EXPERIMENTAL AND MATHEMATICAL MODEL FOR THE EVALUATION OF DYNAMIC RESPONSES OF ISOLATED BLOOD VESSELS

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ABSTRACT
In this study we present the experimental and mathematical model, which can be used for precise assessment of isolated blood vessels dynamic response to different stimuli. Two experimental procedures were applied: Using metal-tubes (instead of blood vessels) to standardize experimental conditions and to evaluate mathematical functions which describe the dynamic behavior of the process; Using isolated blood vessels (rat abdominal aorta) to demonstrate how the changes in process dynamics can be described in detailed quantitative terms by mathematical parameters. Up to now, various studies have observed only the end points of considered change, or so-called the “alternate steady states” of the processes. Unfortunately, in this way, we cannot be able to describe fully and precisely what happens to the process variables in between these alternate steady states.

Our results show that applied experimental model and mathematical procedures enable us to describe precisely (at the high sensitivity level) what happens with the pressure change in between alternate steady states.

Keywords: arterial dynamics response, rat, biomechanical properties, L-arginine.

INTRODUCTION
In this study we present the experimental model and mathematical procedures which can be used to describe precisely the dynamic response of isolated blood vessels to different stimuli. The reason why we used the isolated perfuse segment (rat abdominal aorta) in this study, is because the response obtained with the whole isolated vessels are more closely approximate to in vivo responses than in the experiments with the strip of the vessels (1). Up to now, various studies (2–6) have observed only the end points of considered change, or so-called the “alternate steady states” of the processes. Unfortunately, in this way, we cannot be able to describe fully and precisely what happens to the process variables in between these alternate steady states. This behavior, in between alternate steady states, is referred to as the transient or “alternate steady states”. This behavior, in between alternate steady states, is referred to as the transient or “alternate steady states”.

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1) First - using of metal-tubes (instead of blood vessels) to achieve the standardized experimental conditions and to evaluate mathematical functions which can describe the dynamic behavior of the process.
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First procedure. All experiments were performed using metal-tubes, with length of 40 mm and internal diameter 1, 2, 3 and 5mm. Each of the metal-tubes was placed into the water-bath on which proximal (inflow) and distal (outflow) end we mounted two-glass cannula with equally matched tip diameters. One end of the tube was placed on the proximal cannula and the other one onto the distal cannula. Lumen of the metal tube was perfused with water using a peristaltic pump. The distal cannula was connected to the two-way tap, which allowed two-direction flow of perfusion water through two independent tubes. One of the presence and absence of L-arginine. The influence of L-arginine on the blood vessel is well known, so we have chosen this substance to demonstrate how the changes in dynamics of the process can be described in detailed quantitative terms by mathematical parameters.

SAŽETAK
U ovoj studiji predstavljamo eksperimentalni i matematički model koji može da se koristi za preciznu procenu dinamičkog odgovora izolovano-nog krvnog suda na različite stimuluse. Primjenjene su dve eksperimen-talne procedure: jedna sa upotrebom metaličnih cevica (umenio krvnih sudova) u cilju standardizovanja eksperimentalnih uslova i procese matematičkih funkcija kojima se opisuje dinamičko ponašanje, druga sa upotrebom izolovanih krvnih sudova (abdominalna aorta pacova), da bi se pokazalo kako dinamičke promene u toku samog procesa mogu da budu detaljno opisane kvantitativnim terminima koristeći matematičkih parametara. Do sada, različite studije su izučavale samo krajnje tačke poznamenatih promena, poznate kao „alternate steady states” procesa. Nakon toga, na taj način, nije moguće da se potpuno i precizno opišu što se dešava sa promenljivima veličinama između ovih alternativnih ravnateljskih stanja.

MIŠLJENJE
Nasli rezultati pokazuju da primjenjeni eksperimentalni model i ma-tematičke procedure daju mogućnost da se precizno opiše (na visoko senzitivnom nivou) što se dešava sa promenama pritiska između alter-nativnih ravnateljskih stanja. Eksperimentalni i matematički model za evaluaciju dinamičkog odgovora izolovanih krvnih sudova

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these tubes was at the level of the water bath and metal-tube, and the other one was elevated to a certain height, to allow the change of hydrostatic pressure from \( H_0 (0 \text{ mmHg}) \) to \( H_1, H_2 \) and \( H_3 \) (15 mmHg, 40 mmHg and 60 mmHg respectively).

