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Quantitative Structure–Activity Relationship Studies on Local Anesthetics

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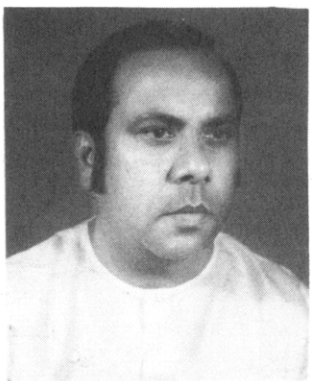
Contents



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I. Introduction

Local anesthetics are the drugs that are used to produce selective analgesia or anesthesia to some part of the body ranging from a small skin wheal to the entire abdomen and lower limbs. Unlike other drugs, local anesthetics are not used to cure any disease, but they are used to place the patient into a state of aloofness and nonresistance to a local surgical operation. They do this by preventing the generation and the conduction of the nerve impulse. Their main site of action is the cell membrane and there is seemingly little direct action of physiological importance on the axoplasm in the concentrations used to produce local anesthesia. They possess the specific ability to block conduction in excitable tissues in a reversible manner. It is suggested that they block the conduction by interfering with the transport of sodium and potassium[1–5] or by modifying the state of intracellular or membrane-bound calcium.[3,6–8]

Although it is widely accepted that the local anesthetics exert their pharmacological action by interacting with the cell membranes, the sites of their action in membranes are still not clearly resolved. Some authors suggest that local anesthetics interact with membrane phospholipids,[9,10] and some suggest that they interact with the proteins associated with the membrane.[11,12] It has also been proposed that local anesthetics act by causing perturbations of the bulk membrane structure.[13,14] However, some recent investigations have indicated that local anesthetics interact with specific receptors in the membrane.[15,16] They are known to affect the functions of a variety of membrane proteins such as $(Na^+–K^+)$-ATPase,[17,18] $(Ca^{2+}–Mg^{2+})$-ATPase,[19,20] adenylate cyclase,[21,22] guanylate cyclase,[23] calmodulin-sensitive enzymes,[24,25] phospholipase A_2,[26] and the channels responsible for increase in cellular permeability to Na^+, K^+, or Ca^{2+} ions.[27–32] Their effects on voltage-sensitive sodium channels have appeared to be fundamental to their local anesthetic activity.[33] It was suggested long ago that local anesthetics increase the surface pressure of the lipid layer that constitutes the nerve membrane and thus close the pores through which ions move.[34] This would cause a general decrease in the resting permeability and would also limit the increase in sodium permeability, the fundamental change necessary for the generation of the action potential. On the other hand, Metcalfe and Burgen[35] suggested that local anesthetics affect permeability by increasing the degree of disorder of the membrane.

Most of the theories discussed above however broadly speak of the mechanism of the blockade of the nerve conduction, and thus no satisfactory theory has been established to discuss the mechanism of drug action at

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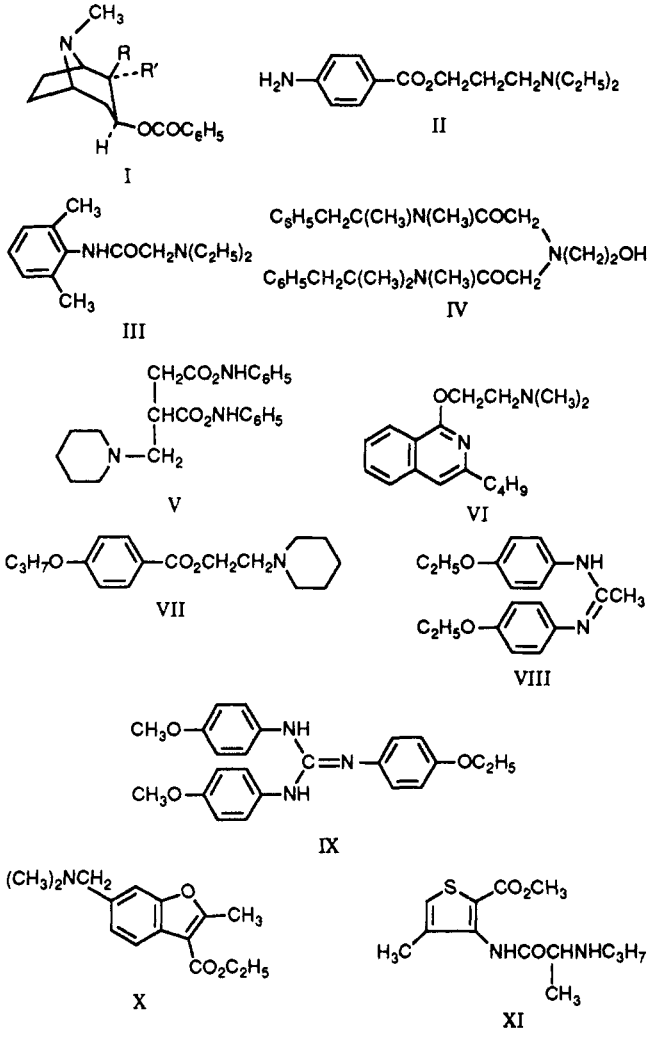
CHART I



the molecular and receptor level. To propose anything on this aspect of local anesthetics would require a thorough knowledge of their structure–activity relationship (SAR). We present here a spectrum of their quantitative structure–activity relationships (QSARs) which give more definitive relationships between the physicochemical and topographical properties of molecules and their biological or pharmacological actions. QSARs have proven their worth in the interpretation of the mechanisms of inhibition of a number of enzyme systems[36] and in elucidating the modes of actions of a variety of drugs acting at the central nervous system.[37,38]

II. A Prelude to Structure–Activity Relationships

Local anesthetics belong to a large number of different types of chemical compound, such as cocaine and related agents (I), procaine (II) and its analogues, aminoacyl anilides like lidocaine (III), non-anilide amides like oxethazaine (IV), aminocarbamates like diperodon (V), amino ethers like dimethisoquin (VI), amino ketones like falicaine (VII), amidines and guanidines like phenacaine (VIII) and acoin-C (IX), and some miscellaneous compounds like furocaine (X) and carticaine (XI) (Chart I). All these different types of chemical compound are found to possess hydrophobic and hydrophilic domains that are separated by an intermediate alkyl chain. The hydrophilic group is usually a tertiary or secondary amine and the hydrophobic domain is an aromatic residue. Linkage to the aromatic group is of either the ester type or amide type, and the nature of this bond determines several pharmacological properties of these agents. The ester link is important because this bond is readily hydrolyzed during metabolic degradation and inactivation in the body.

