Tolperisone: Evaluation of the Lidocaine-Like Activity by Molecular Modeling

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Summary

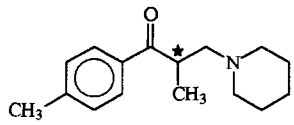
Tolperisone (**1**), a muscle relaxant with lidocaine-like activity, was compared to lidocaine (**2**) by molecular modeling methods. Conformational search analysis has been employed to find the global minima of these compounds along with numerous low energy conformations from which specific conformers were extracted that show good superimposition of the structural features important for protein binding. Two additional compounds, mepi- (**3**) and bupivacaine (**4**), were included in the analysis to validate the method as these ligands show very close structural and pharmacological relationship to lidocaine (**2**) and are assumed to bind to an identical site. As a result we find conformers of all four ligands that have exactly the same position and orientation of the potential sites for hydrogen bonding with the rest of the molecule showing close comparison of the three-dimensional geometry. Semiempirical calculations furthermore reveal good agreement of the electrostatic potentials of these conformations indicating similar interactions with a receptor. We conclude that tolperisone (**1**) and lidocaine (**2**) despite their chemical divergence can still attach to identical protein binding sites.

Introduction

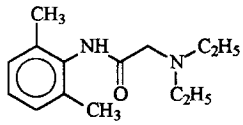
Tolperisone [2,4′-dimethyl-3-piperidinopropiophenone] is known as muscle relaxant for more than 30 years[1]. Pharmacologically this compound belongs to the broad class of local anesthetics with membrane stabilizing activity in the central and the peripheral nervous system. In this respect tolperisone is similar to lidocaine [2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide] a well known drug of broad applicability[2] (Fig. 1). Even though tolperisone displays distinct lidocaine-like activity its classification into one of the electrophysiologically based five antiarrhythmics classes[3,4] has not yet been established. Also, specific binding sites both for muscle relaxants and local anesthetics have not yet been disclosed[2].

Despite the structural heterogeneity of antiarrhythmics there are a few biologically important functional groups common to this class. A structural comparison of tolperisone and lidocaine reveals the existence of a tertiary nitrogen and a carbonyl-group as well as an aromatic ring as their common chemical features[5].

While a carbonyl oxygen and a protonated tertiary nitrogen are sites of hydrogen bonding the phenyl ring is capable of hydrophobic interaction with appropriate sites at a protein interface. Therefore, a hypothetical minimum model for binding[6] of both ligands to a specific receptor or channel protein would include these features as a general chemical formula of the type Ar-X-$(CHR)_n$-$^+NHR_2$ with Ar being a substituted phenyl ring while X is an electron donating group (Fig. 2).



(1)



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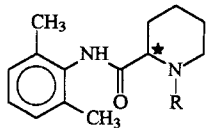

(3) R = CH_3
(4) R = $CH_2CH_2CH_2CH_3$

Fig. 1. Structures of tolperisone (**1**), lidocaine (**2**), mepivacaine (**3**) and bupivacaine (**4**); * denote chiral centers.

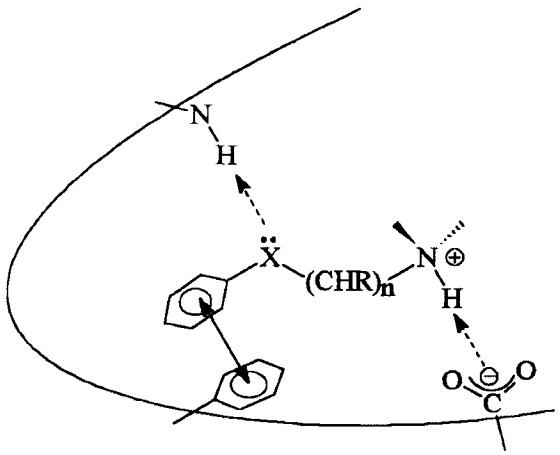

Fig. 2. Hypothetical binding site model for the interaction of muscle relaxants of the general formula Ar-X-$(CHR)_n$-$^+NHR_2$ with proteins (Ar = substituted phenyl ring, X = electron donating group).

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In contrast to the amide structure of lidocaine tolperisone contains a ketone group and a chiral centre α to the carbonyl group. Enolization of the carbonyl group under physiological conditions will result in racemization of the stereogenic centre. Tolperisone is therefore marketed as a racemic (*RS*) mixture. Yet there are indications for different pharmacological effects of the two enantiomers, i.e. D-tolperisone is believed to induce central skeletal muscle relaxation, while the L-stereoisomer might cause either vasodilatation or bronchodilatation[7]. In general the two tolperisone enantiomers can result in different pharmacological effects with one enantiomer displaying the lidocaine-like activity while the stereoisomer is either physiologically inactive or is held responsible for different pharmacology. Specific receptor-ligand-interactions of this type are well known e.g. from blockade of sodium channels[8]. As a consequence of the structural mirror image relationship only one tolperisone enantiomer should exclusively adopt a conformation that is comparable to the biologically active conformation of lidocaine.

