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MATHEMATICAL MODEL OF PLASMA RENIN ACTIVITY AFTER NIFEDIPINE TREATMENT

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Summary

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A mathematical model of plasma renin activity after nifedipine treatment is developed. The system identification of the process is done applying the cyclic coordinate descent as optimization procedure. The model allows predicting the effects of different drug doses and permits the researcher to examine the behaviour of the system under all conceivable conditions.

Key words: mathematical model, plasma renin activity, renin angiotensin system, system identification

INTRODUCTION

A mathematical model is a collection of mathematical relationships which describe a process. Models are by necessity abstractions of the real situations they represent. The power of modeling lies in this abstraction, since a single family of models may present a vast majority of real systems. Mathematical modeling finds out the mathematical relations that characterize the internal structure of the delimited system and formalizes the interdependencies between the input and output variables. A model is considered here as a tool for systems representation in an abstract sense, allowing the simulation and the prediction of the future behaviour of the system.

The purpose of mathematical modeling is to translate the observed phenomena into a set of equations, to determine the parameter values of that model, reflecting in a particular experimental set-up, and then through simulation to predict the behaviour, confirming or disputing previous knowledge or hypotheses. Some of advantages of modeling are:

- trials of tested systems can be accomplished in much shorter time period;
- system performance can be observed under all possible conditions;
- decisions concerning future systems presently in conceptual stage can be examined;
- the investigated phenomenon can be simplified, without affecting its nature, in order to make its qualitative analysis simpler, or possible at all;
- modeling can be used in education to describe, interpret, predict or explain phenomena;
- computer modeling and simulation are often the only feasible or safe techniques to analyze and evaluate a system.

- simulation results can be obtained at lower cost than in real experimentation;

These general advantages give the opportunity to speed up and make less expensive experiments in biology and medicine, aimed at formulation of new drugs in the pre-clinical phase and to decrease the number of experimental animals. Also, using simulation, the results and conclusions could be associated to application of the drugs in people.

In our previous experiments (Ilieva *et al.*, 1995; Tolekova, 2003) we investigated the change in the regulatory kinetics of plasma renin activity (PRA) after blocking of the transmembrane calcium flow through L-type calcium channels. Their dynamic characteristics were investigated employing system and functional analysis (Tolekova, 1998; Tolekova *et al*., 1998; Tolekova *et al*., 2002; Yankov *et al*., 1998a; 1998b). A chronological sequel of these experiments is the creation of a mathematical model of the change in PRA as a function of the applied doses of the drug. The first formulated model is for application of nicardipine (Tolekova *et al.,* 2006).

The aim of the present work was to create a mathematical model of the dynamics of renin (a key enzyme in the regulation of the arterial blood pressure) after the application of different doses nifedipine. The model will be a basis of investigating the enzyme activity of renin from the point of view of the modern system theory.

MATERIALS AND METHODS

Data acquisition

The experiments were carried out on 208 male Wistar rats. PRA was assessed radioimmunologically (DiaSorin-Biomedica Ltd.) after oral application of nifedipine at doses of 10 (n=35), 20 (n=69), 40 (n=69), or 60 (n=35) mg/kg body weight. The values of experimental groups were compared with these of a control group (n=18). PRA was sampled at the intervals showed in Table 1. The animals were housed in polycarbonate cages in temperature (18–23^{o}C) and humidity (40–70%) controlled conditions and 12 h light/dark cycle, with free access to tap water and standard laboratory chow. They received humane care compliant with the Institution's guidelines for humane care of experimental animals of the Trakia University and with the national and European regulatory rules (Decree for protection and humane care of experimental animals 25/10.06.2005, Law on Veterinary Medical Activities G87/11.01.2005, Art 2 (152 and 153) and Council Directive 86/609/ EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes).

The application of treatments started at the same time of the day (at 8 AM). Until the beginning of the experiment the animals remained at the specified conditions in the plastic cages with adaptation purposes. The sampling of blood was performed under general anaesthesia with thiopental sodium at 30 mg/kg, applied intraperitoneally. The animals were immobilized on operation tables and a laparotomy was performed. The arterial and venous kidney vessels were ligated on both sides after which the chest was opened with a sagittal cut. The right heart camera was punctured with a syringe, previously perfused with EDTA solution. Approximately 5 mL of blood were aspired and used in the plasma renin analysis.

Design of the Mathematical Model

In developing a mathematical model of a real system, two basic approaches are possible. The first is based on fundamental understanding of the modeled processes that give rise to the formulation of the mathematical model. The other is based on experimental data and is essentially a data-driven approach (black-box model). Experimental modeling is known in the literature as system identification. System identification is a general term to describe mathematical tools and algorithms that build dynamical models from measured data. A dynamical model in this context is a mathematical description of the dynamic behaviour of a system or process. The identification experiment is performed by applying a specific input signal $U(t)$, to the system measuring the observed output y(t) over a time interval and trying to determine a mathematical relation between them without going into the details of what is actually happening inside the system (Fig. 1). The model is determined from measured signals using some adequate identification methods. For nonlinear models very few results have been obtained and there is no standard algorithm for testing a global identifiability. Various approaches have been proposed, e.g., power series (Pohjanpalo, 1978), differential algebra (Carson *et al.*, 1983), similarity transformation methods (Vajda *et al.*, 1989), stochastic approximation (Petrov, 2005), cyclic coordinate descent (Yankov, 2006).

System identification of PRA

For modeling of the PRA production process, the identification was planned observing the following sequence:

- *Input signal* $U(t)$. A short oral application of nifedipine is considered as Dirac function. The signal amplitude is correlated to the nifedipine dose.
- *Identification time* t_p. The maximum duration time was fixed to 11 hours. This was expected to be sufficient and practical. After this time the system response reaches the steady state level and the system state variables are time independent.
- *Sampling time.* The first two samples were taken at post treatment min 30 and hour 1 and the subsequent ones were taken at every 2 h (Table 1).
- *Output response y(t).* During the experiment, a discrete-time output $\Phi(t) \subset y(t)$ is observed:

$$\Phi(\mathrm{t}) = (\phi_1, \phi_2, \ldots \phi_N)^{\mathrm{T}},$$

where: N – number of samples.