Intraluminal pressure at the constant perfusion flow under different hydrostatic pressure conditions was measured with the System for Biomechanical and Functional Tissue Investigations (ECM, Kragujevac) consisting of (Figure 1):

a) Laboratory equipment, (bottle for oxygenation, two reservoirs with perfusion solution, two peristaltic pumps, water-bath with electrodes and temperature transducer).

b) Hardware (movable transducer holder (MTH), amplifier pressure and temperature signals, A/D converter, pressure transducer, driver for MTH and PC).

c) Software. (Data acquisition, experimental data fitting, statistical analysis)

- This System for Biomechanical and Functional Tissue Investigations allows:
- Recording of pressure/developed during the experiment (electro stimulation, drug effects etc.) by mechanoelectrical transducer and/or pressure transducer.
- Continual addition of drugs into the perfusion medium by micro infusion pump, for drug effects investigations.
- Linear or nonlinear fitting of experimental data with various mathematical formulas.
- Statistical analysis of experimental data.

Metal-tube (\( \Theta = 1.0, 3.0 \) and 5.0 mm) was perfused with constant perfusion flow (7.5 ml min\(^{-1}\)) and treated by increasing of hydrostatic pressure from \( H_0 (0 \text{ mmHg}) \) to \( H_3 (60 \text{ mmHg}) \).

Each of the described protocols was repeated 3–5 times and the developed intraluminal pressure was recorded on a computer, continuously.

Experimental data as the pressure/time curves were fitted using exponential mathematical functions as follows:

\[
y(t) = b_1 \left(1 - e^{-b_2 t}\right)
\]

where \( y \) is the pressure (in mmHg) and \( x \) is the time (s). \( b_1 \) and \( b_2 \) are coefficients of the curve:

- \( b_1 \) has units of pressure and
- \( b_2 \) has units of time and it is destined to play a key role in our understanding of the dynamic behavior of processes.

This function (1) is an exponential one (Figure 2A). The constant \( b_1 \) represents the maximum of developed pressure (where the experimental curve reaches approximate value of the alternate steady state).

The dominant time constant (T) is the value of the cross section point, between the asymptote of the exponential curve and tangent of the exponential curve at the zero point, and can be expressed as:

\[
T = \frac{1}{b_2}
\]

We consider that alternate steady state is established for \( t = 5T \) (\( t=\text{time} \)), because in this case, \( e^{-t/T} = e^{-5T} = 0 \).

At this moment \( y = b_1 \), so we consider that maximum pressure is developed.

Figure 1. Schematic diagram of System for Biomechanical and Functional Tissue Investigations (1. perfusion fluid; 2. peristaltic pump; 3. AD converter and amplifier; 4. organ bath; 5. metallic tube or vessel; 6. transducer; 7. water bath; 8. oxygen bottle; 9. PC. 10. \( H_0, H_1, H_2, H_3 \) hydrostatic levels.)

Protocol:

a) In order to investigate the effects of hydrostatic pressure on intraluminal pressure we defined two experimental protocols:

b) Metal-tube (\( \Theta = 5 \text{ mm} \)) was perfused with constant perfusion flow (10 ml min\(^{-1}\)) and treated by increasing of hydrostatic pressure from \( H_0 (0 \text{ mmHg}) \) to \( H_n (n=15, 40 \text{ and } 60 \text{ mmHg}) \).

Figure 2. A: Exponential curve \( y=b_1 \left(1 - e^{-b_2 t}\right) \) and corresponding positive (\( D_k^+ \)) and negative (\( D_k^- \)) values of distensibility coefficients.

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2. Second procedure. All experiments were performed in accordance to the ethical standard recommended by the Helsinki Declaration. Wister rats of both sexes, weighing between 180–200 g were killed by cervical dislocation.