Molecular modeling experiments of lidocaine by Pattabiraman et.al.[9], LaPlanche et.al.[10] and Marrer[11] have already disclosed energy minimum conformations for this compound whereas tolperisone has not yet been structurally investigated. On the premise that the molecular structure of muscle relaxants is important to biological activity, we have explored the three-dimensional relationship of lidocaine and the tolperisone enantiomers, both as protonated and unprotonated ligands as the tertiary amines common to both structures are easily protonated under physiological conditions (pK_a of lidocaine = 7.8[12]). Special emphasis has been taken to elucidate the requirements for identical binding of these ligands to a common site. In contrast to earlier work on lidocaine[9–11] we have employed geometrical optimization methods on the basis of molecular mechanics utilizing every dihedral angle in the conformational analysis. In addition, contour maps of electrostatic potentials have been used to compare the potential interaction with a charged environment. As a further validation of the computational methods employed two additional compounds were included in the analysis, mepivacaine [2-(*N*-methylpiperidyl)-*N*-(2,6-dimethylphenyl)acetamide] and its *N*-*n*-butylpiperidyl derivative bupivacaine (Fig. 1). Both compounds exhibit strong structural similarity to lidocaine and therefore should yield good geometrical and electrostatic correspondence with this compound.

Results and Discussion

1. Lidocaine

Starting from three different structures of lidocaine that were either taken from known crystallographic data[13, 14] (**Lic3**- and **Lip1**-series) or generated by the HyperChem[15] molecule building tool (**li4**-series) independent structural optimization were accomplished under standard HyperChem MM+ optimization conditions. The resulting conformations then were submitted to a conformational analysis, in which torsion angle pairs a/b, b/c, c/d, d/e, d/f, e/f, g/h and i/j (Fig. 3) were varied from 0° to 360° in intervals of 10° with all other structural parameters held constant, followed by geometrical optimization under total relaxation of the final conformation (see Tab. 1, upper).

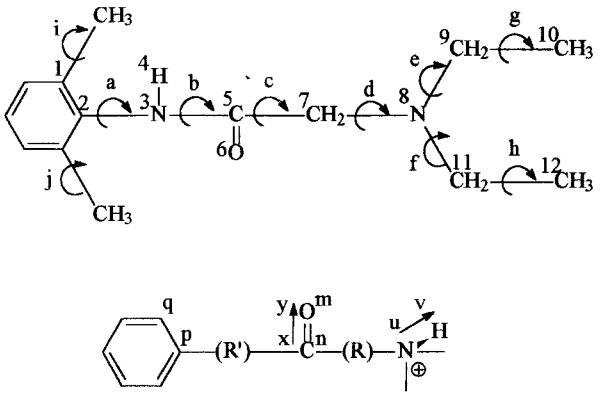

Fig. 3. Upper part: Specification of torsion angles and atoms in lidocaine (**2**). Lower part: Definition of orientation vectors "uv" and "xy" and of unproper torsion angles ("uvxy") and ("mnpq") that describe the orientation of the CO group with respect to the NH-group and the aromatic ring, respectively.

The results obtained for the three lidocaine series illustrate that geometrical optimizations are not sufficient to find global minima but rather lead to various local minima. The derived lowest local minima of the lidocaine series were thus submitted independently to conformational search analyses under MM+ conditions, in which torsion angles "a" through "j" (Fig. 3) were randomly varied to generate new structures followed by energy minimization for each of those structures. By this procedure a large variety of energetically optimized structures is created limited only by the number of torsion angles to be varied and by the totally allowed computing time for the experiment. The resulting lidocaine conformation could be accepted as the global minimum if all three different lidocaine starting structures yield an identical energy minima.

Table 1: Comparison of the lowest energy conformations of lidocaine: (upper): found after successive variation of torsion angle pairs as described in the text followed by geometrical optimization (MM+) with total relaxation. (lower): found after conformational search analysis. For comparison, both the lowest energy *s-cis*- and *s-trans* conformations are listed.

Lidocaine series	Energy (kcal/mol)	N-O distance[1] (Å)	Torsion angle[2] a1[3] (°)	Torsion angle[2] b1[3] (°)
Li4	13.8	3.38	−119.5	23.8
Lic3	17.5	3.40	−123.3	161.1
Lip1	20.0	3.42	125.8	163.5
Li4_cis	13.8	3.38	−119.0	23.7
Li4_trans	18.4	3.39	120.5	160.2
Lic3_cis	13.8	3.41	123.8	−24.4
Lic3_trans	17.7	3.37	117.8	−164.0
Lip1_cis	13.8	3.40	121.1	−25.8
Lip1_trans	17.7	3.36	116.4	−164.3

[1] Distance between tertiary nitrogen and carbonyl oxygen.