The measured data corresponding to $\Phi(t)$ are presented in Table 1. The vector $\Phi(t)$ is used during the identification process. The data in Table 1 are statistically processed (Statistica 6 for Windows, StatSoft Inc). Data interpolation is performed applying spline interpolation (Yankov, 1998a; 1998b).

Determination of the system model

This stage of identification includes the selection of mathematical equations from a set of candidate system descriptions

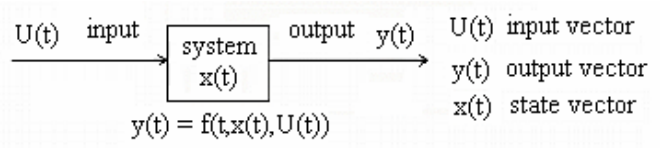

Fig. 1. System description.

Table 1. Plasma renin activity in ng/(mL.h) after treatment with nifedipine at doses of 10, 20, 40 and 60 mg/kg. Data are presented as means ± standard deviation

T (hours)	Dose (mg/kg body weight)			
	10	20	40	60
0	7.58 ± 0.8	7.58 ± 0.8	7.58 ± 0.8	7.58 ± 0.8
0.5	28.3 ± 3.1	39.1 ± 10.8	39.6 ± 13.5	40.2 ± 2.9
1	36.3 ± 9.4	50.7 ± 10.2	51.8 ± 15.7	57.3 ± 1.7
3	27.5 ± 3.4	33.0 ± 4.6	55.7 ± 13.6	62.5 ± 2.6
5	15.4 ± 4.5	26.2 ± 4.3	35.1 ± 5.9	40.1 ± 3.9
7	9.8 ± 1.5	11.9 ± 3.7	20.4 ± 4.7	23.7 ± 1.5
9	7.58 ± 0.8	8.98 ± 2.4	10.7 ± 2.8	14.3 ± 1.7
11	7.58 ± 0.6	7.56 ± 1.7	7.58 ± 1.6	9.5 ± 1.4

within which a model is to be found. PRA follows an oscillation curve. The most appropriate model is a second order ordinary differential equation (ODE):

$$\frac{d^2y(t)}{dt^2} + 2\zeta\varpi\frac{dy(t)}{dt} + \varpi^2 y(t) + K_0 = K_u\varpi U(t) \quad (1)$$

where: $\zeta(d)$ – the damping ratio; $\omega(d)$ – the undamped, natural frequency of the system; $K_0(d)$ – the base level; $K_u(d)$ – the sensibility of the process to the input influence (proportionality coefficient).

The parameters above are unknown and they must be calculated in order to identify the process. All of them are dose (d) dependent and they form the identification vector $Q(d)$:

$$Q(d)=\mathrm{Q}(\zeta(d),\ \omega(d),\ K_0(d),\ K_u(d))$$

Because the structure of the mathematical relation is *a priori* fixed. the parameters of the structure must be fitted to the data applying the algorithms of mathematical optimization (Bazaraa & Shetty, 1979).

The mathematical model is identified using the KORELIA-DYNAMIX program (Yankov, 2006). KORELIA identifies a set of most frequently used algebraic, transcendental and ordinary differential equations up to third order. As identification method, the cyclic coordinate descent (CCD) method is applied. The residuals between experimental data and identified model are minimized applying least square or uniform fitting.

RESULTS

The calculated values of the $\zeta(d)$, $\omega(d)$, $K_0(d)$ and $K_u(d)$ using CCD are presented in Table 2.

Natural frequency ω is dose independent. The coefficients $\zeta(d)$, $K_0(d)$ и $K_u(d)$ are nonlinear toward the nifedipine dose d. They must be identified as a function of dose quantity.

As can be seen from the graphs on Fig. 2, the dependence of the change of the parameter on the applied dose can be modeled with exponential decay curve:

$$F(d) = C_0 \exp(-\frac{d+\Delta d}{D}) + C_{const}, F(d) \in Q(d) \quad (2)$$

The unknown parameters for identification are:

$C_0 = \mathrm{F}(0) - \mathrm{C}_{\mathrm{const}}$
D – dose-constant.
Δd – dose correction parameter;
C_{const} – free term

Applying again the CCD, the calculated values for identification parameters are obtained (Table 3).

Table 2. Identification parameters for equation 1

ODE parameters	Nifedipine dose (mg/kg body weight)			
	10	20	40	60
$\zeta(d)$	1.50	1.39	1.40	1.21
$\omega(d)$	0.60	0.60	0.60	0.60
$K_0(d)$	0.24	0.0	–0.73	– 2.51
$K_u(d)$	61.92	40.42	26.18	15.00

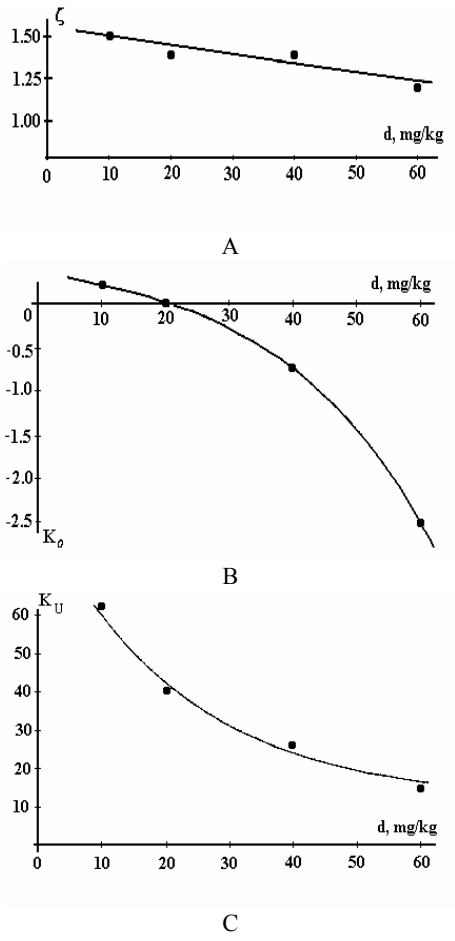

Fig. 2. A. Damping ratio *ζ(d);* B. Constant base level K_0; C. Proportionality coefficient $K_u(d)$.

