FUZZY ESTIMATION OF ISOLATED OVIDUCT RESPONSE TO HISTAMINE

Milan S. Matijević[†], Danijela P. Tadić[†] and Slobodan M. Janković[‡]

[‡]- Medical Faculty, Dep't. of Pharmacology and Toxicology; [†]- Faculty of Mechanical Engineering; University of Kragujevac, Serbia&Montenegro

Summary – The adequate mathematical description of response of isolated oviduct smooth muscle on histamine does not exist. Until now, pharmacological analysis of histamine effects on isolated smooth muscle of human oviduct was limited to statistical estimation of concentration-effect curve. Because of dissipation of discrete experimental effects, statistical mathematical armamentarium is inappropriate for use. In our study we have evaluated contractile response of isolated isthmus from human oviduct to histamine. The isolated preparations were taken from 31 patient, after abdominal hysterectomy with adnexectomy in general anesthesia. Histamine produced concentration-dependent tonic contraction of the isolated preparations. The fuzzy algorithm for evaluation of experimental data and estimation of the response on the agonists was created and the response interval has been contracted to more acceptable range by the proposed algorithm.

Key words: Effective concentration 50 (EC_{50}), Estimation concentration-effect curve, Fuzzy estimation, System identification

Introduction

Systems biology is a new field in biology that aims at system-level understanding of biological processes and systems [1]. It is a recurrent theme in the scientific community. One of the first attempts at system-level research, modeling and understanding biological systems was done by Norbert Wiener (still in 1948), when the term biological cybernetics was coined. System-level understanding of biological systems requires a set of principles and methodologies that links the behaviors of molecules to system characteristics and functions. Ultimately, cells, organisms, and human beings will be described and understood at the system level grounded on a consistent framework of knowledge that is underpinned by the basic principles of physics [1]. The development of this scientific area requires further research [1]: 1) the techniques of identification of a system structure – i.e. identification of the systems, such as genes, metabolism, and signal transduction networks and physical structures, 2) the dynamics of such systems, 3) methods to regulate and control the state of systems (For instance: How can we transform cells that are malfunctioning into healthy cells? How can we control cancer cells to turn them into normal cells or cause apoptosis?), and 4) methods to design and modify systems for desired properties (One futuristic example would be to actually design and grow organs from the patient's own tissue.

Such an organ cloning technique would be enormously useful for the treatment of diseases that require organ transplants). The scope of systems biology is potentially very broad and different sets of techniques may be deployed for each research target. It requires collective efforts from multiple research areas, such as molecular biology, high-precision measurement, computer science, control theory, medicine, bioinformatics, and other scientific and engineering fields. Although the benefits understanding fundamental biological processes and human health are self evident, many problems are only noted and wait to be solved.

Histamine is a wide-spread neurotransmitter and paracrine factor in human organism. Histamine produces tonic contractions of human oviduct affecting the H1 receptors [2-6]. Defining mathematical model which describes histamine effects is very important for understanding the nature of the biological process. The investigation is based upon experimental results and in this phase is important to design method, which would enable certain identification of experiments with results reflecting pure agonist effect, giving chance for precise estimate of EC_{50} value and other pharmacological parameters [7]. Namely, the concentration of an agonist, which produces 50 percent of a maximal response, is well known as effective concentration 50, or EC_{50}. It is indirect measurement of an agonist affinity related to appropriate receptor. In most up to date considerations [8], statical relationship between drug concentration and measured effects (so-called E/[A] curve) was starting point in estimation of EC_{50} value, and in functional characterization of receptors. The estimation of EC_{50} of an agonist depends heavily on the mathematical model used for description of its concentration-response relationship. Another ever-present problem in this type of experiments is significant dispersion of responses from different isolated preparations, making classic statistical analysis difficult to apply.

Our study offers fuzzy algorithm for evaluation of experimental data and estimation of the response on histamine. Potential response interval has been contracted in required frame by proposed algorithm.