[2] Negative values denote a torsion angle of 360° minus the absolute number of the specified angle, e.g. −55.9° = 360° − 55.9° = 304.1°.

[3] Torsion angle a1 depicts atoms 8–7–5–6 of Fig. 3 and angle b1 atoms 6–5–3–4, respectively.

The lower part of Tab. 1 summarizes the result of these experiments showing that independent of the starting conditions a molecular energy of 13.8 kcal/mol is obtained which presumably represents the global minimum even though there are still minor variations in the structural data, e.g. –24.4° instead of –25.8° for the amide bond. The result also demonstrates that there are two almost identical structures that display an apparent enantiomeric relationship because of the pseudo stereoisomerism of the amide nitrogen (see **Li4_cis** and **Lic3_cis** in Tab.1).

The amide bond is independently derived as of *s-cis* geometry for the energy minimum structure although there are also numerous conformations found with *s-trans* configuration that are energetically only slightly above the global minimum.

2. *Protonated Lidocaine*

It is usually assumed for tertiary amines that at physiological pH the protonated (charged) molecule is the biologically active species. For a structural comparison of biologically relevant conformations of compounds (**1**) through (**4**) the protonated structure is thus of superior interest as compared to the free amine base. To investigate the effect of protonation on the structure of lidocaine we have protonated the two mirror imaged structures **Li4_cis** and **Lic3_cis** at the tertiary nitrogen followed by conformational search analyses.

Table 2: Energy minimum structures of protonated *s-cis*- and *s-trans*-lidocaine

Lidocaine series	Energy (kcal/mol) (Å)	N-O distance[1] a1[3] (°)	Torsion angle[2] b1[3] (°)	Torsion angle[2]
Li4p_cis	14.0	3.37	–116.2	23.4
Li4p_trans	17.1	3.37	117.2	–160.4
Lic3p_cis	14.0	3.40	–118.3	26.0
Lic3p_trans	17.1	3.37	–118.2	162.9

[1),2),3)] See footnote of Table 1.

The data of Tab. 2 demonstrate that the global minimum of the protonated lidocaine still displays *s-cis* geometry and that protonation in general does not have a dramatic effect on the molecular geometry as compared to the free tertiary base (Fig. 4). Again, there are a number of *s-trans* structures with reasonable energies found in the conformational search analysis.

3. *Tolperisone*

The two enantiomeric tolperisone structures were separately subjected to conformational search analyses as described for lidocaine to retrieve the global minima. As depicted in Tab. 3 identical energies were received for the two stereoisomers in the protonated and also in the unprotonated state suggesting that global minima were found for both structures. Also, as was observed with lidocaine, protonation does not affect the overall energy minimum structure to a large extent. The resulting structures of the protonated and unprotonated enantiomeric tolperisones show the expected mirror image relationship.

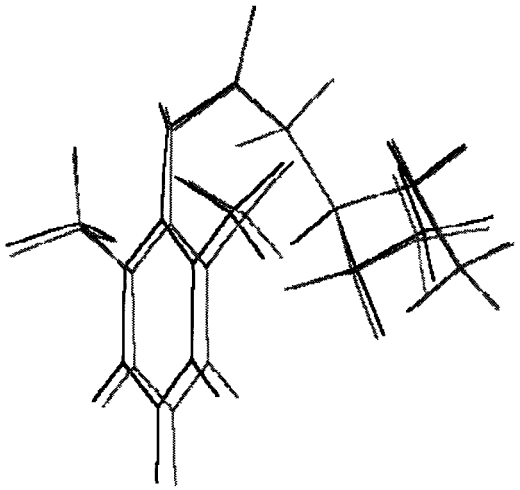

Fig. 4. Structural overlay of energy minimum conformers of protonated (black) and unprotonated (gray) lidocaine, superimposition arranged for maximum fit at the tertiary nitrogen and the carbonyl group.

Table 3: Energy minimum structures of enantiomeric protonated and unprotonated tolperisones

Structure	Tertiary nitrogen	Energy (kcal/mol)	N-O distance[1] (Å)	Torsion angle[2] N~CH_3[4] (°)	Absol. configuration
Tol1_R	unprotonated	16.7	2.90	–171.3	*R*
Tol1p_R	protonated	19.2	3.13	–178.1	*R*
Tol3_S	unprotonated	16.7	2.89	171.1	*S*
Tol3p_S	protonated	19.2	3.12	178.0	*S*

[1),2)] see footnote of Table 1.

[4)] N~CH_3 depicts torsion angle N-CH_2-CH-CH_3 of tolperisone.