The segment of the abdominal aorta was rapidly isolated and transferred to the water-bath on which proximal (inflow) and distal (outflow) end we mounted glass cannula with equally matched tip diameters. The proximal end of the artery is tied to place on the proximal cannula with a silk thread, and the lumen is perfused with Krebs-Ringer physiological solution (KRS), using a peristaltic pump at 9.5 ml min\(^{-1}\). The perfusate was continuously bubbled with a 95% \(O_2\) and 5% \(CO_2\) and the pH adjusted to 7.4 at 37 ºC. The distal end of the artery is then tied onto the distal cannula. The distal cannula was connected to the two-way tap which had connections with two tubes filled with perfusion solution, and allowed the change of hydrostatic pressure from \(H_0\) (0 mmHg) to \(H_1\) (about 60 mmHg). The exterior of the vessel was perfused with KRS from a reservoir using a peristaltic pump at 3 ml min\(^{-1}\), at 37 ºC and aerated with the same gas mixture as the lumen of artery.

The artery is stretched to its approximate in vivo length using a micrometer. The strip diameters were measured immediately after the artery was isolated and suspended in KRS at 37 ºC using the light microscope and microscopically graduated plate. The abdominal arteries were considered to be viable if they contracted when 25 mM KCl was added to the bath as well as if the presence of functional endothelium was verified by dilation with ACh (1 \(\mu\)M), at the end of experiment.

Protocol

As described above, the segment was dissected out from the rat abdominal aorta. After equilibration period (20–30 min) in the water bath, the abdominal artery was perfused with constant perfusion flow at 9.5 ml min\(^{-1}\), and then this segment was treated by increasing of hydrostatic pressure from \(H_0\) (0 mmHg) to \(H_1\) (about 60 mmHg). This protocol was repeated 3–5 times for each segment and a resting period of several minutes (usually 15 min) was allowed between the two activities. The development of pressure was recorded on a computer, continuously using the same system that was described in the first procedure.

After this, we added L-arginine (1 mM, 10 mM and 20 mM) continuously into the perfusion system with micro infusion pump at 100 \(\mu\)l min\(^{-1}\). The same protocol at \(H_0\) and \(H_1\) hydrostatic pressure by constant perfusion flow was repeated 3–5 times for each molar concentration of the L-arginine.

Experimental data, presented as the pressure/time curves, were fitted using exponential mathematical functions (1), as described in the previous section. Then, to define distensibility properties of the blood vessels, we introduced coefficient of distensibility (Dk) as the integral of the difference between two fitted curves, normalized with respect to the applied hydrostatic pressure (H):

\[
D_k = \frac{1}{H} \int_0^\infty \left[ b_1 \left( 1 - e^{-b_2 \cdot x} \right) \right] \left[ b_1 \left( 1 - e^{-b_2 \cdot x} \right) \right] dx \tag{2}
\]

where \(b_2a\) is the coefficient of the first curve (in the absence of drug – control curve) and \(b_2b\) is coefficient of the second curve (from the experiment in the presence of the drugs – test curve).

Solution of the equation (2) is presented as:

\[
D_k = \frac{b_1 b_2 b_2 - b_2 a}{H b_2 a} \tag{3}
\]

The calculated Dk is the area between the test and control curves (Figure 2B). Positive value of Dk indicates the shift of the test curve to the left and faster development of maximal pressure (alternate steady state). On the contrary, negative value of the Dk would indicate the shift of the test curve to the right and slower development of maximal pressure.

Statistical analysis

The data were analyzed using Student’s t-test: where \(p\) value of \(<0.05\) was considered as statistically significant.

Solutions and reagents

The composition of Krebs-Ringer physiological solution contained (in mM): NaCl 117, KCl 4.7, NaHCO\(_3\) 24.8, MgSO\(_4\)\(\times\)7H\(_2\)O 1.2, CaCl\(_2\) 2.5, KH\(_2\)PO\(_4\) 1.2 and D-glucose 11.1 (Merck, Darmstadt). L-arginine (1mM, 10 mM and 20 mM) was prepared in KRS. All drug concentrations are expressed as the final molar concentrations in the intraluminal perfusate.

RESULTS

1. First procedure

As described previously, the experiments in the first procedure were performed in two ways:

a) Metal–tube, diameter of \(O=5\)mm and length 40mm, was perfused with constant perfusion flow of 10ml min\(^{-1}\) and treated by increasing of hydrostatic pressure from \(H_0\) (0mmHg) to three levels \(H_1\) (15mmHg), \(H_2\) (40mmHg) and \(H_3\) (60mmHg) (Figure 3). Experimental pressure-time curve was then fitted using equation 1 and coefficients \(b_1\) and \(b_2\) of the curves were obtained (Figure 4)
Table 1 shows the values of $b_1$ and $b_2$ coefficients of the fitted experimental data at different levels of hydrostatic pressures. There is no statistical significant difference between coefficient $b_2$ under $H_1$, $H_2$ and $H_3$ hydrostatic pressure, but the value of $b_1$ coefficient depends on the hydrostatic level.

