Recovery of Adenine Nucleotide Pool in the Brain after Craniocerebral Trauma in Rats: Comparison of Biochemical Data with Mathematical Model

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A mathematical model is developed, which predicts the maximum deviation of adenine nucleotide content from the normal and the period of its restoration in the brain of animals. This model diminishes labor expenditures and duration of experimental studies. Moreover, it significantly increases the accuracy and reliability of the data. This model can assess the efficiency of pharmacotherapy of craniocerebral trauma aftereffects.

Key Words: *craniocerebral trauma; adenine nucleotide; mathematical simulation*

The modern medical and biological studies need new methods for evaluation of changes in metabolic parameters during pathological states and extreme conditions. Of particular importance is mathematical simulation of these changes carried out with widely varied numerical factors. The optimal use of the factor space determines the model parameters with greater accuracy in comparison with routine multifactor studies. As a result, this approach diminishes laboriousness and duration of experimental studies and significantly increases its accuracy and reliability [5]. Our aim was to compare the biochemical data on restoration of cerebral energy metabolism (adenine nucleotide pool) after craniocerebral trauma (CCT) in rats with theoretical data produced by mathematical model.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (180-200 g) from Rappolovo Animal Breeding Department (Leningrad District). The closed head injury was produced with a load weighing 64 g, which freely fell in a tube with a height of 80 cm and diameter of 1.3 cm [6]. On postinjury days 1, 3, 7, 14, and 21, the cerebral content of free adenine nucleotides (ATP, ADP, and AMP) was assayed with thin layer ascending chromatography on silufol plates and scanned on MPF-4 spectrofluorimeter (Hitachi) [2]. The data were processed statistically using Student's *t* test.

In mathematical model, the contents of ATP, ADP, and AMP were denoted by y_0 for intact control rats and by y_1, y_2, y_3, y_4, y_5 for experimental rats on postinjury days 1, 3, 7, 14, and 21. These values were approximated by $y(t)$ function of adenine nucleotide content at the moment t after injury. Thus,

$$y_i=y(t_i)+\delta_i,$$
$$i=0,\ 1\ \ldots\ 5,$$
$$t_0=0,\ t_1=1,\ t_2=3,\ t_3=7,\ t_4=14,\ t_5=21, \qquad \textbf{(1)}$$

The deviations δ_i are independent stochastic parameters with zero mean value.

The $y(t)$ function is chosen as a response of aperiodic second-order linear system with time constants of $T_1=1/b$ and $T_2=1/c$ to instantaneous pertur-

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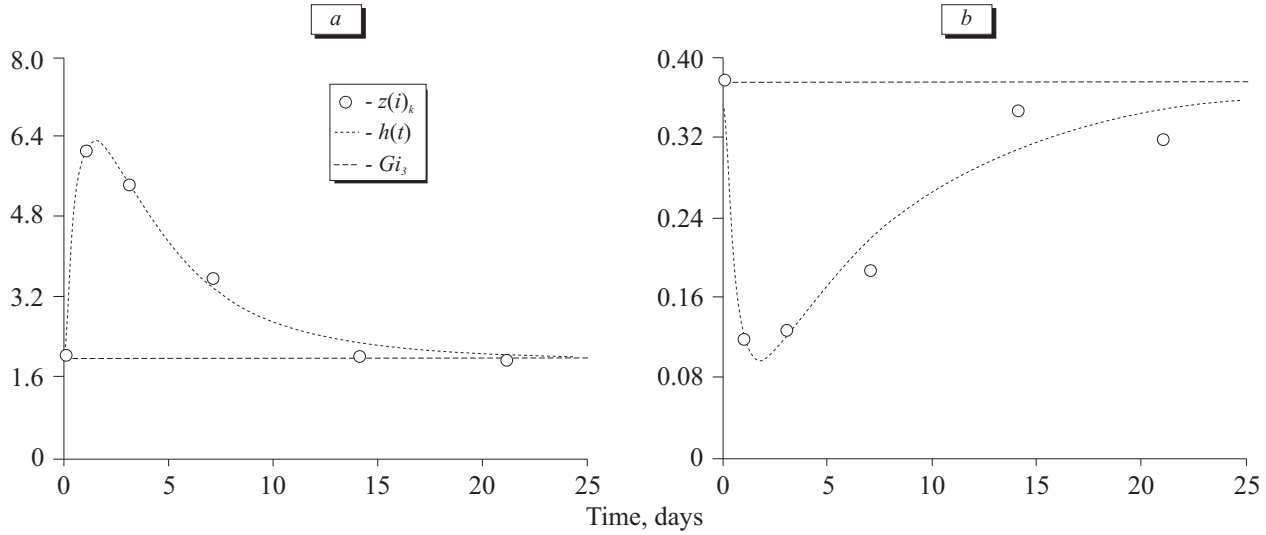

