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Conformation and local anesthetic activity of carbanilates

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Summary. The influence of spatial configuration on intensity, onset, and duration of anesthetic effect has been observed in some carbanilate local anesthetics of fixed conformation. Synthesis of the carbanilates is described.

One approach to gain insight into membrane local anesthetic 'receptor' configurations is by fitting a variety of isomeric drugs to a putative receptor[1]. A number of position isomers and stereoisomers[2,3] as well as optical isomers[4] has already been prepared and pharmacologically tested in the group of pentacaine derivatives[2,5,6] which belongs to the group of carbanilate local anesthetics. The anesthetic activity of these compounds can be related to some of their physicochemical properties[7]. Boots and Boots[8] and Borne et al.[9] called attention to conformational aspects in norbornyl and azabicyclooctyl esters with local anesthetic activity, respectively. The purpose of the present study was to analyze whether isomers of pentacaine with rigid conformation differ in the local anesthetic activity.

A group of diastereoisomeric pentacaine derivatives conformationally firmly fixed by tertiary butyl group was prepared according to the previously described method[10-12]. By Clarke-Eschweiler methylation, dimethylaminoderivatives were prepared from all the 4 possible vicinal aminoalcohols with subsequent addition on 2-hexyloxyphenyl isocyanate[13]. The resulting 2-hexyloxycarbanilates are shown in the table. Structures of the substances were confirmed by IR-spectroscopy ($\nu_{c=o}$ 1730 cm^{-1}, 4 mg in 400 mg KBr (table), SPECORD IR 75), mass spectroscopy (200 °C, 12 and 75 eV, JEOL IMS-100), GC (aminoalcohols only, CHROM-4) and TLC (ethanol:water:ammonia = 4:3.5:0.1).

Pharmacological effects of the drugs were studied on rat sciatic nerve using a modified method of Paterson and Hamilton[14] and on guinea-pig ileum in vitro[15]. Changes in the size of the compound action potential in isolated sciatic nerve induced by the drugs were measured. To minimize the influence of perineurium as a diffusion barrier, the nerve preparations were longitudinally split into 2 bundles. The local anesthetic effect of the isomers in the concentration 1×10^{-3} moles/l was not identical. Following 120 min of application, the isomer No.3 inhibited the compound action potential almost completely, and isomer No.1, by approximately 30% (fig. 1, A). The other 2 isomers possessed intermediate potency. The differences were statistically highly significant ($p < 0.001$). The identical sequence of drugs has been found in quantities characterizing the dynamics of the local anesthetic effect, namely in the rate of action potential inhibition during the application

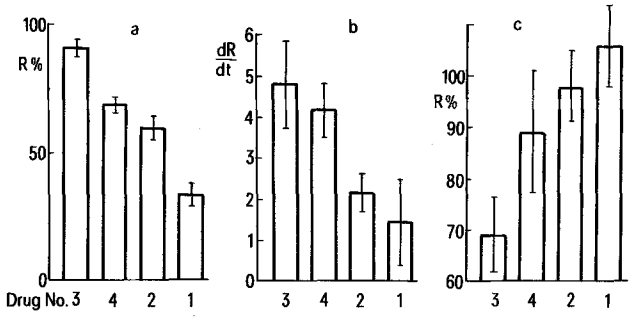


Figure 1. Decrease in compound action potential amplitude in isolated rat sciatic nerve (A), rate of action potential inhibition in the 1st 5 min of drug action (B) (R, reduction of action potential in percent of the effect in the 120th min of application, t, time in min), and inhibition persisting even following 60 min of drug wash-out expressed in percent of the maximal effect (C) induced by the 4 diastereoisomers (see table). Means ± SEM are shown.