Table 1. Effect of increasing level of hydrostatic pressure on $b_1$ and $b_2$ coefficients at constant perfusion flow of 10 ml min$^{-1}$.

<table>
<thead>
<tr>
<th>METAL-TUBE</th>
<th>$b_1 \pm$ SE</th>
<th>$b_2 \pm$ SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ø =1.0 mm</td>
<td>61.05 ± 0.284</td>
<td>3.38 ± 0.37</td>
</tr>
<tr>
<td>Ø =3.0 mm</td>
<td>40.24 ± 0.047</td>
<td>2.981 ± 0.589</td>
</tr>
<tr>
<td>Ø =5.0 mm</td>
<td>63.746 ± 0.0</td>
<td>3.65 ± 0.0</td>
</tr>
</tbody>
</table>

Table 2. Effect of hydrostatic pressure ($H_3=60$ mmHg) on $b_1$ and $b_2$ coefficients at constant perfusion flow of 7.5 ml min$^{-1}$ on metal-tubes of different diameters (Ø=1.0, 3.0 and 5.0 mm).

<table>
<thead>
<tr>
<th>METAL-TUBE</th>
<th>$b_1 \pm$ SE</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ø =1.0 mm</td>
<td>62.36 ± 0.22</td>
<td>3.03 ± 0.35</td>
</tr>
<tr>
<td>Ø =3.0 mm</td>
<td>62.54 ± 0.26</td>
<td>3.16 ± 0.428</td>
</tr>
<tr>
<td>Ø =5.0 mm</td>
<td>61.37 ± 0.61</td>
<td>3.96 ± 0.08</td>
</tr>
</tbody>
</table>

The values of $b_1$ and $b_2$ coefficients of the fitted experimental data for different diameters of metal-tube (Ø=1.0, 3.0 and 5.0 mm) are shown in the table 2. Hydrostatic pressure is about 60 mmHg and perfusion flow is 7.5 ml min$^{-1}$. Experimental data show that coefficient $b_2$ depends on the diameter of the tube, and coefficient $b_1$ has the same value for different tube diameters at certain hydrostatic level.

Table 3. Effect of increasing molar concentration of L-arginine in rat abdominal aorta on $b_1$ and $b_2$ coefficients; and time (s) within which the maximal pressure is developed (taken as 5T).

<table>
<thead>
<tr>
<th>METAL-TUBE</th>
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<th>ST</th>
</tr>
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<tbody>
<tr>
<td>Rat, Abdominal aorta KRS</td>
<td>62.36 ± 0.22</td>
<td>3.03 ± 0.35</td>
<td>1.66 ± 0.109</td>
</tr>
<tr>
<td>Rat, Abdominal aorta KRS + L-Arginine (1mM)</td>
<td>62.54 ± 0.26</td>
<td>3.16 ± 0.428</td>
<td>1.6 ± 0.13</td>
</tr>
<tr>
<td>Rat, Abdominal aorta KRS + L-Arginine (10mM)</td>
<td>61.37 ± 0.61</td>
<td>3.96 ± 0.08</td>
<td>1.26 ± 0.017</td>
</tr>
<tr>
<td>Rat, Abdominal aorta KRS + L-Arginine (20mM)</td>
<td>62.19 ± 0.31</td>
<td>4.2 ± 0.122</td>
<td>1.19 ± 0.017</td>
</tr>
</tbody>
</table>

2. Second procedure

Finally, isolated perfused segments of the rat abdominal aorta were treated with increasing of hydrostatic pressure at constant perfusion flow in the absence and in the presence of L-arginine (1 mM, 10 mM and 20 mM). Experimental pressure-time curves were analyzed using a method described in the Material and method section and the values of $b_1$ and $b_2$ coefficients of the fitted experimental data in the absence and in the presence of L-arginine (Table 3). Our data indicate that coefficient $b_2$, which describes the dynamic behavior of processes, is dependent on the molar concentration of L-arginine.
To describe distensibility properties of the blood vessels, coefficient of distensibility (Dk) was calculated (see equations 2 and 3), (Table 4). 