Fig. 1. The plots of model 0 for $b<c$ (*a*) and $b>c$ (*b*).

bation described by δ-function. Generally, this function is presented as

$$y(t)=a\times(e^{-bt}-e^{-ct})+d, \qquad \textbf{(2)}$$

where a, b, c are positive constants, and $d=y(0)=y(\infty)$. The cases of $b<c$ and $b>c$ are shown in Fig. 1, *a* and Fig. 1, *b*, respectively.

This model was termed as the basic model 0. If one of its constants (b or c) considerably surpasses the other (for example, $c>>b$), one exponent term can be neglected in (2), thereby reducing the formula to the following:

$$y(t)=\begin{cases} a\times e^{-bt}+d, & t>0; \\ d, & t=0. \end{cases} \qquad \textbf{(3)}$$

In automatic control theory, $T_2=0$ corresponds to the first-order element with time constant of $T_1=1/b$. The case of $b>>c$ is described by a similar formula (b is replaced by c, and the sign at a is inversed), which is not specially considered here and which allows negative values of a. The function (3) is referred as the model 1. It described the case, when the examined parameter changes very rapidly by a jump (Fig. 2).

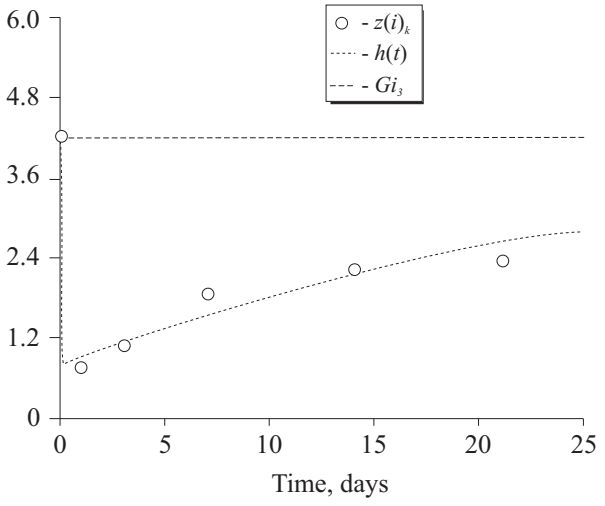

Fig. 2. The plots of model 1 ($a<0$).

When the constants b and c are almost equal, the difference between the exponential terms in basic formula (2) will be small, while the constant a will be large. This case is better described by model 2:

$$y(t)=a\times b\times t\times e^{-bt}+d, \qquad \textbf{(4)}$$

In this model, the negative values of a are permissible, which mean $y(t)\le d$ as in the model 1. In theory, this case is described by multiple roots of the characteristic polynomial $T_1=T_2$.

The above models can fit the experimental data yielding the set of coefficients a, b, c, and d (model 1) or a, b, and d (model 2). Substitution of these coefficients in $y(t)$ formula by their numerical values yields the formula for estimation of the experi-\mental parameters.

The coefficients of the models were calculated by the least square method, which minimizes the error function equal to the sum of squares of the differences between the experimental values y_i and the corresponding theoretical values $y(t_i)$:

$$S=\sum_{i=0}^{n}(y_i-y(t_i))^2 \qquad \textbf{(5)}$$

Combination of (2) or (3) with (5) reduces the problem of model construction to minimization of the error function S, which depends on 4 or 3 arguments in models 1 and 2, respectively.

