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Study of local anaesthetics, part 146*: Correlation between local anaesthesia, coded structural information, and chromatographic properties for homologous series of alkoxysubstituted esters of phenylcarbamic acid using a neural network

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RP HPLC capacity factors were used for the characterisation of the lipophilicity of homologous series of o- and m-alkoxy-substituted pyrolidino-, piperidino-, and N-methylpiperazino esters of phenylcarbamic acid. The mathematical method of a neural network was employed for supplementing of the incomplete original data matrix and for smoothing the biological data. The dependencies of the number of carbon atoms in the alkoxy side chain (resp. LC capacity factors) on the surface anaesthesia for the homologous series have parabolic character. The surface anaesthetic activity of the o-alkoxy-substituted derivatives was higher than that of m-alkoxy-substituted derivatives. m-Alkoxy-substituted esters presented maxima of activity at 6 and o-derivatives at 7 carbon atoms in the alkoxy side chain.

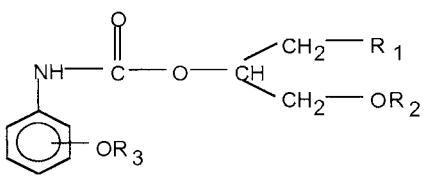
1. Introduction

The chemical structure of basic esters of phenylcarbamic acid can be modified in all parts of molecule: in the lipophilic part, in the hydrophilic part and in the connective chain. Modifications of base structure influence physico-chemical and biological properties of the compounds. The homologous series of o- and m-alkoxy-substituted esters of phenylcarbamic acid studied in this work represent a very important group of local anaesthetics [1–4].

The finding of mathematical relationships between the biological activity, which is a very complex quality, and measurable characteristics (e.g. physico-chemical parameters, structural information) of compounds can be problematic due to the nonlinearity of the dependencies investigated. Standard modelling techniques require a mathematical function known in advance. The advantage of the neural network model is that it does not require knowledge of a mathematical function. The nonlinearity of a single unit transformation and a sufficiently large number of variable parameters ensures adaptation of the neural network to any relation between input and output data [5].

Neural networks are specialised computer systems. They are particularly appropriate for a special class of problems, which may be characterised as follows:

- Pattern recognition, rather than sequential data processing.
- Non-linear capabilities, which can be a problem for other methods.
- Sufficient examples of the target data are available to allow a learning system to be considered.



R_1: pyrolidino-, piperidino-, N-methylpiperazino- group

R_2: $-C_nH_{2n+1}$ n = 1, 2, 3

R_3: $-C_nH_{2n+1}$ n = 3, 4, 5, 6, 7 o-, m- position

The aim of this work was to use an advanced mathematical neural network method for studying the relationships between local anaesthesia of the derivatives of phenylcarbamic acid, structural information, and lipophilicity represented by the RP HPLC chromatographic capacity factors.

2. Investigations, results and discussion

The mechanism of biological activity of a compound is commonly determined by its transport to the receptor and by the interaction with this receptor. It can be assumed, that the interaction with the receptor depends on the type of nitrogen containing substituent and that the lipophilicity of molecule strongly influences the transport of the drug to the receptor [7]. RP chromatography with a C 18 stationary phase can be a suitable method for the characterisation of the lipophilicity of phenylcarbamic acid derivatives.

The neural network was used for supplementing the original data matrix and for the smoothing of the noisy biological data. The structural information of pyrolidino-, piperidino- and N-methylpiperazino-esters of o- and m-alkoxy-substituted phenylcarbamic acids was coded in the following way:

- number of carbon atoms in alkoxy-substituent in connective chain (1–3)
- position of carbon atoms in alkoxy-substituent in lipophilic part (3–7)
- position of this substituent (o: 1, m: 2)
- type of nitrogen containing substituent (pyrolidinyl-: 1, piperazinyl-: 2, N-methylpiperazinyl-: 3)

The training group set of experimental data of 39 local anaesthetics which was used in the learning groups. The original data and the data calculated by the neural network are listed in Table 1. The outcomes of the learning process were evaluated by the following characteristics:

- average sum-of-squares error (SSO) of calculated and experimental outputs,
- gradient of function (grad),
- index of correlation (I_c^2) of calculated and experimental outputs.

The characteristics are listed in Table 2. Index of correlation is 0.9 (1 is the best fit).

