trolled by a software package. The software for recording and analysing membrane currents was developed in our department. Membrane currents were filtered at 2 kHz and stored on disc for subsequent analysis.
Only rod shaped cells with clear cross striation and a membrane potential more negative than −65 mV were selected.
To study outward potassium currents, pipettes with resistances between 2 and 4 mΩ when filled with intracellular solution were used. The whole-cell configuration was established while superfusing the cells with control solution 1. Then, this solution was exchanged by control solution 2 containing 0.5 mmol/l $CdCl_2$ to block the L-type Ca^{2+}-current. According to the stimulation protocol (Fig. 1) Na^+- and T-type Ca^{2+}-currents were inactivated by an prepulse from the holding potential (−70 mV) to −40 mV. Potassium outward currents were elicited as shown in Fig. 1. After assessment of membrane currents in the absence of drug (3 min), the perfusate was changed to one containing the various concentrations of AWD 23-111 and data were collected continuously. Frequency dependent effects of AWD 23-111 on IK were tested by applying 250 ms lasting depolarising pulses from a holding potential of −40 mV to 0 mV or to +40 mV at stimulation frequencies of 0.2, 1 and 2 Hz.
Ca^{2+}-currents were recorded in a similar way but using control solution 1 and the intracellular solution but without cAMP. The L-type Ca^{2+}-current was elicited by depolarising steps from a holding potential of −40 mV to test potentials between −30 and +40 mV at a stimulation frequency of 0.5 Hz for the registration of the I–V-relationship. To test the frequency-dependent effects of AWD 23-111 on ICa 250 ms lasting test potentials to +40 mV were applied at stimulation frequencies of 0.2, 1 and 2 Hz starting from a holding potential of −40 mV.
Action potentials were recorded in the current clamp mode. The cells were superfused by control solution 1 and the intracellular solution contained no cAMP as described above. APs were evoked by 20 ms lasting suprathreshold depolarising currents at stimulus frequencies between 0.2 and 2 Hz during control and after superfusion with AWD 23-111.

4.3. Statistics

Data were expressed as mean ±SEM. Student's paired t-test was used to compare two means. Comparisons between multiple means were performed by two-way ANOVA followed by subsequent pairwise t-test if the null hypothesis could be rejected. A two-tailed probability was considered to be statistically significant. Null hypothesis was rejected at the $p < 0.05$ level.

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Dr. Manuela Klapperstück
Julius-Bernstein-Institut für Physiologie
Martin-Luther-Universität Halle-Wittenberg
Magdeburger Str. 6
D-06097 Halle (Saale)

Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Can diastereoisomerism of alkoxyphenylcarbamates influence their local anesthetic activity?

E. RAČANSKÁ and F. GREGÁŇ

The surface local anesthetic activity (LAA) in the homologous series of racemic (±)-*cis*- and (±)-*trans*-*N*,*N*-dimethyl-2-(2-alkoxyphenylcarbamoyloxy)cyclopentylmethylamonium chlorides was evaluated. The potency was expressed in rabbits as efficiency indices (EI) in comparison to the standard drug cocaine. All tested racemic mixtures of the phenylcarbamates were local anesthetically active and their potency increased with the size of alkoxysubstitution from the propyloxy- to the hexyloxyderivative and then decreased abruptly (cut-off effect). When different mixtures of both diastereoisomers were applied the synergistic effect – i.e. increase of the LAA of one diastereomer when adding the other – was observed. It seems that an optimal racemic ratio of the compounds could increase their local anesthetic efficiency.

1. Introduction

The study of the local anesthetic activity (LAA) of various derivatives of alkoxyphenylcarbamic acid has shown that the intensity of their biological efficiency is determined mainly by size and shape of the alkoxysubstituent [1, 2]. In amphiphilic drugs with long chain, where the majority of local anesthetic belongs to, a nonlinear, rather parabolic relationship to the size of alkoxy group was described [3, 4]. This general pharmacological phenomenon depends on the physico-chemical properties of the local anesthetics. It is obvious that only drugs with an optimal chain length are able to influence adequately their transport properties at the site of action and provide maximal pharmacotherapeutic effect [5].

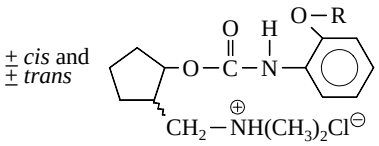
Only a few works exist in the literature dealing with the arrangement of conformationally fixed isomers [6]. In our previous studies we have been interested in spatial configuration of various phenylcarbamates and we have found different LAA of separate diastereoisomers [7]. Recently, we have found surprisingly that separated diastereoisomers have not only a different intensity of LAA, but various mixtures of *cis*- and *trans*-isomers showed also significantly diverse anesthetic effects. This possibly synergistic effect, i.e. increase of anesthetic activity of one diastereoisomer by adding the second one strictly depends on the molar ratio of both stereoisomers [8].

