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# RELATIONSHIPS BETWEEN LIPOPHILIC CHARACTER AND BIOLOGICAL ACTIVITY OF NEW POTENTIAL LONG-ACTING LOCAL ANESTHETICS

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**ABSTRACT**

The partition coefficients (P-values) of certain new potential, stereoselective, reversible, long-acting local anesthetics have been determined in an n-octanol/ phosphate buffer system. These are derivatives of 2-phenoxyethyldialkylamine hydrochloride, and almost all of them are readily soluble in water. Their aqueous solutions have shown different absorption maxima, and these have been used quantitatively to determine the concentration of solute in aqueous phase using the Beer-Lambert equation and the calibration curve, which has been a straight line within the test concentrations. The values of P have been calculated as the ratio of the concentration of solute in the octanol phase divided by the concentration in the aqueous phase; the former value has been obtained from mass balance. The mass balance is confirmed by obtaining the absorption measurements of the organic phase. The guinea pig intradermal wheal test has been used to determine the anesthetic properties of the test agents. They have exhibited better anesthetic profiles than those of the bupivacaine standard. Moreover, they have displayed no apparent side-effects neither locally nor systemically. Although there has been no sharp general correlation between the P-values and duration of action (WT), it is observed, in many cases, that derivatives with low P-values have shorter WT than those with high P-values. Therefore, it seems that the duration of action of these compounds is not influenced only by this property, i.e. P-values, and other factors such as physicochemical, pharmacokinetic, and pharmacodynamic parameters as well as non-specific binding characteristics at adjacent tissues are also involved. Thus, the action of these new compounds is due to the molecule as a whole and not to any one particular physical or chemical property. Furthermore, these are reversible and stereoselective agents.

**Keywords:** Partition Coefficient, determination; Local Anesthesia, duration of action; Local Anesthetics, new derivatives.

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## INTRODUCTION

The rate of diffusion of a drug through capillary endothelial cells has been found to increase with increasing P-values.[1-3] Affinity for the nerve membrane has been related to duration of action.[4-8] Büchi et al.[5-6] have pointed out that binding to non-specific sites generally decreases the concentration of the free active molecules which are able to diffuse and permeate to the site of action, i.e. sodium channel receptors. This phenomenon will eventually affect the duration of action. In certain long-acting local anesthetics, it has been noticed that an increase in hydrophobicity, i.e. P-value, is associated with increased WT.

Moreover, it has been generally accepted that penetration of local anesthetics from the site of application to the site of action, i.e. sodium channel proteins in the lipoidal nerve cell membrane, proceeds through a passive diffusion process and can be regarded as transmission from an aqueous phase into a lipid phase. This implies adequate solubility in the aqueous extracellular fluids on the one hand, and also in the predominantly lipid membrane formed from fatty acid esters, phospholipids, glycoproteins and glycolipids on the other.[9] In addition, it has been reported that P-value progressively increases from the lower to higher homologues in certain series of compounds, and parallels local anesthetic potency until it reaches a maximum. Potency then decreases in the higher homologues. The attainment of maximum potency with a particular member of a homologous series can be explained *inter alia* in terms of the water solubility of the higher homologues, being too low for a sufficient concentration of the drug to reach the biophase. Therefore, a cut-off effect appears, and this can then affect other properties. However, the higher the P-value before the cut-off effect, the smaller the dose or external concentration required to exert a biological effect. This implies a greater activity, which is usually coupled with a longer duration and greater toxicity. Thus, there appears to be, in some series of compounds, a direct relationship between liposolubility and either intensity or duration of action. This conclusion, however, cannot be generalized and is not applicable to other series, since other physicochemical characteristics are involved and cannot be overlooked.[5,6] Furthermore, the partition coefficient has been used frequently in quantitative structure-activity studies[8] and its usefulness in the assessment of transport properties of drugs through biological membranes, extraction of solutes in aqueous-organic liquid systems, measurement of equilibria, and design of controlled-release drug delivery systems is well established.[10-13]

