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Partition coefficients (*n*-octanol/water) of *N*-butyl-*p*-aminobenzoate and other local anesthetics measured by reversed-phase high-performance liquid chromatography

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Abstract

For the determination of the logarithmic partition coefficients between *n*-octanol and water (log $P_{o/w}$) of local anesthetics, the pH of the aqueous phase needs to be adjusted to high values to ensure that the local anesthetics are in the unionized form. Using the shake-flask or the stir-flask method, this high pH may catalyze hydrolysis, leading to increasing amounts of impurities in time. These impurities exclude non-selective quantification methods like UV spectrometry and require repetitive quantitative analysis of both liquid phases resulting in a tedious and time-consuming method. A rapid reversed-phase HPLC method was developed to measure log $P_{o/w}$ of the local anesthetics *N*-butyl-*p*-aminobenzoate, methyl-*p*-aminobenzoate, benzocaine, procaine, mepivacaine, prilocaine, lidocaine, bupivacaine, etidocaine, tetracaine and oxubuprocaine. © 1997 Elsevier Science B.V.

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1. Introduction

Recently, epidural administration of an aqueous suspension of *N*-butyl-*p*-aminobenzoate (BAB) to dogs and humans has been shown to produce ultra-long lasting, up to 6 months, sensory selective nerve block [1–3].

To elucidate the mechanism(s) responsible for the observed long-lasting effects after epidural administration as a suspension knowledge of the physico-chemical properties of BAB is necessary. Water solubility (approximately 140 mg/l) and pK_a (2.52) of BAB are well known [4–6]. A study of the partitioning between polar (aqueous)/non-polar (lipid) phase is indicated because partition coefficients of local anesthetics have been shown to correlate with onset-time, duration of action and potency [7–10].

Partition coefficients (*P*) of individual or small series of local anesthetics have been studied employing mainly the shake-flask and the stir-flask method. Experimental conditions in these studies vary strong-

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ly (liquid phases used, temperature, equilibration time, hydrolysis etc.) and are sometimes incompletely reported, hindering comparison of the results [11–13]. Furthermore, there is often confusion with respect to the values presented, e.g., partition or distribution coefficients [14].

Because HPLC capacity factors are increasingly used to estimate partition coefficients [15,16], the present paper describes a reversed-phase HPLC method and the use of this method to measure and position the logarithmic partition coefficient between *n*-octanol and water (log $P_{o/w}$) of BAB compared with the partition coefficients of the local anesthetics: methyl-*p*-aminobenzoate, procaine, benzocaine, mepivacaine, prilocaine, lidocaine, bupivacaine, etidocaine, tetracaine and oxybuprocaine.

2. Experimental

2.1. Reagents and materials

Analytical or pharmaceutical grade chemicals were used, unless indicated otherwise. *N*-Butyl-*p*-aminobenzoate was a gift of Abbott (Chicago, IL, USA). Benzocaine, mepivacaine HCl, procaine HCl, prilocaine HCl, lidocaine HCl, bupivacaine HCL and oxubuprocaine HCl were supplied by BUFA (Uitgeest, Netherlands), etidocaine HCl by Astra (Uppsala, Sweden), methanol (HPLC-grade), sodium hydroxide, benzophenone, formamide and dihydrogenphosphatedihydrate by Merck (Darmstadt, Germany) and 4-chloraniline, methyl-*p*-aminobenzoate, 4-methylbenzyl alcohol, acetanilide, phenyl benzoate, 2,3-dichloroaniline and biphenyl were obtained from Aldrich (Axel, Netherlands). Phosphate buffer (0.025 *M*) was prepared by dissolving weighed amounts of dihydrogenphosphatedihydrate in distilled water and adjusting the pH to 11.2 with 0.1 *M* sodium hydroxide solution. The pH of the buffer was set at 11.2 to assure that all local anesthetics are in their nonionized form (two units higher than highest pK_a of the local anesthetics tested, e.g., procaine HCl pK_a=9.0).

2.2. Instrumentation

The HPLC system consisted of an autosampler 507, a solvent delivery module 110B, a programmable UV detector module 166 set at 220 nm and an analog interface module 406 connected to an IBM PS2 55SX computer with System Gold chromatography software version 7.11 (Beckman, Mijdrecht, Netherlands).

The HPLC column was a Waters Radial Pack Resolve C_{18} 10 μm column fitted into an RCM 100 holder. The mobile phase consisted of a mixture of methanol–phosphate buffer (2:1, v/v) pH 11.2. The flow-rate of the mobile phase was 1 ml/min. The sample volume injected was 50 μl. The experiments were done in an air-conditioned laboratory (Temperature, 21±1°C).

2.3. Determination of dead time t_0

The dead time was measured by injection of the unretained compound formamide dissolved in mobile phase (n=6).

