საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 11, №3, 2017
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 11, no. 3, 2017

Human and Animal Physiology

Direct Measurement of Contractility of Isolated Small Arteries Preparations

Nino Chikobava[*], Merab Dekanosidze[**], Nodar Mitagvaria[§]

[*]*Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[**]*New Vision University, Tbilisi, Georgia*
[§]*Academy Member, I. Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia*

ABSTRACT. In the present study we offer the system of simple but effective method assigned for studying the metabolites direct action on the smooth muscles of small arteries. The object of study is the ring of the artery isolated from the experimental animal. Before the beginning of experiment the ring preparation (1.5 mm width and up to 500 m in diameter) is prepared under the light microscope. The preparations are connected to mechanotron sensors, located in special chamber with a flowing Krebs Solution. After that, the vascular rings exposed to preliminary stretch and tension (up to 5 mN). For recording the contractile activity of the isolated arterial preparations a tensometric system with a mechanotron (6MX1C) in the isometric mode is used.

The amplified signals from a mechanotron are directed to recorder. With intervals at 15-30 of minutes the studied biologically active substances (or substance) are added to the Krebs solution in desirable concentration and its effect (if any) will be reflected on the recorder. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: arterial preparations, tensometric system, contractility

An objective method for analyzing the function of arterial vessel's smooth muscles is to measure the parameters of contractility of isolated vascular preparations using mechanotronic transducers [1]. This method allows you to measure the degree of relaxation or the level of increase in the tone of the vessels, depending on the nature of the influence of a different type of action on the object under study. By means of such a methodical approach, it becomes possible to analyze some mechanisms of smooth muscle regulation without intervention in these mechanisms of centrogenic neurohumoral signals.

The method makes it possible to evaluate the reactivity of smooth muscles on a wide range of physiologically active substances in their sequential, as well as combined application. Usually, this method was used successfully to study the function of smooth muscles of large vessels. At the same time, the activity of the smooth muscles of small arteries (<500 jm) was assessed only indirectly by their visually recorded reactions of narrowing or widening when vasoactive compounds were applied [2, 3].

© 2017 Bull. Georg. Natl. Acad. Sci.

Object of the Study

In our experiments we tried to perform measurements on ring segments of isolated pial arteries of rabbit. The object of the study is the segment of a small artery, branching off from a larger vascular trunk. By its anatomical position, such a site should be attributed to the category of vasoconstrictors providing nutrition to a particular cortical zone, regulation of blood flow in smaller arteries, including intracerebral arteries, and therefore they might be considered an important link in the mechanisms of blood supply to the cerebral cortex [2, 4].

To make the vascular preparations, the method of preparing the ring segments of isolated artery was used [5, 6]. This method of preparation does not lead to significant violations of the architectural integrity of the vessel as well as the spatial orientation of the smooth muscles of the arteries [7]. The structural integrity of the arterial preparation was controlled under the microscope.

How to Prepare Vascular Preparations

Vessels are extracted from the brain of the animal immediately after it is slaughtered and placed in a standard Krebs-Halitol saline solution. If necessary, the material can be stored in the refrigerator at + 5^0C for 24 hours [8 - 10]. Immediately, before the experiment, under the binocular microscope the ring segment (1.5 mm width and up to 500 m in diameter) must be cut off from the artery. With the aid of a special device [11], the segment of the vessel is placed in a jar (Fig.1-A) (with a Krebs-Heilith solution) of the working chamber of a tensometric device, where it is mounted on two metal hooks (Fig. 1-B), one of which is rigidly attached to the mechatronic rod. The preparation has to be stretched up to the level of 5.1mN which was defined according to data received after smooth muscle contractility testing by standard solutions containing K^+ at a concentration of 80M. The preparation must be heated in Krebs solution for at least 1.5 hours at 37°C.

Registration of Smooth Muscles Mechanical Activity of Vascular Preparations

The registration of the isolated vascular preparations contractile activity can be carried out on a tensometric device with 6MX1C mechanotrones in isometric mode. Electric signals from mechatronics are fed to amplifiers, as the bridge circuits for which it is possible to use the simple, standard bridge circuit.

Calibration of mechatrons is performed in millinewtons (mN) by hanging of the standard weights to horizontally placed rods and mesurements (in mm) of pen deviation from the initial level in the recorders diagram tape. This method of calibration can be acceptable, because mechano-electric sensors of type 6MX1C, are able to provide the precision measurement of linear displacements and forces. Each mechanotron must be calibrated separately. The measuring range is usually 0 - 10.2 mN.

The level of stretch is usually rationed by the maximum contractile responses to the hyper-potassium solution (80 mM) per liter of Krebs solution

Preparation of Solutions, Control of Medium pH and Constancy of Temperature

As a feed solution, a Krebs flowing solution of the following composition (in mMol / L) is used: NaCl - 118.0; KCl: 4.7; $NaHCO_3$ = 14.9; KH_2PO_4 1.18; $MgSO_4.7H_2O$ 1.17; $CaCl_2.2H_2O$ 2.5; Glucose - 11.0. The experiments are carried out under the control of the pH of the flowing medium. PH measurements are made throughout the test, immediately before each exposure by means of pH- or ionometer. In the solution it is necessary to maintain a pH equal to 7.35-7.45.