Accordingly, to find whether a relationship exists between the P-values and the duration of local anesthesia of certain new derivatives of 2-phenoxyethyldialkylamine hydrochloride (Fig. 1), and also if these compounds are stereoselective or not, this investigation has been initiated, the results of which are reported. Such a relationship or dependence is of great importance in quantitative correlations of lipophilicity, i.e. P-value, with drug transport properties, protein binding and biological activity.[8,14]

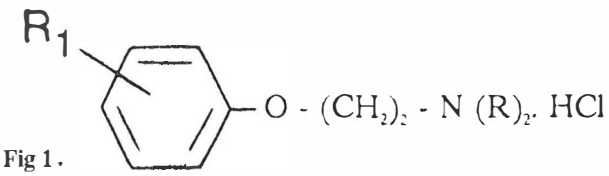

Fig 1.

## MATERIALS AND METHODS

### 1. Determination of apparent partition coefficient

n-Octanol was purified by washing with dilute sulphuric acid, sodium hydroxide (5 M), and deionized water, followed by distillation. For the P-value determination, the drugs were dissolved in octanol-saturated phosphate buffer (pH 7.4) of defined ionic strength (0.09 M); any minor deviation in pH was corrected at this stage. The solutions were shaken efficiently for a minimum period of half an hour to ensure complete dissolution of the test agents. Thereafter, the solutions were also shaken vigorously with buffer-saturated octanol in a shaking water bath at 20±1°C for at least 12 h. After the attainment of the state of equilibrium and complete separation of the two layers, which required a further 24 h, samples from the aqueous phase were diluted suitably, and analyzed spectrophotometrically at the wavelength of maximum absorption (λ max. nm) of each compound, using phosphate saturated buffer as a reference. The values of P were then calculated as the ratio of the concentration in octanol phase divided by the concentration in the aqueous phase, the former value obtained from mass balance and using the Beer-Lambert equation as well as the standard calibration curve. Absorption measurements of the organic phase were taken to check the mass balance.[14-17]

### 2. Determination of duration of local anesthesia

The guinea pig intradermal wheal test was used.[16,18] Guinea pigs of the Dunkin-Hartley strain (250-350 g) were employed. A drug concentration of 0.5% W/V was injected into one of 4 or 6 randomly selected areas on the back of the animal. Bupivacaine hydrochloride solution, 0.5% W/V, was used as the standard and normal saline solution, 0.9% W/V, as the control. A minimum of 2 animals were used for each compound, while the standard solution was injected into 4 animals.

**Table I. Absorption readings of the test agents (Fig. 1), their partition coefficients, and duration of full local anesthesia using bupivacaine as a standard.**

| Compound no. | $R_1$ | R | λ max. | $\varepsilon_{max}$[1] | P[2] | $WT_t$[3] | $WT_s$[4] |
|---|---|---|---|---|---|---|---|
| 1 | 2,4,6-$(Me)_3$ | Me | 272 | 409 | 1.50 | 240 | 120 |
| 2 | 2,6-$(i\text{-}Pr)_2$ | Me | 262 | 311 | 4.78 | 360 | 90 |
| 3 | 2,6-$(Me)_2$ | Et | 262 | 250 | 5.65 | 180 | 120 |
| 4 | 3,5-$(Me)_2$ | Me | 270 | 901 | 6.17 | 240 | 120 |
| 5 | 3-(OMe) | Et | 272 | 2207 | 6.81 | 120 | 120 |
| 6 | 3-(OEt) | Me | 271 | 1874 | 7.27 | 180 | 120 |
| 7 | 4-(t-Bu) | Et | 271 | 1308 | 8.22 | 120 | 120 |
| 8 | 3,5-$(Me)_2$ | Et | 278 | 1031 | 9.13 | 120 | 120 |
| 9 | 2,6-$(t\text{-}Bu)_2$ | Me | 276 | 404 | 10.01 | 1440 | 120 |
| 10 | 4-(s-Bu) | Me | 273 | 1191 | 11.16 | 1440 | 150 |
| 11 | 2,4,6-$(Cl)_3$ | Me | 278 | 520 | 12.01 | 180 | 120 |
| 12 | 2-(t-Bu) | Me | 267 | 1249 | 12.22 | 1440 | 150 |
| 13 | 4-(t-Bu) | Me | 271 | 1304 | 16.78 | 1440 | 150 |
| 14 | 4-(t-Am) | Me | 272 | 1180 | 21.78 | 360 | 120 |
| 15 | 4-(s-Bu) | Et | 275 | 1440 | 25.32 | 360 | 120 |
| 16 | 4-(n-OBu) | Et | 284 | 2224 | 28.55 | 360 | 120 |
| 17 | 4-(n-Bu) | Me | 284 | 2077 | 29.06 | 1440 | 150 |
| 18 | 2,6-$(s\text{-}Bu)_2$ | Me | 263 | 309 | 31.15 | 1440 | 120 |
| 19 | 2-(t-Bu) | Et | 268 | 1358 | 35.29 | 360 | 90 |
| 20 | 2,6-$(t\text{-}Bu)_2$-4-Me | Me | 282 | 322 | 131.82 | 1440 | 150 |

