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MATHEMATICAL MODELS IN QUANTITATIVE PHARMACOLOGY*

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The following problems in the quantitative pharmacology are very important and interesting from applied mathematics point of view:

– Protein folding problem;
– Docking problem;
– Quantitative ligand-receptor interaction.

All of the above problems have been investigated by the members of the "Center for Advanced Bioinformatics Research" of the South-West University "Neofit Rilski" in Blagoevgrad.

In this article, the novel results in the area of the three problems mentioned above will be presented.

Introduction. The quantitative pharmacology is based on receptor occupation theory and formally the main problem is to find the dependence on the physiological effect from concentration of the ligand or on the ligand acting on the given type of receptors.

The steps between ligand-receptor interactions and subsequent biological effect are to some extent unknown and are subject to mathematical and computer modelling. Schematically this is presented in Figure 1.

In this article, it will be considered the mathematical models of the problems mentioned in Figure 1 obtained by the members of the Center for Advanced Bioinformatics Research (CABR) at SWU "Neofit Rilski", Blagoevgrad (`http://bioinfo.swu.bg`).

The problems and results formally could be divided into the following three groups: mathematical models, algorithms and computational results of protein folding, docking problem and quantitative ligand-receptor interaction.

Further, it will be used some terminology which is explained below:

– receptors are the protein molecules which contain more than 100 amino acid residues;
– ligand could be peptides (which contain less than 100 amino acid residues) or non-peptides (any chemical structure that could bind receptor);
– conformation is one of the infinite number of possible 3D arrangements of atoms in peptide (protein) molecule.

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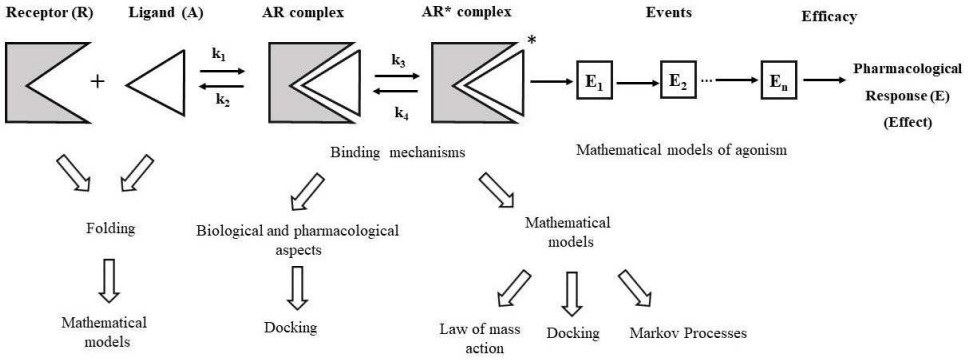

Fig. 1. Mechanism of ligand-receptor interactions

1. Folding problem. The protein and ligand functions depend on their tertiary structure, which in turn depends on their primary structure.

The folding problem (prediction of tertiary structure) of a given ligand or receptor could be formulated as follows:

- Let $F(x)$ be a scoring function estimating qualitatively a conformation x of a ligand or a receptor X defined by its primary structure.
- Let $M(X)$ be the set of all conformations of the ligand or receptor X.
- The problem is:

$$F(x) \to \text{extr}(\max, \min), \quad x \in M(X) \tag{1}$$

So, predicting the tertiary structure – it is to find the optimal solution $x^* \in M(X)$ of problem (1).

It is a common practice for solving problem (1) to use models that simplify the possible conformations search space (i.e. $M(X)$). These models reflect the different global characteristics of the protein structure.

The Hydrophobic-Polar (HP) model [17] describes the protein sequence based on the fact that hydrophobic amino acids must have less contact with water as opposed to the polar amino acids. This leads to the formation of hydrophobic core in the tertiary structure of the proteins.

In the HP models, the amino acids are situated in vertices of the 2D or 3D rectangular lattice.

