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Synthesis and antispasmodic activity of lidocaine derivatives endowed with reduced local anesthetic action

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Abstract—The present structure-activity relationship (SAR) study focused on chemical modifications of the structure of the local anesthetic lidocaine, and indicated analogues having reduced anesthetic potency, but with superior potency relative to the prototype in preventing anaphylactic or histamine-evoked ileum contraction. From the SAR analysis, 2-(diethylamino)-*N*-(trifluoromethyl-phenyl) and 2-(diethylamino)-*N*-(dimethyl-phenyl) acetamides were selected as the most promising compounds. New insights into the applicability of non-anesthetic lidocaine derivatives as templates in drug discovery for allergic syndromes are provided.

The local anesthetic lidocaine is largely used in clinical settings as a short-acting local and regional anesthetic and antiarrhythmic agent. Its major target on excitable cells is the voltage-sensitive sodium channel, which accounts for increased sodium permeability noted during the rising phase of the action potential in peripheral nerves, skeletal muscles, and neuroendocrine and heart cells.[1] Studies suggest that lidocaine also has clinical properties other than local anesthesia and antiarrhythmia.[2] For instance, lidocaine inhibits the function of leukocytes, including eosinophils,[3,4] mast cells[5], and T_H2 lymphocytes,[6] and prevents contraction of smooth muscle airways.[7] These effects suggest the promising possibility of alternative clinical applications than local anesthesia, e.g., as inhibitors of chronic inflammatory and airways obstructive diseases. Several clinical studies revealed that nebulized lidocaine has glucocorticoid-sparing properties in asthmatic patients.[8,9]

Lidocaine and the majority of local anesthetics with clinical applications possess a hydrophobic aromatic ring linked to a hydrophilic tertiary amine by a small intermediate alkyl chain. The aromatic group is the major contributor to the hydrophobic properties of local anesthetics since it is the molecular region primarily involved in cross-membrane transport in nerve cells and, thus, directly related to the efficacy of the local anesthetic. The amide alkyl linker has been described as an essential requirement for local anesthetic activity.[10] We have synthesized, in one-pot procedures, lidocaine derivatives by targeting and making substitutions in the 2,3-dimethylphenyl ring and in the amidealkyl links. A major objective was to assess the putative antispasmodic and anti-allergic properties of the lidocaine derivatives synthesized in order to achieve reduced local anesthetic activity.

The synthesis of lidocaine was first developed in 1948 by Lofgren and co-workers using 2,6-xylidine, diethylamine, 2-chloroacetyl chloride, and acetic acid, having benzene as solvent.[11] We however have employed toluene rather than benzene as solvent,[12] see Scheme 1, as an alternative one-pot procedure. Such a process was carried out in a RC1 calorimetric reactor which allowed the synthesis of industrial scale amounts of lidocaine and its analogues.

In this work, the local anesthetic activity of lidocaine and analogues was evaluated in naive rats by means of

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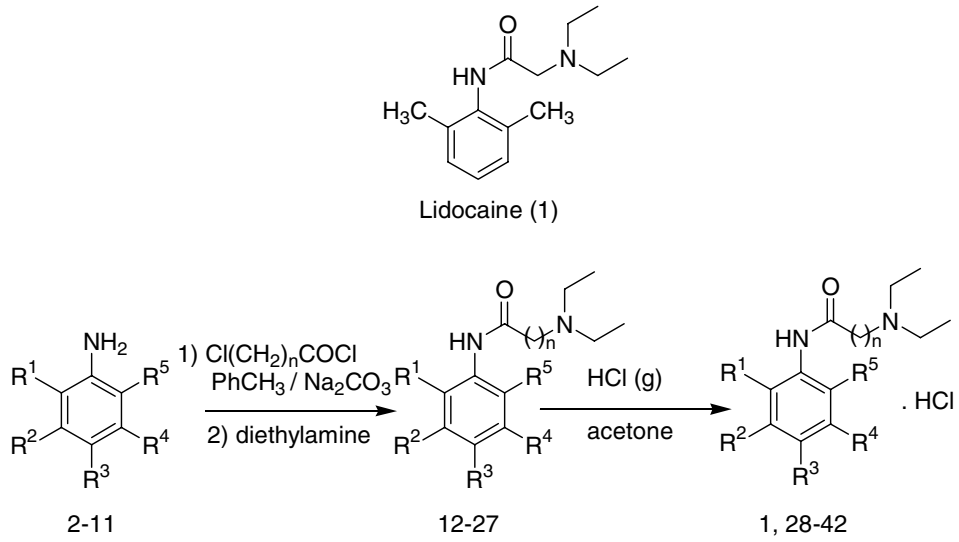