Finally, the time and dose dependent PRA model is described by the system of equations:

$$\begin{cases} \zeta(d) = 15.11\exp(-\dfrac{d+999}{1000}) - 4 \\ K_0(d) = -\exp(-\dfrac{d}{23.81}) + 0.602 \\ K_u(d) = 75.86\exp(-\dfrac{d}{22.22}) + 11.61 \\ \dfrac{d^2y(t,d)}{dt^2} + 1.2\zeta(d)\dfrac{dy(t)}{dt} + 0.36y(t) + K_0(d) = 0.6K_u(d)U(t) \\ \text{initial conditions: } y(0) = 7.58;\ \dfrac{dy(0)}{dt} = 0 \end{cases} \quad (3)$$

The graphics of the experimental data interpolated using cubic spline and generated models of PRA for doses of 10, 20 ,40 and 60 mg/kg are shown on Fig. 3.

Error estimation

As mentioned above the measured values are ϕ_i. The identified values for the same time points are y_i. For each point ϕ_i, the residual (absolute error) is:

$$\Delta y_i = \| y_i - \phi_i \| \ , \ i=1..N$$

And the relative error r_i for each experimental point is:

$$r_i = |\Delta y_i / \phi_i|$$

The maximum absolute error for the identification interval is:

$$\Delta Y_{max} = max| \Delta y_i |$$

The calculated errors for tested doses and the standard deviations in the cor-

Table 3. Identification parameters for equation 2

ODE parameters	Chart	C_0	D	Δd	C_{const}
ζ(d)	Fig. 2a	15.11	1000	999	– 4
K_0(d)	Fig. 2b	–0.25	23.81	0	0.602
K_u(d)	Fig. 2c	75.86	22.22	0	11.64

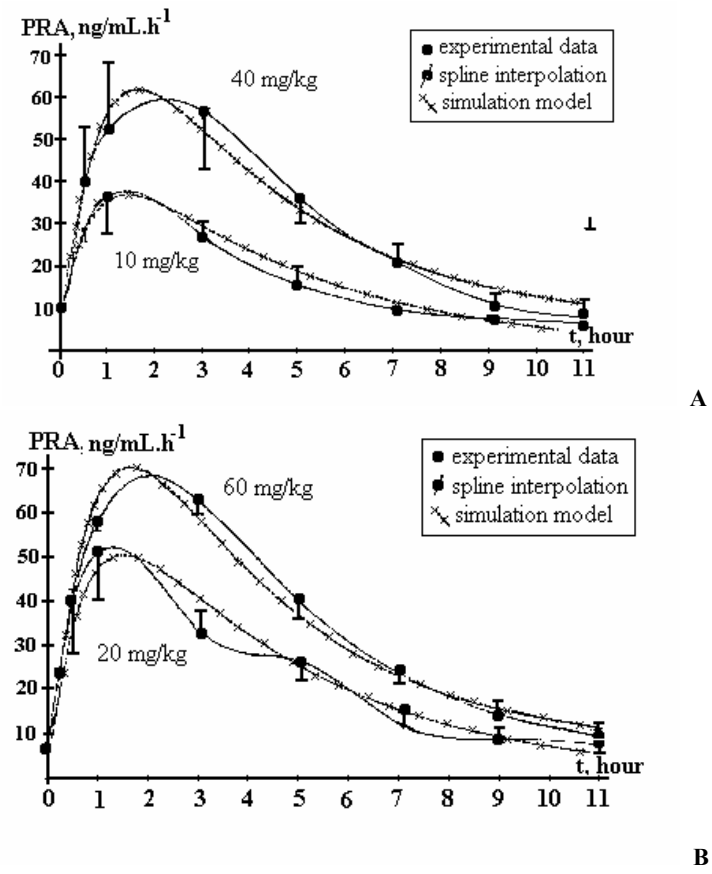

Fig. 3. Experimental data and simulation curves of PRA: A. after nifedipine treatment at doses of 10 and 40 mg/kg b.w; B. after nifedipine treatment at doses of 20 and 60 mg/kg b.w.

responding experimental points are given in Table 4.

Transfer function

The transfer function of a system is the ratio of the Laplace transforms of its output and input, assuming zero initial conditions (Lijung, 1999; Wolkenhauer, 2005). A general second-order transfer function looks as follows:

$$G(s) = \frac{K_U \omega^2}{s^2 + 2\zeta\omega s + \omega^2} \quad (4)$$

Table 4. Maximum absolute and relative error for different doses nifedipine

Dose	T (hour)	PRA experiment	PRA model	Absolute error ΔY_{max}	Standard deviation	Relative error r_i
10	3	27.5	34.012	6.512	3.4	0.2412
20	0.5	39.1	31.890	7.210	10.8	0.1844
40	1	51.8	57.478	5.678	15.7	0.1096
60	1	57.3	61.614	4.314	1.7	0.0752

After substituting the corresponding coefficients from system (3) the dose-dependent transfer function is obtained:

$$G(s,d)=\frac{27.3096\exp(-\frac{d}{22.22})+4.1796}{s^2+1.2(15.11\exp(-\frac{d+999}{1000})-4)s+0.36} \tag{5}$$

State-space representation

State space is an alternative representation of a system that is defined by differential equations. Specifically it is a collection of linear first order differential equations. Let the set of time, dose variables $x_1(t,d)$, $x_2(t,d)$, $x_n(t,d)$, being chosen to describe the dynamic behaviour of a system. These variables are state variables of that system and they satisfy the following conditions:

- at any initial time $t = t_0$, the state variables $x_1(0,d)$, $x_2(0,d)$,... $x_n(0,d)$ define the initial states of the system at the selected initial time.
- once the inputs of the considered system for $t \geq t_0$ and the initial states defined above are specified, the state variables should completely define the future time behaviour of that system.