**Table 4. Effect of increasing molar concentration of L-arginine in rat abdominal aorta on coefficient of distensibility (Dk).**

<table>
<thead>
<tr>
<th>Abdominal aorta of the rat</th>
<th>Dk ± SE</th>
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<tr>
<td>KRS + L-Arginine (1 mM) / KRS</td>
<td>0.024 ± 0.006</td>
</tr>
<tr>
<td>Abdominal aorta of the rat</td>
<td>0.077 ± 0.005</td>
</tr>
<tr>
<td>KRS + L-Arginine (10 mM) / KRS</td>
<td>0.092 ± 0.007</td>
</tr>
<tr>
<td>Abdominal aorta of the rat</td>
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</tr>
<tr>
<td>KRS + L-Arginine (20 mM) / KRS</td>
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</tr>
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**DISCUSSION**

Our results show that b₁ coefficient from the equation 1. depends only on the hydrostatic level, with no difference in b₁ coefficient when metallic-tube diameter increases. This means that maximal developed pressure (alternate steady state) is the same for different tube diameters at the same hydrostatic level. Because of this, any change in diameter of the tube cannot influence the alternate steady state in our experimental design. On the contrary, the tube diameter affects the b₂ coefficient in equation 1. Furthermore, increasing hydrostatic level dose not affect b₂ coefficient. This clearly demonstrates that b₂ coefficient depends only on the tube diameter and that this can describe what happens in between alternate steady states. Increase in the tube diameter leads to increase of the coefficient b₂ suggesting faster development of intraluminal pressure for the constant hydrostatic level.

These considerations are confirmed in our experiments with blood vessels in the presence and absence of L-arginine. Our results show that, in the presence of L-arginine (1–20 mM), there is no difference in b₁ coefficient. This means the maximal developed pressure is the same in the presence and absence of L-arginine. The maximal developed pressure under the constant flow conditions only depends on the hydrostatic level in our experimental design, and thus L-arginine cannot influence the alternate steady state. On the contrary, L-arginine increases the b₂ coefficient in dose dependent manner. This clearly shows the L-arginine influences on the dynamic response during the change of pressure. In the presence of L-arginine the time within the maximum pressure (alternate steady state) decreases from 1.65 seconds to 1.19 seconds (Table 1), indicating a faster pressure change. Interestingly, the effects of L-arginine on the blood vessels dynamic response were achieved with very high doses (1–20 mM). Our previous investigations (7) of L-arginine uptake by the isolated guinea-pig heart indicate that endothelial uptake of L-arginine is saturated and stereo specific process with Km= 183 ± 10 μM and Vmax= 50 ± 10 nM min⁻¹ g⁻¹. Also, these results showed 85 ± 1 % of saturation in the presence of 1 mM of L-arginine. However, in this work 1 mM of L-arginine showed hardly notably effects on blood vessel dynamic response. The reasons for this are, probably in high flow (10 ml min⁻¹) in this work compared with low flow (3 ml min⁻¹) in our previous work, and inversely, small contact area for L-arginine endothelial uptake in this work, compared with large contact area for L-arginine endothelial uptake in the isolated heart with intact coronary circulation.

It is well known that L-arginine is partially essential amino acid, and has numerous biological properties including being exclusive substrate for the formation of nitric oxide (NO). The effects NO are also well known, including smooth muscle relaxation effects in the blood vessels. Taking all this into account, we can conclude that in the presence of L-arginine causing increased production of the NO from endothelial cells, consequent smooth muscle relaxation of isolated blood vessel occurs. This relaxation then changes the distensibility properties of isolated blood vessel, which is quantitatively described by coefficient of distensibility Dk (Table 4). The Dk increases in dose dependent manner in the presence of L-arginine, indicating the shift of the exponential curve to the left.

It is important to underline that Dk, as the area between the test and control curves, increases almost four times, while the b₂ coefficient increases for only about 30% reflecting the time difference within the alternate steady state that is developed for only 0.47 seconds in the presence of arginine (1–20 mM). Approximately 1% change in Dk equals 1ms of the time differences. This makes Dk a very sensitive parameter of transient or dynamic response behavior in the described process.

Our results show that applied experimental model and mathematical procedures enable us to describe precisely (at high sensitivity level) what happens with the pressure change in between alternate steady states.

**REFERENCES**