Isolated Perfused Resistance Vessels and Myogenic Tone*

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THERE are a little information about the re-activity of isolated vascular smooth muscle from resistance vessels, in spite of their important role in the regulation of circulation. The purpose of this paper is to present a method for studying isolated perfused resistance vessels

down to 50 μ o.d. and to present evidence for presence of myogenic tone in the resistance vessels. A preliminary report is also to be presented on the effect of sodium concentration in perfusate on the myogenic tone.

Fig.1. Schematic presentation of the conceptual components for total peripheral resistance. The evidence of myogenic tone in isolated perfused resistance vessels gave a support to the idea of the differentiation of myogenic tone from non-neurogenic tone (basal tone).

Key Words: Resistance Vessel
Vascular Smooth Muscle
Vascular Myogenic Tone
Hypertension
Total Peripheral Resistance
Sodium

Methods of Perfusion of Resistance Vessels

Preparation of vessels and the method of perfusion have been described in detail! A part of the arterial tree was isolated from an organ and a section of the vessel prepared for perfusion by

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Fig.2. Effect of low Na concentration on myogenic tone in isolated resistance vessel perfused with Tris-PSS. Myogenic tone in Tris-Ba PSS (NaCl 130.0, KCl 6.0, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.2, Dextrose 5.5, Sucrose 60.0, Tris 5.0, BaCl_2 1.6, mM in liter; neutralized to pH 7.4 with HCl; equilibrated with oxygen; at 37°C) disappeared when Ba was removed. It reappeared when 1.6 mM Ca added. The perfusate contained normal 130.0 mM Na. Its magnitude increased greatly when the perfusate was changed to Tris-Ca (1.6 mM) PSS containing low Na (65.0 mM). Isosmotic replacement of Na was accomplished by Tris.

tying off all branches, except the terminal one. The vessel segment was cannulated at its proximal end and mounted in a muscle chamber and was perfused with warmed (37°C), aerated (95% O_2 , 5% CO_2) physiological salt solution (PSS, a modified Krebs solution). A constant flow rate was maintained by a modified Sigma motor pump (Model T8). Perfusion pressure was recorded by a pressure transducer, through the side arm of the cannula. Since perfusion was at constant flow rate, a rise in perfusion pressure indicates vasoconstriction and a fall in perfusion pressure indicates vasodilatation. All pharmacological agents were injected into the perfusion fluid by a micro-injector system.

The validity of the method was established because responses to a given stimulating agent

are reproducible and stable over a reasonable period of time. The important role of the terminal minute branch was revealed by the fact that 45 to 90% of the resistance of the whole vessel was due to the resistance in the terminal segment.

The increase of total peripheral resistance in established hypertension has been well documented and the important role of the resistance vessel for maintenance of high blood pressure has been emphasized. The method of perfusion of resistance vessels described above might promise to help to clarify the mechanism of sustained high blood pressure in hypertension. The method may be used in making preparations from any vascular bed and might be applicable to studies of the vascular reactivity in hypertensive vascular diseases.

Na and Myogenic Tone

— Rat Skeletal Muscle Artery —

Fig.3. Effect of alteration of Na concentration on myogenic tone in isolated resistance vessel perfused with modified Krebs solution (NaCl 119.0, KCl 4.7, KH_2PO_4 1.18, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.17, NaHCO_3 14.9, Dextrose 5.5, Sucrose 50.0, CaNa_2EDTA 0.026, CaCl_2 1.6, mM in liter; equilibrated with 95% O_2 and 5% CO_2 ; at 37°C). Na was replaced by sucrose isosmotically. High Na reduced myogenic tone and low Na increased myogenic tone. Note the same level of tone during perfusions with normal Na PSS.

Myogenic Tone in Isolated Resistance Vessels

In situ studies yield only indirect evidence as to whether non-neurogenic vascular tone results from an intrinsic myogenic tendency of the smooth muscle cell to contract or is due to stimulation of the muscle by some vasoactive humoral influence of the environment. With using our method of perfusion, we have observed spontaneous myogenic tone in isolated resistance vessels perfused with PSS² which has rarely been described in numerous reports dealing with large conduit arteries. The presence of myogenic tone in perfused vessels can be demonstrated by (1) a gradual increase in perfusion pressure with time after starting perfusion; (2) a large drop in pressure during perfusion with Ca-free PSS; and (3) vasodilatation by vascular smooth muscle relaxants such as sodium nitrite, papaverine and hydralazine. The residual pressure during Ca-free perfusion or after injection of a supramaximal dose of the smooth muscle relaxants represents the passive structural resistance of the vessel and the

magnitude of the decrease in perfusion pressure reflects the amount of intrinsic myogenic tone. Since the constriction was not eliminated by reserpine pretreatment or by alpha adrenergic blockade and since the vessel was isolated and in an inert physiological salt solution, the contraction is neither of neurogenic nor of humoral origin but is of myogenic origin. Further study showed that myogenic tone was prevalent in smaller resistance vessels of many vascular beds although significant differences were observed among the species studied.³

The observation that most resistance vessels have myogenic tone leads us to infer that the myogenic tone may be responsible for an important component of total peripheral resistance. The isolated perfusion method gave us an actual proof for differentiation of myogenic tone from non-neurogenic tone in situ studies. Fig.1 shows conceptual components of total peripheral resistance and the possibility is to be searched for increase in the magnitude of myogenic tone in

established hypertension with high peripheral resistance.

