Spinal Disposition and Meningeal Permeability of Local Anesthetics

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Purpose. To investigate the spinal disposition, the cerebrospinal fluid (CSF) bioavailability, and the *ex vivo* meningeal permeability of six homologous pipecoloxylidide local anesthetics and to search for correlations with lipophilicity.

Methods. The *ex vivo* meningeal permeability was studied on fresh specimen of meninges (dura mater and arachnoid mater) removed from lumbar and cervical level of rabbit spine following laminectomy. Spinal disposition and CSF bioavailability were investigated using microdialysis sampling after simultaneous injection of an equimolar dose of the six homologs in the epidural or in the intrathecal spaces. In a first step, intrathecal and epidural microdialysis were performed after epidural administration. In a second step, intrathecal microdialysis was performed after intrathecal administration.

Results. Permeability through cervical and lumbar meninges was linearly correlated, and the cervical permeability was around 60% of the lumbar permeability. Apparent permeability data showed a parabolic relationship with the lipophilicity of the derivatives with a marked decrease in permeability for log P above 3. *In vivo* experiments have shown that the absorption rate constant linearly decreased with lipophilicity of the derivatives (0.171 to 0.125 min^{-1}) whereas the intrathecal bioavailability, which was low, increased with lipophilicity (7.2 to 15.9%).

Conclusions. The unexpected increase in CSF bioavailability with a decrease in absorption rate through meninges emphasizes the role of specific competitive clearance and distribution processes in the epidural space.

KEY WORDS: cerebrospinal fluid bioavailability; *ex vivo* spinal meningeal permeability; intrathecal and epidural disposition; local anesthetics; microdialysis.

INTRODUCTION

Epidural local anesthetics are routinely administered for postoperative pain treatment as a continuous infusion through an indwelling catheter because of their relatively short duration of action. Epidural disposition of local anesthetics depends on several processes such as blood uptake in capillary vessel, distribution into epidural fat, and crossing through spinal meninges (i.e., dura and arachnoid mater) to reach their sites of action—the spinal nerves and cord. Permeability of meninges to different drugs has been studied with the chamber diffusion technique (1–6), showing that a passive diffusion mechanism was likely. However, the nature of the relationships between permeability and drug physicochemical properties appeared controversial depending on the nature of meninges (human or animal, frozen or fresh meninges) and of the drugs studied (local anesthetics, opiates, and so forth). As diffusion of epidurally administered drugs through meninges is a prerequisite for their access to cerebrospinal fluid (CSF), *in vivo* investigations of the intrathecal bioavailability are required. Microdialysis is particularly relevant to study the spinal disposition of drugs because it allows sampling in the epidural space, which is a fluid-free space, and sampling in the intrathecal space without altering the dynamics of the CSF that could interfere with the disposition of the drug. This technique has found significant applications in pharmacokinetics (7–10) and more precisely in spinal pharmacokinetics of local anesthetics following epidural and intrathecal administration (11,12) and for the study of opioids following intrathecal (13) and epidural (14) administration. By using the microdialysis technique in rabbits (12), we previously showed that the intrathecal bioavailability of bupivacaine and lidocaine was low (5.5% and 17.7%, respectively) and comparable to those described for other anesthetic or analgesic drugs [i.e., 3.7% for meperidine (15), from 0.3 to 3.6% for morphine (15–17), 2.7% for sufentanil (18), 14% for clonidine (19), 22% for dexmedetomidine (20), and 7% for MPV-2426, a new $\alpha 2$ agonist (21)]. If spinal disposition and intrathecal bioavailability of drugs administered by epidural route has been the subject of several investigations, so far there is no clear understanding of the mechanisms governing drug spinal trafficking and of the influence of drug lipophilicity. Indeed, most of the knowledge is based on inference-driven assumptions rather than on experimental facts.