The experimental methodology

The Fallopian tubes were taken from 31 female patients (one tube from each patient) during abdominal hysterectomy with adnexectomy. All patients were operated in general anesthesia from year 1996 to 1999 in Gynecological Clinic of Clinic Hospital Center “Kragujevac” in Kragujevac, Serbia. The patients were pre-medicated with intramuscular injection of 0.5 mg atropine, 1h before the operation. Drugs used during anesthesia in all operations were: N_2O fentanyl, droperidol, succinilholine (only for induction), thiopental sodium (only for induction) and pancuronium. None of the patients received any drug for a month before operation. All patients (26 to 51 years old, X_{bar}=42.3 years) were in luteal phase of menstrual cycle, and were suffering from grossly myomatous uterus. Their Fallopian tubes were free, macroscopically healthy. The informed consent for the study was obtained from the patients, as well as the approval from local Ethics Committee.

About 20 minutes after taking a Fallopian tube from the patient in the operating room, the isolated preparation was mounted in an isolated organ bath. The preparation of isthmus segment was used. It consisted of whole isthmus with its serosa cut off. Only the isthmus preparations with following used in the experiments measures were: 4 cm in length, wall thickness 1.3 mm and the lumen diameter 1 mm. The preparation was mounted in an organ bath longitudinally, analogous with Magnus preparations of rat ileum.

Opposite walls of the preparation were attached to the bath base and the transducer, respectively [5,6].

The isolated preparations were mounted in 15ml isolated organ bath, filled with De Jalons solution (NaCl 9,0 g, $NaHCO_3$ 0,5 g, glucose 0,5 g, KCl 0,42 g and $CaCl_2$ x $2H_2O$ 0,06 g in liter of solution).

The bath solution was maintained at 37°C and aerated with 100% O_2. One end of the isolated preparation was attached to the bath base, and the other to the lever of the isotonic transducer (T_3 isotonic transducer, Palmer Bio Science, USA). Preparations were placed under a load of 0,5 g. Tonic contractions of isolated preparations were recorded on PC, using AD convertor and original software (Majk elektronik®, Mladenovac, Serbia) (Figure 1).

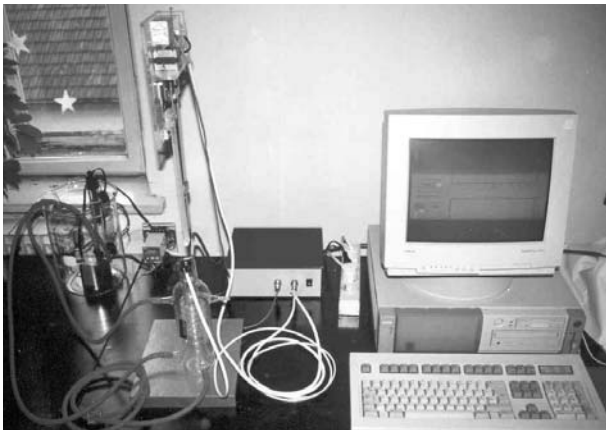

Figure 1: Experimental setup

After mounting in the isolated organ bath the preparations were allowed 45 minutes to equilibrate, i.e. achieving of steady state of biological system with certainty (the tissue is still alive). Excitation of the system was achieved by adding histamine in the organ bath. In this study following agonist was used: histamine dihydrochloride (Sigma Chemical Co., St. Louis, USA). Histamine was added to the bath cumulatively, without bath washings between subsequent doses (7 doses of histamine were added per cumulating). The effect of each dose of histamine was recorded for two minutes. Between two histamine cumulating, at least 45 minutes were allowed to elapse. Histamine concentration was increased 7 times, i.e. for each of 31 experimental samples was recorded 7 responses on the agonist.

The response (contraction) induced by each concentration of histamine was expressed as a percentage of the maximum response induced by histamine and used for construction of concentration-response curves. Hence has been valid physics limitation

$$0 \le E[\%] \le 100$$

The experimental results and modeling

The estimation of significant pharmacological parameter EC_{50} can be based on diverse mathematical models of E/[A] curve. The linear regression model is widely used in experimental pharmacology. However, it is neither precise nor has biological explanation of its parameters. Besides, it cannot discriminate between pure agonist effect and effect of an agonist superimposed on spontaneous changes in the tissue which is investigated.

