STUDY OF RELATIONSHIP BETWEEN SURFACE ANAESTHESIA AND CHROMATOGRAPHIC PROPERTIES OF ALKOXY ESTERS OF PHENYLCARBAMIC ACID BY NEURAL NETWORK METHOD. Part I.

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The advanced mathematical method of neural network was employed for studying of surface anaesthetical activity of three homologous series of 2-, 3- and 4-alkoxy substituted morpholinoethyl, piperidinoethyl and azepanylethyl esters of phenylcarbamic acids. RP-HPLC capacity factors were used for the characterization of the lipophility of tested drugs and they were included to the neural network. The three-layer perceptron, that is trained by the back propagation of errors was successfully used for the supplementing of the incomplete original data matrix and also for the smoothing of the noisy biological data. The dependencies between the surface anaesthesia and the number of C atoms in the side alkoxy chain presented the peak character which is in agreement with the theoretical assumptions. Surface anaesthesia grew up in order from *para* to *ortho* position of alkoxy chain (*para* < *meta* < *ortho*) in individual homologous series. The azepanyl derivatives presented in average the highest surface anaesthesia. This fact is in agreement with the lowest polarity of azepanyl substituent.

The development of biologically active compounds takes place in the two mutually coherent stages. After discovery of a biologically active compound with the new structure, the following phase of activity optimization is commonly characterized by modification of the basic structure to obtain the maximal biological activity[1]. The finding of mathematical relationships between the biological activity, which is the very complex quality, and a measurable characteristics (physical parameters, structural information, etc.) of compounds is often very problematic due to the nonlinearity of dependencies investigated.

Neural networks are specialized computer systems that have similar processing capabilities of the human brain. They process entire patterns rather than individual items of data[2,3]. Once trained, neural networks are quick and have a tolerance to

incomplete or noisy data. This makes them helpful in a real-world environment, such as biological systems considered in this work.

The multilayer perceptron, that is trained by the back propagation of errors, was employed in presented project. Multilayer perceptron consists of a number of interconnected processing elements organized into layers. The outputs of these elements are connected to the inputs of other elements. Relationships between elements are controlled by the weights. A training algorithm measures the error of output[4].

The homologous series of some 2-, 3- and 4-alkoxy substituted ethyl esters of phenylcarbamic acids represent a very important group of compounds because of their high local anaesthetic activity[5–7]. The study of correlation between biological activity, LC capacity factors and some physicochemical properties of these compounds was published previously[8–10]. The aim of this work was to use a neural network method for study of the relationships between anaesthetical activity of above derivatives of phenylcarbamic acid, some coded structural information and lipophility represented by the RP-HPLC chromatographic capacity factors.

EXPERIMENTAL

The experimental details of HPLC system used for the determination of capacity factors of tested drugs were published previously[11]. The drugs studied (see Fig. 1) were prepared according to refs[5–7] and the values of surface anaesthesia were taken also from these papers.

Neural network employed in our experiments had a following architecture. To provide good generalization from training data to testing data, it is desirable to used the smallest number of hidden "neurons" that give satisfactory training performance. In our experiments the best number of hidden "neurons" was determined by training several different sized networks and picking the best. The experiments used four input "neurons" which partly characterized the molecules of local anaesthetics (number of carbon atoms in the side aliphatic chain, position of this chain on the benzene ring, type of the nitrogen containing substituent and LC chromatographic capacity factor). Hidden layer consists of five elements and output one element (logarithm of activity in a surface anaesthesia). All had a

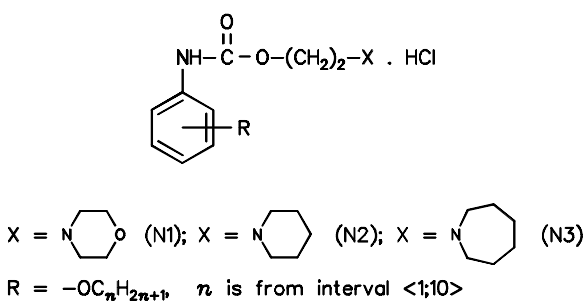

FIG. 1
Structural formulas of tested drugs

feed-forward layered structure with connections only allowed between adjacent layers. Schematical representation of above neural network is shown in Fig. 2.

Program of neural network was written in Turbo-Pascal 7.0. Training process took 180 min on a PC-AT 486 DX, 33 MHz.

RESULTS AND DISCUSSION

The biological activity of compound is determined by its transport to the receptor and by the interaction with this receptor. The transport strongly depends on the lipophility of compound[1]. Reversed phase chromatography (especially with C_{18} stationary phase) can be successfully employed for the characterization of lipophility of tested derivatives of phenylcarbamic acid[8–10].