In the effort to confirm this effect in the similar series of phenylcarbamates we decided to evaluate the intensity of LAA for other diastereoisomeric phenylcarbamates, i.e. (±)-*cis*- and (±)-*trans*-*N*,*N*-dimethyl-2-(2-hexyloxyphenylcarbamoyloxy)cyclopentylmethylamonium chloride (Table 1) and their mutual mixtures. The study has been expected to provide useful information to our understanding of the mechanism of specific membrane action of phenylcarbamate local anesthetics.

2. Investigations, results and discussion

Local anesthetic activity of homologous series of phenylcarbamate amphiphilic compounds shows often a non-linear dependence on their chain length. This so-called "cut-off" effect has been described earlier and could be explained by the necessity of an optimal chain length to cross the membrane lipid bilayers to the inner binding sites of action [3, 9].

Table 1: Structures of the studied compounds, their molar weights (Mr), efficiency indices of relative surface local anesthetic activity (SLLA) and their LD_{50} values



Compd.	R	Mr	SLLA (cocaine = 1)	LD_{50} (mg · kg^{-1})
c^3 t^3	$-(CH_2)_2CH_3$	356.9	1.8 2.4	200–300
c^4 t^4	$-(CH_2)_3CH_3$	370.0	9.2 7.8	200–300
c^6 t^6	$-(CH_2)_5CH_3$	398.8	142.2 166.8	400–500
c^7 t^7	$-(CH_2)_6CH_3$	413.0	156.1 153.8	200–300
c^8 t^8	$-(CH_2)_7CH_3$	427.0	125.0 144.9	200–300
c^{10} t^{10}	$-(CH_2)_9CH_3$	455.1	≤10.0	*

* insoluble in water

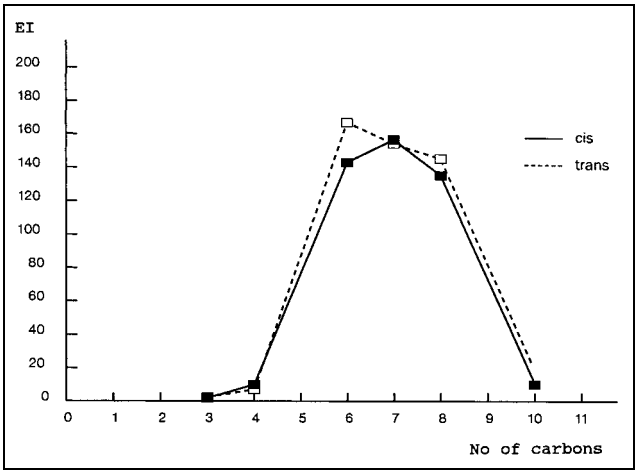


Fig. 1: Influence of number of carbons in alkoxysubstituent on efficiency indices (EI) of local anesthetic activity in phenylcarbamate series

The purpose of this study was to verify the above described effect in the homologous series of *cis*- and *trans*-isomers of alkoxysubstituted phenylcarbamates (three to ten carbons). Our experiments showed an exponential increase in LAA from 3 to 7 carbons. Then the biological activity decreased (Table 1, Fig. 1). The highest efficiency index of LAA was found for the *trans*-hexyloxyderivative ($t6 = 166.8$), while the index of the decylderivative was less than 10. A similar effect was found for the *cis*-isomers series. This confirms the suggestion that this process is size dependent and proportional to the molecular weight of the substances. Individual stereoisomers differ not only in their physicochemical properties but in their biological activity as well. Our previous studies have shown that usually *cis*-isomers have higher LAA than their *trans*-counterparts [7, 8] but some exceptions have also been found [10]. However, when two parts of diastereoisomers in various molar concentration were mixed, both surface and also infiltration local anesthetic actitivies were changed. This indicated the possibility of synergism or antagonism which could increase or decrease, resp. the intensity of anesthetic action. Our previous results have shown that the highest synergic effect was reached when 25% of the molar concentration of the *cis*-isomer was added to the *trans*-form [8].