The constant temperature of solutions during the experiment is maintained on the level of 37±0.5^{0}C by means of an ultrathermostat, which drives the heated water through special flasks with water jackets and a thermostated chamber connected to a common circulatory system (Fig. 1B).

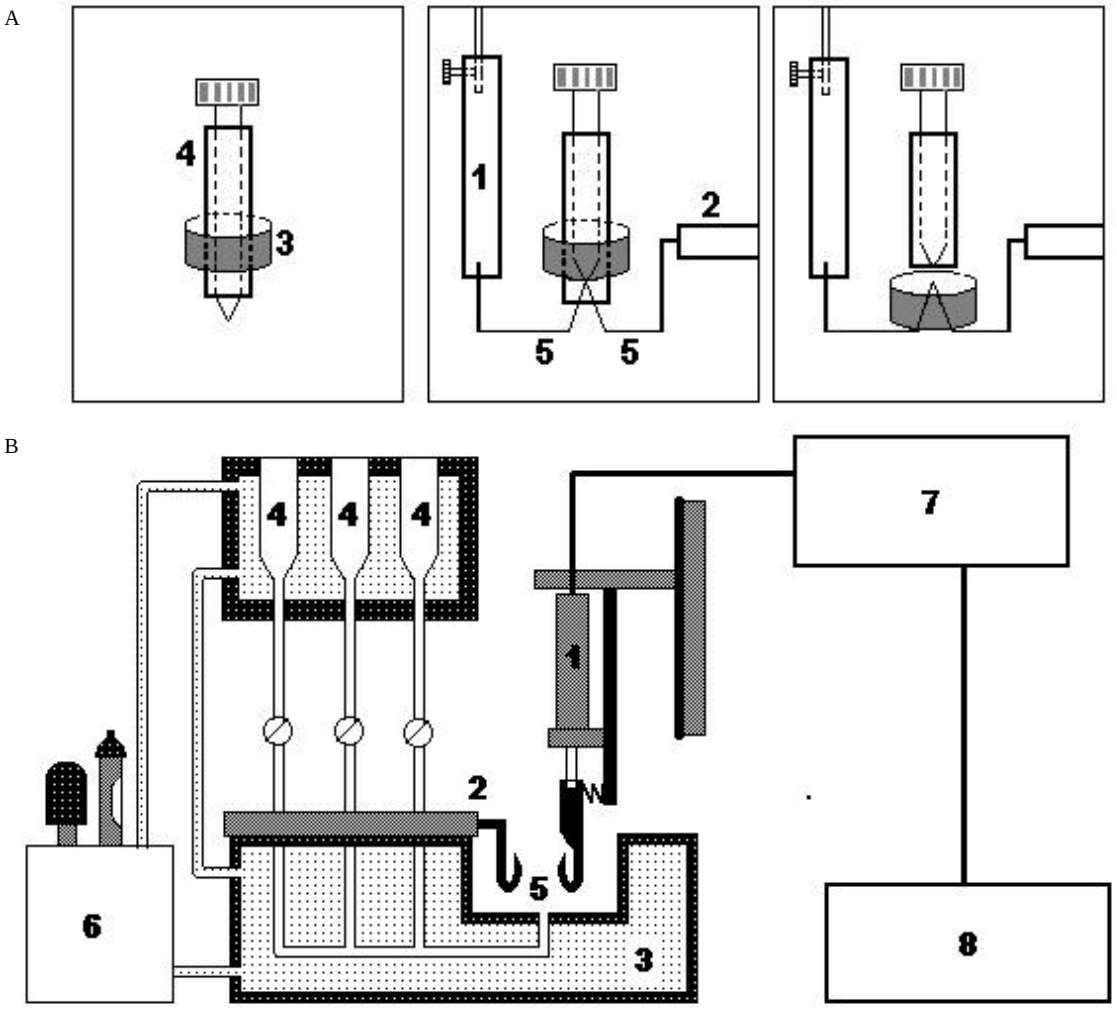

Fig. 1. A – Stages of the vascular segment attachment to the hooks of the tensometric mechanotron. ***Designations***: 1 - Mechanotron, 2 – Part of the tension and calibration mechanism, 3 - Ring segment of the artery, 4 - Temporary holder, 5 - Hooks.
B - The block diagram of the device: ***Designations***: 1 - Mechanotron; 2 - Tension and calibration mechanism; 3 - Thermostatic chamber; 4 - Flasks with Krebs solution; 5 – Hooks in Operating chamber; 6 - Ultratermostat; 7 - Amplifier unit; 8 – Recorder.