Up to now, the most precise known mathematical model of concentration-response relationship was Richard's logistic model, successfully validated in many studies [9]

$$E = \frac{\alpha}{(1+\delta \cdot e^{-\ln(10)\cdot p\cdot(\log_{10}([A])-\log_{10}(EC_i))})^{1/\delta}} \quad (1)$$

This model belongs to the category of logistic models, and it has four parameters: α - upper asymptote; $pEC_i = -log_{10}(EC_i)$ - point of inflection of the logistic curve; p - slope of the curve; and δ - coefficient of asymmetry. In order to determine the parameters of Richard's model, one has to use numeric method, assuming that convergence of the solution depends on the choice of numeric algorithm and initial vectors of the parameters [10]. Hence, Richard's model isn't widely used in pharmacological calculations and studies.

However, regardless of choice of a model and its possibilities to give parameter identification, the problem of validity of experimental results, has not been addressed yet. Usually, from experimental set one takes mean values (as valid) for finite calculation of pharmacological parameters, but the problem of dispersion of experimental results remains. We could raise the question whether mean values of response adequately represent process behavior, when standard deviations even higher than the mean value of the response are often calculated? The dispersion of experimental results is illustrated in Figure 2. The response of preparation is approximated by cubic spline for each experimental sample, and then are estimated EC_{50} values for all experiments, based upon this mathematical model of response. The diversity of EC_{50} values from different experimental trial is evident (see Figure 2) and could not be explained by random errors of measurement.

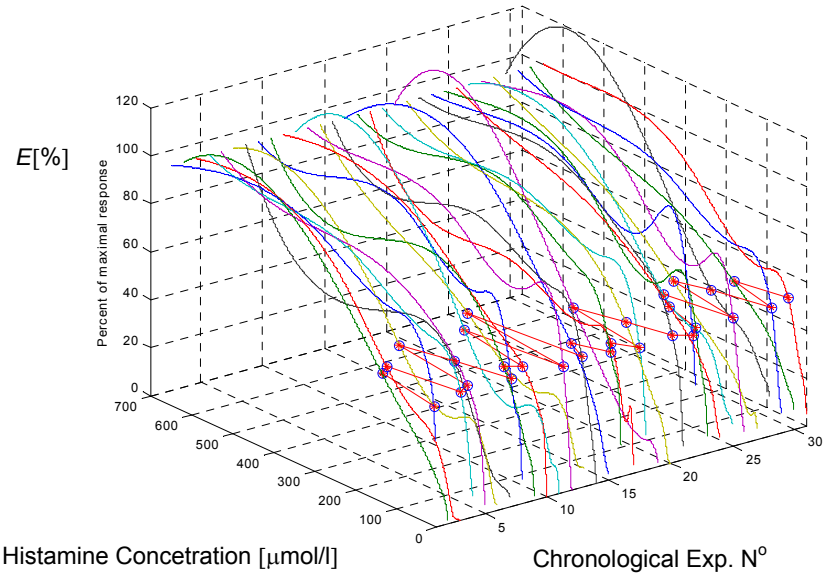

Figure 2: Estimation of E/[A] curve and EC_{50} values by cubic spline in independent experiments

The causes of significant dispersion of consecutive experimental responses we could find in insufficiently detailed analysis of process nature and in the supposed structures of mathematical models which was function of only one argument up to now. The construction of more qualitative models which include patient characteristics (e.g. age, bodily weight, high, etc.), would contribute to better mathematical explanation of

experimental results. Nevertheless, the conventional practice requests pharmacological calculations which can be easily applied to recorded responses. Hence, this area is still dominated by simple statistical methods.

The fuzzy algorithm of response estimation

The values of the preparation responses on agonist we can sort within arbitrary wide intervals ΔE. Interval width respects necessary level of precision. The extension of interval ΔE increases success of simple models in regard to estimation of preparation response zone to arbitrary agonist concentration. Considering responses induced by the same concentration in different experimental trials, it seems that taking into account responses in different intervals without the same "specific weight" is possible.