2.4. Determination of the retention time t_R of reference compounds

Reliable data on local anesthetics were not available (Table 2). Therefore a more general calibration was established using the following reference compounds: acetanilide, 4-methylbenzyl alcohol, 4-chloraniline, 2,3-dichloroaniline, benzophenone, diphenylamine, phenyl benzoate and biphenyl covering the log $P_{o/w}$ range of 1.0–4.0 [17]. Reference compounds, in combinations of four, were dissolved/mixed with mobile phase immediately before injection and retention times measured (each compound n=6).

2.5. Determination of the t_R of the local anesthetics

Local anesthetics, in combinations of four, were dissolved/mixed with mobile phase immediately before injection and retention times measured (each compound n=6).

2.6. Calculation of log $P_{o/w}$ values

After determination of the dead time (t_0) and the retention times of the reference compounds and the

Table 1
Measured t_R values and log $P_{o/w}$ [17] of the reference compounds used for calibration

Compound	log $P_{o/w}$	t_R
Acetanilide	1.0	1.63
4-Methylbenzylalcohol	1.6	2.06
4-Chloraniline	1.8	2.07
2,3-Dichloraniline	2.8	3.23
Benzophenone	3.2	5.05
Diphenylamine	3.4	5.28
Phenylbenzoate	3.6	7.16
Biphenyl	4.0	10.76
Formamide (t_0)	–	1.18

local anesthetics, the capacity factor (k) of the reference compounds and local anesthetics were calculated according the expression:

$$k = (t_R - t_0)/t_0 .$$

A calibration graph was established of log $P_{o/w}$ of the reference compounds versus the calculated log k values and the correlation calculated by least-squares linear regression. Log $P_{o/w}$ of the local anesthetics was derived from the linear response function of the calibration graph. All calculations were performed using the statistical software SPSS 6.0 (SPSS, Chicago, IL, USA).

3. Results

The dead time of the chromatographic system, as assessed by the injection of formamide was 1.18±0.06 min.

All reference compounds and local anesthetics were clearly eluted separately by the system and all eluted within 14 min (Tables 1 and 2).

Despite the high pH of the mobile phase combined with a silica-based stationary phase the repeatability and accuracy of the retention times were good (all standard deviations ≤4%). No column deterioration was noted for the duration of the experiments. The calibration graph of log k of the reference compounds versus log $P_{o/w}$ is shown in Fig. 1.

The chromatograms of the local anesthetics procaine, tetracaine and oxybuprocaine showed two peaks. The height and surface of the peak with the shortest retention time (most water soluble compound) increased with increasing time between preparation of the solution and injection, indicating base catalyzed hydrolysis of the compounds. The retention times, calculated capacity factors and log $P_{o/w}$ of the local anesthetics are presented in Table 2. The standard deviation of all log $P_{o/w}$ values is <3%.

Log $P_{o/w}$ calculated from the retention data obtained by the HPLC method correlate significantly with data of Ref. [11] ($r=0.99$, $F=3298.4$, $P=0.01$)

Table 2
Retention times (t_R), calculated capacity factors (log k) and log $P_{o/w}$ of the tested local anesthetics

Compounds	t_R(min)	log k	log $P_{o/w}$	Reported log $P_{o/w}$ values				
				Shake-flask/stir-flask values				HPLC
				log $P_{o/w}$ Ref. [11]	log $P_{o/w}$ Ref. [12]	log $P_{o/w}$ Ref. [13]	log $P_{o/w}$ Ref. [18]	log $P_{o/w}$ Ref. [19]
Methyl-*p*-aminobenzoate	1.59	−0.49	0.89	1.12				
Benzocaine	1.84	−0.25	1.44	1.65	2.15	2.12	1.93	
Procaine	3.05	0.20	2.51			2.00	0.16	2.27
N-Butyl-*p*-aminobenzoate	3.23	0.24	2.61	2.70	2.70			
Mepivacaine	3.43	0.28	2.69			2.11		
Prilocaine	3.53	0.30	2.73					
Lidocaine	5.77	0.59	3.40			2.56	3.24	3.13
Bupivacaine	9.73	0.86	4.05			3.50		4.85
Etidocaine	10.99	0.92	4.19			3.86		
Tetracaine	12.20	0.97	4.32			3.76	4.25	
Oxubuprocaine	13.54	1.02	4.38					

Values reported are shown in the last five columns.

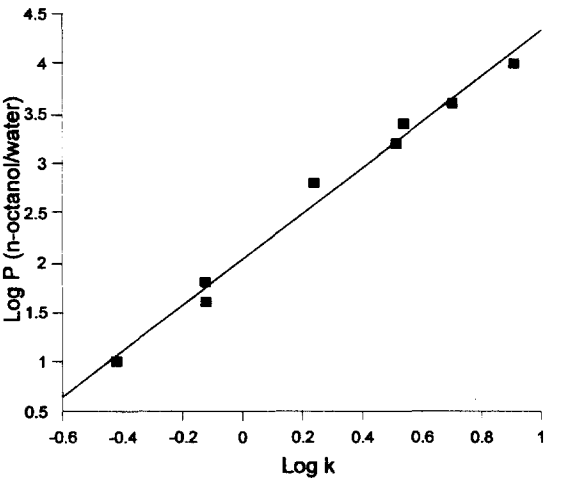

Fig. 1. Calibration curve constructed by plotting log k of each reference compound versus their log $P_{o/w}$; $y=2.31+2.03x$; $r=0.9915$.

and Ref. [13] ($r=0.89$, $F=19.95$, $P=0.0066$). Correlation with Ref. [12] was not tested. There is no significant correlation with data from Ref. [18] except in the case the value of procaine (0.16) is omitted as an outlier (Fig. 2).