However, comparison of N-O distances of the energetically favored protonated structures of lidocaine and tolperisone reveal the poor geometrical agreement of those functional groups necessary for hydrogen bonding. In order to evaluate this divergence we have included mepivacaine and bupivacaine in our investigation. Both compounds are well known local anesthetics with substantial structural and pharmacological analogy to lidocaine[2] which consequently should exhibit good agreement of the biologically active conformation.

4. *Mepivacaine and Bupivacaine*

Both mepi- and bupivacaine contain a stereogenic centre as in tolperisone and additionally, if protonated at the amino-nitrogen, show *cis/trans* stereochemistry with respect to the vicinally substituted piperidine ring. With the assumption that protonation will occur at physiological pH, calculations have been limited to the protonated structures. Conformational

search analysis of these compounds were accomplished as described above and result in the expected bisequatorial substitution pattern for the energy minimum structures (see Tab. 4).

Table 4. Comparison of lowest energy *s-cis* and *s-trans* conformations of protonated mepivacaine and bupivacaine.

Structure	Energy (kcal/mol)	N-O distance[1)] (Å)	Torsion angle[2)] a1[5)] (°)	Torsion angle[2)] b1[5)] (°)
mepi_cis	20.0	2.97	–62.6	22.3
mepi_trans	21.1	3.33	109.6	–158.7
bupi_cis	30.2	3.01	–65.6	22.6
bupi_trans	31.7	3.37	113.2	–158.7

[1),2)] See footnote of Table 1.

[5)] Torsion angles are analogous to a1 and b1 (see footnote of Tab.1).

Again, protonation does only slightly affect the structure. Also, in accordance with the results obtained for lidocaine, the global minima of mepi- and bupivacaine, respectively, show *s-cis* configuration of the amide bond while a number of *s-trans* configurations are found at energies slightly above the global minimum.

5. *Comparison of the Protonated Structures*

Analogous formation of hydrogen bonding of lidocaine, tolperisone and mepi/bupivacaine to a common binding site would assume identical three-dimensional geometry of the carbonyl groups and the protonated NH, i.e. of the geometrical orientation vectors "uv" and "xy" that make up the improper torsion angle "uvxy" as described in Fig. 3 (lower).

Comparison of the energy minimum structures of ligands (**1**) through (**4**) yet expose an insufficient correspondence of the N-O distances and of the orientation vectors "uv" and "xy". However, binding is most likely to occur not with conformations of lowest energy. Ligands rather will constantly undergo rotation at σ-bonds and thus occupy higher energy levels from which docking to a binding site is attained. Under this aspect it becomes necessary to consider all the various conformations (local minima) that can be generated within a given energy limit above the global minimum. Considering rotational barriers as, e.g., of gauche interaction and in accordance with literature data[16] it is conceivable to accept 5 kcal/mol as the upper limit for such an analysis. From the numerous conformations found by conformational search analysis within this limit for the four ligands one subsequently can extract those cases that show identical values of the critical geometrical parameters for binding. In order to evaluate all these conformers we have therefore compared the N-O distances and the unproper torsion angles "uvxy". This procedure is similar to the directional characterization of ligands as used in the CAVEAT-program[17]. The result of this analysis is shown in Fig. 5 in which each datapoint represents a conformation characterized by its N-O distance and torsion angle.

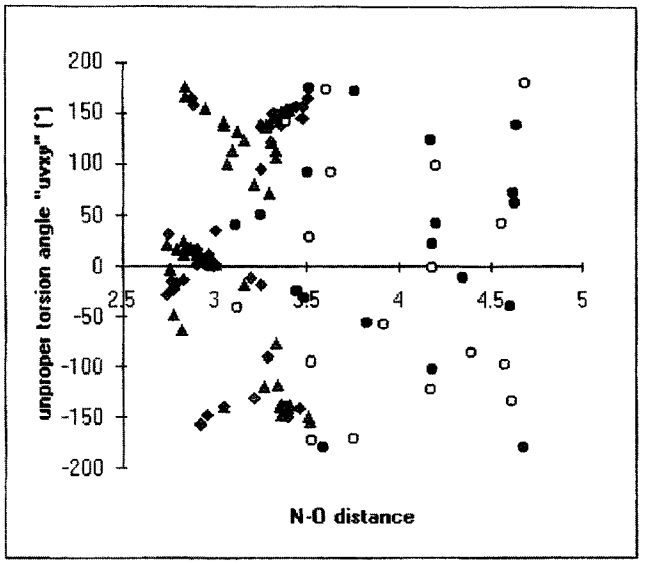

Fig. 5. Comparison of all conformations of ligands (**1**)–(**4**) with energies in the range of up to 5 kcal/mol above the respective global minimum by a two-dimensional plot of N-O distances and the unproper torsion angle "uvxy" (red triangles: lidocaine **Li4**-series, red diamonds: lidocaine **Lic3**-series, cyan triangles: mepivacaine, cyan diamonds: bupivacaine, yellow circles: (*R*)-tolperisone, green circles: (*S*)-tolperisone).