Therefore, the state variables of a system are defined as a minimal set of variables:

$X(t,d) = (x_1(t,d), x_2(t,d), \ldots x_n(t,d))^{\mathrm{T}}$

such that knowledge of these variables at any initial time t_0, plus information on the dose input excitation subsequently applied, are sufficient to determine the state of the system at any time $t > t_0$, for any dose d.

The nifedipine model is described by second order ODE, therefore two variables $x_1(t,d)$ and $x_2(t,d)$, are necessary. Let substitute:

$$\begin{vmatrix} x_1(t,d)=y(t,d) \\ x_2(t,d)=\frac{dy(t,d)}{dt} \Rightarrow \frac{dx_2(t,d)}{dt}=\frac{d^2y(t,d)}{dt^2} \end{vmatrix}$$

And the state space form is:

$$\frac{dx_2(t,d)}{dt}=-2\zeta(d)\varpi(d)\frac{dy(t,d)}{dt}-\varpi^2(d)y(t.d)-K_0(d)+K_u(d)\varpi(d)U(t) \tag{6}$$

For most practical purposes, analysis of the system is conducted by local linearization of the nonlinear system near a particular operating point $X_O(x_{O1}, x_{O2})$ in the state space. Thus the linearization allows using well established tools from linear systems theory.

The state space model (6) can be linearized about an operating point (x_{O1}, x_{O2}) using the Jacobi matrix $J(t,d)$:

$$\frac{dX(t,d)}{dt} = J(t,d).(X(t,d) - X_0)$$

For the nifedipine model the state-space mathematical model can be represented in matrix form:

$$\begin{bmatrix} \frac{dx_1(t,d)}{dt} \\ \frac{dx_2(t,d)}{dt} \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -0.36 & -18.132\exp(-\frac{d+999}{1000}) + 4.8 \end{bmatrix} \begin{bmatrix} x_1(t,d) - x_{O1} \\ x_2(t,d) - x_{O2} \end{bmatrix} + \begin{bmatrix} 0 \\ 45.516\exp(-\frac{d}{22.22}) + 6.966 \end{bmatrix} U(t) \quad (7)$$

CONCLUSIONS

Mathematical modeling has recently become a powerful tool for better understanding and simulating of processes. It can be used to describe, interpret, predict or explain. Simulation results can be obtained at lower cost than real experimentation reducing the time and number of animals during the test.

Using a specialized software the mathematical model of PRA as a function of nifedipine dose is obtained. The model shows, that the natural frequency of PRA system is dose independent. The parameters $\zeta(d)$, $K_0(d)$ and $K_u(d)$, have non-linear exponential dependence on the nifedipine dose. The model is filled out with dose-dependent transfer function in Laplace domain and state space linearized model in matrix form. The proposed PRA model provides a means to study the role of various drugs and doses for regulation of renin-angiotensin system and human cardiovascular status.

In future the efforts will be oriented to formulation of PRA models after treatment with different drugs (Tolekova *et al.*, 1995; 1996; Ilieva *et al*., 1994; 1995), frequence and stability analysis of obtained models and comparison of received models of studied drugs from the point of view of system theory.

REFERENCES

Bazaraa, M. S. & C. M. Shetty, 1979. *Nonlinear Programming. Theory and Algorithms,* John Wiley and Sons, New York, Chichester, Brisbane, Toronto.

Carson, E. R., C. Cobelli & L. Finkelstein, 1983. The Mathematical Modeling of Metabolic and Endocrine Systems, Wiley, New York.

Ilieva, G., K. Trifonova, A. Tolekova & A. Logofetov, 1994. Plasma renin activity in tachistin-stimulated hypercalcemia and under the effect of chlorazine. *Experimental Medicine and Morphology*, **32,** No 3–4, 1–7 (BG).

Ilieva, G., A. Tolekova, E. Slavov, A. Parvanova & A. Logofetov, 1995. The change of electrolyte balance and renin-angiotensin-aldosterone system after peroral and intraperitoneal treatment with nifedipine. In: *Acid-base and Electrolyte Balance, Molecular, Cellular, and Clinical Aspects.* Papers from the Second G. A. Borelli Conference, July 8–9, eds. Natale Gaspare de Santo & Giovambatista Capasso. Istituto Italiano per gli Studi Filosofici, Naples, 335–340.

Lijung, L. 1999. *System Identification: Theory for the User,* 2nd edn, Prentice Hall ECS Professional, Upper Saddle River, NJ.

Petrov, N., 2005. Stochastic approximation for estimation of biological models. *Trakia Journal of Sciences*, **2**, 17–21.

Pohjanpalo, H., 1978. System identifiability based on the power series expansion of the solution, *Mathematical Biosciences*, **41**, 21–33.

Tolekova, A., G. Ilieva, M. Tzaneva, & M. Ganeva, 1995. Effect of acute and chronic acetylsalicylic acid administration on renin-angiotensin-aldosterone system and stomach mucosa in rats. *Endocrine Regulations* (*Bratislava*), **29**, No 2, 115–120.

Tolekova, A., G. Ilieva & A. Parvanova, 1996. Comparison of the effects on RAAS of the combination of ACE inhibition with calcium channel blockade. In: *Annual Proceedings of the International Medical Association Bulgaria (IMAB) (scientific papers*), **2**, No 1, 209–210.

Tolekova, A., 1998, Functional analysis of changes in PRA after some pharmacological influences. In: *Proceedings of the 50th Anniversary of the Union of Scientists in Bulgaria,* Plovdiv, **2**, 11–14.

Tolekova, A., K. Yankov & V. Spasov, 1998. Dynamic parameters of plasma renin activity after blocking of l- and t- type voltage-dependent calcium channels. In: *Proceedings of Ninth National Conference "Modern tendencies in the development of fundamental and applied sciences*", June 5–6 1998, Stara Zagora, Bulgaria, 198–203 (BG).

Tolekova, A. & K. Yankov, 2002. System analysis of plasma renin activity upon condition of pharmacological and physiological stimulation. In: *Proceedings of Jubilee Scientific Conference*, October 18–20 2002, Stara Zagora, Bulgaria, vol. 1, *Biomedical Sciences*, 61–65 (BG).