The structural information of piperidinoethyl esters, morpholinoethyl esters and azepanylethyl esters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids was simply coded by the following way: (i) position of the alkoxy chain on the benzene ring (*ortho* – 1, *meta* – 2, *para* – 3); (ii) number of C-atoms in this chain (from one up to ten); (iii) type of nitrogen containing substituent, see Fig. 1 (morpholino – 1, piperidino – 2, azepanyl – 3).

The experimental data were divided into a training set of 38 local anaesthetics which was used in the learning process, and the testing set of 10 local anaesthetics. The learning of the neutral network is accomplished by repeated cycling through the training data, presenting patterns at the input elements and indicating associations of all

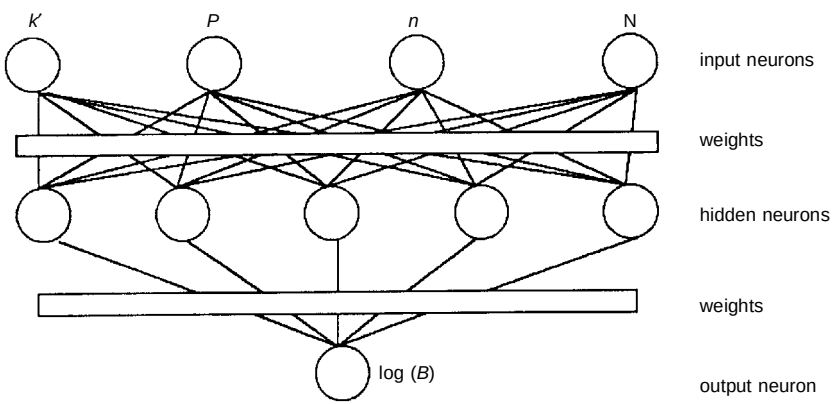

FIG. 2

Schematical presentation of neural network employed in this work. N represents the type of nitrogen containing substituent, *n* is the number of C atoms in a side alkoxy chain, *P* is the position of this chain on the benzene ring and *k'* is the LC capacity factor and log (*B*) is the logarithm of infiltration anaesthesia

elements. In the testing process the output (local anaesthesia) is calculated according to the weights obtained in the training process.

The results of learning and testing process were evaluated by the following characteristics: (i) average sum-of-squares error (SSO) of calculated and experimental outputs; (ii) the gradient of function (*grad*); (iii) index of correlation (I_c^2) of calculated and experimental outputs. These characteristics of our system are listed in Table I.

Indexes of correlation both for training and testing process were around 0.9 and this is relatively high value for the noisy biological system (one is the best fit).

TABLE I
The results of training and testing process

Quantity	Training process	Testing process
SSO	0.026	0.029
grad	0.00013	–
I_c^2	0.892	0.905

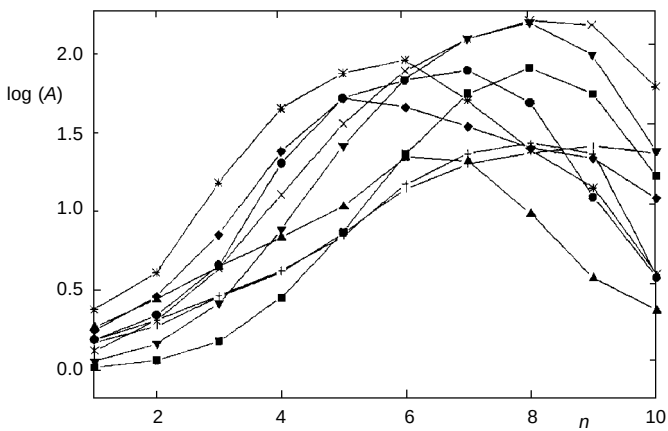

FIG. 3
The dependencies between log (*B*) and the number of C atoms *n* in the side alkoxy chain R (see Fig. 1) of tested local anaesthetics. Abreviations such as (*o*-N1): *o*- means *ortho* position of alkoxy chain R and N1 indicates the N-containing substituent (in this case morpholinyl). ■ *o*-N1, ● *m*-N1, ▲ *p*-N1, ▼ *o*-N2, ◆ *m*-N2, + *p*-N2, × *o*-N3, ✶ *m*-N3, | *p*-N3