Two regions of overlapping datapoints can be identified in the area of positive angle values, one at about 3.4 Å distance and +150° torsion angle and the other at 2.9 Å and +15°. Each of these clusters is mirror imaged by similar values at negative torsion angles. Datapoints at a given N-O distance and opposite sign of torsion angle are conformations of mirror image relationship. As a consequence, discrimination between enantiomers by this method is not possible. Thus, for further analysis we can focus on either side of the N-O axes and to this end we will discuss below only data sets of positive torsion angles.

Table 5: Comparison of conformations with N-O distances in the range of 3.3–3.4 Å and unproper torsion angles of "uvxy" 140–152°: tolperisone (**Tol1**), lidocaine (**Li4** and **Lic3**), mepivacaine (**Mepi**), bupivacaine (**Bupi**), arranged in ascending order of N-O distances.

Structure	Energy (kcal/mol)	N-O distance[1)]	Amide bond (Å)	Torsion angle[6)] "uvxy" (°)	Torsion angle[6)] "mnpq" (°)	Absol. config.
Mepi_4a	21.4	3.33	*trans*	146	36	*S*
Bupi_8	32.5	3.34	*trans*	147	33	*S*
Mepi_3	21.1	3.35	*trans*	148	37	*S*
Mepi_6	23.4	3.36	*trans*	147	35	*R*
Li4_2_13	17.1	3.37	*trans*	140	33	–
Lic3_14	17.6	3.37	*cis*	140	–63	–
Bupi_9	32.6	3.37	*trans*	149	37	*S*
Bupi_5	31.7	3.37	*trans*	150	36	*S*
Mepi_5a	21.4	3.37	*trans*	151	–34	*S*
Li4_10	17.8	3.38	*cis*	144	–52	–
Lic3_12	17.3	3.38	*trans*	146	32	–
Tol1_15	23.6	3.39		141	–4	*R*
Mepi_7	23.6	3.40	*trans*	152	–34	*R*

[1)] See footnote of Table 1.

[6)] See Fig. 6 for specification of torsion angles.

One can further deduce from Fig. 5 that the amide compounds lidocaine, mepi- and bupivacaine all fall into a N-O distance range of 2.7–3.5 Å while tolperisone data points appear in the region of 3.1–4.7 Å. Consequently, in order to compare structural similarities between the amide compounds and the enantiomeric tolperisones, only conformers that intercept with an N-O distance margin of 3.1–3.5 Å have to be considered. As can be seen from Fig. 5 the only data-cluster in this region is located at 3.3–3.4 Å distance and 140–155° torsion angle value. Conformations that fall into this area are listed in Tab. 5. They all have very similar geometry of the orientation vectors, i.e. they display almost identical geometrical orientation of the protonated N-H group with respect to the carbonyl group.

Of the various ligands with amide structures shown in Tab. 5 all but two show *s-trans* configuration of the amide bond. Furthermore, with the exception of the *s-cis* structures there are two groups of structures that both have the same vectorial alignment of the carbonyl group relative to the phenyl ring but only differ in the sign of the unproper torsion angle "mnpq" (about +35° or –35° respectively). Overlaying conformers of lidocaine, mepivacaine and bupivacaine within such a set of identical angle values nicely demonstrates the excellent fit of the entire three-dimensional structures which proves the analytical capability of the modeling method employed. This is exemplified in Fig. 6 with each one of the amide structure ligands of positive "mnpq" angle value.

Fig. 6. Molecular superposition of lidocaine (**Lic3_12**, cyan), mepivacaine (**Mepi_3**, green), and bupivacaine (**Bupi_9**, yellow) with identical N-O distances and unproper torsion angles "uvxy" and "mnpq" respectively, superimposition arranged as in Fig. 4.

Within the considered cluster at 3.3–3.4 Å and 140–155° also one conformer of the (*R*)-tolperisone enantiomer can be found (see **Tol1_15** in Tab. 5). Despite the fact that this is an excellent agreement of the N-O distance and the geometrical alignment of the orientation vectors "uv" and "xy", the structure of tolperisone still displays two major differences with respect to the amides. Molecular superpositioning of tolperisone and the amide structures with respect to the N-O distances can only be achieved by attaching the nitrogen and oxygen atoms themselves rather than the entire protonated N-H group and the carbonyl group (Fig. 7). This leads to a rotation of the "uv"- and "xy"-vectors of tolperisone as compared to the other ligands by about 50° in the plane of the CO bond while still maintaining the unproper torsion angle "uvxy".