Tolekova, A., 2003. Study of plasma renin activity after some physiological and pharmacological influences. Ph.D. Thesis, Sofia (BG).

Tolekova, A. & K. Yankov, 2006. Model of a plasma renin activity after nicardipine treatment. *Journal of Information, Control and Management Systems* (*Slovakia*), **4**, 203–212.

Vajda, S., K. Godfrey & H. Rabitz, 1989. Similarity transformation approach to identifiability analysis of nonlinear compartmental models. *Mathematical Biosciences*, **93**, 217–248.

Wolkenhauer, O., 2005. *Systems Biology Dynamic Pathway Modelling*. http:// www.sbi.uni-rostock.de/dokumente/t_sb.pdf (March 9 2008: date last accessed).

Yankov, K., 1998a. Software utilities for investigation of regulating systems. In: *Proceedings of 9th National Conference "Modern Tendencies in the Development of Fundamental and Applied Sciences",* June 5–6 1998, Stara Zagora, Bulgaria, 401–408.

Yankov, K., 1998b. Evaluation of some dynamic characteristics of transient processes. In: *Proceedings of the 12th International Conference Saer '98,* St. Konstantin Resort, September 19–20 1998, Varna, Bulgaria, 113–117.

Yankov, K., A. Tolekova & V. Spasov, 1998a. Mathematical modelling of drug-induced changes in plasma renin activity, *Pharmacia*, **45,** No 1, 26–29.

Yankov, K. & A. Tolekova, 1998b. Functional analysis of biological data. In: *Proceedings of 50-th Anniversary of the Union of Scientists in Bulgaria, Plovdiv,* Vol.2, 53–56.

Yankov, K., 2006. System identification of biological processes. In: *Proceedings of 20-th Inernational Conference "Systems for Automation of Engineering and Research (Saer-2006),* St. Konstantin Resort, September 22–24, Varna, Bulgaria, 144–149.

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The application of treatments started at the same time of the day (at 8 AM). Until the beginning of the experiment the animals remained at the specified conditions in the plastic cages with adaptation purposes. The sampling of blood was performed under general anaesthesia with thiopental sodium at 30 mg/kg, applied intraperitoneally. The animals were immobilized on operation tables and a laparotomy was performed. The arterial and venous kidney vessels were ligated on both sides after which the chest was opened with a sagittal cut. The right heart camera was punctured with a syringe, previously perfused with EDTA solution. Approximately 5 mL of blood were aspired and used in the plasma renin analysis.

Design of the Mathematical Model

In developing a mathematical model of a real system, two basic approaches are possible. The first is based on fundamental understanding of the modeled processes that give rise to the formulation of the mathematical model. The other is based on experimental data and is essentially a data-driven approach (black-box model). Experimental modeling is known in the literature as system identification. System identification is a general term to describe mathematical tools and algorithms that build dynamical models from measured data. A dynamical model in this context is a mathematical description of the dynamic behaviour of a system or process. The identification experiment is performed by applying a specific input signal $U(t)$, to the system measuring the observed output y(t) over a time interval and trying to determine a mathematical relation between them without going into the details of what is actually happening inside the system (Fig. 1). The model is determined from measured signals using some adequate identification methods. For nonlinear models very few results have been obtained and there is no standard algorithm for testing a global identifiability. Various approaches have been proposed, e.g., power series (Pohjanpalo, 1978), differential algebra (Carson *et al.*, 1983), similarity transformation methods (Vajda *et al.*, 1989), stochastic approximation (Petrov, 2005), cyclic coordinate descent (Yankov, 2006).

System identification of PRA

For modeling of the PRA production process, the identification was planned observing the following sequence:

- *Input signal* $U(t)$. A short oral application of nifedipine is considered as Dirac function. The signal amplitude is correlated to the nifedipine dose.
- *Identification time* t_p. The maximum duration time was fixed to 11 hours. This was expected to be sufficient and practical. After this time the system response reaches the steady state level and the system state variables are time independent.
- *Sampling time.* The first two samples were taken at post treatment min 30 and hour 1 and the subsequent ones were taken at every 2 h (Table 1).
- *Output response y(t).* During the experiment, a discrete-time output $\Phi(t) \subset y(t)$ is observed:

$$\Phi(\mathrm{t}) = (\phi_1, \phi_2, \ldots \phi_N)^{\mathrm{T}},$$

where: N – number of samples.

The measured data corresponding to $\Phi(t)$ are presented in Table 1. The vector $\Phi(t)$ is used during the identification process. The data in Table 1 are statistically processed (Statistica 6 for Windows, StatSoft Inc). Data interpolation is performed applying spline interpolation (Yankov, 1998a; 1998b).

Determination of the system model

This stage of identification includes the selection of mathematical equations from a set of candidate system descriptions

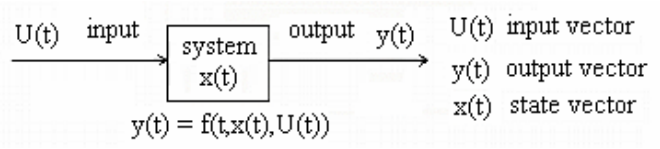

Fig. 1. System description.

Table 1. Plasma renin activity in ng/(mL.h) after treatment with nifedipine at doses of 10, 20, 40 and 60 mg/kg. Data are presented as means ± standard deviation

T (hours)	Dose (mg/kg body weight)			
	10	20	40	60
0	7.58 ± 0.8	7.58 ± 0.8	7.58 ± 0.8	7.58 ± 0.8
0.5	28.3 ± 3.1	39.1 ± 10.8	39.6 ± 13.5	40.2 ± 2.9
1	36.3 ± 9.4	50.7 ± 10.2	51.8 ± 15.7	57.3 ± 1.7
3	27.5 ± 3.4	33.0 ± 4.6	55.7 ± 13.6	62.5 ± 2.6
5	15.4 ± 4.5	26.2 ± 4.3	35.1 ± 5.9	40.1 ± 3.9
7	9.8 ± 1.5	11.9 ± 3.7	20.4 ± 4.7	23.7 ± 1.5
9	7.58 ± 0.8	8.98 ± 2.4	10.7 ± 2.8	14.3 ± 1.7
11	7.58 ± 0.6	7.56 ± 1.7	7.58 ± 1.6	9.5 ± 1.4

within which a model is to be found. PRA follows an oscillation curve. The most appropriate model is a second order ordinary differential equation (ODE):

$$\frac{d^2y(t)}{dt^2} + 2\zeta\varpi\frac{dy(t)}{dt} + \varpi^2 y(t) + K_0 = K_u\varpi U(t) \quad (1)$$

where: $\zeta(d)$ – the damping ratio; $\omega(d)$ – the undamped, natural frequency of the system; $K_0(d)$ – the base level; $K_u(d)$ – the sensibility of the process to the input influence (proportionality coefficient).

