leakage (~600ml/24h). The patient was otherwise euthyroid and without medication both before and after the operation. The lymphatic segment was removed with special care to minimize trauma and was immediately placed in a chilled (4°C) Krebs buffer solution (for composition see below) and brought to the laboratory. With the tissue submerged in the buffer solution, the connective tissue was removed from the lymphatic using an operative microscope. The vessel was cut into 8 uniform segments which were mounted in organ

baths for investigation of isometric contraction (for method, see 1).

These lymphatic truncal segments were repeatedly stretched in the baths for one hour, and then they were left (about 15 min.) to stabilize to a basal tension. The bath fluid was exchanged to a K^+ (124mM) Krebs buffer solution, thereby giving a "maximal" contraction. This procedure was repeated until reproducible responses were obtained. In only two segments did potassium induced contractions exceed 1mN. In the other 6 segments, no contraction was recorded although the basal tension was changed between 1 and 5mN. No further experiments were performed on these 6 "non-contracting" segments.

Drugs and buffer solutions

Dilutions of 1-noradrenaline hydrochloride (Sigma) was made in NaCl containing 1mM ascorbic acid. A stock solution of U-46619 (a gift from Upjohn) was made up in absolute ethanol (5mg/ml). The final dilution of U-46619 was made with phosphate buffer at neutral pH just before use. The Krebs buffer solution was composed of (in mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ and glucose 11. The composition of the K^+ (124mM) buffer solution was similar apart from exchanging all NaCl with equimolar amounts of KCl. The concentrations are given as final molar concentrations in the organ baths.

Analysis of data

Agonist-induced contractions were related to the maximal amplitude of the previous K^+ (124mM)-induced contraction. The maximum contraction, E_{max} , and the negative logarithm of the concentration giving half maximal contraction, pEC_{50} , were determined.

RESULTS

For the two lymphatic segments used, the distance between the metal holders as measured at basal tension (1.2 and 3.5mN, respec-

tively) was 2mm and the length was 1mm for both segments. The potassium-induced contractions recorded before the agonists were added, were 7.2 and 1.0mN, respectively.

Noradrenaline and U-46619 induced tonic and phasic contractions (Fig. 1). At a noradrenaline concentration of $10^{-5}M$ the phasic contractions had a frequency of 5 min^{-1} . The highest frequency, 9 min^{-1} , was recorded with $3 \times 10^{-10}M$ of U-46619 present in the bath. The concentration-response curves are plotted semi-logarithmically in Fig. 2. Noradrenaline had a mean E_{max} of 50% of the previous K^+ (124mM)-induced contraction, and the mean pEC_{50} -value was 6.7. The E_{max} and pEC_{50} -values of U-46619 were 142% and 9.5%.

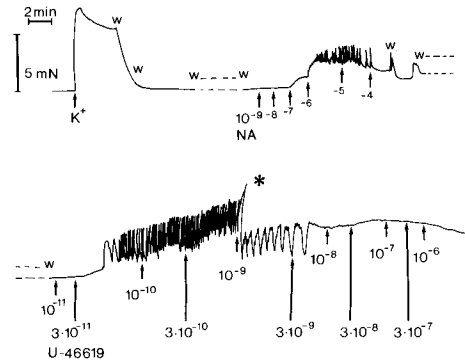


Fig. 1. Tracing from a single thoracic duct segment. Noradrenaline concentrations higher than $10^{-9}M$ are shown as log concentrations. After the cumulative addition of noradrenaline several washouts with fresh Krebs buffer solution (W) were made during one hour before U-44069 was added cumulatively. K^+ indicates the previous potassium (124mM)-induced contraction. * Indicates a 100% decrease in the sensitivity of the amplifier.

DISCUSSION

This investigation was made on only two segments of the thoracic duct from one patient. Therefore, no definitive statements are possible regarding differences in potency or intrinsic activity to the drugs tested. Nonetheless, the ability of noradrenaline to induce con-

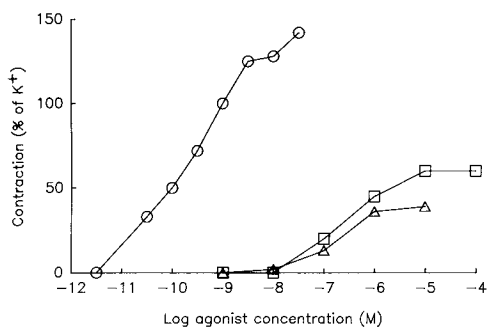


Fig. 2. Concentration-response curves for U-44069 (○) and noradrenaline (□ and Δ; □ is the same vessel segment on which U-44069 was tested) in the human thoracic duct. The contractile responses are expressed as percentage of the previous K⁺-(124mM)-induced contraction.

traction is greater in the thoracic duct than in lymphatics taken from the superficial groin (1) and the lower leg (2). In the present investigation, the frequency of the phasic contractions induced by noradrenaline is in the lower range of those in the lower leg (5-13 min⁻¹) (2). The thromboxane A₂-mimetic U-46619 also seems to be more potent on the thoracic duct (pEC₅₀=9.5) than in lymphatic segments obtained from the groin (pEC₅₀=8.1) (4).

At basal tension the circumference of the tested segments of the thoracic duct were about four times longer than lymphatics previously studied (1,2,4). This finding means that there was no problem in handling or mounting the segments in the organ baths. If the vessel lumen is overly narrow, these highly fragile lymphatic vessels can easily be injured by the metal holders in the organ bath. Still, only 2 out of the 8 segments were able to contract after the addition of K⁺ (124mM) Krebs buffer solution. This discrepancy may indicate that the distribution of smooth muscle is non-uniform along the thoracic duct.

The strong response of the thoracic duct compared to the very weak response of the superficial groin lymphatics to noradrenaline suggest a more important role for postjunctional α-adrenoceptors in the former. Postjunctio-

nal α-adrenoceptors seem also to play a role for the contractility of the lymphatics in the lower leg (2) but not in the groin (1).

Because this investigation was done on a segment of the thoracic duct taken from a patient three weeks after the removal of a papillary thyroid cancer, we cannot exclude the possibility that intra- or perioperative stress or the malignancy itself influenced the results.

REFERENCES

1. Sjöberg, T, L Norgren, K-E Andersson, et al: Contractile response in isolated human groin lymphatics. *Lymphology* 20 (1987), 152.
2. Sjöberg, T, S Steen: Contractile properties of lymphatics from the human lower leg. *Lymphology* 24 (1991), 16.
3. Sjöberg, T: Contraction-mediating receptors in human peripheral vessels with special reference to veins and lymphatics. Thesis. 1989 In: *Bulletin Nr 72 from the Department of Surgery, Lund University, Sweden*.
4. Sjöberg, T, K-E Andersson, L Norgren, et al: Antagonism of thromboxane receptor induced contractions in isolated human groin lymphatics. *Lymphology* 22 (1989), 135.

Trygve Sjöberg, Ph.D.
Department of Cardiothoracic Surgery
University Hospital of Lund
S-221 85 Lund
SWEDEN