Fig. 7. Structural overlay of all conformers with N-O distance in the range of 3.3–3.4 Å and unproper torsion angles of 140–152° (tolperisone: green, all other compounds: cyan, the carbonyl oxygen (red) and the protonated nitrogen (blue) are color coded), superimposition arranged as in Fig. 4

Despite this fact location and orientation of hydrogen bonding sites in these molecules are still in good agreement and the depicted conformations of these molecules should still allow binding to identical sites at a protein interface. This is particularly true as hydrogen bonding will occur with free electron pairs that stick out with a sp^2-geometry from the oxygen atom. Furthermore, it is important to notice that the C=O and N-H groups, respectively, of all the conformers shown in Fig. 7 each lay in identical plains.

As a second restriction of structural identity the orientation of the phenyl ring is almost planar to the carbonyl group in tolperisone but is tilted about 35° in the amide structures. The result of this effect can be seen in Fig. 8 which displays two distinct views of structural overlays of each one of the four different ligands indicating the deviation of the phenyl ring

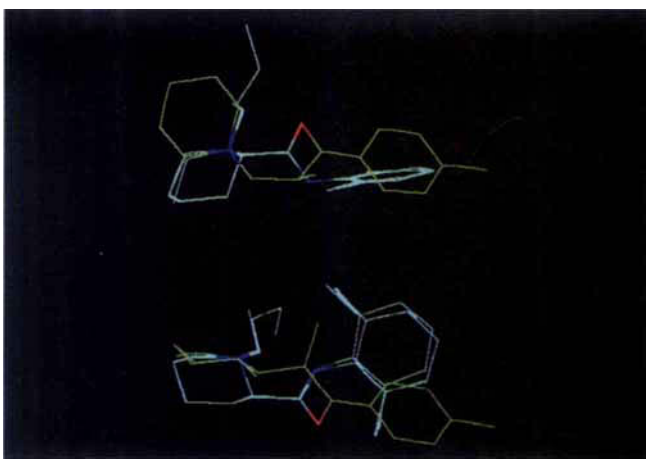

Fig. 8. Different structural views of superimposed molecules of tolperisone (**Tol1_15**), lidocaine (**Lic3_12**), mepivacaine (**Mepi_3**) and bupivacaine (**Bupi_9**), for color codes see legend to Fig. 9, superimposition arranged as in Fig. 4, hydrogen atoms omitted.

orientations. However, as hydrophobic interaction of aromatic rings is a rather global phenomenon and involves electron clouds of aromatic ring structures rather than a specific single point interaction, the rotated phenyl ring of tolperisone as compared to the amide structures is still acceptable for binding to an identical site.

Besides the (*R*)-tolperisone conformer (see **Tol1_15** in Tab. 5) there exists also a data cluster in which the (*S*)-stereoisomer is accumulated with a number of lidocaine, mepi- and bupivacaine structures. The respective (*S*)-tolperisone shows a 3.41 Å N-O distance and a torsion angle "uvxy" of 140° reflecting the mirror image relationship between the two tolperisone stereoisomers. Therefore, structural overlays as shown in Fig. 7 and 8 and as described in Tab. 5 are also found for (*S*)-tolperisone.

It is worth mentioning that even though the calculations were done in vacuo the data cluster at N-O distance of about 3.4 Å and a torsion angle values "uvxy" of roughly 140° closely resembles a conformation of lidocaine (N-O distance of 3.5 Å and torsion angle of 139°) that has been found to be one possible preferred conformation in solution[10]. This finding justifies the assumption made throughout this work that the calculated conformations are also representatives of solution structures.

With respect to the conformational analogy between the amide compounds lidocaine, mepi- and bupivacaine it is furthermore of interest that another region of overlapping datapoints exists at about 2.9 Å and small torsion angle values "uvxy" (Fig. 5). The majority of conformations at this cluster is of *s-cis* amide structure which again can nicely be overlaid. However, there is no conformer of tolperisone which is in reasonable vicinity of this data cluster. Possibly these datapoints depict a three-dimensional geometry that displays another active conformation of lidocaine, mepi- and bupivacaine not accessible by tolperisone.

6. QSAR Data and Semiempirical Calculations

Further evidence for identical binding of lidocaine, mepi-/bupivacaine and tolperisone comes from QSAR-data that have been calculated[18] using the conformations depicted in Tab. 5. Of the three most studied physicochemical properties the hydrophobic (log P) and steric properties (refractivity) are listed in Tab. 6 along with some additional data of interest. QSAR studies have been carried out on a variety of general anesthetics resulting in an optimum hydrophobicity close to log P = 2.3, regardless of the class of anesthetics[19]. As shown in Tab. 6 log P values of tolperisone, lidocaine and mepivacaine are well in this range while bupivacaine is of somewhat higher value. In addition, despite a variation of the molecular shape of the ligands measurements of global molecular parameters (surface area, volume, refractivity) also reflect the similarity of these ligands.