The parameters above are unknown and they must be calculated in order to identify the process. All of them are dose (d) dependent and they form the identification vector $Q(d)$:

$$Q(d)=\mathrm{Q}(\zeta(d),\ \omega(d),\ K_0(d),\ K_u(d))$$

Because the structure of the mathematical relation is *a priori* fixed. the parameters of the structure must be fitted to the data applying the algorithms of mathematical optimization (Bazaraa & Shetty, 1979).

The mathematical model is identified using the KORELIA-DYNAMIX program (Yankov, 2006). KORELIA identifies a set of most frequently used algebraic, transcendental and ordinary differential equations up to third order. As identification method, the cyclic coordinate descent (CCD) method is applied. The residuals between experimental data and identified model are minimized applying least square or uniform fitting.

RESULTS

The calculated values of the $\zeta(d)$, $\omega(d)$, $K_0(d)$ and $K_u(d)$ using CCD are presented in Table 2.

Natural frequency ω is dose independent. The coefficients $\zeta(d)$, $K_0(d)$ и $K_u(d)$ are nonlinear toward the nifedipine dose d. They must be identified as a function of dose quantity.

As can be seen from the graphs on Fig. 2, the dependence of the change of the parameter on the applied dose can be modeled with exponential decay curve:

$$F(d) = C_0 \exp(-\frac{d+\Delta d}{D}) + C_{const}, F(d) \in Q(d) \quad (2)$$

The unknown parameters for identification are:

$C_0 = \mathrm{F}(0) - \mathrm{C}_{\mathrm{const}}$
D – dose-constant.
Δd – dose correction parameter;
C_{const} – free term

Applying again the CCD, the calculated values for identification parameters are obtained (Table 3).

Table 2. Identification parameters for equation 1

ODE parameters	Nifedipine dose (mg/kg body weight)			
	10	20	40	60
ζ(d)	1.50	1.39	1.40	1.21
ω(d)	0.60	0.60	0.60	0.60
K_0(d)	0.24	0.0	–0.73	– 2.51
K_u(d)	61.92	40.42	26.18	15.00

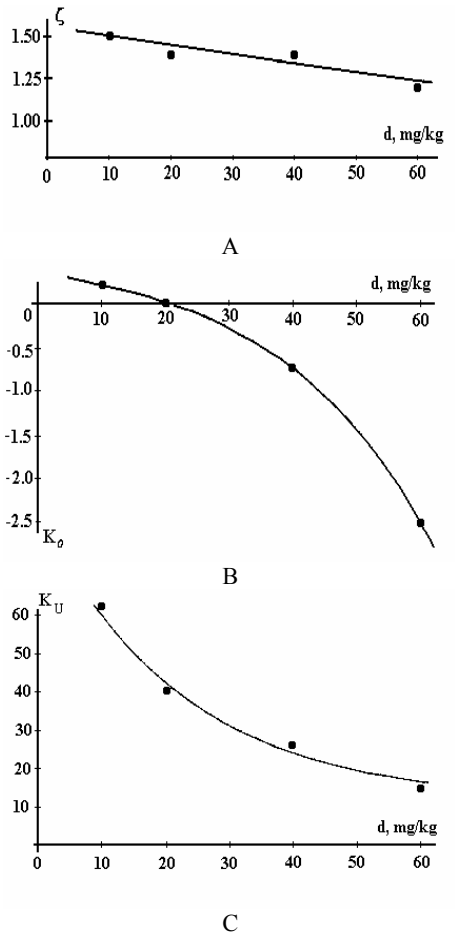

Fig. 2. A. Damping ratio *ζ(d);* B. Constant base level K_0; C. Proportionality coefficient K_u(d).

Finally, the time and dose dependent PRA model is described by the system of equations:

$$\begin{cases} \zeta(d) = 15.11\exp(-\dfrac{d+999}{1000}) - 4 \\ K_0(d) = -\exp(-\dfrac{d}{23.81}) + 0.602 \\ K_u(d) = 75.86\exp(-\dfrac{d}{22.22}) + 11.61 \\ \dfrac{d^2y(t,d)}{dt^2} + 1.2\zeta(d)\dfrac{dy(t)}{dt} + 0.36y(t) + \mathrm{K}_0(d) = 0.6K_u(d)U(t) \\ \text{initial conditions: } y(0) = 7.58;\ \dfrac{dy(0)}{dt} = 0 \end{cases} \quad (3)$$

The graphics of the experimental data interpolated using cubic spline and generated models of PRA for doses of 10, 20 ,40 and 60 mg/kg are shown on Fig. 3.