The optimal conformation of protein folding in the HP model is the optimal solution x^* of (1) with scoring function $F(x)$.

It is proved that the protein folding problem in the HP model for 2D and 3D is NP-hard [8].

Folds in HP model. The processes, related to the protein folding, are very complex and only a minority of them are explained and understood by scientists. For this reason, the simplified models, such as Dill's HP model, have become one of the main tools for study of the proteins [17]. The HP model is based on the observation that the hydrophobic interaction between the amino acids is the driving force in the protein folding

process. In this model, the 20 amino acids are reduced to two types – H (hydrophobic) and P (hydrophilic). The energy of the conformation is defined as the number of contacts between hydrophobic amino acids (H-H contacts), which are not neighbours in the protein sequence. The optimal conformation is the conformation with lowest energy value, defined as a negative of the number of H-H contacts, i.e. it is $x^* \in M(X)$ which minimizes $F(x)$. In these models $M(X)$ is the set of foldings in 2D or 3D lattices and these folding are named "self-avoiding paths".

Conformations of the proteins in the HP models are limited to self-avoiding paths in the lattice models. The self-avoiding path is a sequence of moves in the lattice, which do not pass through the same position more than once. In a cubic lattice, such a path is simply a sequence of moves from (x, y, z) node to one of the six neighbour nodes. The goal is to find the path that minimizes the energy.

Let us mention again that, the HP model of protein folding consist of:

- A given amino acid sequence, $S = s_1, s_2, \ldots, s_n$ (sequence of letters over the {H, P} alphabet):
- Assignment: Each amino acid must occupy one lattice point.
- Non-overlapping: No two amino acids may share the same lattice point.
- Connectivity: Every two amino acids that are consecutive in the protein's sequence must also occupy adjacent lattice points.
- Find a solution of problem (1). This solution is a path satisfying assignment, non-overlapping and connectivity.

For solving the protein folding problem by the HP model, it is proposed a number of optimization algorithms, including Evolutionary algorithms [34], Monte Carlo algorithms [36], and Ant-Colony Optimization algorithms (ACOs) [55].

An integer programming formulation of HP models. The key ingredients of the problem are as follows: a sub lattice (arbitrary) and a sequence of H, P letters are used below for creating a problem on graphs, which could be solved as an integer programming problem or by means of graph theory only [In the 2D case, the sublattice is a square, and for 3D, a cube with nodes painted in black (set V_b) and white (set V_w) alternately]. Let $G_c = (V_c, E_c)$ be a graph with $V_c = V_b \cup V_w = \{1, 2, \ldots, m\}$, where node i corresponds to the ith node of the sublattice (under an arbitrary numeration of the sublattice nodes) and the edge $(i; j) \in E_c$ if i and j are neighbours in the sublattice. The simple paths (each node is visited at most once) in G_c are called *self-avoiding paths.*

Let S be a sequence of n letters on $\{0, 1\}$ alphabet (0 – for P, and 1 – for H). Let $G_S = (V_S, E_S)$ be a graph associated with S with a node set $V_S = \{1, 2, \ldots, n\}$ and $(i, j) \in E_S$ if and only if $|i - j| > 2$ and $S[i] = S[j] = 1$. Let $G = G_S \cup G_c$ be a complete bipartite graph with node set $V_S \cup V_c$. The matching (in this case one-to-one mapping of V_S to V_c) $M = \{e_1, e_2, \ldots, e_n\}$ with $|M| = n$ is *feasible* if the covered nodes in V_c define a self-avoiding path. Define function $z(e_i, e_j) = z_{ikjl} = 1$ if $(i, j) \in E_S$, $(k, l) \in E_c$, and $z_{ikjl} = 0$ otherwise. Finally, define

$$v(M) = \sum_{e_i, e_j \in M} z(e_i, e_j). \tag{2}$$

Let $\widetilde{M}$ be the set of feasible matchings. Then, the problem of finding the optimal folding over the chosen lattice is as follows:

$$\text{Folding problem on graph: For } M \in \widetilde{M} \text{ find } v = \max v(M).$$

