Action model of Angiotensin II receptors on smooth muscle ileum preparations

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Abstract: This paper presents an approach using control theory methods to monitor the effect of Angiotensin II (Ang II), AT1 and AT2 receptor subtypes on the visceral smooth muscle in ileum preparations. Since AT1 and AT2 are involved in feedback during the regulatory action of Ang II, it is convenient to block them with a suitable antagonist. In this way, three transients were obtained, which were identified by a second-order differential equation. The individual identification vector was calculated for each of the processes. The analysis of the models showed that when the PD 123319 blocker was applied, the amplitude of the responses to smooth muscle contractions increased. From this, it can be concluded that the AT2 type receptors have an AT1 antagonistic effect during the contractile process.

1. Introduction
The octapeptide Angiotensin II (Ang II) is the main effector of the Renin-angiotensin system (RAS), which plays an important role in a number of processes in the human body. The physiological effects of Ang II are carried out at the cellular level by means of two separate angiotensin receptor subtypes, AT1 and AT2 [1,2]. With their help, the octapeptide participates in the regulation of blood pressure and water-electrolyte homeostasis in the body, maintains vascular smooth muscle tone, and last but not least regulates ion fluxes acting on the visceral smooth muscle. Therefore, knowledge of the mechanisms of action and the role of the two receptor subtypes of Ang II is of particular importance in the control and treatment of a number of diseases. By participating in feedback, the AT1 and AT2 receptors carry out the regulatory action of Ang II. Obtaining transients by blocking the action of one of the two feedbacks (blocking a receptor) using a suitable preparation, makes it possible to create models and their subsequent identification with mathematical software. Separate blocking of receptors is also required by the fact that most studies related to the action of octapeptide are focused on the signaling mechanisms involved in the activation of AT1 receptors. The classical understanding of counteracting AT2 receptors to AT1-mediated responses has not yet been fully confirmed [3,4]. In this regard, Hadjibodzheva and team present many results related to time parameters of Ang II - stimulated contractile activity in smooth muscle preparations, isolated from experimental animals. A significant contribution of the authors is the established differences in the action of AT2 receptors in different preparations from isolated rat organs. In the case of the stomach, for example, AT2 has been shown to have an AT1 receptor antagonist effect on participation in the contractile process, while in jejunum the two subtypes of receptors act synergistically [4].

The aim of this article is applying the control theory methods to investigate the effect of both types of receptor subtypes AT1 and AT2 on the visceral smooth muscle in preparations of ileum of healthy
rats. For this purpose, the transients, obtained in the experiment will be identified by a second-order differential equation.

2. Materials and methods

2.1 Experimental data
The data, used in the study were provided by P. Hadjibozheva [3]. The experiment was performed on isolated smooth muscle preparations of ileum (10-12 mm) of healthy rats, aged 5-7 months, Wistar line. In order to analyze the role of the AT1 and AT2 receptor subtypes in the implementation of smooth muscle contraction, a separate blocking of the feedback, in which they participate, was performed. Blockade of AT1 receptors was performed with the antagonist Losartan, and on AT2 with PD 123319. 15 minutes after blocking, the contractile agent Ang II was treated and reactions were monitored.

2.2 Used software
The computer program ISOSYS-Advanced 1.0 (Experimetria Ltd., Hungary) recorded the smooth muscle responses of ileum preparations. The processing and storage of the data for subsequent analysis is performed with specialized software from the KORELIA family [5]. The processes are identified by a second-order differential equation [6]. The graphoanalytical solutions in the phase plane are presented with the software product Mathcad. The vector function ‘Rkadapt’ was used to solve the Ordinary differential equation (ODE) by the Runge-Kuta method in Mathcad, and the ‘eigenvals’ function was used to calculate the eigenvalues of the state matrix.

3. Model identification
The identification of the three processes is performed with a second-order differential equation:

\[
\frac{d^2y(t)}{dt^2} + 2 \cdot \zeta \cdot \omega \cdot \frac{dy(t)}{dt} + \omega^2 \cdot y(t) = K_u \cdot \omega^2 \cdot U(t)
\]

where:
- \( U(t) \) – the input signal
- \( \zeta \) (z) – the damping ratio
- \( \omega \) – the natural frequency of the system.
- \( K_u \) – the sensibility of the system to the input influence.
- \( \omega^2 \cdot K_u = G_{SS} \) – the steady state gain of the system.