Compounds	n	Substituents
1	1	R^1, $R^5 = CH_3$; R^2, R^3, $R^4 = H$
28	1	R^1, $R^2 = CH_3$; R^3, R^4, $R^5 = H$
29	1	R^1, $R^3 = CH_3$; R^2, R^4, $R^5 = H$
30	1	R^1, $R^4 = CH_3$; R^2, R^3, $R^5 = H$
31	1	R^2, $R^3 = CH_3$; R^1, R^4, $R^5 = H$
32	1	R^2, $R^4 = CH_3$; R^1, R^3, $R^5 = H$
33	2	R^1, $R^5 = CH_3$; R^2, R^3, $R^4 = H$
34	2	R^1, $R^2 = CH_3$; R^3, R^4, $R^5 = H$
35	2	R^1, $R^3 = CH_3$; R^2, R^4, $R^5 = H$
36	2	R^1, $R^4 = CH_3$; R^2, R^3, $R^5 = H$
37	2	R^2, $R^3 = CH_3$; R^1, R^4, $R^5 = H$
38	2	R^2, $R^4 = CH_3$; R^1, R^3, $R^5 = H$
39	1	$R^1 = CH_3$; R^2, R^3, R^4, $R^5 = H$
40	1	$R^1 = CF_3$; R^2, R^3, R^4, $R^5 = H$
41	1	$R^2 = CF_3$; R^1, R^3, R^4, $R^5 = H$
42	1	$R^3 = CF_3$; R^1, R^2, R^4, $R^5 = H$

Scheme 1. Synthesis of lidocaine and its analogues by one-pot process.

blockade of the withdrawal reflex toward pinching on cutaneous sites previously injected with increasing doses of the test substance.[13,14] As expected, intradermal injection of lidocaine (1) induced a dose-dependent blockade of the nocifensive response. This blockade was progressively longer with 1 in doses ranging from 0.5 to 4 μmol/site, reaching 163 ± 2 min (mean ± SEM) at the highest dose (Table 1).

A torsional angle ($\theta = 44°$) between the aromatic ring and the plane of the aliphatic linker has been shown to be essential to the anesthetic activity of lidocaine.[16] Lidocaine exhibits an intramolecular hydrogen bond between the xylidine hydrogen atom and the nitrogen atom of the amide group in the side chain which helps to freeze the desired conformation.[17] This may facilitate its interaction with the sodium channel binding site. Our findings indicated that the substitution pattern in the aromatic ring clearly affected the anesthetic activity, in line with literature reports.[16]

As shown in Table 1, compounds **28**–**31** and **39** were generally less potent than lidocaine as local anesthetics. The duration of complete blockade of the nocifensive response appeared even shorter for those analogues in which the 2- and 6-dimethyl groups were replaced by 2-, 3- or 4-trifluoromethyl groups in the aromatic ring. At the highest dose level (4 μmol/site), the values for compounds **40**, **41**, and **42** were 24 ± 1, 18 ± 6 and 21 ± 2 min (mean ± SEM), respectively (Table 1).