To better understand the spinal trafficking of drugs, and especially the factors involved in drug transfer through spinal meninges and in intrathecal bioavailability, we performed in rabbits a comparative study of the epidural and intrathecal disposition and of the intrathecal bioavailability of six homologous amide local anesthetics from the pipecoloxylidide series whose structure differed by the length of the alkyl chain (from CH_3 to C_6H_{13}) linked on the nitrogen of the piperidine ring. Correlations of *in vivo* data with lipophilicity and with apparent permeability data obtained *ex vivo* in a model of isolated meninges in diffusion chambers were investigated.

MATERIALS AND METHODS

Chemicals

Six amide local anesthetic homologs having only structural difference in the length of the alkyl chain (from CH_3 to C_6H_{13}) linked on the nitrogen of the piperidine ring (Fig. 1) were kindly supplied by Astra (Astra Pain Control, Södertälje, Sweden). Methyl, propyl, and butyl derivatives are marketed local anesthetics, namely mepivacaine, ropivacaine, and bupivacaine.

A mock CSF solution (NaCl 8.2 g/l, $NaHCO_3$ 2.1 g/l, KCl 0.22 g/l, $MgCl_2 \cdot 6H_2O$ 0.08 g/l, $CaCl_2 \cdot 2\ H_2O$ 0.3 g/l, D-glucose 0.7 g/l, urea 0.2 g/l) was used as medium for the *ex vivo* experiment on isolated meninges as previously described (3).

A Ringer's solution (NaCl 8.6 g/l, KCl 0.33 g/l, $CaCl_2 \cdot 2\ H_2O$ 0.3 g/l) was used as perfusion fluid during the microdialysis experiments. All others reagents were of analytical grade.

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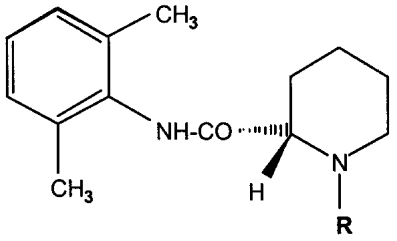

R	Product
$-CH_3$	Mepivacaine
$-C_2H_5$	
$-C_3H_7$	Ropivacaine
$-C_4H_9$	Bupivacaine
$-C_5H_{11}$	
$-C_6H_{13}$	

Fig. 1. Structure of homologous local anesthetics from the pipecoloxylidide series.

Determination of Physicochemical Descriptors

Partition coefficients (log P) were calculated using Crippen fragmentation method in ChemOffice software from CambridgeSoft Corp (Cambridge, MA, USA) (22). pK_a, reported previously for methyl, propyl, and butyl derivatives, was estimated by a nonlinear regression for the other compounds using Sigmaplot 2001 (SPSS, Chicago, IL).

Chromatographic Analysis

The separation and quantification of the local anesthetics in the dialysate (CSF, epidural samples) or in the *ex vivo* samples were carried out using a high-pressure liquid chromatography method with UV absorbance detection (λ = 205 nm) derived from a previously published method (23). Samples were immediately injected onto the chromatographic system. The chromatographic system consisted of a Waters Model 600 pump (Waters Assoc., Milford, MA, USA) equipped with a Waters Model 717 automatic injector, a UV PAD Waters Model 996 Spectromonitor detector, and a Waters Millenium 32 data acquisition system. The analytical chromatographic column was a Lichrospher RP-B Merck (Darmstadt, FRG) (length, 125 mm; internal diameter, 3 mm). The flow rate was 0.5 ml/min, and the temperature was maintained at 30°C. The mobile phase A consisted of a mixture of acetonitrile and pH 2.1, 0.01 M sodium dihydrogenphosphate (10:90), and the mobile phase B consisted of pure acetonitrile. A 0–45% mobile phase B and 100–55% mobile phase A in 20 min were used for the chromatographic analysis of the different drugs studied.