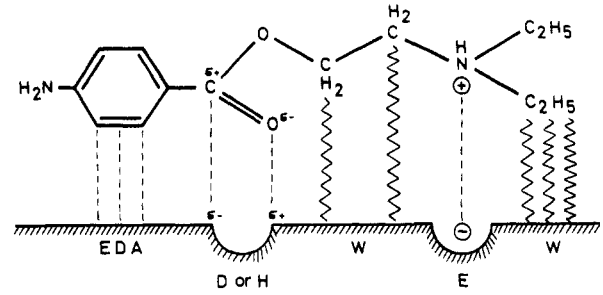
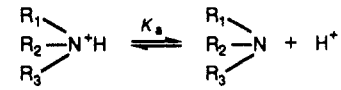


Figure 1. Büchi's hypothetical model of the binding of a local anesthetic with the receptor: EDA, electron donor–acceptor binding; D, dipole binding; H, hydrogen binding; W, van der Waals binding; E, electrostatic binding.

Since all the commonly used local anesthetics contain a tertiary or secondary nitrogen atom, they exist either as uncharged tertiary or secondary amines or as the positively charged substituted ammonium cation which can ionize as



Since the dissociation constant of this cation is very small, only a small fraction of the dose of any local anesthetic applied remains in the uncharged form. But that small fraction is important since the drug usually has to diffuse through connective tissues and other cellular membranes to reach its site of action and it is believed that it can do so only in the form of the uncharged amine. Once an anesthetic has reached its site of action, the active form of the molecule is supposed to be the cationic[39] which is aquired due to the low pH of the medium of the nerve fibers, obeying Henderson–Hesselbalch equation

$$\mathrm{pH} = \mathrm{p}K_a + \log ([\mathrm{B}]/[\mathrm{BH}^+]) \qquad (1)$$

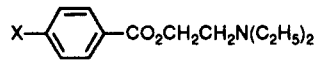
where B represents the uncharged form and BH^+ the charged form of the amine. It is therefore accepted that local anesthetics exert their principal action in the cationic form. According to Zipf,[40] procaine and other local anesthetics bind in a nonspecific way to suitable reactive sites of the membranous protein and lipid structures. A binding model[41] is presented as shown in Figure 1.

All these basic premises regarding local anesthetics have been discussed by many authors[42] but without any recourse to QSAR studies. An analysis of QSAR studies may open a new vista of thought regarding the modes of action of local anesthetics.

III. QSAR Results and Discussions

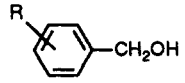
When Hansch had just started correlating biological activities of drug molecules with physicochemical properties, the local anesthetic activity of a series of paracaines (Table I) was found[43] to be well related to

TABLE I. Local Anesthetic Activity and Physicochemical Properties of Some Paracaines (2-(Diethylamino)ethyl 4-Substituted-benzoates)



entry	X	σ	π	log (1/C)
1	OC_2H_5	−0.25	0.54	1.92
2	$N(CH_3)_2$	−0.60	−0.08	1.72
3	OCH_3	−0.27	0.11	1.22
4	NH_2	−0.66	−1.52	1.13
5	Cl	0.23	0.80	1.05
6	OH	−0.36	−0.27	0.90
7	$NHCOCH_3$	−0.02	−0.98	0.28
8	NO_2	0.78	0.04	0.13

TABLE II. Local Anesthetic Activity and Physicochemical Properties of Benzyl Alcohols



entry	R	$\sum E_R$	log P	log (RBR)
1	3,5-I_2,2-OH	0.41	1.76	1.48
2	3,5-Br_2,2-OH	0.41	1.34	1.18
3	3,5-Cl_2,2-OH	0.33	0.98	0.88
4	5-Pr,2-OH	0.20	0.96	1.30
5	5-I,2-OH	0.29	0.61	1.00
6	5-Br,2-OH	0.29	0.40	0.70
7	5-Cl,2-OH	0.25	0.22	0.31
8	5-Et,2-OH	0.20	0.46	0.53
9	5-Me,2-OH	0.20	−0.04	0.40
10	2-OH	0.17	−0.54	0.00
11	6-Me,2-OH	0.20	−0.04	−0.05
12	4-Me,2-OH	0.20	−0.04	0.26
13	6-Br,3-OH	0.29	0.40	0.30
14	3-OH	0.17	−0.61	−0.70
15	3-Br,4-OH	0.29	0.22	−0.10
16	4-OH	0.17	−0.85	−1.52

the hydrophobic constant π and the Hammett constant σ of the substituents (eq 2). The latter is an electronic

$$\log (1/C) = 0.579\pi - 1.262\sigma + 0.961$$
$$n = 8,\ r = 0.933,\ s = 0.265 \qquad (2)$$

parameter which, if positive, denotes the electron-withdrawing character and, if negative, the electron-donating character of the substituent.[44] In eq 2, C refers to the molar concentration of the drug leading to a desired effect, n is the number of data points, r is the correlation coefficient, and s is the standard deviation. Now the correlation expressed by this equation expresses that more a hydrophobic and electron-donating group will lead to a more pronounced increase in the activity. These compounds were studied by Galinsky et al.[45] These authors related the activity with the infrared absorption frequency of the substituent which is of course related to σ. They also noted that the more electron-releasing group gave the more pronounced effect on the activity. Hansch and Fujita[43] found the activity to be correlated with σ alone as

$$\log (1/C) = 0.917 - 0.882\sigma$$
$$n = 8,\ r = 0.669,\ s = 0.498 \qquad (3)$$

and argued that considering the difficulty in quantitative testing for local anesthetic eq 3 was quite satisfying.