TABLE 1. Effect CCT on Adenine Nucleotide Content in Rat Brain (Data of Biochemical Tests, $M\pm m$, n=10)

Parameter, group		Day 1	Day 3	Day 7	Day 14	Day 21
ATP, μmol/g	intact			3.68±0.14		
	CCT	0.87±0.16*	1.12±0.15*	1.83±0.17*	2.12±0.13*	2.27±0.13*
ADP, μmol/g	intact			0.61±0.11		
	CCT	1.72±0.14*	1.84±0.12*	1.27±0.13*	1.15±0.11*	0.92±0.14*
AMP, μmol/g	intact			0.31±0.11		
	CCT	0.97±0.08*	1.13±0.05*	0.84±0.03*	0.71±0.04*	0.41±0.05*

Note. *p<0.05 compared to intact rats.

The coefficients for $y(t)$ function were calculated by Microsoft Access software. The experimental data were processed with original program module designed on VBA language (Visual Basic for Applications). This module fitted the experimental parameters for any of two models and yielded some other characteristic values like the moment of the greatest deviation of a parameter from the norm, the amplitude of this deviation, *etc*. To minimize S, we used the most simple and stable gradient-descent method for any combination of exponent index, stimulation factor, and stability. In the following, we used that model of the three, which had the least value of the minimum error function S_{min}. The following values were calculated for each model: t_{max}, the moment of most great deviation of a parameter from the norm, $f_{max}=y(t_{max})-d$, the value of this deviation, and the moments t_{50}, t_{20}, t_{10}, and t_5, which corresponded to the moments when the parameter retuned to 50, 20, 10, and 5% deviation from the norm. To this end, the following equation was solved:

$$\underset{t>t_{max}}{|y(t)-d|=h\times d} \qquad (6)$$

where h=0.5, 0.2, 0.1, and 0.05, respectively.

RESULTS

The state of energy metabolism, which rapidly changes during CCT, is a very important criteria for evaluation of tissue tolerance to extreme stimulation. The universal energy suppliers are adenine nucleotides, whose content can be used to assess directivity of tissue metabolism. Examination of the content of adenylic component in rat brain after moderate CCT revealed a decrease in ATP content and accumulation of its hydrolysis products (Table 1). On the first postinjury day, the cerebral content of ATP dropped by 76%. Then, ATP content somewhat increased, but remained below the normal by 70, 50, and 42% on postinjury days 3, 7, and 14, respectively (p<0.05). The maximum increase in cerebral contents of ADP and AMP was documented on postinjury day 3. In comparison with intact rats, the contents of ADP and AMP increased by 3 and 3.6 times (p<0.05). After moderate CCT, the parameters of adenine nucleotide pool in rat brain significantly differed from those in intact rats during all examination period. CCT-induced energy shortage is a severe membrane-damaging factor, leading to disturbances in activity of ATP-dependent ionic pumps, deterioration of plastic processes aimed at repair of membrane injuries, and dephosphorylation of membrane proteins [1,3,4].

Our mathematical model predicts the values of maximum deviation of adenine nucleotide content in the brain of animals from the normal values and the terms of its recovery, which can avoid the use of laborious biochemical analyses. In experimental rats, cerebral ATP, ADP, and AMP returned to normal within 5% accuracy on postinjury days 68, 45, and 49, respectively (Table 2). These results favor the use of this model in similar studies. Moreover,

TABLE 2. Predicted Normalization Periods (Days) of Adenine Nucleotide Content in Rat Brain after CCT (for 50, 20, 10, and 5% Deviation from Normal)

Parameter	ATP	ADP	AMP
Model	1	0	0
a	-2.79	1.44	1.09
b	0.04	0.084	0.087
c		1.813	1.216
d	3.657	0.636	0.299
t_{max}	0	1.78	2.335
F_{max}	0.872	1.82	1.125
S_{min}	0.132	0.043	0.015
t_{50}	11	18	23
t_{20}	34	29	33
t_{10}	51	37	41
t_5	68	45	49

the model can assess the efficiency of pharmacological treatment of CCT.

Thus, parameters of energy metabolism recovered within 45-68 days after CCT. The corresponding biochemical assay of adenylic components should be performed on postinjury days 30, 45, and 60, which would pronouncedly complicate the study. The mathematical model and calculation of restoration period for the disturbed parameters of energy metabolism simplifies the assay and limits it to routine analyses performed during postinjury weeks 3-4. The time course of recovery of other parameters can be reasonably calculated with the described mathematical model.

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