Microdialysis Conditions

Microdialysis was performed using a CMA/102 microinjection pump coupled to a microdialysis probe CMA/20 (membrane length of 10 mm, 0.5 mm outer diameter, molecular weight cutoff 20 kDa) (CMA Microdialysis, Stockholm, Sweden). Dialysates (sample volume = 1 μl) were collected in vials containing 100 μl of etidocaine (1 μg/ml) (external standard of HPLC) and a 50-μl aliquot was injected onto the chromatograph. Retrodialysis, using lidocaine as internal standard, was applied to calibrate the microdialysis probes. Thus, the probe was perfused throughout the experiment, at a flow rate of 1 μl/min with lidocaine (100 μg/ml) containing Ringer's solution. During the experiment, the relative loss of lidocaine was determined in each sample and used to correct the dialysate concentrations. Before and at the end of the *in vivo* experiment, the probes were tested *in vitro* in order to verify the lack of significant deterioration.

Animals

Experiments were performed on female New Zealand albino rabbits weighing 3.1 ± 0.3 kg that were housed individually with free access to food and water in a temperature-controlled room (22 ± 2°C). Animals were fasted from the night before the experiments. The study was approved by the Committee of Laboratory Investigation and Animal Care of our institution and achieved according to the guidelines for laboratory animal experiments (French Ministry of Agriculture authorization no. 007223).

Ex vivo Experiment

Meningeal maters of rabbits were sampled in order to determine their apparent permeability to the local anesthetics tested. Under general anesthesia, posterior processes of vertebrae were dissected, and laminectomies were performed at lumbar as well as cervical levels of spine. When spinal cord was revealed, rabbit received a large dose of thiopental, then spinal cord, embedded with meninges, over a 3-cm length was sampled at cervical and lumbar levels. Then, meninges (dura mater and arachnoid) were dissected and kept in their physiological anatomic relationships and were placed in the center of a Plexiglas thermostated diffusion chamber system with a 0.6-cm^2 connecting port (Costar Corporation, Buckinghamshire, UK). Eight milliliters of mock CSF were introduced in fluid reservoirs on either side of the meningeal tissue. Oxygen (95%) and carbon dioxide (5%) were bubbled in each fluid reservoir. The temperature was kept at 37°C during all the experiments.

An equimolar solution (2.2 mM) of the six compounds was added to the fluid reservoir on the dura mater side of the diffusion cell at time 0. The diffusion of the compounds was studied by sampling 100 μl simultaneously in the donor and in the receiver compartments at 15-min intervals for 90 min. The flux was determined by measuring the amount of each compound in the receiver compartment at each sample time. The data were plotted vs. time, and the value of the slope obtained with the use of least-squares linear regression is equal to the drug's flux through the meninges.

The apparent permeability coefficient (P) was calculated as follows :

$$\text{Papp (cm/min)} = \frac{Q}{(A \times C)}$$

where Q is flux through the meninges (mmol/min), A is meningeal surface area (cm^2), and C is un-ionized concentration in the donor compartment (mM) calculated from experimental concentrations corrected according to the Henderson–Hasselbach equation and the pK_a. The experimental concen-

trations mentioned above were those measured in the donor compartment and not the theoretical concentrations (i.e., 2.2 mM), as we have taken into account an adsorption phenomenon of our compounds onto the Plexiglas diffusion cell. The magnitude of the adsorption was dependent on the lipophilicity of the drugs (e.g., around 20% for bupivacaine).

In vivo Experiment

The animals were sedated with intermittent intravenous 1% thiopental throughout the experiment and euthanized with thiopental at the end of the experiment. After L5-L6 laminectomy under epidural procaine anesthesia (24), microdialysis probes and an injection catheter were inserted in the epidural or in the intrathecal spaces as previously described (12), and drug administrations were performed after a 120-min time interval.