In a later study, Hansch and Kerley[46] found the local anesthetic action of a series of benzyl alcohols (Table II) to be significantly correlated with the hydrophobicity of the compound (characterized by octanol–water partition coefficient P of molecule) and a radical parameter E_R (eq 4). The E_R denotes the radical delocalizing

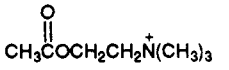
$$\log (\text{RBR}) = 1.329\ (\pm 0.45) \log P - 3.607\ (\pm 4.0)E_R + 0.086\ (\pm 0.92)$$
$$n = 16,\ r = 0.927,\ s = 0.308 \qquad (4)$$

ability of the substituent, but in eq 4 it is statistically not significant from the point of view of the 95% confidence intervals which are given for each variable within parentheses. Nonetheless, Hansch and Kerley[46] suggested that radical delocalization by the substituent would decrease the anesthetic action, probably due to the greater ease of oxidation of the drugs in the test system (frog skin).[47] However, we do not find any firm role of electronic character of substituents. Since dropping the E_R from the equation does not lead to any significant change in the correlation (eq 5), one can say

$$\log (\text{RBR}) = 0.984\ (\pm 0.26) \log P + 0.051\ (\pm 0.20)$$
$$n = 16,\ r = 0.905,\ s = 0.337 \qquad (5)$$

that the hydrophobicity of benzyl alcohols plays a major role in their anesthetic action. The RBR in equations refers to the relative biological activity of compounds as compared to that of 2-hydroxybenzyl alcohol.[47]

Many of the local anesthetics bear a structural resemblance to acetylcholine (XII) and thus they are supposed to combine with an acetylcholine receptor in the neuronal membrane.[48] Galinsky et al.[45] reasoned

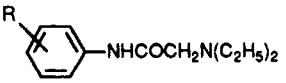


XII

that the carbonyl bond order of paracaines of Table I should reflect their ability to compete with acetylcholine for the receptor. Bond orders were not calculated per se but rather were inferred from carbonyl stretching (infrared absorption) frequencies.

Local anesthetics are also known to form charge-transfer complexes with thiamine.[49] It was recognized that thiamine participates in some manner in nerve conduction,[50] although its role could not be precisely defined. Yoneda and Nitta[51] approached the problem by calculating the charge-transfer parameters with the help of Hückel molecular orbital (HMO) method for a series of 2-(diethylamino)acetanilides (Table III). Although Yoneda and Nitta[51] narrated that there was a rough parallelism between the local anesthetic action

TABLE III. Charge Transfer Index and Local Anesthetic Activity of Some 2-(Diethylamino)acetanilides



entry	R	δE^a	activity[b]
1	2-Cl,6-Me	0.4350	0.8
2	2,6-$(Me)_2$	0.5397	1.05
3	2-Me	0.4000	1.4
4	H	0.3706	1.8
5	2-Cl	0.3689	2.0
6	3,4-$(Me)_2$	0.4117	2.1
7	2-Cl,4-Me	0.4254	2.3
8	2-Me,3-Cl	0.3531	2.7
9	2-Me,5-Cl	0.3341	3.25

[a] In unit of molecular orbital parameter.[51] [b] Concentration with 1 h anesthetic duration (%).[51]

TABLE IV. Local Anesthetic Action and Molecular Properties of Some Nonspecific Compounds[54]

no.	compd	α	I, eV	V_W, 10^2 Å^3[a]	log (MBC), mM	χ[b]
1	methyl alcohol	8.2	10.85	0.326	3.09	1.000
2	ethyl alcohol	12.9	10.48	0.480	2.75	1.414
3	acetone	16.2	9.69	0.587	2.60	1.732
4	isopropyl alcohol	17.6	10.16	0.634	2.55	1.732
5	propanol	17.5	10.20	0.634	2.40	1.914
6	urethane	23.2	9.00	0.767	2.00	2.769
7	ethyl ether	22.5	9.53	0.801	1.93	2.414
8	butanol	22.1	10.04	0.788	1.78	2.414
9	antipyrine	29.8	7.70	1.675	1.78	
10	pyridine	24.1	9.32	0.775	1.77	2.500
11	chloroform	21.4	11.42	0.663	1.50	
12	hydroquinone	29.4	8.10	0.934	1.40	3.288
13	aniline	31.6	7.70	0.897	1.30	2.893
14	benzyl alcohol	32.5	8.80	1.020	1.30	3.432
15	acetanilide	30.5	8.40	1.234	1.17	
16	pentanol	26.8	9.85	0.942	1.20	2.914
17	phenol	27.8	8.50	0.866	1.00	2.893
18	toluene	31.1	8.82	0.952	1.00	2.893
19	benzimidazole	40.2	8.24	1.024	0.80	3.466
20	hexanol	31.4	9.75	1.096	0.56	3.414
21	nitrobenzene	32.5	9.92	0.996	0.47	3.804
22	quinoline	42.1	8.50	1.175	0.30	3.966
23	8-hyroxyquinoline	44.7	8.10	1.243	0.30	4.376
24	heptanol	36.0	9.70	1.250	0.20	3.914
25	2-naphthol	45.4	8.10	1.286	0.00	4.359
26	methyl anthranilate	48.9	8.10	1.307	0.00	4.423
27	octanol	40.6	9.65	1.404	−0.16	4.414
28	thymol	47.3	8.70	1.432	−0.52	4.608
29	*O*-phenalthroline	57.8	8.00	1.552	−0.80	5.448
30	ephedrine	50.2	9.10	1.589	−0.80	5.253
31	procaine	67.0	8.10	2.192	−1.67	7.668
32	xylocaine	72.5	8.00	2.273	−1.96	7.578
33	diphenhydramine	79.5	8.50	2.458	−2.80	8.270
34	tetracaine	79.7	7.76	2.508	−2.90	8.629
35	phenyltoloxamine	79.9	8.80	2.758	−3.20	8.254
36	quinine	93.8	8.00	2.783	−3.60	9.705
37	eserine	82.4	8.50	2.315	−3.66	7.969
38	caramiphen	87.0	8.80	2.759	−4.00	10.224
39	dibucaine	103.6	8.25	3.193	−4.20	11.182

[a] From ref 55. [b] From ref 56.

of these compounds and the calculated stabilization energy (δE) of the complexes formed with thiamine, we found quantitatively the existence of a poor correlation (r = 0.72) between them. The calculated quantity of charge transfer (δQ) was observed to have no correlation at all with the activity.[51] It would be therefore unreasonable to assume that there would be an involvement of charge-transfer phenomenon in the action of local anesthetics. The calculation of δE and δQ was made on the basis of the most probable orientation of the acetanilide ring and the pyrimidine ring of the thiamine.