Converting the HP problem to an optimization problem on graphs allows building various integer programming models. Most of them involve introducing binary variables, let's say x_{ik} for modelling the feasible matchings from above as 0–1 solutions to simple linear constraints:
(Assignment)

$$\sum_{i=1}^{m} x_{ik} = 1, \qquad k = 1, \ldots, n. \tag{3}$$

(Non-overlapping)

$$\sum_{k=1}^{n} x_{ik} \leq 1, \qquad i = 1, \ldots, m. \tag{4}$$

The objective function could be expressed by linearization of $z_{ijkl} = x_{ik}x_{jl}$ and/or by partitioning the sum of z in subsums in different ways. We will not present all possible integer programming models, but for the sake of completeness, we add the following constraints to finish modelling the self-avoiding paths:

$$\begin{gathered} x_{ik} \leq \sum_{j \in n(i)} x_{jk+1}, \\ i = 1, \ldots, m, \ k = 1, \ldots, n-1. \end{gathered} \tag{5}$$

where $n(i)$ is the set of neighbours of the ith node.

Getting back to the HP folding problem and its conversion to a problem of finding matching that maximizes the number of overlapping edges, one could find a lot of similarity with another problem known as Contact Map Overlap that is (in our case): for a given two graphs G_S, G_c find an embedding (matching) of V_S in V_c that maximize the number of common (overlapped) edges.

A suitable platform for building integer programming models is the so called alignment graph $G = (V_c \otimes V_S, E)$ with $E = \{i, k, j, l\}$, $(i, j) \in E_c$, $(k, l) \in E_S$. By decomposing the sum (2), we could obtain different integer programming models like the next one: without loss of generality assume that the set EVEN is of smaller cardinality and they are assigned to V_b. Let $y_{ij} \in \{0, 1\}$, $i \in V_b$, $j \in V_w$, corresponds to the sum of z arcs between rows i and j. Thus the problem is to maximize:

$$\sum y_{ij}, \tag{2'}$$

subject to additional (to (3), (4), (5)) constraints allowing y_{ij} to be equal to 1:

$$v_i = \sum_{k \in EVEN} x_{ik}, \quad i \in V_b, \tag{6}$$

$$v_j = \sum_{k \in ODD} x_{jk}, \quad j \in V_w, \tag{7}$$

$$v_j \geq y_{ij} \leq v_i. \tag{8}$$

The model above is a mixed linear integer programming problem with x_{ik} binary and by using appropriate solvers like

CPLEX (`www.ibm.com/software/products/en/ibmilogcpleoptistud`) or

GUROBI (`http://www.gurobi.com/documentation/`)

it allows finding optimal folds for sequences of up to 100 elements on a computer with average capabilities. A challenge for the reader is to prove that the number of binary variables could be reduced from 0.5 to 0.25 nm.

A new classifier for protein fold class recognition. The proteins are large biomolecules, which play a key role in many vital functions in living organisms. Up to date, the number of experimentally determined 3D protein structures in Protein Data Bank (www.rcsb.org) is > 125 000 and the comparison of protein structures or protein fold classification and identification is becoming a hot topic in computational biology. The proteins can be classified into one of four major structural classes: α, β, α/β, $\alpha+\beta$ [35]. The structure classification of proteins (SCOP) groups the proteins according to their structures and amino acid sequences, thus providing detailed information about the structural relationship between all recognized proteins [19, 44]. According to SCOP, the four structural classes are divided into folds. The protein fold classification process determines the fold that the query protein belongs to.

Proteins can be classified according to their structural classes and folds. Classification according to the structural classes is called first level classification, and classification according to folds is called second level classification. There are many studies related to the first level classification [4, 18], but in this article we focus on the second level one, namely protein folds classification. One of the basic studies related to the fold classification was performed by Ding and Dubchak [18].