In a first step of the experiment, a microdialysis probe was placed in the intrathecal space, then a microdialysis probe and an injection catheter were inserted in the epidural space. An equimolar solution of the six compounds (2.2 mM) was injected in the epidural space (1 ml over 30 s), and the epidural and intrathecal spaces were sampled simultaneously. The study was performed in 8 animals, and a series of 8 and 6 concentration–time profiles were obtained in the epidural and in the intrathecal spaces, respectively.

In a second step of the experiment, a microdialysis probe and an injection catheter were inserted in the intrathecal space. An equimolar solution of the six compounds (2.2 mM) was injected in the intrathecal space (0.1 ml over 30 s), and the intrathecal space was sampled. A series of seven intrathecal concentration–time profiles were obtained.

Microdialysis sampling was achieved every 2 min for 90 min in the epidural and intrathecal spaces.

Pharmacokinetic Analysis

Intrathecal and epidural population pharmacokinetic parameters of local anesthetics were determined by using the population pharmacokinetic software P-Pharm (version 1.5, Innaphase, Champs sur Marne, France). Intrathecal concentrations following intrathecal administration and epidural concentrations following epidural administration were fitted according to a biexponential model. Intrathecal concentrations following epidural administration were fitted according to a triexponential model with a first-order input. The distribution of the random effect was assumed as normal and the residual error variance as heteroscedastic. Initial population parameter estimates were derived from the mean of the individual parameter values obtained by using a stripping algorithm. Individual parameters for each data set (Bayesian estimates) were obtained from the current population parameters and the individual data.

The maximum free epidural and intrathecal concentration (C_{max}) and the corresponding time (T_{max}) were derived from raw data. Because both epidural and intrathecal administrations were not performed in the same animals, a mean CSF bioavailability (F-csf) was determined following

$$\text{F–csf} = \frac{\text{Mean AUC-csf-epi}}{\text{Epidural dose}} \times \frac{\text{Intrathecal dose}}{\text{Mean AUC-csf-it}}$$

where AUC-csf-epi is the area under the unbound CSF concentration–time curve after epidural administration and AUC-csf-it is the area under the unbound CSF concentration–time curve after intrathecal administration.

Clearance parameters were CL_E, defined as the elimination clearance, and CL_I, defined as the intercompartmental distribution clearance. V_1 and V_{ss} were the central and the steady-state volume of distribution, respectively. K_{12} and K_{21} were the distribution rate constants and K_{10} the elimination rate constant. $T_{1/2\ \alpha}$ and $T_{1/2\ \beta}$ were the apparent distribution and elimination half-lives.

Statistics

All data are presented as mean ± SD. Student's *t* test was used to compare individual means. A p value less than 0.05 was considered as statistically significant.

RESULTS

Physicochemical Descriptors

Physicochemical descriptors data are reported in Table I. The length of the alkyl chain linked to the nitrogen of the piperidine ring markedly influences the lipophilicity of the compounds leading to a variation of log P from 2.62 for the methyl derivative to 4.7 for the hexyl derivative. The log P of the hexyl derivative was close to the critical log P value for which reduced drug absorption/permeability may be encountered (25). pK_a values were in accordance with data in the literature (26) for the compounds for which ionization has been studied (methyl, propyl, and butyl derivatives).

Permeability

The results of the *ex vivo* experiment are summarized in Table I. Permeability through cervical and lumbar meninges were linearly correlated ($r^2 = 0.9976$); the cervical permeability being around 60% of the lumbar permeability.

Comparison with data in the literature, only available for the butyl derivative (bupivacaine), indicates that in our model

Table I. Physicochemical Descriptors and Apparent Permeability Coefficient (Mean ± SD, 10^{-3} cm/min) of Homologous Pipecoloxylidide Local Anesthetics Through Cervical and Lumbar Spinal Meninges (Dura and Arachnoid Mater) in Rabbits

R	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_6H_{13}
Log P	2.62	2.96	3.44	3.86	4.28	4.7
p*Ka*	7.60	7.85	8.00	8.10	8.19	8.26
Cervical Papp	96 ± 18	255 ± 53	213 ± 45	97 ± 24	36 ± 14	—
Lumbar Papp	158 ± 47	419 ± 126	361 ± 94	169 ± 36	78 ± 9	—

Papp: apparent permeability coefficient.