Instead of considering specific interaction at the atomic level, Agin et al.[52] assumed that all molecules acting as local anesthetics interacted with the receptor in a similar way and that the controlling factor in the interaction could be expressed in terms of molecular properties. They therefore derived an approximate expression (eq 6) relating the anesthetic action to molecular polarizability (α) and the ionization potential (I). In this expression, MBC refers to the minimum

$$\log (\text{MBC}) = \log C_s - K\alpha I \qquad (6)$$

concentration in an external solution necessary to completely block excitability and C_s to the minimum blocking concentration at the surface. The derivation of this expression was based on an equation derived by Casimer and Polder[53] for the interaction energy between a neutral molecule and a conducting wall. The parameter K occurring in eq 6 was primarily a function of interaction distances but as a first approximation it was considered to be a constant. The validity of the relation given by eq 6 was observed, when log (MBC) data studied by Agin et al.[52] for a fairly large series of miscellaneous anesthetics (Table IV) were plotted against αI and a straight line was obtained.[54] But in a quantitative analysis of these data, Agin et al.[52] had already shown, obtaining eqs 7 and 8, that the ionization potential had only a marginal effect in the action of anesthetics and that the major role was played by the polarizability. Since polarizability is related to molecular volume, Handa et al.[55] were also able to show the existence of a significant correlation between log (MBC) and the van der Waals volume V_w (eq 9). In eq 9, the

$$\log (\text{MBC}) = 0.082\ (\pm 0.005)\alpha - 3.67\ (\pm 0.23)$$
$$n = 39,\ r = 0.987,\ s = 0.34 \qquad (7)$$

$$\log (\text{MBC}) = 0.010\ (\pm 0.000)\alpha I - 3.99\ (\pm 0.17)$$
$$n = 39,\ r = 0.994,\ s = 0.24 \qquad (8)$$

$$\log (\text{MBC}) = 2.650\ (0.123)V_w - 3.765$$
$$n = 39,\ r = 0.962,\ s = 0.568 \qquad (9)$$

datum within parentheses is the standard error and not the 95% confidence interval for coefficient of variable. An equation similar to eq 9 was obtained by Kier et al.,[56] using molecular connectivity index (χ) derived from the numerical extent of branching or connectivity

in the molecular skeleton (eq 10). In fact this χ was also related significantly with α (eq 11).[56] In their correlation study, Kier et al. had not included entries 9, 11, and 15 of Table IV and assigned no reasons for doing so.

$$\log (MBC) = 3.55 - 0.762\chi$$
$$n = 36, r = 0.983, s = 0.390 \quad (10)$$
$$\alpha = 1.60 + 9.26\chi$$
$$n = 36, r = 0.990, s = 3.59 \quad (11)$$

A few of the compounds of Table IV, namely 2-naphthol, thymol, ephedrine, procaine, tetracaine, phenyltoloxamine, quinine, and dibucaine, were studied by Hersh[57] in order to find the nature and extent of interaction of local anesthetics with the surface of the cell membrane. He used monolayers of a synthetic dipalmitoyl lecithin in his experiment and observed that the minimum blocking concentration of each of these compounds lowered the surface tension of lecithin–water interface by approximately the same amount. A linear relationship was developed between the log of the rate of change of the surface pressure with concentration, log (Δp/MBC), and the product of the mole refraction and ionization potential, RI. This relationship suggested that the mode of interaction that causes the lowering of the surface tension involves the London (dispersion) interaction energy. This study by Hersh led to the support of the hypothesis that the site of action of local anesthetics is at the cell membrane and that the interaction between nonpolar groups is of primary importance. There are however also reports describing ionic interactions between tertiary amine local anesthetics and phospholipids.[58,59] A model incorporating both nonpolar and ionic interactions between local anesthetics and the site of action has been suggested by Blaustein and Goldman.[58]

Besides decreasing the surface pressure of the membrane, local anesthetics were also found to change various other physical properties of lipid or membranes, such as phase transitions of lipid membrane,[60] lipid polymorphism,[61] and order parameter of total brain lipid or synaptosomal membranes.[62-65]

It has been also reported[66] that local anesthetics inhibit mitochondrial electron transport at several points along the respiratory chain and that an inhibition effect is located at cytochrome c oxidase which catalyzes the electron transfer from cytochrome c to molecular oxygen. It has been observed that the level of oxidase inhibition varies with the effectiveness of anesthetic molecules.[67] For a small group of nonspecific local anesthetics (Table V), Casanovas et al.[68] showed the existence of a linear correlationship between the anesthetic activity of infiltration (AAI) and the affinity for the enzyme ($1/K_i$) (eq 12). On the other hand, the affinity

$$(1/K_i) = 0.026(AAI) - 0.015$$
$$n = 8, r = 0.99 \quad (12)$$
$$\log (1/K_i) = 1.03 \log P - 2.42$$
$$n = 7, r = 0.99 \quad (13)$$
$$\log (1/K_i) = 1.00 \log P - 2.35$$
$$n = 5, r = 0.999 \quad (14)$$

of the molecules for the enzyme was shown to be well correlated with their octanol–water partition coefficient (log P) (eq 13). In the derivation of eq 13, parethoxycaine was not included, as it behaved as an outlier. For only the last five compounds (Table V) which possessed similar ionization state, a still better correlation was found to exist between the affinity and log P (eq 14). Thus eqs 12–14 led Casanovas et al. to suggest that local anesthetics elicit their effect through the inhibition of cytochrome c oxidase and that their interaction with the enzyme involves hydrophobic interaction.[68]

TABLE V. Anesthetic Activity of Infiltration (AAI), Cytochrome c Oxidase Binding ($1/K_i$), and Physicochemical Properties of Some Nonspecific Local Anesthetics

no.	compd[a]	log P	pK_a	AAI	$1/K_i$, mM^{-1}
1	procaine	1.36	9.05	1	0.078
2	parethoxycaine	2.22	8.08	7	0.086
3	carticaine	1.70	7.45	6.3	0.167
4	bupivacaine	1.60	7.27	10.1	0.189
5	lidocaine	1.29	7.92	4.2	0.093
6	prilocaine	1.11	7.60	4.5	0.054
7	pramocaine	2.09	6.24	17.7	0.555
8	quinisocaine	2.50	6.30	55.8	1.410

[a] For structures of compounds see ref 68.

In a latter communication,[69] Casanovas et al. tried to study the effect of dissociation constant (pK_a) of these anesthetics on their infiltration activity and affinity for the enzyme, but since pK_a was almost collinear with log P and since it entered the correlation with a negative coefficient, nothing more, except that the neutral form of the molecule would be more important in the anesthetic action, could be added to the already drawn conclusion.