Sodium and Myogenic Tone

Sodium has an intimate relation to production and acceleration of hypertension. Conflicting results of altered Na concentration on vascular muscle contraction even in normal animals have been reported. The current study examines the role of changes in Na concentration in perfusate on myogenic tone of isolated resistance vessels.

After myogenic tone was stabilized in normal PSS, perfusate was changed from normal to low Na PSS or to high Na PSS.

Reduction of Na Concentration: Lowering the Na concentration from the control to 15–65 mM increased greatly the magnitude of myogenic tone either in Tris PSS as shown in Fig.2 (4 preparations: Na was replaced by Tris) or in bicarbonate buffer PSS (3 preparations: Na was replaced by sucrose). Reduction of Na from 134 mM to 109 mM was enough to produce the detectable change in myogenic tone (Fig.3).

Increase of Na Concentration: Increasing the Na to 159 mM replaced by sucrose in bicarbonate buffer PSS had the opposite effect and decreased the magnitude of myogenic tone in four experiments of two preparations (Fig.3).

There are now ample evidence that the entry of Ca from the extracellular space into the vascular smooth muscle may be essential for maintenance of myogenic tone: the magnitude of myogenic tone was directly dependent on Ca concentration of perfusate²; addition of EDTA and Mn to perfusate lowered it⁴; Ba and Sr which are not present in normal cells could substitute Ca for its maintenance⁵. Thus the results support the idea that decrease in extracellular Na may increase Ca influx and increase in extracellular Na may decrease Ca influx⁶.

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DISCUSSION:

Chairman: YOSHIKI MASUYAMA, Tokyo Univ.

In 1967, Dr. Uchida reported with Dr. Bohr the new method for studying the reactivity of isolated resistance vessels, thereafter he has reported very interesting results about myogenic tone and the reactivity, using a series of various isolated vessels.

Dr. IKEDA (Tokyo University) asked the following three points; 1) the range of diameter of resistance vessels, 2) relationship between vascular contraction and tone and 3) the role of myogenic tone on hypertension. The answers were as follows: 1) The vascular portion which determines total peripheral resistance had been mentioned in the classical paper by Haddy. It has been shown that pressure drop is large at the vessels of 50–500 μ o.d. in vascular beds. 2) Myogenic tone is one of the factors which initiate vascular smooth muscle contraction, besides nerve stimulation and vasoactive substances. 3) The role of myogenic tone of the vessels on the pathogenesis of hypertension cannot be explained by these experimental results. It could be speculated that there might be some difference of myogenic tone in hypertensive and normotensive animals.

Dr. MOTOMURA (Kyoto University) asked the effect of the total ionic strength in perfusate on myogenic tone, especially when Na concentration was altered. Dr. Uchida answered: Similar results were obtained both when Na replacement was performed isotonicly by sucrose in Krebs PSS and by Tris in Tris PSS perfusate. The entry of extracellular Ca into the cell appeared to be essential for the maintenance of myogenic tone. Therefore, Na might affect on Ca influx by Na-Ca exchange.

Dr. IIMURA (Sapporo Med. Coll.) asked; 1) the effect of Ca on myogenic tone, when Ca concentration in perfusate was gradually reduced and 2) the changes of vascular reactivity to noradrenaline when myogenic tone was altered by Na or Ca concentration. He had observed that basal perfusion pressure was higher in animals fed by thyroid powder than controls and there was inverse correlation between basal perfusion pres-

sure and pressor responses to noradrenaline. Dr. Uchida answered: 1) The effects of gradual changes in Ca concentration on myogenic tone were not observed. There found neither myogenic tone nor responses to noradrenaline during Ca-free perfusion. 2) Conflicting results have been reported about the effect of Na concentration on vascular smooth muscle, depending on the different vascular beds and kinds of the responses to stimuli or perfusate. It had been reported that the responses to noradrenaline was opposite in F-component, using bound Ca, to those in S-component, related to Ca entry. (Bohr, D. F. 1971)

Dr. NOZAKI (Kyoto Univ.) asked; 1) the nature of oscillation in the tracing and 2) relationship between myogenic tone and flow rate in such a constant flow perfusion experiment, from the point of autoregulation. Dr. Uchida answered; 1) The oscillation in the tracing is due to the

spontaneous contraction of the vascular smooth muscle. 2) Myogenic tone was not produced by raising perfusion pressure in the preparations without myogenic tone. In preparations with myogenic tone, the relationship between myogenic tone and perfusion pressure is not yet determined, because the technical limitation and the difficulty to differentiate active contraction and passive distension.

Myogenic tone and vascular reactivity to vasopressor substances would be compared in hypertensive and normotensive animals by using this experimental method. However, the variability of individual preparations would make some difficulties to compare those from these different animals. Though it remains some steps to transfer the results obtained by the in vitro experiment to the in vivo state, these data using isolated resistant vessels might be very valuable for the study on the pathogenesis of hypertension.