Table 1: Experimental data and data calculated by the neural network for piperidino-, pyrolidino- and N-methylpiperazino-esters

$n(R_2)$	P_{R3}	pyrolidino-			piperidino-			N-methylpiperazino-		
	$n(R_3)$	log k	log (A)		log k	log (A)		log k	log (A)	
			exp.	cal.		exp.	cal.		exp.	cal.
1	**o-**									
	3				0.25	1.17	1.11	n	n	0.04
	4				0.34	1.52	1.68	0.55	n	0.18
	5				0.45	1.89	2.02	0.63	0.79	0.75
	6				0.56	2.47	2.16	n	n	1.53
	7				0.55	n	2.20	0.83	1.92	1.98
	m-									
	3				0.16	1.80	1.65	n	n	0.02
	4				0.26	2.07	2.01	0.42	n	0.08
	5				0.34	2.04	2.16	0.53	0.08	0.36
	6				0.47	n	2.23	0.67	1.26	1.06
	7				0.58	n	2.25	0.77	1.64	0.74
2	**o-**									
	3	n	n	0.54	n	n	1.49			
	4	0.34	1.19	1.07	0.45	n	1.80			
	5	0.46	n	1.67	0.49	n	2.01			
	6	0.56	2.10	2.00	0.51	2.09	2.12			
	7	0.66	2.10	2.15	0.55	2.07	2.19			
	m-									
	3	n	n	1.32	n	n	1.79			
	4	0.29	1.35	1.83	0.29	2.04	2.06			
	5	0.39	2.24	2.08	0.39	2.12	2.18			
	6	0.55	2.25	2.20	0.51	2.33	2.23			
	7	0.62	3.19	2.24	0.60	2.19	2.25			
3	**o-**									
	3	n	n	0.57	n	n	1.24			
	4	0.43	0.95	1.15	0.49	1.84	1.77			
	5	0.53	1.87	1.70	n	n	2.06			
	6	0.65	2.09	2.03	0.71	2.09	2.18			
	7	n	n	2.17	0.82	1.04	2.23			
	m-									
	3	n	n	1.81	n	n	1.81			
	4	0.50	2.30	2.02	0.37	2.08	2.08			
	5	0.64	2.28	2.17	0.46	2.47	2.27			
	6	0.73	2.20	2.23	0.57	2.31	2.23			
	7	0.68	1.86	2.24	0.70	2.37	2.25			

$n(R_2)$: number of carbon atoms in substituent in connective chain, $n(R_3)$: number of carbon atoms in alkoxy side cain in lipophilic part, P_{R3}: position of this chain, k: value of capacity factor, A: surface anaesthesia, n: not measured

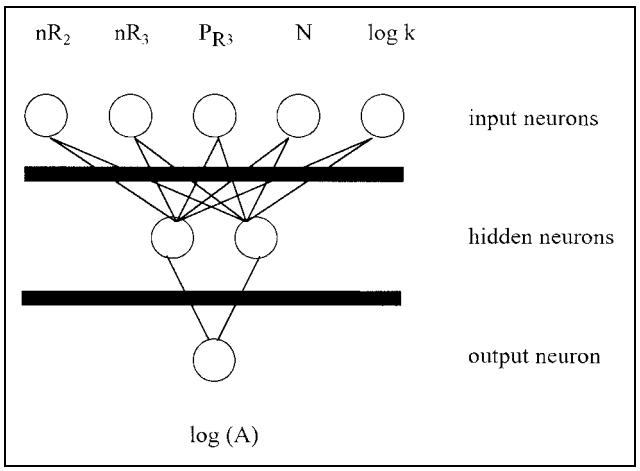


Fig. 1: Schematic presentation of neural network used in work. nR_3: number of carbon atoms in alkoxy side cain in lipophilic part, P_{R3}: position of this chain, nR_2: number of carbon atoms in substituent in connective chain, N: type of nitrogen containing substituent, log k: logarithm of capacity faktor, log (A): surface anaesthesia

The dependencies of logarithm of surface anaesthesia of pyrolidino- and piperidino-derivatives on the number of carbon atoms in the alkoxy-substituent in the lipophilic part (resp. LC capacity factor) for homologous series have parabolic character, which is in agreement with theoretical assumptions (Fig. 2). The increasing part of the curve could be determined by increasing biological activity with increasing lipophilicity (number of carbon atoms in the alkoxy-substituent in lipophilic part). Derivatives with o-alkoxy-substitution on the benzene ring presented maxima of surface anaesthesia for about seven carbon atoms for pyrolidino- and piperidino-derivatives. The local anaesthetic activity of m-alkoxy-substituted derivatives was