TABLE II
The input data and data calculated by the neural network

Position	*n*	N1			N2			N3		
		k′	log (*A*)		*k′*	log (*A*)		*k′*	log (*A*)	
			exp	calc		exp	calc		exp	calc
ortho	1	0.49	n.m.	0.01	1.25	n.m.	0.05	1.72	n.m.	0.12
	2	0.55	n.m.	0.05	1.32	n.m.	0.16	1.81	n.m.	0.31
	3	0.64	n.m.	0.17	1.50	n.m.	0.41	2.06	0.55	0.63
	4	0.77	0.30	0.44	1.80	1.10	0.87	2.47	1.02	1.09
	5	0.90	n.m.	0.85	2.15	1.46	1.40	2.92	1.77	1.54
	6	1.15	1.45	1.34	2.76	1.96	1.82	3.58	1.80	1.87
	7	1.43	1.84	1.72	3.30	2.00	2.07	4.34	2.06	2.07
	8	1.80	1.58	1.88	4.08	1.98	2.17	5.20	n.m.	2.18
	9	2.27	1.82	1.71	5.21	1.97	1.96	6.28	n.m.	2.15
	10	2.90	1.26	1.18	6.82	1.43	1.34	7.41	n.m.	1.75
meta	1	0.47	n.m.	0.19	1.05	n.m.	0.25	1.66	n.m.	0.38
	2	0.51	n.m.	0.34	1.18	0.20	0.46	1.79	n.m.	0.61
	3	0.59	0.85	0.65	1.41	1.04	0.84	1.92	1.43	1.17
	4	0.67	1.20	1.29	1.74	n.m.	1.37	2.35	1.76	1.64
	5	0.82	1.64	1.70	2.03	1.90	1.70	2.94	1.81	1.86
	6	1.09	1.86	1.81	2.65	1.92	1.64	3.56	1.78	1.94
	7	1.28	1.94	1.87	3.37	1.83	1.51	4.50	n.m.	1.68
	8	1.61	1.61	1.66	4.72	n.m.	1.37	5.69	1.18	1.36
	9	2.10	n.m.	1.05	5.95	n.m.	1.30	6.90	n.m.	1.11
	10	2.63	0.48	0.54	7.40	n.m.	1.04	8.41	n.m.	0.56
para	1	0.38	n.m.	0.27	1.02	n.m.	0.19	1.69	n.m.	0.17
	2	0.45	n.m.	0.44	1.18	n.m.	0.31	1.82	n.m.	0.27
	3	0.54	n.m.	0.64	1.42	n.m.	0.46	1.97	0.70	0.45
	4	0.64	n.m.	0.82	1.80	0.67	0.61	2.41	n.m.	0.60
	5	0.81	0.78	1.01	2.21	1.13	0.82	2.95	0.68	0.84
	6	1.01	1.34	1.32	2.79	1.40	1.15	3.79	0.92	1.12
	7	1.28	1.58	1.29	3.66	1.28	1.34	4.83	1.32	1.27
	8	1.62	0.78	0.95	4.58	n.m.	1.40	6.00	n.m.	1.34
	9	2.08	n.m.	0.54	5.94	n.m.	1.33	7.50	n.m.	1.38
	10	2.67	n.m.	0.33	8.00	n.m.	0.55	9.21	n.m.	1.33

Abbreviations: n.m. not measured, *n* number of C-atoms in the side alkoxy chain, *k′* LC capacity factor, log (*A*) logarithm of surface anaesthesia, N1 morpholino ester, N2 piperidino ester, N3 azepanyl ester.

Above procedure was used for supplementing of the incomplete original data matrix and also for the smoothing of the noisy biological data. The original data and data calculated by the neural network are listed in Table II.

Figure 3 shows that the dependencies between the logarithm of surface anaesthesia and the number of C-atoms in the side alkoxy chain for the homologous series have the peak character with one maximum. The first part of the dependence (from the beginning up to the maximum) could be determined by the increasing of biological activity with the increasing of lipophility (number of C-atoms in the alkoxy chain). The descending part of dependence can be explained both by the stereoeffect and very high lipophility of drug caused by the long alkoxy chain, which plays role in the transport of drug through the cell membrane. As seen from Fig. 2 the maximum of log (*A*) lied around eight carbon atoms in the alkoxy chain for the *ortho* and *para* substituted drugs, respectively around six carbon atoms for *meta* derivates. Surface anaesthesis grew up in order from *para* to *ortho* position of alkoxy chain (*para* < *meta* < *ortho*). The azepanyl derivatives (N3) presented in average the highest surface anaesthesia. This fact is in agreement with the lowest polarity of this N containing substituent. The comparison of results reached by the classical bilinear and quadratic regression[12] and results calculated by proposed neural network demonstrates the suitability of neural network method for solving this problem.

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