Semiempirical calculation of the conformers listed in Tab. 5 demonstrate their closely related charge distribution and electrostatic potential suggesting a similar interaction of the molecules with a potential protein binding site. The calculated Mullikan population-derived charges of the atoms involved in the orientation vectors "uv" and "xy" are very comparable (see Tab. 6). Molecular superimposition of the resulting conformers still yields a good geometrical match of the orientation vectors, i.e. of the potential binding groups. Furthermore, contour maps of electrostatic potentials of the depicted conformations (Tab. 5) demonstrate their similar electron distribution. This is exemplified by a two-dimensional contour plot for lidocaine, tolperisone and mepivacaine (Fig. 9).

Table 6. QSAR data and net charges of conformers depicted in Tab. 5, arranged in alphabetical order.

Structure	Surface area (Å^2)	Volume (Å^3)	Hydration energy (kcal/mol)	log *P*	Refractivity (Å^3)	Net charges (Mullikan) at orientation vectors "uv" and "xy"[7]			
						O	C	N	H
Bupi_5	543.1	932.0	0.683	3.572	86.61	–0.300	0.283	–0.000	0.235
Bupi_8	521.4	918.5	0.524	3.572	86.61	–0.299	0.283	0.000	0.235
Bupi_9	536.5	927.8	0.636	3.572	86.61	–0.299	0.284	0.000	0.234
Li4_10	443.7	754.4	–0.811	2.133	70.35	–0.299	0.282	–0.002	0.256
Li4_2_13	462.4	779.3	–0.269	2.133	70.35	–0.355	0.289	0.002	0.265
Lic3_12	466.0	779.6	–0.219	2.133	70.35	–0.294	0.272	–0.003	0.246
Lic3_14	442.9	758.4	–0.785	2.133	70.35	–0.296	0.278	–0.004	0.257
Mepi_3	462.6	791.5	–0.323	2.365	72.74	–0.296	0.277	0.003	0.241
Mepi_4a	468.7	793.0	–0.327	2.365	72.74	–0.300	0.279	0.003	0.240
Mepi_5a	467.0	791.0	–0.325	2.365	72.74	–0.269	0.264	0.004	0.241
Mepi_6	467.3	788.5	–0.482	2.365	72.74	–0.351	0.293	0.015	0.255
Mepi_7	473.6	795.7	–0.346	2.365	72.74	–0.277	0.262	0.006	0.244
Tol1_15	494.9	820.1	1.491	2.433	74.25	–0.333	0.286	–0.001	0.234

[7] net charges derived from semiempirical calculations, see Fig. 6 for specification of orientation vectors