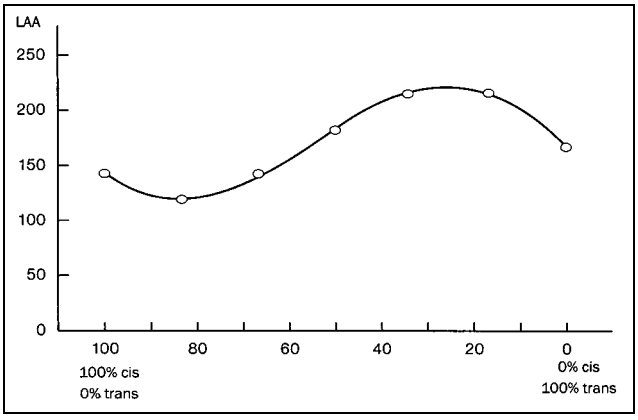


Fig. 2: Dependence of the local anesthetic activity (LAA) on molar fraction of *cis*- and *trans*-isomers of the hexyloxysubstituted compound (c^6, t^6)

Table 2: Surface local anesthetic activity of diastereoisomeric hexyloxyderivative mixtures expressed as concentrations evoking anesthesia lasting 20 min (with their ranges) and relative indices of anesthetic activity in comparison to cocaine

cis : *trans* molar ratio (%)	$EC_{20\,min}$ ($mol \cdot l^{-1}$)	Efficiency index (SLLA)
100 : 0	7.04×10^{-5} (6.80–7.22)	142.2
84 : 16	8.77×10^{-5} (8.38–8.96)	114.0
66 : 34	6.58×10^{-5} (6.20–6.96)	152.0
50 : 50	5.40×10^{-5} (5.24–5.53)	185.2
34 : 66	5.21×10^{-5} (5.08–5.34)	192.0
16 : 84	4.50×10^{-5} (4.38–4.71)	222.2
0 : 100	6.0×10^{-5} (5.81–6.13)	166.8
cocaine	1.0×10^{-2}	1.0

Each value was obtained from 3–6 separate measurements in the least three different concentrations

In the present study the observed indices of LAA of individual *cis*- and *trans*-isomers did not differ significantly (142.8 vs. 166.8). However, when the mixtures of both isomers in various molar concentrations were applied, the intensity of LAA has changed (Table 2). When the molar concentration ratio of *cis*- and *trans*-isomers was 84 : 16 (%) the index of local anesthetic efficiency was the lowest (114.0). When the ratio was reversed, the efficiency index was the highest (222.2). Dependence of LAA on molar ratios of the mixtures of the isomers could be expressed by a sinusoidal curve (Fig. 2). On the other hand a qualitatively different, two peaks curve was found in our previous experiments for phenylcarbamates with the six carbon ring. In this case the highest index of LAA was found when *cis* : *trans* percentual mixture ratio was 16 : 84, i.e. reversed [8].

It seems that the problems of diastereoisomerism in a series of phenylcarbamates are complex and connected mainly with conformational changes at the regions of sodium channels that bind to the tertiary amine moieties of local anesthetic. Although we cannot answer these specific questions now, the existing synergism between individual stereoisomers intimate some attribute which could influence the mechanism of local anesthetic efficiency.

3. Experimental

The surface local anesthetic activity of all compounds was estimated on rabbits cornea according to the method of Vrba and Sekera [11]. Different concentrations of the compound were applied into the conjuctival sac for 30 min. Then, corneal sensitivity was tested by hair esthesiometer repeatedly in 3 min intervals. Full anesthesia occurred if no response was elicited by 6 consecutive stimulations. Each compound was tested in 3–6 separate measurements with a least three different concentrations. Calculated $EC_{20\,min}$ express the time of full local anesthetic lasting 20 min and/or efficiency indices the relative anesthesia activity to the standard drug cocaine (cocaine = 1).

Acute toxicity was estimated on mice after s.c. administration of 1% solutions of tested compounds. The mortality of the animals was recorded 24 h after the application and expressed as LD_{50} in $mg \cdot kg^{-1}$ [12].

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Doc. E. Račanská
Faculty of Pharmacy
Odbojárov 10
83434 Bratislava
Slovak Republic

Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

In vitro effect of imipenem on *Acinetobacter baumannii*

A. HOŠTACKÁ

Imipenem at suprainhibitory concentrations (2×, 4× or 8× MIC) induced postantibiotic effects (PAEs) (suppression of bacterial growth after a short time exposure of bacteria to antimicrobials) against two of three *Acinetobacter baumannii* strains. The highest concentration tested demonstrated the longest delay of bacterial regrowth (1.7 h (strain 5570) or 3.9 h (strain 6070)). All. *A. baumannii* strains showed changes in surface hydrophobicity and serum sensitivity after treatment with imipenem. The antibiotic at 8× MIC reduced hydrophobicity of the strains most significantly (from 42.3%–72.0%) as compared to controls (without antibiotic). Susceptibility of the treated bacteria to serum bactericidal activity has also been lowered. Though imipenem suppressed bacterial growth and decreased surface hydrophobicity of the bacteria, it increased survival of bacteria after incubation with serum. These different alterations observed in the studied strains should be taken into account when evaluating the effects of imipenem.