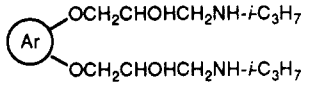
However, since most of the compounds studied were observed to act as competitive inhibitors competing with cytochrome c for the active site of the enzyme and since cytochrome c binding involves electrostatic interaction, it was also suggested by Casanovas et al.[68,69] that binding of local anesthetics with the enzyme would also involve the electrostatic interaction. Thus these authors speculated the involvement of both electrostatic and hydrophobic interactions in the binding of local anesthetics with cytochrome c oxidase and consequently assumed that local anesthetic action deals with cytochrome oxidase associated phospholipids. Some such conclusions were drawn by Boulanger et al. also when studies were made on binding of local anesthetics with lipidic membranes.[70] Boulanger et al. suggested that there are at least two binding sites for each of the charged and uncharged forms of drugs in membranes. However, the model suggested by Casanovas et al. for the interaction of local anesthetics with cytochrome c oxidase is based upon the ability of molecules to penetrate the lipid layer surrounding the oxidase protein and permits both hydrophobic and electrostatic types of interaction between enzyme associated phospholipids and anesthetics.

Local anesthetics have been shown to affect several parameters of mitochondrial function including valinomycin-induced potassium uptake and basic protein-induced swelling.[71] Johnson et al. observed a close correlation between these two effects for some nonspecific local anesthetics and suggested that the primary action of local anesthetics on mitochondria is related to an inhibition of membrane configurational or conformational changes. (The former refers to a rear-

rangement of membrane protein or lipoprotein units and the latter to changes in the tertiary or quaternary structure of an individual unit.) These authors also noted a close parallelism between the potency of local anesthetics on mitochondria and their effect on nerve conduction and proposed that the mechanism of action of local anesthetics on nerves may be directly related to a stabilization of membrane structure rather than an inhibition of sodium, potassium, or calcium movements, as is generally believed.[71] Since lipid solubility appears to be a major determinant of local anesthetic activity on nerves,[41,42b] Johnson et al.[71] also demonstrated qualitatively the existence of a good correlation between the action of local anesthetics (certain procaine analogues) on mitochondrial processes and their lipid solubility.

Remko and Scheiner[72] recently made a model calculation on the interaction of amine terminus of a local anesthetic molecule (both ionized and un-ionized) with the phosphate group (phospholipids), amide (peptide of lipoprotein), and a number of ions present in the cellular environment (viz., Na^+, K^+, Ca^{2+}, and Cl^-) and concluded: (1) that the protonated amine forms a very strong complex with the phosphate anion in which the charge is transferred from the amine to the phosphate, (2) that the protonated and, to a lesser degree, the unprotonated amine group forms H bonds with the peptide, which are stronger than normal interpeptide links and that the anesthetics are therefore capable of interferring with the normal H-bond patterns of lipoproteins, inducing conformational changes and thereby disturbing the conduction system of the nerve cell, and (3) that the Na^+, K^+, Ca^{2+}, and Cl^- ions form quite strong complexes with the anesthetic model, competing with the binding of drugs to their sites of action. These conclusions are however based on model calculations and do not include such important factors such as the lipid solubility and pH. The tertiary amine terminus of local anesthetics was modeled by ionized and unionized trimethylamine, and phosphate and amide were represented by phosphate monoanion and formamide, respectively.

Besides the lipophilic character, the stability of the cationic form was also shown to be important in the anesthetic activity of a series of bis[2-hydroxy-3-(isopropylamino)propyl] ethers of dihydroxyarenes (XIII).[73]



XIII

Zaagsma and Nauta[73] found the local anesthetic activity of these compounds to be correlated with log P and $pK_{a(m)}$, the mean of pK_a of monoprotonated and diprotonized species, as

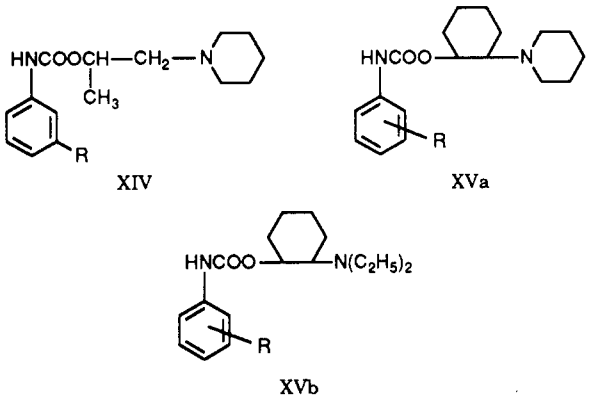
$$-\log EC_{50} = 3.719\ (\pm 2.289)pK_{a(m)} - 1.186\ (\pm 0.424)(\log P)^2 - 31.411$$
$$n = 10,\ r = 0.932,\ s = 0.207 \qquad (15)$$

Since these ethers may be present in monoprotonized as well as diprotonized species, the observed pK_a is supposed to be the mean of the pK_a of two species. At pH 7.40 at which pK_a was measured, the diprotonized species of a compound was assumed to be 97.48%, monoprotonized species 2.51%, and uncharged form only 0.016%.[73] The positive coefficient of $pK_{a(m)}$ in eq 15 suggests that the less dissociated species will give more activity. Thus one can say that a compound of this series acting as a local anesthetic would be most effective in its diprotonized form ($pK_a > 9$).[73] The parabolic character of the equation in log P however suggests that the optimum activity of the compound will be attained when it is equally distributed in the lipid and the aqueous phases. Notwithstanding these conclusions, eq 15 must be interpreted with great caution, as it is based upon an insufficient number of data points.

Pešák et al.[74] correlated the relative surface local anesthetic activity (relative to cocaine) of a series of 3-substituted carbanilates (XIV) with log P and Hammett constant σ as

$$\log A_s = 1.086 \log P - 0.909\sigma - 2.436$$
$$n = 8,\ r = 0.972,\ s = 0.292,\ F_{2,5} = 42.1 \qquad (16)$$

But for another series of carbanilates (XVa and XVb)



XIV XVa XVb

where substituents were at 2,6-positions or only at 2-position, a spectral parameter f was found necessary to achieve a statistically reasonable correlation (eq 17).

$$\log A_s = 0.414 \log P - 0.446\sum\sigma - 3.246f + 0.587$$
$$n = 14,\ r = 0.854,\ s = 0.24,\ F_{3,10} = 8.95 \qquad (17)$$

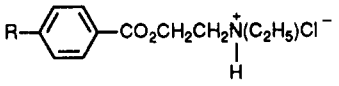
The parameter f is related to π–π^* transition band in UV absorption spectra of the compound. Although eq 17 cannot be taken to be very reliable, since it uses relatively insufficient data for three variables and has even then comparatively a low value of r, it corroborates eq 16 in suggesting that while the lipophilic character of molecules plays a dominant role in local anesthetic actions, the electronic characters of substituents also produce considerable effects on the activity, and this is exactly what eq 2 and Galinsky et al.'s observations[45] had suggested for paracaines.

