Department of Chemistry[1], Faculty of Natural Sciences, Matej Bel University, Banska Bystrica, Slovak Republic; Department of Chromosome Biology[2], Max F. Perutz Laboratories, University of Vienna, Austria; Department of Genetics[3], Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Local anesthetic activity of mixtures of *cis*- and *trans*-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates

F. Gregan[1], J. Gregan[2,3], M. Skorsepa[1]

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Juraj Gregan, Max F. Perutz Laboratories, University of Vienna, Department of Chromosome Biology, Dr. Bohr-Gasse 1, 1030 Vienna, Austria
juraj.gregan@univie.ac.at

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In a previous study, we synthesized two homologous series of racemic stereoisomeric *cis*- and *trans*-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates with alkyl chain lengths ranging from C_1 to C_8 and analyzed their local anesthetic activity. Here, we show that the local anesthetic activities of mixtures of *cis*-1 and *trans*-1 stereoisomers are higher than the sum of activities calculated for the individual stereoisomers at all molar fractions. We conclude that an appropriate ratio of *cis*- and *trans*-stereoisomers is necessary to achieve the maximum anesthetic activity of the studied stereoisomeric carbamates.

1. Introduction

Over the past century, local anesthetics have been used to prevent acute pain and to ameliorate chronic pain associated with various conditions. Despite their remarkable efficacy, the risk of toxicity associated with local anesthetics remains a recurring issue (Dillane and Finucane 2002; Borgeat and Aguirre 2010). Therefore, it is important to develop new local anesthetic compounds as well as to improve existing ones. The interaction between two anesthetic drugs can be: (i) additive, (ii) synergistic, or (iii) antagonistic (infra-additive) according to whether their combined effect is equal to, greater than, or less than the sum of their individual effects. The identification of synergistic drug combinations is essential because these combinations allow lower doses of each drug to be used, therefore potentially reducing the side-effects caused by the individual drugs and making them particularly attractive for clinical use (Hendrickx et al. 2008; Shafer et al. 2008). In our previous studies, we focused on the effect of stereoisomerism on the local anesthetic activity of selected carbamate compounds (Gregan et al. 2011) The stereochemistry of local anesthetics can affect their potency by altering various characteristics of the drugs, such as their binding to target molecules, rate of metabolism and lipophilicity (Gregan et al. 1995; Blesova et al. 1985; Whiteside and Wildsmith 2001; Remko and Scheiner 1991). Therefore, it is important to study individual *cis*- and *trans*-isomers as well as the two isomers in combination.

2. Investigations, results and discussion

In our previous study, we synthesized two homologous series of racemic *cis*- and *trans*-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates with alkyl chains ranging in length from methyl- to octyl- and determined their local anesthetic activity (Gregan et al. 2011; Blesova et al. 1985). In our current study, we prepared three series of stereoisomeric *cis*- and *trans*-carbamates with propyl-, hexyl- and octyl- groups (Fig. 1) and determined the Surface Local Anesthetic Activity (SLAA) of these compounds and their mixtures in water solution. For each pair of stereoisomers, we tested nine mixtures with molar fractions ranging from 100% *cis*-1 (0% *trans*-1) to 0% *cis*-1 (100% of *trans*-1) (Fig. 2). The experimentally measured SLAA values and corresponding molar fractions of the studied stereoisomers are indicated in Fig. 2. The dependence of the SLAA values of the *cis*- and *trans*-stereoisomers on the molar fraction was nonlinear, exhibited two maxima and was similar for all three carbamates (Fig. 2). We next compared our experimentally measured SLAA values with the sums of the SLAA values calculated for the individual *cis*-1 and *trans*-1 stereoisomers present in the mixture. The dependence of the sums of the calculated SLAA values for the *cis*-1 and *trans*-1 isomers on their molar fractions was linear for all three carbamates, with R = propyl, hexyl or octyl (Fig. 2, Table 1). The correlation coefficients for the linear dependences are shown in Table 2. If the SLAA values for the mixtures of the *cis*-1 and *trans*-1 stereoisomers were additive, the dependence of the experimentally measured SLAA values on the molar fraction of stereoisomers should also be linear. However, our experimental data show a non-linear distribution with two maxima (Fig. 2). For all molar fractions, the experimental SLAA values were greater than the sums of the calculated values for the individual *cis*-1 and *trans*-1 isomers. At maximum 1 (Max1), the experimental SLAA value was 52% higher for the propyl derivate, 18% higher for the hexyl derivate and 40% higher for the octyl derivate (Fig. 2, Table 1). At maximum 2 (Max2), the experimental SLAA value was 52% higher for the propyl derivate, 23% higher for the hexyl derivate and 30% for the octyl derivate (Fig. 2, Table 1).

Although the molecular mechanism underlying this effect is not known, similar effects have been observed for several other compounds (Chen et al. 2011; Macht 1929; Gregan et al. 1995b; Racanska and Gregan 1999; Kiuchi et al. 1993).