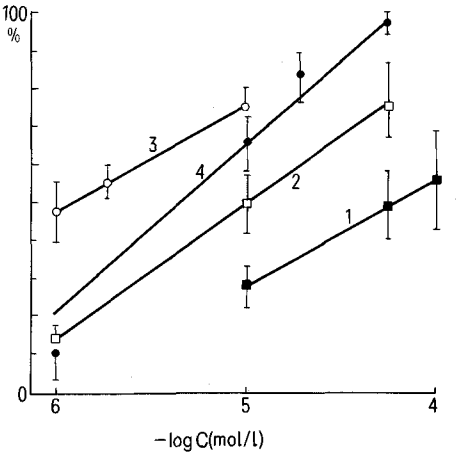
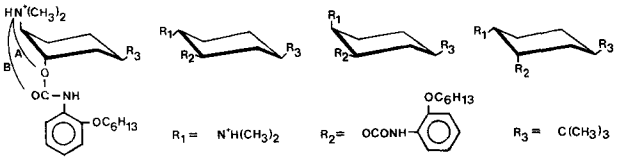


Figure 2. Dependence of inhibition of guinea-pig ileum twitches induced by transmural electrical stimulation on concentration (C) of the 4 diastereoisomers (see table).

Conformationally fixed diastereoisomeric pentacaine derivatives*

Isomer No.*	1	2	3	4
				
A (10^{-10} m)**	3.7	≥2.9	≤2.9	≤2.9
B (10^{-10} m)**	3.2-5.6	≥1.0-5.2	≤1.0-5.2	≤1.0-5.2
m.p. °C				
Oxalate	128-130	50	-	-
Hydrochloride	169-172	72-78	163-166	95-97

* All compounds are racemic isomers, hence, the structures do not show absolute configurations. ** The distances were measured on Dreiding stereomodels.

(fig. 1,B) and in the time course of action potential recovery during drug wash-out (fig. 1,C). The same drug sequence has been found also in an inhibitory effect of the isomers (10^{-6}-10^{-4} moles/l) on both phasic and tonic contractions of guinea-pig ileum induced by transmural electrical stimulation (fig. 2) or by acetylcholine or norepinephrine (5×10^{-6} moles/l). Obviously, the type of nerve fibers and/or excitable membrane does not play any essential role in the observed differences in the biological effects of the isomers.

Büchi[16] pointed to the importance of distance between positively charged nitrogen of amino group and carbon of carbonyl group in local anesthetics with classical structure[17] in their interaction with membrane binding sites.

In the 1st approximation, we may say that the activity of the studied diastereoisomers, which might be considered to be fixed conformers of mobile cyclohexane derivatives, increases with decreasing distance between the N and O atoms (1 < 2 < 4 < 3). As known, in vicinal di-substituted cyclohexanes the distance between diequatorial (trans) substituents is somewhat greater than between the axial-equitorial (cis) ones. Thus, in the isomer No. 2 the distance is greater than in the cis isomers Nos 3 and 4. As the results of syn-axial interactions the bulky (solvated) axial ammonium group in isomer No. 3 is more pressed towards the oxygen function than is the sterically less demanding oxygen in the isomer No. 4, making thus the distance shorter in the isomer No. 3 than in No. 4.

Another factor affecting the activity might be basicity (and hence the lipophilicity) of the studied compounds, which is likely to vary a little with the spatial relationship of the neighbouring groups.

The results indicate that the biological activity of the studied type of derivatives depends not only on configuration but also on the actual conformation in which the given compound exists.

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An immunohistochemical study on the distribution of histiocytes containing S-100 protein-like antigen in cutaneous T-cell lymphoma/leukemia; a preliminary report[1]

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The immunohistochemical distribution of histiocytes containing S-100 protein-like antigen in the skin lesions of cutaneous T-cell lymphoma/leukemia is investigated. Marked hyperplasia of these histiocytes is found in two cases of mycosis fungoides.

S-100 protein[2] is generally considered to be a specific protein of the nervous system. However, many authors have recently demonstrated that several nonneuroectodermal cells contained S-100 protein-like antigen which could not be distinguished immunologically from S-100 protein of the nervous system[3-5]. In human lymphoid tissues, S-100 protein-like antigen is present in interditating reticulum cells (IDC)[6] and in Langerhans cells[5]. These types of histiocytes are closely similar in fine structure[7] and several authors suggest that they are of the same origin and play an important role in the maturation of T-lymphocytes[7-9]. Although these special types of histiocytes could usually be recognized by electron microscopic observations[10], the immunohistochemical staining using antibody against S-100 protein makes it possible to identify them easily at the light microscope level. In the present study, we investigated the