For another series of paracaines (Table VI), the anesthetic activity was found[75] to have some correlation with parachor (Pcr) (eq 18) but no correlation with hydrophobic parameter at all (eq 19). Equations 18 and 19 express that all the derivatives must be reaching

$$\log A = 0.287 - 0.027\ (\pm 0.031)\mathrm{Pcr} - 0.00024\ (\pm 0.00022)\mathrm{Pcr}^2$$
$$n = 10,\ r = 0.79,\ s = 0.37,\ F_{2,7} = 5.71 \qquad (18)$$

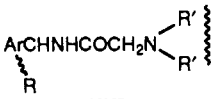
$$\log A = -0.158 - 0.018\ (\pm 0.755)\pi - 0.034\ (\pm 1.032)\pi^2$$
$$n = 10,\ r = 0.03,\ s = 0.60,\ F_{2,7} = 0.003 \qquad (19)$$

TABLE VI. Relative Local Anesthetic Activity (*A*) and Physicochemical Parameters of Paracaines



entry	R	Pcr	π	*A*
1	NH_2	45.5	−1.23	1.0
2	NHC_2H_5	128.5	0.08	10.0
3	OH	29.8	−0.67	0.5
4	OC_2H_5	115.3	0.38	1.4
5	CH_3	55.3	0.56	0.8
6	H	15.5	0.00	0.85
7	F	26.1	0.14	0.25
8	Cl	55.2	0.71	0.33
9	Br	68.0	0.86	0.5
10	NO_2	75.7	−0.28	0.1

the site of action equally well and approximately in equal concentration irrespective of their lipophilic character, and that the difference in their activity may be due to the difference in their interactions with the receptor site. Since parachor is given by the product of molar volume and the fourth root of the surface tension, eq 18, although not very significant, indicates the involvement of some kind of dispersion interaction between anesthetics and the receptor. The dispersion or the polar interaction was found[76] to produce a more dominant effect than the hydrophobic interaction in the local anesthetic action of a series of *N*-[(*N*′,*N*′-disubstituted-amino)acetyl]arylamines (XVI) (compare eq 20 with eq 21). In eq 20, the molar refraction, MR, is



XVI

representative of dispersion or polar interaction and also of a global steric effect since the correlation expressed is parabolic. The anesthetic action of these compounds

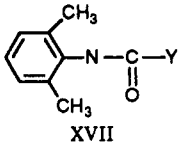
$$\log (1/C) = 0.497\ (\pm 0.176)\mathrm{MR} - 0.0028\ (\pm 0.0015)\mathrm{MR}^2 - 20.018\ (\pm 7.192)$$
$$n = 12,\ r = 0.951,\ s = 0.161,\ F_{2,9} = 42.26 \quad (20)$$

$$\log (1/C) = 0.625\ (\pm 0.899)\log P - 0.057\ (\pm 0.162)\ (\log P)^2 + 0.063\ (\pm 1.139)$$
$$n = 12,\ r = 0.797,\ s = 0.314,\ F_{2,9} = 7.83 \quad (21)$$

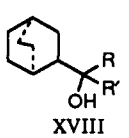
was also shown[77,78] to have a significant correlation with Kier's zero-order valence molecular connectivity index ($^0\chi^v$) (eq 22). However, since MR in general has been

$$\log (1/C) = 2.99\,{}^0\chi^v - 0.117({}^0\chi^v)^2 - 17.5$$
$$n = 12,\ r = 0.964,\ s = 0.14,\ F_{2,9} = 58 \quad (22)$$

found to be related to connectivity indices,[79] eq 22 conveys the same meaning as eq 20. In any case, these examples do not undermine the role of lipophilicity in local anesthetic action. In addition to many examples discussed earlier, it was shown for a series of lidocaines (XVII)[80] and for certain mono- and diaryl-2-quinuclidinylcarbinols (XVIII)[81] that there existed good



XVII



XVIII

linear correlations between their local anesthetic activities and log *P* (eqs 23 and 24, respectively). In eq

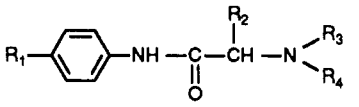
$$\log \mathrm{AAS} = 1.382\ (\pm 0.181)\log P - 5.80\ (\pm 0.77)$$
$$n = 15,\ r = 0.88,\ s = 0.247,\ F_{1,13} = 54.97 \quad (23)$$

$$\mathrm{LA} = 0.260\ (\pm 0.088)\log P - 0.132$$
$$n = 18,\ r = 0.84,\ s = 0.23,\ F_{1,16} = 38.75 \quad (24)$$

23, AAS refers to anesthetic activity on the surface, and in eq 24, LA stands for anesthetic activity relative to that of propranolol,[82] a prominent local anesthetic. For lidocaines, it was however observed that their anesthetic activity of infiltration (AAI) was not so well correlated with log *P* (eq 25),[80] and for another series of lidocaines

$$\log \mathrm{AAI} = 0.550\ (\pm 0.144)\log P - 2.63\ (\pm 0.70)$$
$$n = 15,\ r = 0.65,\ s = 0.383,\ F_{1,13} = 14.67 \quad (25)$$

(XIX), it was shown[83] that their local anesthetic potency



XIX

was greatly a function of electronic parameter (eq 26).

$$\log (1/C) = 2.082 - 1.322\ (\pm 0.280)\sigma_{246}$$
$$n = 11,\ r = 0.84,\ s = 0.46,\ F_{1,10} = 22 \quad (26)$$

The correlation expressed by eq 26 was however further improved by the inclusion of parameters representing the solubility (*S*) and the lipophilicity (π) of the compounds (eq 27). But eq 27 can be misleading, since

$$\log (1/C) = 1.942 - 1.111\ (\pm 0.069)\sigma_{246} - 0.001\ (\pm 0.0001)S + 0.299\ (\pm 0.043)\pi_{246}$$
$$n = 11,\ r = 0.999,\ s = 0.11,\ F_{3,7} = 185 \quad (27)$$

there are now three parameters just for 11 data points. Moreover, the solubility parameter has too low of a coefficient to be counted. However, the effect of π can not be ignored, but one thing to be pointed out is that although π and σ both were used for substituents at positions 2, 4, and 6, there was hardly any compound in the entire series that had a group other than Cl at 2- and/or 6-position. It was therefore only the substituent at 4-position whose physical and electronic properties were responsible for the local anesthetic activity of this series of lidocaines.