Used Impacts

To analyze the contractility of the vascular preparation of small arteries, the pharmacological substances and metabolites used (for example, noradrenaline, histamine, serotonin, acetylcholine, adenosine, hypocapnicidal solution, etc.) should be added to the Krebs solution. The solution must be prepared before the experiment. The duration of the exposure and the concentration of the substance used, depend on the task and are selected by the experimenter. The substances have to be introduced into the jar of the working chamber with a 15-30 minute interval.

Conclusion

The described technique allows studying the direct action of metabolic regulation factors at the level of isolated vascular preparations and makes it possible to assess their regulatory significance in relation to other regulatory factors.

ადამიანისა და ცხოველთა ფიზიოლოგია

მცირე არტერიის იზოლირებული პრეპარატის კონტრაქტილობის პირდაპირი გაზომვა

ნ. ჩიქობავა[*], მ. დეკანოსიძე[**], ნ. მითაგვარია[§]

** ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, თბილისი, საქართველო*
*** ნიუ ვიჟენის უნივერსიტეტი, თბილისი, საქართველო*
§ აკადემიის წევრი, ი.ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

წარმოდგენილ სტატიაში შემოთავაზებულია მარტივი, მაგრამ ეფექტური მეთოდი, რომელიც საშუალებას იძლევა შევისწავლოთ არტერიული სისხლძარღვების გლუვ კუნთებზე მეტაბოლიტების უშუალო, პირდაპირი მოქმედების ეფექტი. კვლევის ობიექტს წარმოადგენს ექსპერიმენტული ცხოველის არტერიის იზოლირებული რგოლი. ცდების დაწყებამდე არტერიული რგოლის პრეპარატის (სიგანე 1,5მმ, დიამეტრი - 500 მკმ-მდე) მომზადება ხდება სინათლის მიკროსკოპის გამოყენებით. პრეპარატი ფიქსირდება მექანოტრონის სენსორებზე მოთავსებულ სპეციალურ კამერაში, რომელშიც უწყვეტად გაედინება კრებსის ხსნარი. ამის შემდეგ სისხლძარღვის რგოლი განიცდის წინასწარ დაჭიმვას (5 მილინიუტონის დონემდე). იზოლირებული სისხლძარღვოვანი პრეპარატის (რგოლის) კონტრაქტილური აქტივობის რეგისტრაციისთვის გამოიყენება მექანოტრონებიანი (6MX1C), იზომეტრულ რეჟიმში მომუშავე ტენზომეტრული სისტემა. მექანოტრონიდან მიღებული სიგნალი ძლიერდება და მიეწოდება რეგისტრატორს. ბიოლოგიურად აქტიური შესასწავლი ნივთიერებები (ან ნივთიერება) 15-30 წუთის ინტერვალებით სასურველი კონცენტრაციით ჩაედინება კრებსის ხსნარში და მისი მოქმედების ეფექტი აისახება რეგისტრატორზე.

REFERENCES

1. Berlin GS., Petrov AG., Kharkevich DA., Shorr VA. (1979) On the possibility of using mechanotron transducers in experimental biological studies. *Bulletin of Experimental Biology and Medicine*, **88**, 11: 626-629.
2. Baramidze DG., Levkovich Yu.N., Mchedlishvili GI. (1984) Dynamics of local vascular reactions in conditions of increased brain activity. *Sechenov Physiological Journal of the USSR*, **69**: 1058-1064.
3. Wahl M., Kushinsky W. (1977) Influence of H^+ and K^+ on adenosine-induced dilatation at pial arteries of cats. *Blood Vessels*, **14**: 285-293.
4. Mchedlishvili GI., Baramidze DG. (1984) Physiological mechanisms of regulation of microcirculation in the cerebral cortex. *Sechenov Physiological Journal of the USSR*, **70**, 11: 1473-1483.
5. Vedernikov Yu., Ignatenko A. (1981) About the presence of specific receptors for bradykinin in the smooth muscle of arteries and veins. *Bulletin of Experimental Biology and Medicine*, **91**: 14-15.
6. Orlov RS., Aivar Yu.P. (1979) The effect of potassium ions on the contractile activity of the smooth muscles of the renal arteries. *Sechenov Physiological Journal of the USSR*, **65**: 1040-1045.
7. Rogoza AN. (1975) Investigation of mechanical parameters of small blood vessels. *Problems of Space Biology*, **3**: 165-176.
8. Belsky E.D. (1973) Spontaneous contractile activity of the smooth muscles of the subcutaneous vessels with temperature effects. *Sechenov Physiological Journal of the USSR*, **59:** 1536-1540.
9. Carrier O., Murphy J.C., Tenner T. (1973) Effect of cold storage on calcium – related responses and electrolyte content of rabbit aortic strips. *Europen Journal of Pharmacology* **2**: 225-233.
10. Linder M., Alksue J. (1978) Prevention of persistent cerebral vessels muscle contraction in response to whole blood. *Stroke*, **9**: 472-477.
11. Klimin V.G. (1989) Influence of factors of metabolic regulation on the tone of smooth muscles in the arteries of the cerebral cortex. *Author's abstract. Cand. Thesis*, Kazan.

Received May, 2017