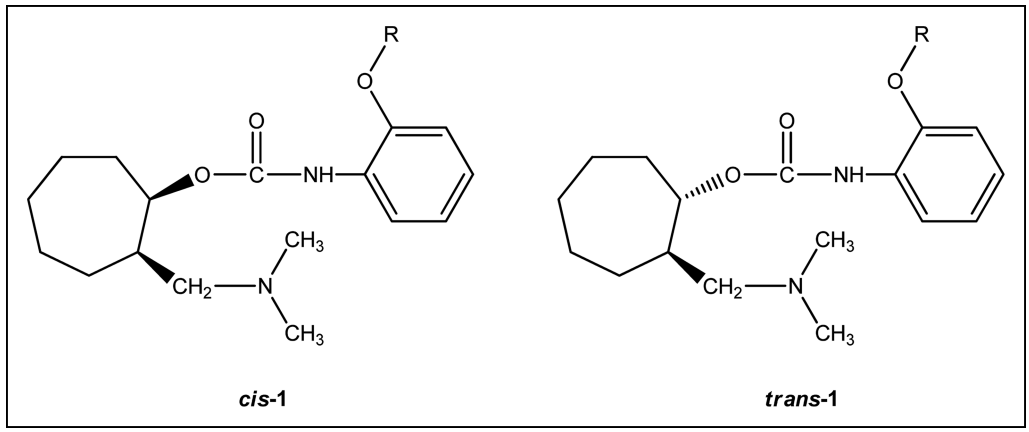

Fig. 1: Structures of the studied stereoisomeric *cis*- and *trans* carbamates (R = alkyl chain with 3, 6 or 8 carbons).

Table 1: Experimental and calculated SLAA values for varying molar fractions of *cis*-1 and *trans*-1 carbamates in the mixture

$M_{\%}^{cis-1}$	$M_{\%}^{trans-1}$	$P3_{calc}^{cis-1}$	$P3_{calc}^{trans-1}$	$\Sigma P3_{calc}$	$P3_{exp}$	$P6_{calc}^{cis-1}$	$P6_{calc}^{trans-1}$	$\Sigma P6_{calc}$	$P6_{exp}$	$P8_{calc}^{cis-1}$	$P8_{calc}^{trans-1}$	$\Sigma P8_{calc}$	$P8_{exp}$
100	0	89.0	0	89.0	89	289.0	0	289.0	289	178.0	0	178.0	178
85	15	75.7	8.0	83.7	117	245.7	29.0	274.7	310	151.3	15.8	167.1	210
75	25	66.8	13.3	80.1	122	216.8	48.3	265.1	314	133.5	26.3	159.8	224
60	40	53.4	21.2	74.6	93	173.4	77.2	250.6	280	106.8	42.0	148.8	183
50	50	44.5	26.5	71.0	83	144.5	96.5	241.0	260	89.0	52.5	141.5	165
35	65	31.2	34.5	65.7	90	101.2	125.5	226.7	250	62.3	68.3	130.6	160
25	75	22.3	39.8	62.1	94	72.3	144.8	217.1	267	44.5	78.8	123.3	160
10	90	8.9	47.7	56.6	69	28.9	173.7	202.6	240	17.8	94.5	112.3	140
0	100	0	53.0	53.0	53	0	193.0	193.0	193	0	105.0	105.0	105

$M_{\%}^{cis-1}$ ($M_{\%}^{trans-1}$); Molar fraction of *cis*-1 (*trans*-1) isomer in the mixture (%).
$P3_{calc}^{cis-1}$, $P6_{calc}^{cis-1}$, $P8_{calc}^{cis-1}$ ($P3_{calc}^{trans-1}$, $P6_{calc}^{trans-1}$, $P8_{calc}^{trans-1}$); Calculated SLAA values for *cis*-1 (*trans*-1) isomers in the mixture; P3: R = propyl, P6: R = hexyl, P8: R = octyl.
$\Sigma P3_{calc}$, $\Sigma P6_{calc}$, $\Sigma P8_{calc}$; Sums of the calculated SLAA values for the *cis*-1 and *trans*-1 isomers; P3: R = propyl, P6: R = hexyl, P8: R = octyl.
$P3_{exp}$, $P6_{exp}$, $P8_{exp}$; Experimental SLAA values for the *cis*-1 and *trans*-1 isomers in the mixture; P3: R = propyl, P6: R = hexyl, P8: R = octyl.

3. Experimental

3.1. Surface Local Anesthetic Activity (SLAA)

Equation (1) was used to calculate the Surface Local Anesthetic Activity, SLAA (P), for the individual *cis*-1 (*trans*-1) stereoisomers in the mixture (see Fig. 2 and Table 1)

$$P = \frac{SLAA \times M_{\%}}{100_{\%}} \quad (1)$$

where P is the calculated SLAA value of the individual stereoisomers in the mixture of the *cis*-1 and *trans*-1 stereoisomers, SLAA is the Surface Local Anesthetic Activity of the individual isomers, and M is the molar fraction of the individual stereoisomers in the mixture.