Since the effects of local anesthetics on voltage-sensitive sodium channels have appeared to be fundamental to their local anesthetic activity,[33] a series of nonspecific local anesthetics (Table VII) were studied for their ability to inhibit opening of sodium channels by agents like batrachotoxin (BTX).[84] BTX-like agents enhance the function of sodium channels by binding to sites of the latter. They also induce phosphoinositide breakdown in brain synaptoneurosomes.[85,86] Therefore local anesthetics of Table VII were also studied for their effects on binding of [^{3}H]BTX-A 20α-benzoate to sodium channels in guinea pig cortical synaptoneurosomes and on BTX-elicited phosphoinositide breakdown.[84] All such effects of compounds were found to be mutually correlated[84] and each one of them significantly corre-

TABLE VII. Local Anesthetics and Their Inhibitory Effects on BTX-Elicited Sodium Flux, BTX-Elicited Phosphoinositide Breakdown, and Binding of [^{3}H]BTX-A 20α-Benzoate to Sodium Channels along with their V_w and log P Values

		−log IC_{50}				
no.	compd	Na flux	phosphoinositide breakdown	BTX binding	V_w, 10^2 Å^3	log P
1	dibucaine	6.00	5.68	5.85	3.233	1.030
2	tetracaine	5.57	5.35	5.47	2.532	0.690
3	euprocin	5.54	5.48	6.13	3.676	0.755
4	bupivacaine	4.89	4.85	5.27	2.809	3.550
5	dimethisoquin	6.15[a]	5.48	5.47	2.693	2.315
6	quinacrine	5.17	4.36	5.48	3.476	6.840
7	phenacaine	5.16	4.64	5.77	2.802	3.130
8	QX-572	4.04	4.08	5.41[c]	2.861	−2.730
9	QX-314	3.92	4.07	4.01	2.631	−1.875
10	diphenhydramine	4.35	4.00	5.22	2.506	4.200
11	lidocaine	3.85	3.96	3.62	2.297	0.295
12	diphenhydramine methiodide	3.48[a]	3.14[b]	4.19	2.636	−0.580
13	piperocaine	4.50	4.02	4.89	2.475	2.940
14	prilocaine	4.07	3.37	4.27	2.085	−0.640
15	cocaine	4.32	2.85[b]	4.31	2.640	1.240
16	etidocaine	5.09	4.92	5.46	2.703	2.065

[a] Not used in deriving eq 28. [b] Not used in deriving eq 29. [c] Not used in deriving eq 30.

lated with van der Waals volume (V_w) and the lipophilicity of the compounds (eqs 28–30).[87] Although inhibition of sodium flux:

$$-\log IC_{50} = 1.146\ (\pm 0.690)V_w + 0.197\ (\pm 0.169)\log P - 0.041\ (\pm 0.036)(\log P)^2 + 1.610$$
$$n = 14,\ r = 0.82,\ s = 0.45,\ F_{3,10} = 6.20 \quad (28)$$

inhibition of phosphoinositide breakdown:

$$-\log IC_{50} = 1.259\ (\pm 0.674)V_w + 0.182\ (\pm 0.163)\log P - 0.056\ (\pm 0.035)(\log P)^2 + 1.283$$
$$n = 14,\ r = 0.84,\ s = 0.44,\ F_{3,10} = 7.35 \quad (29)$$

inhibition of BTX binding:

$$-\log IC_{50} = 1.172\ (\pm 0.685)V_w + 0.334\ (\pm 0.228)\log P - 0.051\ (\pm 0.042)(\log P)^2 + 1.617$$
$$n = 15,\ r = 0.85,\ s = 0.45,\ F_{3,11} = 7.97 \quad (30)$$

correlations expressed by eqs 28–30 are based on relatively insufficient data, they indicate that these local anesthetics might involve dispersion interaction because of V_w playing a role in activities. Further, since correlations are parabolic in log P, it was suggested that hydrophobic character would help the molecule to cross the lipid barrier and reach the binding sites.[87] No mutual correlation was found to exist between V_w and log P. In deriving eqs 28–30 certain compounds as indicated in Table VII were not included, as they were found to misfit in correlations. It was found difficult to assign any reason for these outliers.

IV. Overview

It appears quite difficult to point out any unified theory, from the analysis of QSAR, regarding the mechanism of action of local anesthetics on molecular level. What QSARs firmly do establish is that the local anesthetics involve both hydrophobic as well as polar interactions in binding with the sites of action. While any correlation between the activity and log P would be suggestive of lipophilicity providing the facility to the molecule to cross the lipid phase and reach the binding site, the correlations expressed by eqs 2, 5, 16, 17, 23, 24, and 27 indicate that there may be a direct hydrophobic interaction between the substituent of local anesthetic molecules and the binding sites, as the change in log P values and consequently in activity is due to change in the nature of the substituent. Although many of these correlations as those expressed by eqs 2, 16, 17, and 27 are based upon relatively insufficient data and therefore should be interpreted with great caution, the involvement of hydrophobic interaction cannot be ignored, as local anesthetic action is supposed to be a membrane phenomenon and lipid is a major constituent of the membrane.

The polar interaction of local anesthetics with binding sites is supported by eqs 3, 7, 9, 16–18, 20, 26, and 27. Equations 3, 16, 17, 26, and 27 express that an electron-donating group present at an aromatic ring position favors the activity of local anesthetics. This electron donation by the substituent of the aromatic ring appears to affect the bond order of carbonyl bond which presumably participates in the polar interaction with the binding sites. This idea gets support from the study of Galinsky et al.,[45] where the local anesthetic action of paracaines was shown to be correlated with the carbonyl stretching (infrared absorption) frequencies. It was indicated long ago by Löfgren[88] that all effective local anesthetics are characterized by a highly reactive carbonyl group in which the electron cloud at the oxygen atom is sufficiently dense to attach the molecule to the binding sites. This hypothesis was further extended by Büchi and Perlia[89] and got the support from the work of many other authors.[74,83,90,91] It is assumed that in case of aromatic esters, the conjugation of the aromatic ring creates a resonance effect between the carbonyl group and the ring, resulting in the shift of electrons from the ring to the carbonyl oxygen.[92]

Equations 7, 9, 18, and 20 suggest the involvement of purely dispersion interaction between the local anesthetic molecules and the binding sites. This interaction depends upon the size of the molecule or portion of the molecule approaching the binding sites. Here the carbonyl group participates in the interaction.