Error estimation

As mentioned above the measured values are ϕ_i. The identified values for the same time points are y_i. For each point ϕ_i, the residual (absolute error) is:

$$\Delta y_i = \| y_i - \phi_i \| \ , \ \text{i=1..N}$$

And the relative error r_i for each experimental point is:

$$r_i = |\Delta y_i / \phi_i|$$

The maximum absolute error for the identification interval is:

$$\Delta Y_{max} = max| \Delta y_i |$$

The calculated errors for tested doses and the standard deviations in the cor-

Table 3. Identification parameters for equation 2

ODE parameters	Chart	C_0	D	Δd	C_{const}
ζ(d)	Fig. 2a	15.11	1000	999	– 4
K_0(d)	Fig. 2b	–0.25	23.81	0	0.602
K_u(d)	Fig. 2c	75.86	22.22	0	11.64

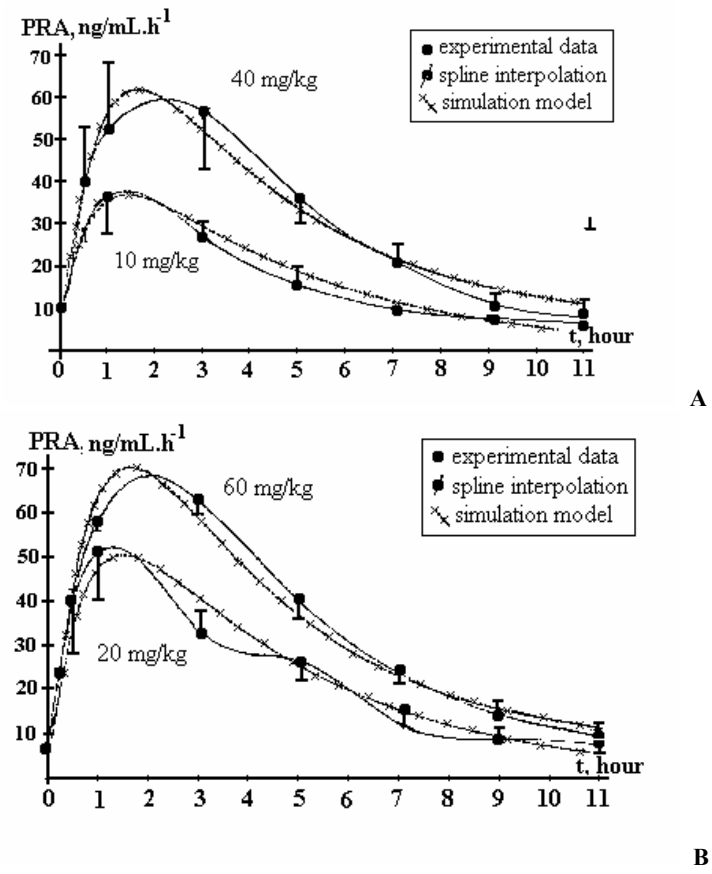

Fig. 3. Experimental data and simulation curves of PRA: A. after nifedipine treatment at doses of 10 and 40 mg/kg b.w; B. after nifedipine treatment at doses of 20 and 60 mg/kg b.w.

responding experimental points are given in Table 4.

Transfer function

The transfer function of a system is the ratio of the Laplace transforms of its output and input, assuming zero initial conditions (Lijung, 1999; Wolkenhauer, 2005). A general second-order transfer function looks as follows:

$$G(s) = \frac{K_U \omega^2}{s^2 + 2\zeta\omega s + \omega^2} \quad (4)$$

Table 4. Maximum absolute and relative error for different doses nifedipine

Dose	T (hour)	PRA experiment	PRA model	Absolute error ΔY_{max}	Standard deviation	Relative error r_i
10	3	27.5	34.012	6.512	3.4	0.2412
20	0.5	39.1	31.890	7.210	10.8	0.1844
40	1	51.8	57.478	5.678	15.7	0.1096
60	1	57.3	61.614	4.314	1.7	0.0752

After substituting the corresponding coefficients from system (3) the dose-dependent transfer function is obtained:

$$G(s,d)=\frac{27.3096\exp(-\frac{d}{22.22})+4.1796}{s^2+1.2(15.11\exp(-\frac{d+999}{1000})-4)s+0.36} \tag{5}$$

State-space representation

State space is an alternative representation of a system that is defined by differential equations. Specifically it is a collection of linear first order differential equations. Let the set of time, dose variables $x_1(t,d)$, $x_2(t,d)$, $x_n(t,d)$, being chosen to describe the dynamic behaviour of a system. These variables are state variables of that system and they satisfy the following conditions:

- at any initial time $t = t_0$, the state variables $x_1(0,d)$, $x_2(0,d)$,... $x_n(0,d)$ define the initial states of the system at the selected initial time.
- once the inputs of the considered system for $t \geq t_0$ and the initial states defined above are specified, the state variables should completely define the future time behaviour of that system.

Therefore, the state variables of a system are defined as a minimal set of variables:

$X(t,d) = (x_1(t,d), x_2(t,d), \ldots x_n(t,d))^{\mathrm{T}}$

such that knowledge of these variables at any initial time t_0, plus information on the dose input excitation subsequently applied, are sufficient to determine the state of the system at any time $t > t_0$, for any dose d.

The nifedipine model is described by second order ODE, therefore two variables $x_1(t,d)$ and $x_2(t,d)$, are necessary. Let substitute:

$$\begin{vmatrix} x_1(t,d)=y(t,d) \\ x_2(t,d)=\frac{dy(t,d)}{dt} \Rightarrow \frac{dx_2(t,d)}{dt}=\frac{d^2y(t,d)}{dt^2} \end{vmatrix}$$

And the state space form is:

$$\frac{dx_2(t,d)}{dt}=-2\zeta(d)\varpi(d)\frac{dy(t,d)}{dt}-\varpi^2(d)y(t.d)-K_0(d)+K_u(d)\varpi(d)U(t) \tag{6}$$

For most practical purposes, analysis of the system is conducted by local linearization of the nonlinear system near a particular operating point $X_O(x_{O1}, x_{O2})$ in the state space. Thus the linearization allows using well established tools from linear systems theory.

The state space model (6) can be linearized about an operating point (x_{O1}, x_{O2}) using the Jacobi matrix $J(t,d)$:

$$\frac{dX(t,d)}{dt} = J(t,d).(X(t,d) - X_0)$$

For the nifedipine model the state-space mathematical model can be represented in matrix form:

$$\begin{bmatrix} \frac{dx_1(t,d)}{dt} \\ \frac{dx_2(t,d)}{dt} \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -0.36 & -18.132\exp(-\frac{d+999}{1000}) + 4.8 \end{bmatrix} \begin{bmatrix} x_1(t,d) - x_{O1} \\ x_2(t,d) - x_{O2} \end{bmatrix} + \begin{bmatrix} 0 \\ 45.516\exp(-\frac{d}{22.22}) + 6.966 \end{bmatrix} U(t) \quad (7)$$

CONCLUSIONS

Mathematical modeling has recently become a powerful tool for better understanding and simulating of processes. It can be used to describe, interpret, predict or explain. Simulation results can be obtained at lower cost than real experimentation reducing the time and number of animals during the test.