3.2. Chemistry

The starting compounds for the synthesis of the studied racemic *cis*- and *trans*-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates with alkyl chain lengths, R = propyl, hexyl and octyl (Fig. 1) comprised 2-alkoxyphenylisocyanates with alkyl chain lengths, R = propyl, hexyl and octyl, respectively, *cis*-2-dimethylaminomethylcycloheptanol (b.p. 83 – 84 °C/0.5 torr, m.p. 6 °C, $n_D^{20} = 1.4798$) and *trans*-2-dimethylaminomethylcycloheptanol (b.p. 91 °C/0.5 torr, $n_D^{20} = 1.4765$) (Gregan at al. 2011, 1995; Cizmarik et al 1976; Forro et al 1998; Forro and Fulop 1999). The starting *cis*- and *trans*-aminoalcohols were purified by column chromatography using silica gel with an eluent of ethylacetat:methanol (1:1). The investigated *cis*-1 and *trans*-1 carbamates were purified by recrystallization from ethylacetate (Gregan et al. 2011) and distilled. The purity of all compounds was verified by TLC on Merc Silica Gel 60 F_{254} plates using ethylacetate:methanol (1:1) as the mobile phase (detection under UV light at 254 nm).

Table 2: Regression coefficients for the linear relationships between the sums of the calculated SLAA values and the molar fractions (x) of *cis*-1 and *trans*-1 carbamates using the equation: SLAA = $ax + b$

P	a	b	R*
P3	−0.3603	89.058	1.000
P6	−0.9603	289.06	1.000
P8	−0.7301	178.03	1.000

P3: R = propyl, P6: R = hexyl, P8: R = octyl.
* R = Correlation coefficient.

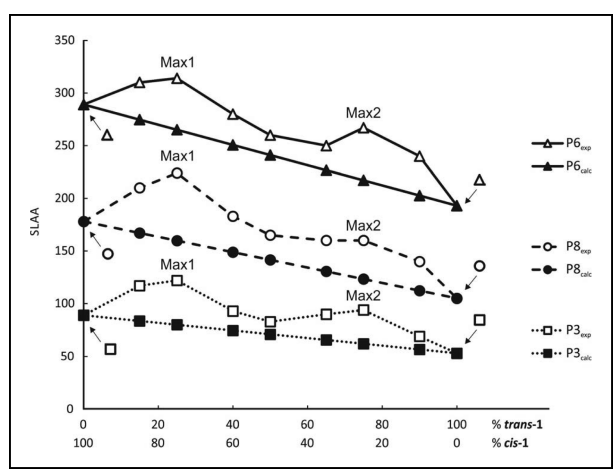

Fig. 2: Dependence of the experimental SLAA values (△, ○, □) and calculated SLAA values (▲, ●, ■) on the molar fractions of *cis*-1 and *trans*-1 stereoisomers in the mixture of studied carbamates. P3: R = propyl, P6: R = hexyl, P8: R = octyl.

The *cis*- and *trans*-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates (Fig. 1) were prepared by the reaction of *cis*- and *trans*-dimethylaminomethylcycloheptanol with 2-alkoxyphenylisocyanates in anhydrous toluene under argon atmosphere, as previously described (Gregan et al. 2011) For the biological assays, hydrochlorides of these basic carbamates were used, and *cis*-2-dimethylaminomethylcycloheptanol was prepared by the selective reduction of 2-dimethylaminomethylcycloheptanone with lithium tri(sec-butyl)borohydride (L-selectride) in anhydrous THF at –70 °C (Gregan et al. 1995; Forro et al. 1998; Forro and Fulop 1999; Perlia 1987). The *trans*-2-dimethylaminomethylcycloheptanol was prepared by the reduction of 2-dimethylaminomethylcycloheptanone with sodium borohydride in water (Gregan et al. 2011; Forro et al. 1998; Forro and Fulop 1999; Perlia 1987). These reductions were previously described for analogical *cis*- and *trans*-2-dimethylaminomethylcyclohexanol (Gregan et al. 1995). 2-Alkoxyphenylisocyanates with propyl-, hexyl- and octyl-groups were prepared from freshly distilled 2-alkoxyanilines by reaction with fosgene in anhydrous toluene (Cizmarik et al. 1976; Gregan et al. 1997). Experimental physico-chemical data for *cis*- and *trans*-2-dimethylaminomethylcycloheptanol are not available in the literature, with the exception of the ^{1}H NMR spectra and optical rotations (Forro et al. 1998; Forro and Fulop 1999).

3.3. Pharmacology

The studied carbamate stereoisomers were tested in the hydrochloride form. The Surface Local Anesthetic Activity indices (SLAA) of the analyzed compounds were determined on rabbit cornea according to the method of Vrba and Sekera (1959). Various concentrations of the compounds were applied to the conjunctival sac for 30 min. Afterwards, corneal sensitivity was repeatedly tested using a hair aesthesiometer at 3-min intervals. Full anesthesia was considered to occur if no response was elicited by 6 consecutive stimulations. Each compound was tested in at least three independent experiments. The surface local anesthetic activity index (SLAA) was calculated as the ratio of equieffective concentrations of the standard and the compound (dimensionless value) (Racanska and Gregan 1999). All biological tests were performed in compliance with regulations for biological testing on animals.

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