Definition of classifier problem. Suppose that a target function $f : X \subset \mathbb{R}^n \to Y = \{1, 2, \ldots, k\}$ is partially known on subset $\overline{X} \subset X$ of instances (training examples). The set X is referred to as feature space and the elements of Y as labels. Now let $\overline{f}$ be a function that is consistent with f, i.e. $\overline{f}(x) = f(x), \;\; x \in \overline{X}$. The case $\overline{f} \neq f(x)$, $x \in X$ is often called a generalization error. Thus, the classification problem could be mathematically defined as a problem of finding $\overline{f}$ with minimum generalization errors. Problems with $k = 2$ are sometimes called binary classification and for $k > 2$ multi group classification. For a finite label set Y, we can w.l.o.g. restrict the class of function to be the step function like defined on a set of mutually exclusive sets A_i. Formally $\overline{f} = \sum_i \chi A_i(x)$, where $\chi A_i(x) = 1$ if $x \in A_i$ and 0 otherwise.

Thus, the problem of learning $\overline{f}(x)$ is a geometrical problem of finding suitable (consistent) sets A_i with less computational burden.

At the end of this part of the present article, the more important results of the members of the CABR will be mentioned very briefly:

- new efficient heuristic algorithm for HP model, presented as original integer programing model [57];
- new mixed integer programing formulation of the problem (1) as HP model, exact algorithm and two heuristic algorithms [59];
- new multi group classifier with better true positive results for protein fold classification. The time complexity of the classifier allows obtaining results for huge dimension (8000) of the feature space in affordable time [60].

2. Docking problem. Protein-protein interactions and protein-ligand interactions are a critical step in the study of the biological function of proteins. Molecular docking is a significant technique in structural biology and computer-aided drug discovery.

Determination of the protein structure by experimental methods often has limitations. For that need computational approach as molecular docking play a vital role [58].

The docking problem could be defined as follows:

- Let X be a ligand and $M(X)$ be the set of all possible conformations x of X;
- Let Y be a receptor, $\widetilde{y}$ be the homology structure of Y and $\overline{y}$ be crystallographic structure.
- Let $F(x, y)$ be a scoring function estimating (characterizing) quantitatively the ligand-receptor interaction.
- The problem is:

$$F(x, y) \to \text{extr } (\max, \min)$$
$$x \in M(X), \quad y \in \{\overline{y}, \widetilde{y}\}$$

The members of the CABR has been working for many years in the field of opioid receptors and the main tasks in the course of this research are the design and synthesis of selective ligands. There is a wide variety of synthesized compounds, analogues of endogenous peptides and, in particular, enkephalins whose biological activity is determined. Design and synthesis is a long and expensive process. The possibilities that computer methods provide for faster screening of existing compounds and chimeric structures are great, and we have focused our attention on that.

Usually the docking problem could be presented as an optimization problem which follows the next few steps:

- Defining the scoring function $F(x, y)$. Each software has its own scoring functions.
- One conformation of the receptor Y has to be chosen and it is denoted by y_0. The structure of the receptor could be theoretically modelled or obtained from PDB (Protein Data Bank), e.g. crystal structure;
- The set of ligands $X_1,\ X_2, \ldots, X_n$ is chosen (at least 10);
- The structure of the ligands $X_1,\ X_2, \ldots, X_n$ could be obtained by solving the folding problem when X_i $(i = 1, \ldots, n)$ is a peptide or crystallography in the case of non-peptides in order to obtain corresponding conformations $x_1,\ x_2, \ldots,\ x_n$.
- For any x_i $(i = 1, \ldots, n)$ the value of the function $F(x_i,\ y_0)$ is calculated and let x_{i0} be a conformation for which $F(x_{i0}, y_0) = \max\limits_i F(x_i,\ y_0)$;
- The ligand conformation x_{i0} is define as a solution to the corresponding docking problem.