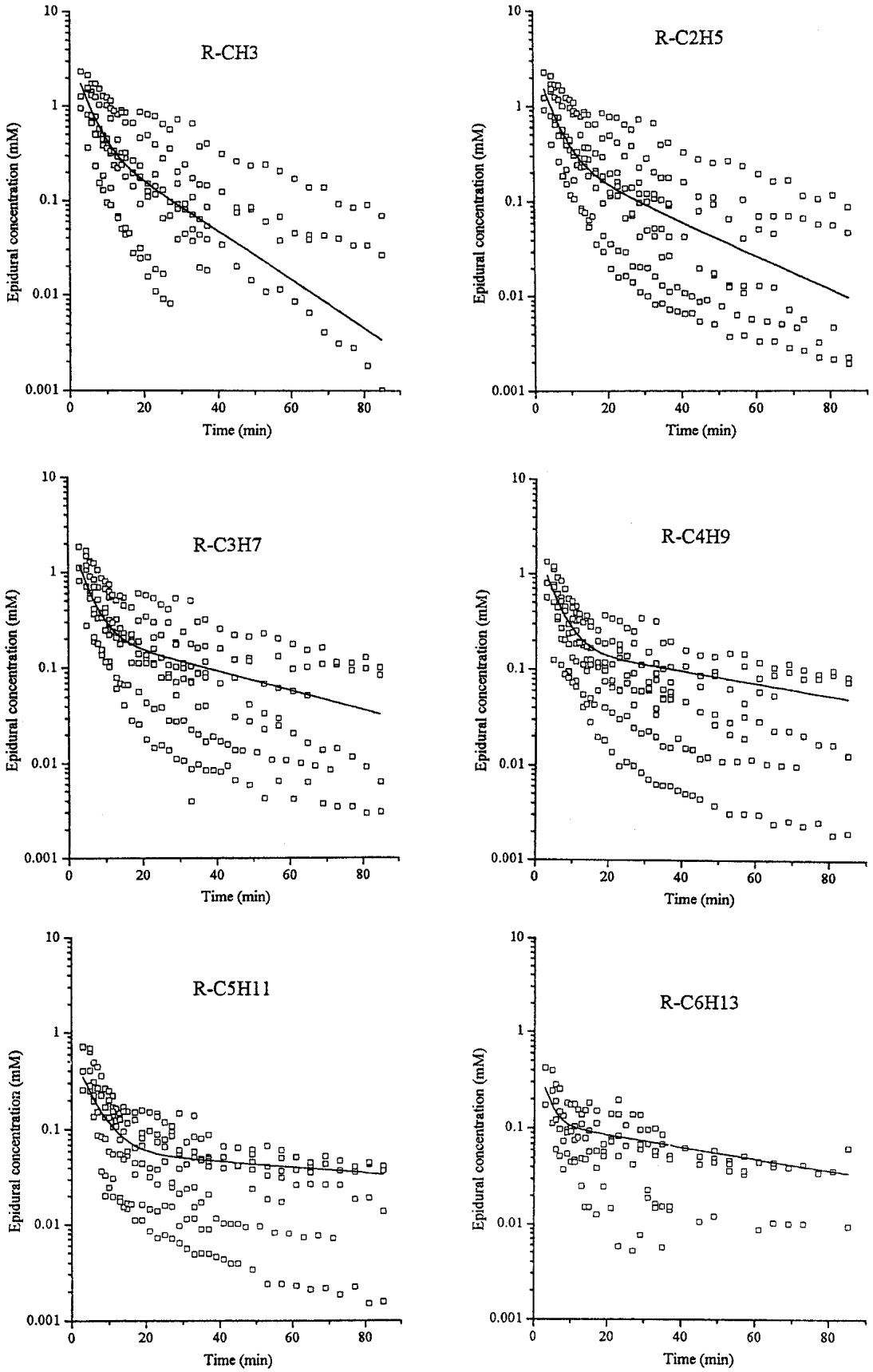

Fig. 2. Measured and population-predicted epidural concentrations of homologous pipecoloxylidide local anesthetics after epidural administration of an equimolar dose (2.2 μM) in rabbits.