In this paper, for the sake of clarity, we present estimation algorithm using concrete example of interval width in response discretization. The mentioned algorithm was successfully used in the area of product distributive control systems [11,12].

We assumed width of interval of discretization of ΔE=5%, in our case. Since the possible response values range from 0 to 100%, we can say that the current point of response belongs to one of 20 possible intervals

$$E \in \{E_1, \quad E_2, \quad \text{K} \quad E_{20}\} \tag{2}$$

For each of the seven tested histamine concentrations, the preparation response in different experimental trials is described by discrete random variable $\tilde{E}$

$$\tilde{E}_i = (E_j, p(E_j)),\ p(E_j) = u_j/31,\ i = \overline{1,7}, j = \overline{1,20}; \tag{3}$$

where u_j is total number of response within interval ΔE_j ($j = \overline{1,20}$).

The discrete random variable is transformed to the fuzzy variable with discrete distribution of possibility, by the algorithm which was defined by Dubois and Prade [12]. The random variable $\tilde{E}$ is transformed to the random variable $\tilde{E}'$

$$\tilde{E}_i = (E_j, p(E_j)) \rightarrow \tilde{E}_i' = \left(E_j', p\left(E_j'\right)\right) \tag{4}$$

where $p\left(E_j'\right)$ is row in decreasing order of row $p(E_j)$, and then discrete membership function is

$$\mu_1' = 1, \mu_j' = j \cdot p\left(E_j'\right) + \sum_{k=j+1}^{n=20} p\left(E_j'\right) \tag{5}$$

The calculation of (5) repeats for each agonist concentration ($i = \overline{1,7}$).

Discrete fuzzy variable is defuzzified by moment method [13, 11]

$$E_i = \frac{\sum_{j=1}^{n=20} \mu_j' E_j'}{\sum_{j=1}^{n=20} \mu_j'} = \frac{\sum_{j=1}^{n=20} \mu_j E_j}{\sum_{j=1}^{n=20} \mu_j},\ i = \overline{1,7} \tag{6}$$

According to the proposed methodology, the obtained value E_i is estimated response value for agonist concentration $[A]_i$ ($i = \overline{1,7}$). The algorithm outputs are estimations of the preparation response for only those agonist concentrations for which are executed the

experimental trial. Such obtained responses solve problem of choice of representative experimental results from experimental set, and make a relevant base for future computation (the inputs for parameter identification of mathematical process model). The outputs of proposed algorithmic approach are presented on Fig. 3. As the process is causal, because of the future more precise estimation of E/[A] curve, the point (0,0) is included in results on Fig.3. Because of the methodology of evaluation and presentation of experimental results, the last interval is transformed to the point with ordinate 100%.

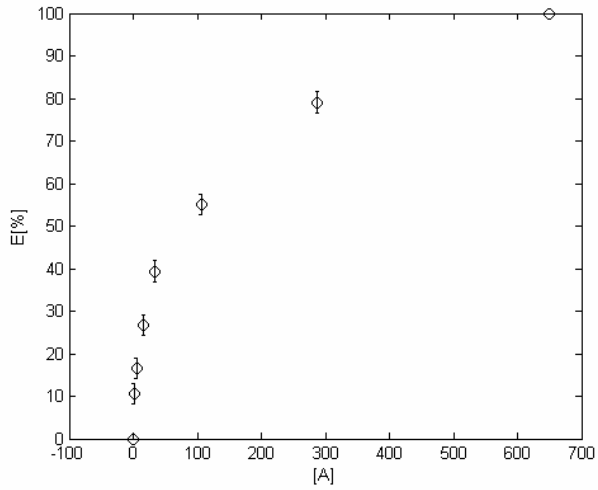

Figure 3: The estimated intervals of response and the centers of intervals

Figure 4. shows the comparative review the mean experimental responses and the mean estimated intervals. The difference between them exists, and the proposed methodology, as well as the estimated values, are more adequate then the mean experimental results. Figure 4. shows significant dispersion of experimental results, too. The statistical methods are problematic because of the relatively small sample, and because the standard deviations were approximately equal or higher then the mean value of experimental results. The proposed procedure is also based on the methodology which uses large samples, but it pays more attention to those experimental results which are within the area of main accumulation of results. Respectively, the proposed approach to a greater extent suppresses influence of the experimental results which “are bounced”.