![Figure 1. Spline interpolation of muscle contractions: A) Ang II - AT1 and AT2 operate; B) Ang II - AT1 receptors operate; C) Ang II - AT2 receptors operate.](image)
Figure 1 shows a second-order differential model of the three processes. The identification vector \( Q \) is:

\[
Q_i = Q \left( \zeta_i, \omega_i, K_{ui} \right)
\]

The calculation of the identification parameters of the studied processes was performed with the KORELIA-Ident program and the obtained values are presented in Table 1. Reaching the maximum value of Coefficient of determination \( R^2 \) is chosen as an optimization criterion.

**Table 1.** Values of the identification parameters for the three studied processes.

| Obtained model with receptor | Parameter | Coefficient of determination \( R^2 \) | Absolute error \( \alpha \) | Quadratic error \( \beta \) |
|-----------------------------|-----------|--------------------------------|-----------------|-----------------|
| \( AT1, AT2 \)              | \( \zeta \) | 0.0245 | 37 | 0.022 | 0.96 | 0.084 | 0.026 |
| \( AT1 \)                   | \( \omega \) | 0.0058 | 168 | 0.006 | 0.91 | 0.174 | 0.068 |
| \( AT2 \)                   | \( K_s \) | 0.0348 | 96.92 | 0.111 | 0.99 | 0.111 | 0.024 |

Transfer function: By the Laplace transform, the transfer function of the processes is obtained in algebraic form the second-order differential equation.

\[
G(s) = \frac{G_{ss}}{s^2 + 2\zeta\omega s + \omega^2}
\]

For all three cases, the transfer function is on Table 2.

**Table 2.** Transfer functions of smooth muscle contractions at Ileum.

| CASE | \( AT1, AT2 \) | \( AT1 \) | \( AT2 \) |
|------|----------------|----------|----------|
| Transfer function | \( s^2 + 0.0642s + 0.0006 \) | \( s^2 + 0.0087s + 0.0003 \) | \( s^2 + 0.1148s + 0.00112 \) |

The transfer function can be exploited for computing the response to an arbitrary input signal.

4. Model in Mathcad environment

By substitution:

\[
y_1 = y(t)
\]

\[
y_2 = \frac{dy_1(t)}{dt}
\]

The second-order differential equation (1) reduces to a system of two first-order differential equations:

\[
\begin{bmatrix}
\frac{dy_1}{dt} \\
\frac{dy_2}{dt}
\end{bmatrix} = 
\begin{bmatrix}
0 & 1 \\
-\omega^2 & -2\zeta\omega
\end{bmatrix}
\begin{bmatrix}
y_1 \\
y_2
\end{bmatrix}
\]

The initial conditions for the three processes are presented with the matrix:
Initial values required for modeling are:

\[
t_0 = 0 \quad // \text{starting point}
\]
\[
t_{11} = 135 \quad // \text{the final moment}
\]
\[
t_{12} = 550 \quad // \text{the final moment}
\]
\[
t_{13} = 280 \quad // \text{the final moment}
\]
\[
N_1 = 2000 \quad // \text{number of points in the interval}
\]

The simple differential equation is solved in the program with the vector function:

\[
D(t, Y) = \begin{pmatrix}
Y_1 \\
-w^2 Y_0 - 2z w Y_1
\end{pmatrix}
\]

The numerical solution is with the operator:

\[
S := \text{Rkadapt}(Y_0, t_0, t_i, N_1, D)
\]

4.1. Phase plane

The phase plane is determined by the spatial variables \(y_1\) and \(y_2\) (Fig.2).