Usually the verification of the docking procedure is based on experimental data concerning the pharmacological effect E_i $(i = 1, \ldots, n)$, measured in the interaction between ligands X_i, $(i = 1, \ldots, n)$ and given receptor R.

If F_i $(i = 1, \ldots, n)$ are the optimal values of the scoring function F for ligand X_i, $(i = 1, \ldots, n)$, then if there is a "good" correlation between $(F_1, \ldots, F_n)$ and $(E_1, \ldots, E_n)$, then it is possible to conclude that the docking procedure is successful.

In order to solve correctly and successfully the docking problem we should use proper structure of the receptors (Y). Depending on the way Y is obtained we have theoretical model [20], homology model [49, 21, 52] and crystal structure [50, 51, 53]. X are selective

δ-opioid ligands [20, 21, 49, 50], enkephalin and dalargin analogues [51] and selective ligands of cannabinoid receptor known in the literature [52, 53].

Scoring function $F(x, y)$ could be any scoring function of the docking software and in our case they are GoldScore, ChemScore and ASP scoring function (`www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/`).

GoldScore is a function of molecular mechanics based on a force field:

$$GoldScore = S_{hb\_ext} + S_{vdw\_ext} + S_{hb\_int} + S_{vdw\_int}$$

where S_{hb_ext} is protein-ligand energy of hydrogen bonds (external H-bond), S_{vdw_ext} is protein-ligand energy of Van der Waals bonds (vdw), (external vdw); S_{hb_int} is intermolecular hydrogen bonds of the ligand (internal H-bond); S_{vdw_int} is intramuscular Van der Waals bonds the ligand (internal vdw).

The ChemScore function evaluates the changes in the total free energy that is produced by binding the ligand to the receptor:

$$ChemScore = \Delta G_{binding} + E_{clash} + E_{int} + E_{cov}$$

where $\Delta G_{binding}$ is the free energy of binding of the ligand to the protein; E_{clash} is the energy of the clashes between the atoms of the ligand and the protein; E_{int} is the ligand internal energy; E_{cov} is the energy of covalently binding the ligand to the protein.

The ASP (Astex Statistical Potential) function calculates the atom-atom potential obtained from the DB of protein-ligand complexes and can be compared with other such potentials estimates. The ASP function compares accurately with ChemScore and GoldScore functions. Traditionally, scoring functions are based on force fields or regressions where the parameters are derived from a plurality of experimentally related structures. The ASP function uses a different approach: information on the frequency of interactions between ligand-protein structures in the PDB is used to generate statistical potential. The ASP scoring function differs from other statistical potentials in the choice of the so-called reference state, which is the expected number of contacts.

$$ASP\ Fitness = -C_s S(map) - c_{int} E_{int} - c_{clash} E_{clash},$$

where $S(map)$ is the overall evaluation of all combinations of the p and ligand l atoms; c_{int} is scaling factor; E_{int} is internal energy; E_{clash} is crash energy.

At the end of this part the more important results of the member of the CABR will be mentioned:

- It is solved the docking problem $Y = DOR$ (delta-opioid receptor) with homology modelled structure, scoring function $F(x, y) = ASP$ and $X_1, X_2, \ldots, X_{11}$ are met- and leu-enkephalin and their analogues [50];
- It is solved the docking problem $Y = MOR$ (mu-opioid receptor) with a crystal structure, scoring function $F(x, y) = ChemScore$ and $X_1, X_2, \ldots, X_{13}$ are met- and leu-enkephalin and dalargin their analogues [51];
- It is solved the docking problem $Y = CB1$ (cannabinoid receptor type 1) with homology modelled and crystal structures, scoring function $F(x, y) = ASP$ and $X_1, X_2, \ldots, X_8$ are selective CB1 ligands from the literature [52, 53].