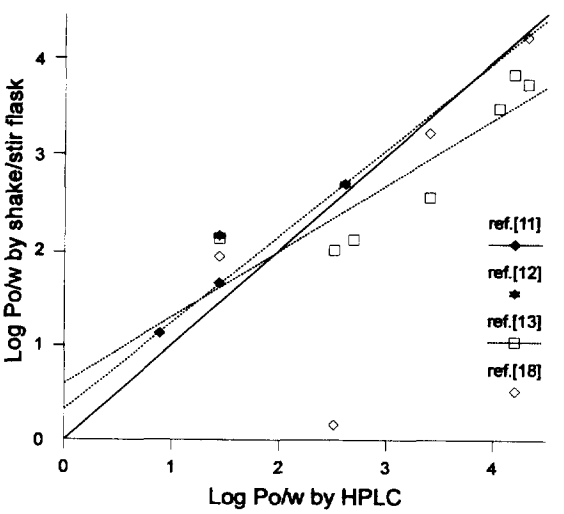

Fig. 2. Plot of log $P_{o/w}$ calculated from the retention data obtained by the HPLC method presented versus log $P_{o/w}$ measured by the shake-flask/stir-flask method (see also Table 2).

4. Conclusion

The relative order of log $P_{o/w}$ values of the local anesthetics obtained in our study is generally according to the order reported in other studies (Table 2). Absolute values differ substantially.

Using the shake-flask or the stir-flask method for the measurement of the partition coefficients of local anesthetics, the pH of the aqueous phase needs to be adjusted to high values to assure that the compounds are in the unionized form. A high pH may however, next to the disadvantage of being sensitive to a priori impurities, cause an additional experimental problem because local anesthetics undergo base catalyzed hydrolysis leading to increasing amounts of more hydrophilic impurities in time (in our HPLC-study noted by the occurrence of two peaks in the chromatogram). Correspondingly, the partitioning equilibrium is shifted in favour of the aqueous phase leading to low experimentally measured log $P_{o/w}$ values, especially when combined with non-selective quantification methods like UV spectrometry [15,16]. This effect becomes most pronounced for log $P_{o/w}$ values near the upper range of these methods; a trend noticed when comparing our values to those obtained using the shake-flask or the stir-flask method (Fig. 2).

Comparison with the values obtained with HPLC using a mobile phase buffered at pH 4.5 (Table 2, Ref. [19]) demonstrates a large difference between the values obtained for bupivacaine (4.05 versus 4.85). One explanation may be that the amount of TEA added to the acidified mobile phase did not completely suppress silanophilic interactions between the ionized bupivacaine and the active silanol groups.

A critical comment regarding the method presented may be that better correlations between chromatographic retention data and log P are found when homologous or structurally similar compounds are tested [15,16,20,21]. Subdividing dissimilar compounds into structurally similar classes and regressing the data separately generally improves the correlation; the error obtained by not dividing is at maximum 0.2–0.3 log P units [22–24]. Excellent results have however also been reported for non-congeners [20,23,25–28]. Our set of local anesthetics tested contains ester- and amide-type local anes-

thetics. Despite this obvious difference, structural similarity has to exist because all substances tested act pharmacodynamically by binding to the same receptor(s). Although the true log $P_{o/w}$ values of the compounds tested are unknown the excellent linearity of the calibration line and the matching order of hydrophobicty compared to reported values and the correlation with log $P_{o/w}$ values obtained by the shake-flask/stir-flask method (Fig. 2) strongly indicate that the experimental conditions chosen (i.e., high pH of eluent [15,21,29–31] and low concentration of methanol [15,23,24] to suppress ionization and silanophilic interactions, combined with a HPLC-column containing silica with a low activity surface [31]) resulted in a reliable HPLC-method for the estimation of log $P_{o/w}$ of local anesthetics.

BAB has a low partition coefficient compared with lidocaine and bupivacaine, the clinically most often used local anesthetics. From the established general relationship between log $P_{o/w}$ and pharmacodynamic parameters [7–10], BAB, applied as a solution, is expected to have a low potency and a slow onset and short duration of action. Extrapolation to the effects after epidural administration as a suspension means that the suspension-formulation has to be responsible for the ultra-long lasting action. Pharmacologic studies, employing solutions of BAB, are necessary to confirm this profile.

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