Table 2: Results of the training process

	Training process
SSO	0.1049
grad	0.00018
I_k^2	0.8781

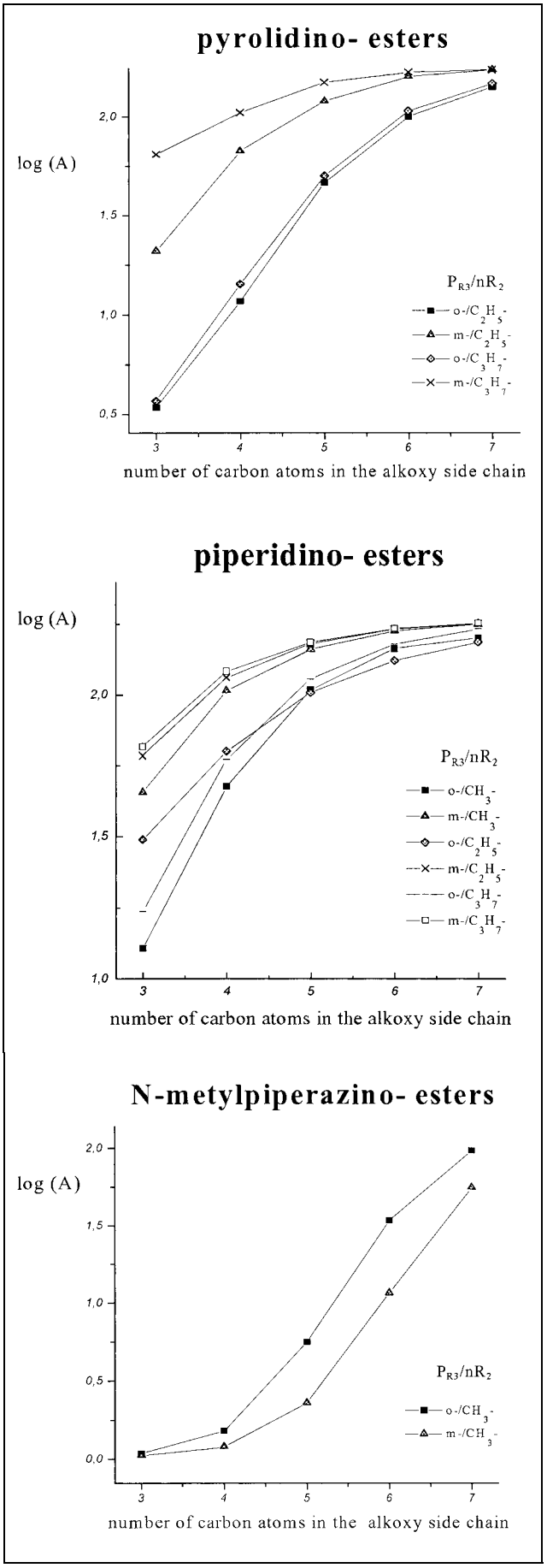


Fig. 2: Relationships between log (A) and number of carbon atoms in alkoxy side chain on benzene ring for o- and m-substituted basic esters phenylcarbamic acid. (A): surface anaesthesia

higher than that of o-substituted drugs and the location of maxima is at six carbon atoms in the alkoxy side chain. In the case of N-methylpiperazino-esters of phenylcarbamic acid (Fig. 2) the anaesthetic effect increases with an increasing of number of carbon atoms (from 3 to 7) in the alkoxy chain. The length of the alkoxy-substitution in the connective chain have no significant effect on the surface anaesthetic properties of the derivatives. The local anaesthetic activities of propyl-derivatives are not significantly higher than those of the ethyl- and methyl-substituted derivatives.

The coefficients of the regression equations are given in Table 3. They show significant dependencies between log k, nR_3, log A. The RP HPLC method is suitable for modelling of penetration of drug through the cell membrane.

Table 3: Coefficients of regression dependencies: $\log k = a + b \cdot n(R_3)$; $\log A = a + b \cdot \log k + c \cdot (\log k)^2$

$n(R_2)$	P_{R3}	a	b	c	r^2
Pyrolidino-ester					
2	o-	−0.0643	0.1038		0.9966
		−2.2904	1.1530	−0.0737	0.9958
	m-	−0.1727	0.1159		0.9800
		−0.9327	0.9872	−0.0767	0.9950
3	o-	−0.0090	0.1095		0.9990
		−2.3413	1.2023	−0.0795	0.9980
	m-	0.2450	0.0715		0.9174
		0.7382	0.4650	−0.0359	0.9916
Piperidino-esters					
1	o-	0.0688	0.0688		0.9990
		−1.5717	1.1668	−0.0899	0.9982
	m-	−0.1568	0.1046		0.9968
		0.1248	0.6776	−0.0538	0.9872
2	o-	0.3276	0.0316		0.9960
		0.1033	0.5893	−0.0418	0.9988
	m-	−0.1413	0.1070		0.9986
		0.5976	0.5290	−0.0415	0.9896
3	o-	0.0449	0.1111		0.9994
		−1.1553	1.0455	−0.0806	0.9931
	m-	−0.0723	0.1087		0.9952
		0.7048	0.4933	−0.0391	0.9850
n-Methylpiperazino-esters					
1	o-	0.1727	0.0932		0.9992
		−0.3663	−0.0657	0.0590	0.9794
	m-	−0.0569	0.1192		0.9950
		1.1887	−0.0751	0.1193	0.9945

$n(R_2)$: number of carbon atoms in substituent in connective chain, P_{R3}: position of the substituent on benzene ring, k: value of capacity factor, A: surface anaesthesia

3. Experimental

3.1. Drugs

The derivatives of phenylcarbamic acid were prepared according to the literature [1–4]. The values of surface anaesthesia were taken also from these papers.