3. Quantitative ligand-receptor interactions. Usually model of agonism are functional curves $F(x, p)$ which in best way approximate the experimental data of the dose-response relationship between a given ligand X and a given type of receptor R. Also the model can include some proposition concerning some steps of the process of the

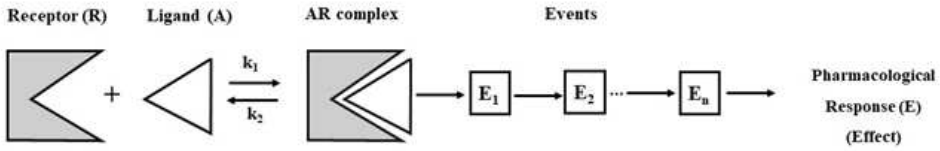

Fig. 2. Ligand-receptor interaction

ligand-receptor interaction. The steps are shown on Figure 2.

May be in all models of agonism the response (effect) is a function of the concentration x of the ligand X of the concentration of the AR complex, i.e. pharmacological response $E = F(x, p)$, $p = (p_1 \dots p_n)$ parameters.

The models are generally divided into three large groups: empirical, mechanistic, and hybrid [22–25, 27, 46–48]. One of the methodical approaches to building such models is the axiomatic approach. In this approach, a sequence of statements (the axiomatics of the model) are considered to be true and implications are derived from them, characterizing the ligand-receptor interactions.

Examples of such models are the Operational Model (OM) of Black & Leff [9], the Theoretical Hyperbolic Model (THM) of Milanov & Pencheva [41] and others [1–3, 7, 10–15, 22–39].

The THM [41] is based on the mechanisms of the ligand-receptor interaction described on Figure 2.

In THM the primary objective is to provide explicit formulas for affinity and efficacy of the agonist, which are important for its quantitative characteristics. Let's note that even before the publication of [41], where the total THM is exposed, the formulas for efficacy for special cases are used in [42, 43, 45] and others. We are shown that when using these models, the theoretical efficacy τ introduced by Black & Leff [9] is equivalent to Stephenson's e_A efficacy [54].

The quantitative analysis of receptor-mediated effects is based on experimental data "concentration-effect", where the independent variable – the concentration of the ligand is associated with the dependent variable – pharmacological response.

Therefore, the developed model aims to describe more fully the nature of the partial agonism at *in vitro* experiments in pharmacology.

The activity of the agonist can be measured by the parameters: *affinity*, reflecting the agonist-receptor interaction kinetics and *efficacy*, characterizing the receptor-response transition, referred to as the stimulus-response.

In this transition, the isolated tissue is involved with its specific mechanism that is independent of the drug and its structure. It can be defined by the receptor concentration and a function that reflects the stimulus-response relationship.

At first glance, the simplest approach to determining the rate of the ligand binding to its receptors may seem to be measurable by the rate at which it acts on isolated tissue, but problems arise. The first one is related with the fact that the relationship between the effect of the tissue and the proportion of the receptors occupied by the ligand is often unknown and can not be determined.

The second one is that the rate at which the ligand acts on the isolated tissue is often determined by the diffusion of the ligand molecules passing through the tissue rather than by binding to the receptors.

The following assumptions for the development THM of Milanov & Pencheva [41] are used:

Axiomatics THM. (a) the interaction between a drug and a receptor is a bimolecular reaction, which according to the law of mass action is expressed as:

$$Y(A) = \frac{X(A)}{[R_0]} = \frac{[A]}{[A] + K_A},$$

where $Y(A)$ is the portion of the receptor that is occupied by agonist [43]; $X(A)$ – the number of the occupied receptors; $[R_0]$ – the total number of the receptors; K_A – dissociation constant.