Molecular modeling studies have shown that the removal of one methyl group in the aromatic ring of lidocaine decreases the energy barrier to rotation, so that the critical torsional angle in compound **39** is essentially 0°. The reduced anesthetic potency of compounds **28**–**31** and **39** must be a consequence of non-ideal torsional angles between the aromatic ring and the plane of the aliphatic side chain. Recent molecular modeling studies of voltage-gated sodium channels and docking studies with lidocaine and analogues have proposed that the 2,6-dimethyl-phenyl moiety interacts with Tyr1586 in the in-

Table 1. Duration of local anesthetic activity after an intradermal injection of lidocaine (**1**) or analogue (0.5–4.0 μmol/site)

Compound	Dose (μMol/site)			
	0.5	1.0	2.0	4.0
1	37 ± 4.0	74 ± 2.0	103 ± 4.0	164 ± 3.0
28	7 ± 1.0	16 ± 2.0	25 ± 2.0	34 ± 3.0
29	17 ± 1.0	32 ± 1.0	63 ± 2.0	92 ± 2.0
30	19 ± 1.0	28 ± 1.0	40 ± 1.0	54 ± 1.0
31	11 ± 1.0	20 ± 1.0	32 ± 1.0	55 ± 2.0
32	44 ± 2.0	91 ± 4.0	134 ± 2.0	181 ± 1.0
33	72 ± 1.0	107 ± 3.0	194 ± 4.0	311 ± 3.0
34	22 ± 1.0	49 ± 4.0	91 ± 3.0	139 ± 2.0
35	35 ± 1.0	89 ± 3.0	166 ± 4.0	239 ± 4.0
36	22 ± 2.0	47 ± 5.0	72 ± 1.0	101 ± 2.0
37	26 ± 1.0	48 ± 2.0	80 ± 2.0	107 ± 3.0
38	88 ± 2.0	118 ± 2.0	240 ± 2.0	377 ± 2.0
39	17 ± 2.0	28 ± 3.0	48 ± 1.0	63 ± 2.0
40	8 ± 1.0	13 ± 2.0	15 ± 2.0	24 ± 1.0
41	17 ± 5.0	19 ± 5.0	18 ± 6.0	18 ± 6.0
42	19 ± 4.0	19 ± 4.0	19 ± 4.0	21 ± 2.0

Duration to full recovery of sensory block measured in minutes (mean ± SEM) from at least six animals after treatment by increasing doses of lidocaine (1) or analogues in rats.

ner pore of the sodium channel. The alkylamine heads are close to Phe1579 and may be either interacting in a cation-π fashion (if a salt, i.e., trialkylammonium compound, is present). Alternatively, the heads are involved in hydrophobic interactions with the phenylalanine ring through van der Waals contacts with the alkyl side chain, which is in accord with the molecular mechanisms reported for lidocaine.[18–23] The changes in the torsion angle (θ) of lidocaine derivatives would result in the alkylamine side chain being in unfavorable positions for binding, thus reducing important interactions with the sodium channel.

Prior research suggested that 3–7 atoms in the side chain favor anesthetic activity, whilst larger or smaller linkers would reduce it.[1] As illustrated in Table 1, compounds **33**–**38**, which have an extra carbon when compared to compounds **28**–**32**, showed higher local anesthetic activity, e.g., **38** blocked mechanical nociception for 88 ± 2, 240 ± 2 and 377 ± 2 min (mean ± SEM) at 0.5, 2, and 4 μmol/site doses, respectively, compared to the values for analogue **32** of 44 ± 2 min, 134 ± 2 min and 181 ± 1 min (mean ± SEM), respectively. The better results for 33–38 may arise from increased lipophilicity or stronger interactions of the pharmacophoric tertiary amine with aminoacid residues in the sodium channel binding site. It has been observed that an increase in the length of the side chain facilitates the formation of the interaction with the sodium channel.[16] Not surprisingly, the structural modifications in this series of compounds led to higher *C*Log *P* values (Table 2),[24] confirming previous findings and supporting the anesthetic activity observed in our experiments.[21,28,29]

Many studies have shown that lidocaine inhibits directly the contraction of intestinal and respiratory smooth muscles evoked by various contracting stimuli, including histamine, acetylcholine, and depolarization caused by elevated concentrations of extracellular potassium.[7,30–32] The mechanism of action relies on the decrease of intracellular levels of calcium by barring the influx and mobilization of intracellular stocks in smooth muscle cells.[7] Accordingly, we noted that guinea pig ileum contraction triggered by either allergen or histamine was inhibited by lidocaine in a comparable fashion (Table 3),[33] suggesting that the inhibition of anaphylactic spasms by lidocaine may result from a direct effect on the contractile function of smooth muscle cells. Remarkably, despite reduced anesthetic potency, analogues **28**–**30**, **39**, and **40** were clearly more potent than lidocaine in blocking the contraction of the isolated guinea pig ileum triggered by either allergen or histamine provocation (Table 3). Analogues **31**, **41**, and **42** also presented reduced anesthetic potency but were not further evaluated for antispasmodic effects, since behavior such as tremors, piloerection, and prostration was observed during the screening for anesthetic activity.