[1]Molecular absorbance coefficient; [2]Mean of partition coefficient; [3]Duration of full, reversible local anesthesia (min) of the test compound; [4]Duration of action of the bupivacaine standard agent (min).

Each site of injection was examined for local anesthesia by gently pricking it 6 times with a needle after the elapse of 5 min, 10 min, 30 min, 1 h, and thereafter at hourly intervals until 6 h after the injection had been given. Compounds which exhibited a long duration of anesthesia, i.e. over 6 h were further assayed on two additional animals. The assessment of anesthesia was not made until 6 h had elapsed so as to reduce the effect of too much pricking on the back of the animals. Failure to react to pain either by skin twitching and/or squeaking was regarded as a positive indication of local anesthesia. Negative responses were indicated by sensitivity to pain evidenced by squeaking and/or skin twitching, while a normal response was taken as the reaction of the animal to the same prick with needle on an uninjected site. A score of 6/6 showed total local anesthesia and a score 0/6 no local anesthesia. All values in between showed partial anesthesia, which was considered as no anesthesia.

## RESULTS

The results are shown in Table I. All compounds were used as hydrochloride salts and dissolved first in phosphate-saturated buffer, save compounds 9, 18 and 20, which were initially dissolved in buffer-saturated octanol. Absorption measurements were taken for the aqueous layer. After initial experiments, it was found that the result, i.e. P-value, does not change significantly between 8 and 12 h of vigorous shaking of the two phases, and leaving the mixture standstill for 12-24 h for complete separation of the two layers and ensuring the attainment of the state of equilibrium. Therefore, measurement of the absorbance of the aqueous buffer layer was made after 12 h of shaking the two phases and leaving the system standstill for 24 h. Figures presented are average of three experiments performed in duplicate.

Moreover, all test compounds showed full, reversible local anesthesia and, generally, their anesthetic profiles were better than those of bupivacaine standard. In the first set of experiments, a prolonged duration of action of over 6 h was observed by compounds 9, 10, 12, 13, 17, 18 and 20. These were assessed further on two additional animals. Complete recovery from anesthesia was observed in both animals injected by compounds 10, 12 and 13, while only one guinea pig completely recovered at 24 h after injection of the others, i.e. compounds 9, 17, 18 and 20. The remaining animal showed no response to pricking at 24 h for compounds 9, 18 and 20 or altered response for compound 17. The test was

not continued after 24 h at this stage. Later on, compounds 9, 10, 17 and 18 were further assayed, and they all produced long-lasting, reversible local anesthesia for 120 h with no obvious signs of local or systemic side-effects. Figures reported are mean values of at least two experiments, and for the prolonged-acting compounds, only a duration of action of 24 h was reported.

## DISCUSSION

The transition of the agent from the extracellular phase into the biophase proceeds by mechanisms which are not precisely known. In particular, it is very difficult to match the test conditions to the biophase in the experimental determination of P-values. Nevertheless, partition between an aqueous phase and n-octanol can be regarded as an experimental model giving the best correlations with biological results. n-Octanol was selected as the ideal standard, non-aqueous solvent for the partitioning experiments,[8,12,19-22] since its properties tend to approximate those of actual membrane components. Besides, it has a more appropriate rate of hydrophilic properties than other non-aqueous solvents, and the hydroxyl group also provides hydrogen bonding capability. The pH of the aqueous buffer phase (pH = 7.4) was selected so as to match the pH of the biological system.