(b) the occupied receptors $X(A)$ generate stimulus S which is quantitatively defined as:

$$S = e_A \frac{X(A)}{[R_0]} = \frac{e_A[A]}{[A] + K_A} = \varepsilon_A \frac{[R_0][A]}{[A] + k_A} = \varepsilon_A X(A),$$

this stimulus leads to some observed (measured) effect [12], e_A – Stephenson's efficacy [12], ε_A – Furchgott's intrinsic efficacy [30];

(c) the best approximation function of the experimental concentration-response data is a function of the type:

$$E^A = \frac{L_1^A[A]}{[A] + L_2^A},$$

where L_1^A and L_2^A are numerical constants;

(d) E_m^T – potential maximum response of the tissue; it depends only on the given tissue; there are drugs which produce this maximum response (full agonist) or one close to it;

(e) the relation between stimulus S and response E^A is a property of the tissue and not of the drug (drug-independent property);

(f) equal stimuli lead to equal tissue responses.

Assumption (a) is a generally accepted relation and follows from the law of mass action. It describes the equilibrium stage of the process of binding and gives a quantitative characteristic of the number $X(A)$ of the AR complex. Assumption (a) is valid only under the suggestion $[R_0] \ll [A]$, i.e., $X(A)$ is very small compared to $[A]$.

Assumption (b) introduces the concept of stimulus S, which is quantified by the efficacy of Stephenson [54] e_A and the relative number of occupied or activated complexes $Y(A)$.

In THM, the efficacy is denoted by e_A. The biological meaning of S can be different for different tissues, and depending on the type of effect that is measured for different types of receptors.

Assumption (c) originates from the empirical observation that the best approximation curves of the experimental data (most of them, if not all), are hyperbolas or semi logarithmic hyperbolas (in a logarithmic scale) [5–7, 16, 28–31, 43, 56].

Assumptions (a), (b) and (c) present in the most general form the complex transducer process from receptor occupancy to their converting into a measurable response, subject to the transducer function in (c).

In assumption (d) E_m^T is a very important tissue characteristic and depends on the pattern of the tissue-response. Assumptions (e) and (f) follow Stephenson's definition of the stimulus–response mechanism [54] and are generally accepted in quantitative pharmacology.

By using assumption (b), the stimulus S can be expressed by the efficacy e_A and p_{AR}:

$$S = e_A.p_{AR} = e_A.\ \frac{[A]}{K_A + [A]}.$$

For parameters K_A and e_A and for each agonist the following Eq.9 are held:

$$e_A = \frac{C_2^A.L_1^A}{C_1^A - L_1^A}\ , \qquad K_A = \frac{C_1^A.L_2^A}{C_1^A - L_1^A} \tag{9}$$

The expressions for the constants C_1^A and C_2^A refer to agonist A acting on a given tissue, the same type of receptor (assumption (a) and specific mechanism, given that the best approximation of the experimental data is performed with the least squares method by using the modeling function specified in assumption (c). On the other hand, the stimulus-effect relationship is a drug-independent property (assumption (e)). Therefore, there exist two constants C_1 and C_2 such that the following equation hold for each agonist A:

$$C_1^A = C_1^1 = C_1, \quad C_2^A = C_2^1 = C_2. \tag{10}$$

The constants C_1^1, C_2^1 and C_1^2, C_2^2 are the same for a given tissue and receptor type and for the particular mechanism, i.e., they do not depend on the agonist A, acting on them.

From Eq. 10 for the response E_1^A and the parameters K_A and e_A for each agonist A it follows

$$E^A = \frac{C_1.S}{S + C_2}, \quad K_A = \frac{C_1.L_2^A}{C_1 - L_1^A}, \quad e_A = \frac{C_2.L_1^A}{(C_1 - L_1^A)} \tag{11}$$

We will also use the terms $e_{rel} = e_A/C_2$, i.e. e_{rel} does not depend on C_2.

