Device for Investigation of Mechanical Tension of Isolated Smooth Muscle Vessels and Airway Segments of Animals

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Abstract. For the purpose of testing and the search for new drug compounds, designed to heal many human diseases, it is necessary to investigate the deformation of experimental tissue samples under influence of these drugs. For this task a precision force sensor for measuring the mechanical tension, produced by isolated ring segments of blood vessels and airways was created. The hardware and software systems for the study of changes in contractile responses of the airway smooth muscles and blood vessels of experimental animals was developed.

1. Introduction
Blood vessels play an important role in the regulation of blood pressure and blood flow distribution. Cell-to-cell communication provides coordination of processes in complex organisms. It is important to understand the mechanisms of regulatory processes of vessels and their interaction in vessel networks in health and disease. However, vessel responses in vivo depend on a complicated interaction between the different cell types of the vessel wall, factors released from nerve endings, metabolites, hormones and mechanical factors like shear stress from the surrounding tissue [1]. The complexity of the system limits the interpretation of experimental results in mechanistic terms. Therefore, in vitro methods have been introduced, where many of these factors can be controlled. The endothelium provides vasodilator effects of circulating hormones. It metabolizes various vasoactive substances, coverts angiotensin I to angiotensin II and secretes the potent vasodilators prostacyclin and EDRF (NO) and the vasoconstrictor peptide endothelin-1. The balance between these mediators determines the responses of the cardiovascular system in diseases such as hypertension, atherosclerosis and myocardial infarction 2]. Moreover, it may tonically suppress vessel constriction even without direct activation [3, 4]. The impaired endothelial function is a major cause of many cardiovascular disorders [5].

Investigation of the vessel segment reactions to the application of external stimuli such as vasoactive substances, pressure, etc., requires different methods. Several methods for the study of vessel segments have been developed. One method employs ring vessel segments threaded on two hooks or wires and the other cannulated vessel segments fixed on two pipettes. In in vitro experiments, vessels are studied in physiological saline solution. Ca²⁺ is an important ion, because it plays a central role in the contraction [6]. H⁺ is another important ion, because small changes in pH induce considerable changes in diameter of vessel.
The fundamental mechanisms for regulation of contractile activity of vascular smooth muscle and the airways are currently actively investigated to find new drugs to correct pathological states. Smooth muscle hyperactivity is a key mechanism of cardiovascular diseases and pathological conditions associated with the development of an inflammatory response under the influence of radiation exposure to the body [7-9]. Research of contractile activity of smooth muscle in biomedical experiments is not conceivable without the use of high-precision force sensors available, that can measure mechanical tension of small diameter bronchi and blood vessels. So, the actual task is to develop a hardware and software system for medical and biological experiments in this field.

The most common method of measuring contractile force is by the use of a force transducer. Such transducers provide real time, continuous readings and can be combined with software to create plots and data analyses. It is very easy to calibrate such devices. Advancements in technology development now allow to measure even microscale forces accurately.

Capacitive sensors can directly measure various quantities - motion, chemical composition, electric fields and many other variables, which can be converted into a movement or dielectric constant. The principle of operation of capacitive sensors for measuring mechanical quantities is based on the measurement of displacements. Movement of the one electrode with respect to the two fixed ones creates a variable capacitor.

Theoretically, reducing the value of clearance can increase the sensitivity of the sensor to high values. However, it is necessary to consider the electrical and mechanical conditions, which limit the maximum sensitivity. One of the major advantages of the capacitive sensor is that to move one of the plates, a very small value of the force is required. Another advantage is stability and weak influence of the ambient temperature and pressure, as well as high reliability and linear response.

2. Materials and methods
For investigation of mechanical tension of isolated smooth muscle vessels and airway segments of animals the capacitive sensor is used. A microcontroller based device using ATMega-16 microcontroller for the capacitance force sensor has been designed and developed. The block diagram of the device is shown in Figure 1.

![Figure 1. Block diagram of the device](image)

To accurately measure the capacitance changes AD7745 chip is used, which is a capacity-digital converter with high resolution. The measured capacitance is connected directly to the input of the chip. The structure includes a 24-bit ADC with 0.01% linearity. Measurement accuracy is ± 4 fF.

Calibration of the device was made using weights of a known mass. The capacitance value for each sample was measured. The results of calibration are shown in Figure 2.
Figure 2. Calibration curve of the device

Form of the curve confirms the efficiency of the device. Next, the device was tested on the real segments vessels. Material for the study was provided by the Department of Biophysics and Functional Diagnostics Siberian State Medical University, Russia, Tomsk. We used mature outbred Wistar rats weighing 180-240 g (n = 13) and guinea pigs weighing 380-420 gr (n = 13). Trachea and bronchi were purified from the surrounding tissue in the bath with Krebs solution at room temperature, after which the ring segments were prepared - width 1.5-2 mm; diameter - 1-1.5 mm.

If necessary, the epithelium was mechanically removed, by rotation of the wooden spatula in the lumen segment for 1 min before performing the experiment. Before the study the intact segments were tested in Krebs potassium solution (40 mM). Contractile response values were evaluated as a percentage of the amplitude of the reference contraction. Increasing the KCl concentration leads to smooth muscle cell membrane depolarization, opening of voltage-dependent calcium channels, and a subsequent contraction of bronchial smooth muscle. Injection of L-cysteine reduces the mechanical tension of segments Figure 3.

![Graph of KCl concentration vs. tension](image)

**Figure 3.** Effect of L cysteine on bronchus segments tension of guinea pig. The arrows show the injection of L cysteine in concentrations 100 μm, 1000 μM, 10 mM and 100 mM.

There is a difference in contractility of the guinea pigs intact bronchial segments and with impaired epithelium Figure 4. Injection of L-cysteine has a relaxing effect on the whole concentration range. Similar experiments were performed using rat trachea segments Figure 5.
A comparison of the relaxing segment responses with intact epithelium and nude one showed that the epithelium contributes to the regulation of modulating tension of the bronchus smooth muscle wall.

3. Results and discussion

The developed device allows to measure the tension of the airways and blood vessels segments with good accuracy. The operation principle is based on the use of capacitive sensor. The contractile properties of airway smooth muscle determine the responsiveness in isometric conditions. The responses to exogenous stimulus are dependent on the intrinsic tone of the airway preparation. When this intrinsic tone is low, contraction is observed, in the case of high tone, relaxation appears. Thus, hydrogen sulphide donor L-cysteine, in concentrations 0.1-100mM exerts a relaxing action on isolated segments of rats and guinea pigs airways. Bronchial epithelium of guinea pigs decreases the effect of hydrogen sulfide donor on the relaxation, while the tracheal epithelium of rats enhances dilation. According to the literature, the relaxation of smooth muscles of the airways depends on opening of different types of potassium channels. These results show that hydrogen sulfide is very important in the development of pathological disorders of the airways and contribute to finding effective ways of pharmacological correction of these conditions.

4. Conclusions

The device is developed for measuring the mechanical tension, produced by isolated ring segments of blood vessels and airways. The device allows to measure the value of forces to within 0.1 mN. The pathophysiology of animal airway was measured using the device. The functional properties of airway smooth muscle are very important in understanding the properties of the airways. There are important differences between species. Different influence of epithelium in rats and pigs can be linked to the density of potassium channels in the membrane of the epithelium and smooth muscle cells. Nevertheless, studies in vitro offer the opportunity to test the side effects of novel pharmacological compounds. The developed model allows to study the effects of drugs, inflammatory mediators, and epithelial cells on the responsiveness of airway smooth muscle and blood vessels in vitro.

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