With all said and done about the nature of binding, the question arises about the nature and location of sites of action. QSARs do not provide much insight into this aspect. However, one can examine various existing

theories of mechanism of local anesthetic action in the light of QSARs and see if the latter can help sort out any unifying theory for the sites of action.

The first theory is based upon the hypothesis that local anesthetics induce changes in the membrane organization. This hypothesis suggests that the primary event takes place by hydrophobic binding between the agent and certain membrane constituents, either lipids or proteins or both. Seeman considered the possibility that lipids and/or proteins may be actively involved in the events.[13] Since QSARs have shown the involvement of both hydrophobic and polar interactions in local anesthetic action, the participation of both lipid and protein constituents of the membrane cannot be ruled out.

The second and current hypothesis suggests the existence of specific receptors at or in sodium channels.[15,93,94] Local anesthetics are supposed to block propagated action potentials by their actions at sodium channels. An early hypothesis regarding the receptor was that since many local anesthetics bear a structural resemblance to acetylcholine (XII), they combine with acetylcholine receptor in neuronal membrane,[48] and as already discussed, Galinsky et al.[45] reasoned that the carbonyl bond order of paracaines (Table I) should reflect their ability to compete with acetylcholine for the receptor. However, no more QSAR studies are available to support this hypothesis.

The modulated receptor hypothesis is that local anesthetics bind to a state or states of Na^+ channel.[94] The channel state and membrane potential determine the interactions of molecules with the channel. The studies have suggested that local anesthetics bind preferentially to the inactivated state of the Na^+ channel and stabilize this state and that according to their structure they can access this site via hydrophilic or hydrophobic pathways. It has been discussed[95,96] that there exist two major pathways for drug access to binding sites: a hydrophilic pathway through the cytoplasmic side of the open channel for quaternary ammonium derivatives, protonated molecules, and other sufficiently hydrophilic species, and a hydrophobic pathway, accessing the channel even in its closed state, for hydrophobic drug species. It has been suggested that amine-type local anesthetics, quaternary blocking agents as well as neutral species act at the same site of Na^+ channel.[96] This unifying theory explains the differences observed between these three structural classes of agents in terms of access to the binding site by means of differing pathways. An interesting aspect of this hypothesis is that the hydrophobic interaction between receptor and agent is more important than the polar interaction.[96] All these discussions about the sites of action are well supported by QSAR studies which have shown that there would be hydrophobic and/or polar interactions between the local anesthetic and the binding sites. Equation 30, which has not been discussed so far, has special significance in this respect. It correlates the inhibition by some local anesthetics of BTX (batrachotoxin) binding to Na^+ channels with molecular size and the hydrophobicity of the molecule. Since inhibition of BTX binding was related with local anesthetic action of the compounds,[84] it was suggested that local anesthetics should bind to BTX binding sites in Na^+ channel and that this binding should involve the dispersion interaction. Since eq 30 is parabolic in log P, it was discussed that hydrophobic character of the molecule would help the molecule to cross the lipid barrier and reach the binding sites.[87]

Attempts have been made to show that local anesthetics exhibit stereoselectivity,[97-101] but this stereoselectivity has not been established to be totally unambiguous. The differences in structural requirements exhibited by local anesthetics for the production of resting and frequency-dependent block of Na^+ channels may be due to differences in the conformations of channel states or in access pathways to binding sites or due to some combination of both. Local anesthetic potency varies considerably between tissues and according to experimental conditions.[97,102]

Regarding the active form of the local anesthetic molecule, it is assumed that the most active form is the cationic form,[39,42] but why then are neutral drugs such as benzocaine ($H_2NC_6H_4CO_2C_2H_5$) a powerful local anesthetic and anionic drugs such as phenobarbital (5-ethyl-5-phenylbarbituric acid) an atypical anesthetic? QSARs do provide some answer to this paradox. Since QSARs have shown that there can be both hydrophobic as well as polar interactions between the anesthetic molecules and the binding sites, the neutral molecules may elicit their effect through purely hydrophobic binding while charged species elicit their effect through purely polar binding. There are pharmacological evidences for the existence of different sites of action for neutral and charged species.[42c] It has been inferred however that neutral agents, i.e., those that do not carry a positive charge to a significant degree at physiological pH, probably act in a more nonspecific way outside the Na^+ channels and thus exert their blocking effect through a disordering of the general normal membrane structure, for instance, through expansion or phase transitions.[103] This nonspecific interaction outside the Na^+ channel can also lead to the increase in the surface pressure of lipid layer of membrane,[34] consequently the pores through which ions move may be closed.

As already discussed in the Introduction, local anesthetics are supposed to affect the functions of a variety of enzymes. However, QSAR discusses a lone example of cytochrome c oxidase inhibition for which also eq 12, which correlates the anesthetic activity of infiltration to the affinity for the enzyme of only eight compounds, hardly provides any sound basis to assume that local anesthetic action is based upon the inhibition of this enzyme. It is therefore hard to believe that local anesthetic action would be based upon the inhibition of any enzyme, although local anesthetics do inhibit many enzymes at high doses.

Now all the above discussions, presented in the light of QSAR studies and existing hypotheses about the local anesthetic action, do not yet point out any unequivocal theory about the mechanism of action of local anesthetics. QSAR studies however give much credence to the modulated receptor hypothesis, according to which the local anesthetics bind to a state or states—preferably an inactivated state—of Na^+ channel, which is accessible to them, depending upon their nature, via hydrophobic or hydrophilic pathways. Na^+ channels possess both the pathways. Thus both hydrophobic as well as polar natures of molecules, which appear in QSAR study to govern the local anesthetic activity,

would help the molecule reach the binding sites in the channel. It would also not be unreasonable to assume that there can be two binding sites in the channel, one for the hydrophobic or neutral molecule to have hydrophobic binding and one for the charged species to have polar or ionic interaction. It would also be logical, on the other hand, to speculate only one binding site but with two different subsites, one hydrophobic and one polar, so that any molecule whether hydrophobic or polar may fully interact with it. A molecule having both hydrophobic and hydrophilic moieties may have stronger interaction with it.

All the above speculations however still need verification and support. Further QSAR studies may provide better understanding and computer-assisted molecular modeling of local anesthetic–receptor interaction may lead to a more vivid picture of the mechanism of action of local anesthetics. So far no study on molecular modeling of local anesthetic action has been reported.

V. Acknowledgments

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