It should be emphasized that lidocaine prevents the anaphylactic release of pro-spasmodic substances, including histamine, stored in tissue mast cells in a mechanism closely related to the blockade of intracellular calcium influx.[31,32,35] In addition, recent studies of our group demonstrated that **40** was 28-fold more potent than lidocaine in inhibiting allergen-evoked cutaneous histamine release,[13] suggesting that the mast cell stabilizing properties of this compound are also contributing for its better efficacy against allergen-evoked ileum contraction. In line with this interpretation we noted that analogues **29** and **40** were more potent in inhibiting allergen- than his-

Table 2. Calculated *C*Log *P* values obtained for lidocaine and its derivatives

Compound	*C*Log *P*
1 (lidocaine)	1.95
28	2.55
29	2.60
30	2.60
31	3.20
32	3.25
33	2.25
34	2.85
35	2.90
36	2.90
37	3.50
38	3.55
39	2.10
40	2.14
41	3.59
42	3.59

Table 3. Antispasmodic effect of lidocaine and analogues on guinea pig ileum contraction induced by histamine or allergen

Compounds	Histamine (10 μM)	Allergen (10 μg/ml)
1 (lidocaine)	1.41 ± 0.12	1.43 ± 0.09
28	0.38 ± 0.06*	0.33 ± 0.00*
29	0.31 ± 0.06*	0.19 ± 0.04*
30	0.14 ± 0.02*	0.12 ± 0.01*
39	0.16 ± 0.01*	0.16 ± 0.01*
40	0.10 ± 0.01*	0.05 ± 0.01*

Results are expressed as mean of IC_{50} (mM) values ± SEM.
* $P < 0.05$ as compared to lidocaine IC_{50} values (Student's t test).

tamine-induced contraction, whereas analogues **28**, **30**, and **39** were shown to be equieffective against both stimuli (Table 3).

The separation of anesthetic and antispasmodic activities of these compounds attests to the diversity of possible interactions by substances of this type. It has been reported that the IC_{50} value for blockade of sodium currents after treatment with the analogue **40** (also named JMF2-1) (25.4 mM) was remarkably higher than that of lidocaine (0.18 mM), which is consistent with the weak anesthetic capacity of this analogue.[13] These findings are also consistent with the interpretation that nebulized JMF2-1 might be a way of achieving the anti-asthma effects of lidocaine without the anesthetic effects.

In summary, various lidocaine analogues have been found to exhibit reduced local anesthetic activities but superior potencies to inhibit allergen- and histamine-evoked intestinal contraction than lidocaine itself. Changes in the aromatic ring substitution of lidocaine led to enhanced potency and selective antispasmodic activity. Changes in the torsional angles between the aromatic ring and the plane of the aliphatic side chain and increased lipophilicity of these compounds are likely to account for the observed results. These findings reinforce the concept that the anesthetic and antispasmodic activities of lidocaine are dissociated. Selected analogues obtained in this study can be considered as new templates in drug discovery for smooth muscle disorders, with fewer expected side effects on cardiac and neuronal excitability.

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Supplementary data

Details of compound characterization have been included as supporting material. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.122.

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derivatives were generated using the Build Module, available in Sybyl 6.8.[25] The geometries of the compounds were energy-optimized using Tripos standard force field and conformational analysis was performed using systematic search, varying all torsional angles at 30°. The lowest-energy conformer of each compound was then reoptimized with the semi-empirical molecular orbital method AM1 using the MOPAC package.[26] Log *P* values were calculated with *C*Log *P* software.[27]

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