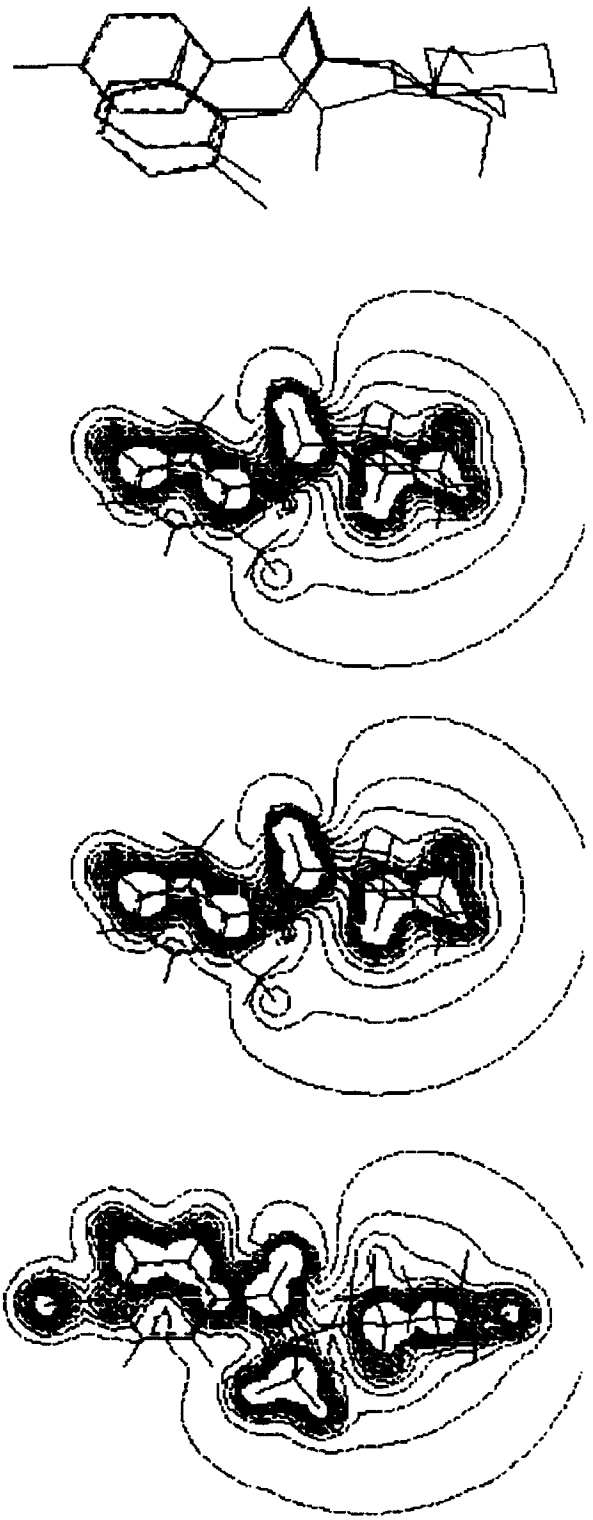

Fig. 9. Contour plots displaying electrostatic potentials of mepivacaine (**Mepi_3**), lidocaine (**Lic3_12**) and tolperisone (**Tol1_15**) (lower three pictures, for details see computational methods). Conformers are taken from a structural overlay as shown in the top picture, superimposition arranged as in Fig. 4.

Conclusion

Despite the chemical divergence of lidocaine, tolperisone, mepi- and bupivacaine we can demonstrate that there exist conformations for all four ligands that allow correct overlay of the protonated tertiary nitrogen and the carbonyl oxygen common to these structures and which are believed[5] to play an important role in ligand binding via hydrogen bonding. These conformations all have energies of less than 5 kcal/mol above the respective global minimum structures and therefore are easily accessible under physiological conditions. They were extracted from numerous conformations of the four ligands derived from conformational search analysis by a procedure similar to the active analog approach [20] however without any geometrical constrains on the investigated structures.

Because of the identical distance and orientation of the assumed major protein binding groups (N-H, C=O) and of a sufficient agreement of the overall structure and because of the similar electrostatic potential pattern observed, all four ligands can adopt a conformation that permits an approach to a protein surface in much the same way connecting to a binding site that fits all four ligands. These conformations may be regarded as the active binding conformations. Further reorientation at the site will then lead to an induced fit different for the specific ligands and responsible for variations in their binding affinity. In conclusion, our investigation shows lidocaine-like structural features for tolperisone which can cause the observed lidocaine-like activity in physiological experiments. The reported results should serve as an element to understand pharmacophore requirements for muscle relaxants and local anesthetics, respectively, the identification of which may lead to rational design of new ligands.

The local anesthetics lidocaine, mepi- and bupivacaine can additionally be superimposed with a different geometrical orientation that cannot be adopted by tolperisone. Therefore, this particular three-dimensional geometry of the amide compounds could represent a different active conformation that binds to sites not accessible to tolperisone. In this respect it is of interest to note that despite its lidocaine-like activity tolperisone does not exert the antiarrhythmic effect of lidocaine at the heart muscle[1].

The computational method described does not allow differentiation between enantiomeric tolperisones as conformational analysis results in almost identical but mirror imaged conformations of lidocaine. Differentiation between stereoisomers on the basis of molecular modeling will only be achievable if the geometry of the binding site is known and can be taken as a template to decide between the stereoisomers. Alternatively, chiral lidocaine-like ligands that are known to bind exclusively or preferentially with one configuration could be taken as a tool to investigate the stereochemistry of tolperisone binding. Molecular modeling experiments in this direction are under investigation in our laboratory.

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Computational Methods

Geometrical optimizations were carried out with the molecular modeling software HyperChem[15] by MM+ force field[21] and semiempirical AM1 methods[22] (calculations in vacuo, bond dipole option for electrostatics, Polak-Ribiere algorithm, RMS gradient of 0.1 kcal/(Å mol) as termination condition for the geometrical optimization). Conformational search analysis was performed using the respective module of ChemPlus[15] which is an addition package to HyperChem. The method involves random variations of user-specified torsion angles to generate new structures followed by energy minimization of each of those (random variation of max. 8 torsion angles at a time, structures with atoms closer than 1 Å or with torsion angles within 10^0 of previous or with change of chiral centres are skipped, structures are considered duplicates if energies are within 0.1 kcal/mol). It should be noted that all calculations have been performed in vacuo neglecting the interaction of the ligands with solvent (i.e. in this instance the physiological body fluid).

Two published crystal structures of lidocaine[13, 14] as well as a structure generated by the HyperChem molecule building tool were independently taken as starting points for the geometrical optimizations. The other ligands were created with help of the HyperChem model builder. Tolperisone enantiomers were considered separately. Mepivacaine and bupivacaine were set up with two equatorial substituents and in contrast to tolperisone were allowed to change chirality during the conformational search analysis in order to record conformers of the stereoisomers at a time. Energy surfaces were calculated by stepwise variations of two torsion angles with help of a torsion angle driver. Contour plots of electrostatic potentials were plotted in the plane of the orientation vectors "uv" and "xy" with the following grid control: horizontal and vertical grid points = 45; plane offset = 0 Å; starting value = 0; increment = 0.05. All figures of molecules were taken directly from HyperChem.

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