A pharmacological interpretation of the parameters and their calculation. The parameters L_1^A and L_2^A are functions of the experimental data (assumption (c)). They are determined by the least squares method for a function of the class specified in the assumption (c) and by the found $L_1^A \approx E_m^A$, $L_2^A = [A_{50}]$. From the assumption (d) it follows that $C_1 \approx E_m^T$, and the effect E^A could be presented as a function of S as follows:

$$\frac{E^A}{E_m^T} = \frac{S}{S + C_2}. \tag{12}$$

Without limitation, we can consider $C_2^1 = 1$, $C_2^2 = 1$, i.e.

$$\frac{E^A}{E_m^T} = \frac{S}{S + 1}. \tag{13}$$

Stephenson [54] suggests that the function $f(S)$ is an unknown, monotone and con-

tinuous function of the stimulus S so that the answer can be represented by:

$$E^A = E_m^T f(S), \tag{14}$$

where $f(S)$ is an unknown function. In THM the response E^A is a hyperbolic function of S, which is monotone and continuous in nature, i.e., it satisfies the assumptions in [54].

Following Eq. 12, the parameter C_2 could be considered as stimulus S, which elicits $0.5E_m^T$, because if $S = C_2$, then $E^A = (E_m^T C_2)/2C_2 = 0.5E_m^T$. So, C_2 could be used as a quantitative measure of the stimulus S in a given tissue. After replacing the coefficients L_1^A, L_2^A and C_1 with E_m^A, $[A_{50}]$ and E_m^T, L_1^A, we obtain the following explicit expressions:

$$K_A = \frac{[A_{50}]\ E_m^T}{E_m^T - E_m^A}, \quad e_{rel} = \frac{E_m^A}{E_m^T - E_m^A}.$$

These formulas are correct only when $E_m^A < E_m^T$ or $E_m^A \approx E_m^T$. This means that A is a partial agonist or almost full agonist.

The parameters E_m^A and $[A_{50}]$ can be calculated from experimental data such as E_m^T (respectively λ_A, μ_A) by using the experimental data of some full agonist or otherwise [30].

In the Operational model of Black and Left [9] the "transducer ratio" τ is introduced. Furchgott [26] redefined Stephenson [54] stimulus $S = e_A.[AR]$ by introducing new parameter $\varepsilon_A = e_A/[R_0]$. Stimulus S can be related through a series of steps to final pharmacological effect adopted the following form

$$\frac{E}{E_m} = \frac{S}{\beta + S}.$$

On the other hand, (Operational model)

$$\frac{E}{E_m} = \frac{[AR]}{K_E + [AR]} = \frac{S}{\varepsilon_A.K_E + S}.$$

Without loss of generally $\beta = 1$ and consequently $K_E\varepsilon_A = 1$ or $[R_0].K_E.\varepsilon_A = [R_0]$. From this it follows that

$$e_A = \varepsilon_A.[R_0] = \frac{[R_0]}{K_E} = \tau.$$

This means that the Stephenson efficacy e_A and the Black and Leff "transducer ratio" τ are equivalent.

At the end of this part the more important results of the members of the CABR will be mentioned:

- the formulas for e_A and K_A are presented for the first time in [40];
- the constants for e_A and K_A are calculated based on experimental information of enkephalin and dalargin analogues [42];
- proposed approach for the implementation of artificial neural networks in the study of ligand-receptor interactions [43];
- complete exhibition of THM for partial agonism [41];
- based on the del Castillo-Katz mechanism [16]

$$A + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} AR \underset{k_4}{\overset{k_3}{\rightleftarrows}} AR^*$$

are derived similar formulas for e_A and K_A. These results are presented in PhD thesis [61] and they are not published yet.

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МАТЕМАТИЧЕСКИ МОДЕЛИ В КОЛИЧЕСТВЕНАТА ФАРМАКОЛОГИЯ

Петър Б. Миланов

От гледна точка на приложната математика следните задачи в количествената фармакология са много важни и интересни:

– задачата за нагъване на протеини;
– задачата за докинг;
– количествената взаимовръзка лиганд-рецептор.

Всички горепосочени проблеми са изследвани от членовете на „Центъра за съвременни биоинформатични изследвания“ към Югозападния университет „Неофит Рилски“ в Благоевград.

В тази статия, ще бъдат представени нови резултати в областта на трите проблема, споменати по-горе.