Using a specialized software the mathematical model of PRA as a function of nifedipine dose is obtained. The model shows, that the natural frequency of PRA system is dose independent. The parameters $\zeta(d)$, $K_0(d)$ and $K_u(d)$, have non-linear exponential dependence on the nifedipine dose. The model is filled out with dose-dependent transfer function in Laplace domain and state space linearized model in matrix form. The proposed PRA model provides a means to study the role of various drugs and doses for regulation of renin-angiotensin system and human cardiovascular status.

In future the efforts will be oriented to formulation of PRA models after treatment with different drugs (Tolekova *et al.*, 1995; 1996; Ilieva *et al*., 1994; 1995), frequence and stability analysis of obtained models and comparison of received models of studied drugs from the point of view of system theory.

REFERENCES

Bazaraa, M. S. & C. M. Shetty, 1979. *Nonlinear Programming. Theory and Algorithms,* John Wiley and Sons, New York, Chichester, Brisbane, Toronto.

Carson, E. R., C. Cobelli & L. Finkelstein, 1983. The Mathematical Modeling of Metabolic and Endocrine Systems, Wiley, New York.

Ilieva, G., K. Trifonova, A. Tolekova & A. Logofetov, 1994. Plasma renin activity in tachistin-stimulated hypercalcemia and under the effect of chlorazine. *Experimental Medicine and Morphology*, **32,** No 3–4, 1–7 (BG).

Ilieva, G., A. Tolekova, E. Slavov, A. Parvanova & A. Logofetov, 1995. The change of electrolyte balance and renin-angiotensin-aldosterone system after peroral and intraperitoneal treatment with nifedipine. In: *Acid-base and Electrolyte Balance, Molecular, Cellular, and Clinical Aspects.* Papers from the Second G. A. Borelli Conference, July 8–9, eds. Natale Gaspare de Santo & Giovambatista Capasso. Istituto Italiano per gli Studi Filosofici, Naples, 335–340.

Lijung, L. 1999. *System Identification: Theory for the User,* 2nd edn, Prentice Hall ECS Professional, Upper Saddle River, NJ.

Petrov, N., 2005. Stochastic approximation for estimation of biological models. *Trakia Journal of Sciences*, **2**, 17–21.

Pohjanpalo, H., 1978. System identifiability based on the power series expansion of the solution, *Mathematical Biosciences*, **41**, 21–33.

Tolekova, A., G. Ilieva, M. Tzaneva, & M. Ganeva, 1995. Effect of acute and chronic acetylsalicylic acid administration on renin-angiotensin-aldosterone system and stomach mucosa in rats. *Endocrine Regulations* (*Bratislava*), **29**, No 2, 115–120.

Tolekova, A., G. Ilieva & A. Parvanova, 1996. Comparison of the effects on RAAS of the combination of ACE inhibition with calcium channel blockade. In: *Annual Proceedings of the International Medical Association Bulgaria (IMAB) (scientific papers*), **2**, No 1, 209–210.

Tolekova, A., 1998, Functional analysis of changes in PRA after some pharmacological influences. In: *Proceedings of the 50th Anniversary of the Union of Scientists in Bulgaria,* Plovdiv, **2**, 11–14.

Tolekova, A., K. Yankov & V. Spasov, 1998. Dynamic parameters of plasma renin activity after blocking of l- and t- type voltage-dependent calcium channels. In: *Proceedings of Ninth National Conference "Modern tendencies in the development of fundamental and applied sciences*", June 5–6 1998, Stara Zagora, Bulgaria, 198–203 (BG).

Tolekova, A. & K. Yankov, 2002. System analysis of plasma renin activity upon condition of pharmacological and physiological stimulation. In: *Proceedings of Jubilee Scientific Conference*, October 18–20 2002, Stara Zagora, Bulgaria, vol. 1, *Biomedical Sciences*, 61–65 (BG).

Tolekova, A., 2003. Study of plasma renin activity after some physiological and pharmacological influences. Ph.D. Thesis, Sofia (BG).

Tolekova, A. & K. Yankov, 2006. Model of a plasma renin activity after nicardipine treatment. *Journal of Information, Control and Management Systems* (*Slovakia*), **4**, 203–212.

Vajda, S., K. Godfrey & H. Rabitz, 1989. Similarity transformation approach to identifiability analysis of nonlinear compartmental models. *Mathematical Biosciences*, **93**, 217–248.

Wolkenhauer, O., 2005. *Systems Biology Dynamic Pathway Modelling*. http:// www.sbi.uni-rostock.de/dokumente/t_sb.pdf (March 9 2008: date last accessed).

Yankov, K., 1998a. Software utilities for investigation of regulating systems. In: *Proceedings of 9th National Conference "Modern Tendencies in the Development of Fundamental and Applied Sciences",* June 5–6 1998, Stara Zagora, Bulgaria, 401–408.

Yankov, K., 1998b. Evaluation of some dynamic characteristics of transient processes. In: *Proceedings of the 12th International Conference Saer '98,* St. Konstantin Resort, September 19–20 1998, Varna, Bulgaria, 113–117.

Yankov, K., A. Tolekova & V. Spasov, 1998a. Mathematical modelling of drug-induced changes in plasma renin activity, *Pharmacia*, **45,** No 1, 26–29.

Yankov, K. & A. Tolekova, 1998b. Functional analysis of biological data. In: *Proceedings of 50-th Anniversary of the Union of Scientists in Bulgaria, Plovdiv,* Vol.2, 53–56.

Yankov, K., 2006. System identification of biological processes. In: *Proceedings of 20-th Inernational Conference "Systems for Automation of Engineering and Research (Saer-2006),* St. Konstantin Resort, September 22–24, Varna, Bulgaria, 144–149.

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