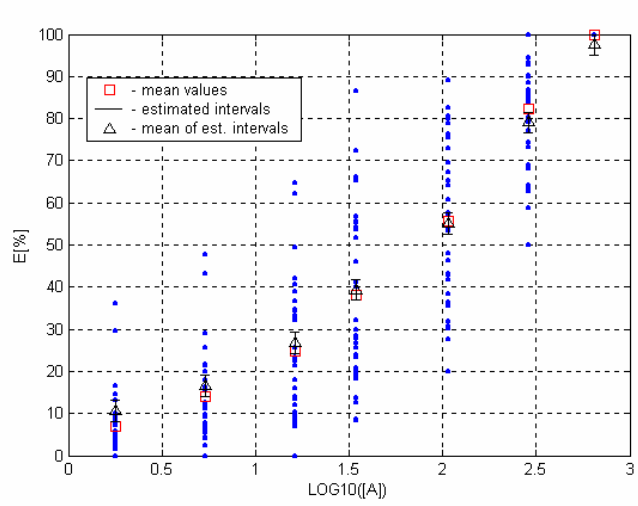

Figure 4: Review of all recorded experimental results, its mean values, the estimated intervals and its mean values

Conclusion

Today, the pharmacological models are subject of interest for researchers in different areas of science. The complexity of biochemical, biomechanical and other processes which influences behavior of isolated biological systems is evident. Today, by virtue of numerous results of medical statistics we have a successful development of phenomenological models on the level: cause – consequence. Nevertheless, more and more we need models which may provide better understanding of effects of different substances. It is clear that the construction more complex models, which recognize more of existing influences, will contribute to this matter.

In our study we have proposed the fuzzy algorithm for evaluation of experimental data from isolated oviduct isthmus and estimation of its responses to histamine. The response interval has been contracted to more acceptable range by the proposed algorithm.

References

1. Kitano H. Perspectives on systems biology, New Generation Computing 2000; 18(3): 199-216.
2. Janković S.M, Protić B.A. Neurohumoral regulation of Fallopian tubes motility, Med Pregl 1995; 48: 395-398.
3. Janković S.M, Milovanović D.R, Janković S.V. Schild's equation and the best estimate of pA_2 value and dissociation constant of an antagonist, Croatian Med J 1999; 40: 67-70.
4. Janković S.M, Varjačić M, Protić B. Relaxant Effect of Oxytocin on Isolated Human Oviduct, Croat Med J 2001; 42: 511-516.
5. Janković S.M, Protić B.A, Janković S.V, et al. Relaxant effects of oxytocin and 8-l-lysine vasopressin on isolated human Fallopian tubes, Epitheor Klin Farmakol Farmakokinet EI 1996; 10: 33-37.
6. Janković S.M, Varjačić M, Janković S.V. Different roles of histamine receptor subtypes in ampullar & isthmic segments of human Fallopian tube, Indian J Med Res 1998; 107: 224-230.
7. Kitchen I. Textbook of in vitro Practical Pharmacology. 1st ed. Oxford 1984: Blackwell Scientific Publications
8. Van der Graaf P.H. Schoemaker R.C, Analysis of asymmetry of agonist concentration-effect curves. J Pharmacol Toxicol Methods 1999; 41: 107-115.
9. Richards F.J. A flexible growth function for empirical use. J Exp Botany 1959; 10: 290-300.
10. Ratkowsky D.A. Models with one x variable, sigmoidally shaped curves. In: Ratkowsky D.A. Handbook of Nonlinear Regression Models. 1st ed. New York: Marcel Dekker, 1990: 123-147.
11. Matijević M, Jakupović G, Car J. Computer Aided Measurement and Control. 1st ed. Kragujevac 2005: University of Kragujevac
12. Dubois D, Prade H. Fuzzy sets and statistical data. EJOR 25 1986: 345-356.
13. Graham I. Uncertainty and Expert Systems. Bristoll 1991: University Bristoll Press.