3.2. HPLC analysis

The chromatographic system (Hewlett Packard series 1100) consisted of a quaternary pump equipped with a injection valve (Rheodyne), and a diode array detector. The analytical chromatography column was a Separon SGX C18 (3.2 × 150 mm I.D., particle size 5 µm). The mobile phase was a mixture of CH_3OH and sodium acetate solution (6.8 g · l^{-1}) (9:1, v/v). The flow rate of the mobile phase was 0.5 $cm^3 \cdot min^{-1}$, and the column temperature was maintained at 25 °C. The chromatograms were scanned at 240 nm. The injection volume was 10 µl. The concentration of the analysed drug solutions was 1 mg · cm^{-3}. A solution of $NaNO_2$ was used for determination of dead time (c = 1 mg · cm^{-3}).

3.3 Neural network method

The neural network employed in the presented experiments had the following architecture (Fig. 1): five input "neurons" characterised the drug molecule; number of carbon atoms in alkoxy-substitution in lipophilic part of molecule, position of this substitution, number of carbon atoms in alkoxy-substitution in connective chain, type of nitrogen containing substituent in hydrophilic part of molecule and LC chromatographic capacity factors. To provide good generalisation it is desirable to use the smallest number of hidden "neurons" that give satisfactory training performance. The optimal number of hidden "neurons" was two (according to the correlation index between calculated and measured data). The output was the logarithm of activity in surface anaesthesia. The program of neural network was written in Turbo-Pascal 7.0.

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Fundamentals and predictions of resolution of enantiomer mixtures by crystallization in the example of phase diagrams of atenolol and atenolol hydrochloride salt

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Dedicated to Prof. G. Heinisch, Innsbruck, on the occasion of his 60th birthday

Enantiomers as well as racemates of atenolol and atenolol hydrochloride were investigated thermoanalytically (thermomicroscopy, DSC, TGA), by X-ray diffractometry and spectroscopy (FTIR, FTRaman). The binary phase diagrams ((*R*)-, (*S*)-) of both substances were constructed and are used in the following discussion to consider the possibilities of separating enantiomers by direct crystallization. While a solid solution according to Roozeboom type I is formed between (*R*)- and (*S*)-atenolol, the hydrochloride crystallizes as a solid solution according to Roozeboom type II, whereby an enantiomer enrichment can be achieved.

1. Introduction

Atenolol INNp, 2-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]acetamide, represents a β_1-selective adrenoceptor antagonist (e.g. Tenormin®) and is one of the most widely used beta-blockers. Ninety percent of them are sold as racemic mixtures [1] consisting of equal moles of the (*S*)- and (*R*)-enantiomers, although there is a clinical advantage in administration of optically pure (*S*)-enantiomers relative to administration of the racemates [2]. Mainly the (*S*)-enantiomer of atenolol has hypotensive activity and activity on bradycardia [3, 4]. The administration of the inactive enantiomer will not substantially increase the desired pharmacological response but may unnecessarily increase the toxicity and adverse side effects. This has resulted in much interest in the production, isolation and purification of (*S*)-atenolol [5–7].

Asymmetric or stereoselective synthesis suffers either from low yield or insufficient enantiomeric excess rates. Separation of the enantiomers via diastereomers or diastereomeric salts is an expensive and time-consuming process that is also accompanied by technical problems. Atenolol shows a very small difference in the solubility between racemate and enantiomer [8]. Therefore, it is difficult to isolate the optically active atenolol utilizing the difference in solubility. Through, the formation of salts of atenolol with different Brønsted's acids, this difference could be increased and hence a method for isolation and purification of enantiomerically enriched atenolol could be achieved [8].

The aim of this work was to investigate the conditions underlying this behaviour, such as melting reaction and stability in the binary systems of (*R*)- and (*S*)-atenolol as well as (*R*)- and (*S*)-atenolol hydrochloride. The importance of resolution of racemates for the commercial production of enantiomerically pure chiral substances has recently been reported by Li and Grant [9]. To follow the route of crystallization prior knowledge is needed including the curve course of the enantiomers in the binary system and their thermodynamical stability in case of polymorphism [10, 11]. This information determines those parameters which can be appropriately obtained by construction of the binary phase diagram. Its curve course