4.2. Nullclines of the process

The process nullclines are constructed in the \(y_1, y_2\) coordinate system. From the system

\[
\begin{vmatrix}
0 & 1 \\
-\omega^2 & -2\zeta \omega
\end{vmatrix}
\begin{vmatrix}
Y_1 \\
Y_2
\end{vmatrix} = 0
\]

after matrix multiplication:

\[
M := A * Y + B \rightarrow \begin{vmatrix}
Y_2 \\
-\omega^2 - 2\zeta \omega
\end{vmatrix} = 0
\]

Two solutions are obtained relative to \(y_2\):

\[
M_0 \text{solve, } y_2 \rightarrow 0
\]
\[
M_1 \text{solve, } y_2 \rightarrow \frac{-w}{2z} \cdot y_1
\]

These solutions determine that for all three processes the first nullline coincides with the abscissa axis (Fig.3). The second nullcline is a line passing through the beginning of the coordinate system and intersecting the first at the special point.
The coordinates of $y_1$ for the different processes are represented by the dependence:

$$\frac{w}{2z}y_1 \text{ solve, } y_1 \rightarrow 0$$

The slope value of the nullclines is calculated by the formula:

$$a = \frac{w}{2z}$$

(10)

**Figure 3.** Nullclines of the processes.

### 4.3. Eigenvalues of the state matrix

The eigenvalues of the state matrix are calculated with the function ‘eigvals’:

$$ev(w, z) := eigvals(A(w, z)) \rightarrow \begin{bmatrix} -wz - w\sqrt{z^2 - 1} \\ -wz + w\sqrt{z^2 - 1} \end{bmatrix}$$

(11)

**Table 3.** Eigenvalues of the state matrix.

| Active receptor | AT1 and AT2 | AT1 | AT2 |
|-----------------|-------------|-----|-----|
| Eigenvalues     | -0.011      | -0.004 + 3.836i.10^-3 | -0.012 |
|                 | -0.053      | -0.004 - 3.836i.10^-3 | -0.103 |

### 5. Discussion

From the values of the identification parameters presented in Table 1 it can be seen that in two of the models the damping ratio has values greater than 1, which speaks for fast establishment of the process without over-regulation. When the AT2 receptor is blocked, the system is characterized by high sensitivity $K_u$ and low values of the $G_{SS}$ gain. Also, the damping ratio in this process is less than 1, leading to the appearance of oscillations. With the inclusion of AT2 in the feedback, the system is stabilized and the sensitivity $K_u$ drops to 37. This means that a change in the administered dose of Ang II will not lead to a cyclic change in the body’s response. From the values of the identification parameters for the three studied processes it can be concluded that the AT2 type receptors operate antagonistically to AT1 when participating in the contractile process. The resulting $G_{SS}$ gain indicates that the AT1 type receptors are involved in negative feedback, while the AT2 type receptors are involved in positive
feedback. These results are also confirmed by the eigenvalues obtained in the three processes. In the models obtained with the simultaneous action of the two receptor subtypes, as well as when connected to feedback only AT2, the real parts are negative and \( \zeta > 1 \), which means that the special points are of the stable node type - in this case the coordinate point (0,0). Under the action of AT1 receptors, the real parts are again negative, but \( \zeta < 1 \) and there is an imaginary part, which leads to oscillations with frequency \( \omega \). The special points are of the stable focus type.

The angular coefficient of nullclines in Ang II induced contractile activity involving AT2 is -0.009, while in the other two processes, - 0.01. In absolute value, the second coefficient is higher. On the graph, the line that defines it is steeper, which is a sign of higher speed of ongoing processes.

6. Conclusion
The paper identifies models describing the effect of the Ang II receptors AT1 and AT2 on visceral smooth muscle of Ileum preparations in rats. Specific receptors are involved in feedback, determining their role in the regulatory mechanisms of the renin-angiotensin system of living organisms. The three processes involving AT1 and AT2 were identified by a second-order differential equation, and the individual identification vector was calculated. Based on the obtained models, the transfer functions are derived, phase trajectories and nullclines for the three processes are constructed. From the results obtained, it can be assumed that the AT1 and AT2 receptors are involved in negative and positive feedback, respectively, in regulating the action of the octapeptide Ang II. In the presence of both receptor subtypes, an extension of the relaxation period was observed in Ang II - induced contractions.

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