The assessed compounds have shown full, potential, reversible, long-lasting local anesthesia of 2-24 h, simply by changing either the aromatic substituent or the dialkylamino moiety or both, with no apparent untoward effects. These have shown P-values higher than one, and generally derivatives with butyl substituents in the aromatic nucleus, irrespective of its position, number and type, have had comparatively high P-values, and exhibited a prolonged action for 24 h or over, particularly the dimethylamino analogues. Substituting a tert-amyl group (compound 14) for tert- or sec-butyl group in the 4-position of the benzene ring (compounds 13 and 10) or a chloro-group (compound 11) for the methyl radical (compound 1) in the 2, 4, 6-positions of the aromatic nucleus, has increased P-value, but decreased WT. This may suggest that there is an optimum group size for the prolonged anesthetic action to be observed, and in this class of compounds, it is most probably either tert- or sec-butyl group as evidenced from the biological studies. Furthermore, the P-value of the tert-butylphenoxy analogue (compound 13) is higher than the sec-butyl derivative (compound 10), while their WT are similar, i.e. 24 h. This fact gives further support to the afore-mentioned conclusion and also justifies additional physical, chemical and biological studies to be carried out on these two compounds in particular to select the best of this series. Moreover, though the 3,5-dimethylphenoxyethyldimethylamine analogue (compound 4) has a lower P-value than the 3,5-dimethylphenoxyethyldiethylamine derivative (compound 8), it has exhibited a longer WT. This observation can give additional support to our previous conclusion regarding the requirement of an optimum group or groups (mainly shape, size and partly position and number) in the anesthetic molecule for the induction of long-lasting action, i.e. the anesthetic drug-receptor interaction is stereospecific. Similarly, there has been no increase in WT, when the dimethylamino group is replaced by the diethylamino radical in any of the assayed compounds, e.g. compounds 13 and 7, compounds 10 and 15, and compounds 12 and 19, and, generally, the former derivatives are longer-acting than the latter ones.

It has been reported, in certain series of compounds, that there is a positive correlation between lipophilicity, i.e. P-value, and strength of binding to specific and non-specific sites that, in turn, affect WT.[4-8,23-28] In addition, the higher the rate of interaction to non-specific acceptors, the shorter the duration of action; while the stronger the binding to specific active sites, the higher the duration of the biological action.[5,6] Accordingly, the longer duration of action of these compounds in comparison to that of the standard may be attributed to the fact that their rate of binding to non-specific acceptors at the biophase and at the adjacent tissues to the active receptor sites are lower than that of bupivacaine standard. This leads to potentiating the degree of interaction of the drug to sodium channel receptors by increasing the concentration of the free drug molecules, which are able to diffuse and ultimately reach the site of action. Furthermore, it is well known that compounds with ether function have higher chemical stability than both of their ester and anilide counterparts. Therefore, the prolonged action of these agents may also be ascribed to their low rate of degradation due to enzymes or physiological pH.

In conclusion, the absence of a positive relationship between P-values and WT indicates that this parameter does not exclusively condition the anesthetic properties and other physicochemical, pharmacokinetic, and pharmacodynamic factors as well as specific and non-specific binding characteristics are also involved. Therefore, the molecule as a whole is responsible for the induction of the biological action. The absence of such a correlation has also been described by other workers.[28,29] Moreover, these compounds appear to be structurally specific and the stereochemistry of the drug molecule has great influence on the extent and rate of interaction to the active receptor sites and also on the rate of clearance from the biophase. This is further supported by the fact that they act at low concentrations

comparable to that of the bupivacaine standard, and a minor modification in their chemical structures leads to a significant change in their duration of action. Finally, since their biological action is reversible, they do not seem to act as neurolytics, but block nerve transmission by interacting strongly with sodium channel proteins in the lipoidal nerve cell membrane, resulting eventually in blocking or interrupting, in certain manner, the transmission of nervous stimuli or interfering with the function of the axonal membrane.

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