the apparent permeability coefficient was higher than values previously reported with meninges of monkeys ($1.6 \pm 0.14\ 10^{-3}$ cm/min) (4) and with human meninges ($26.7 \pm 4.2\ 10^{-3}$ cm/min) (6). Such a difference may be related to animal species and/or study design differences (3). However, the values reported with meninges of monkeys were underestimated by a factor around 6 because bupivacaine ionization was not considered in the calculation of the apparent permeability (27).

Spinal Disposition

The individual epidural concentration–time profiles of the six local anesthetics after epidural administration in rabbits are presented in Fig. 2, and the pharmacokinetic parameters are summarized in Table II. The epidural concentration–time curves showed a biphasic decline for all the drugs administered. After epidural administration, the elimination clearance (CL_E) displayed a 2-fold increase with the lipophilicity of the local anesthetics whereas the intercompartmental distribution clearance (CL_I) displayed a 25-fold increase. The central volume of distribution and, to a much higher extent, steady-state volume of distribution increased with the lipophilicity. The epidural apparent elimination half-life displayed a 4-fold increase from methyl to hexyl derivative.

The individual CSF concentration–time profiles of the local anesthetics after intrathecal administration are illustrated by Fig. 3 and the pharmacokinetic parameters are presented in Table III. The CSF concentration–time curves showed a biexponential decline. It should be noticed that the more lipophilic local anesthetic (hexyl-pipecoloxylidide) was only detected in the intrathecal space of few animals as a scattered profile. The magnitude of elimination and intercompartmental distribution clearance and of distribution volumes was lower than those found in the epidural space. However, a same trend was observed with regard to the relationship with lipophilicity. The intrathecal apparent elimination half-life increased 6-fold from methyl to pentyl derivative with values that were quite close to those observed in the epidural space.

The individual CSF concentration–time profiles of the local anesthetics after epidural administration are illustrated by Fig. 4, and the corresponding biopharmaceutic parameters are presented in Table IV. The CSF concentration–time curves showed a maximum at around 7 min followed by a biexponential decline. It should be noticed that the hexyl-derivative was not detected in the intrathecal space. The absorption rate constant decreased from methyl to pentyl derivative whereas the intrathecal bioavailability displayed a 2-fold increase.

DISCUSSION

Spinal disposition and intrathecal bioavailability of drugs administered by epidural route has been the subject of several investigations, but so far there is no clear understanding of the mechanisms governing drug trafficking in the spinal area and of the influence of physicochemical properties of drugs, most of the knowledge being based on inference-driven assumptions rather than on experimental facts. As a parameter involved in drug transfer from epidural space to intrathecal space, meningeal permeability has to be investigated. The apparent permeability on *ex vivo* models of isolated meningeal tissue was around 2-fold higher at lumbar level of the spinal cord than at cervical level for all compounds, emphasizing the importance of the thickness of the membrane in determining the permeability of drugs. Indeed, Sato *et al.* have measured the thickness of the human dorsal meninges (thoracic, 0.45 mm; lumbar, 0.3 mm) and identified an inverse relationship between thickness and permeability (28). Moreover, earlier reports have showed the same decrease in permeability with the increase of the thickness of the meninges (1,6).

Paracellular transport of ionized forms is not expected to contribute significantly to the total drug transport through spinal meninges because the inner reticular layer of the arachnoid mater bordering the intrathecal space represents the main barrier between the epidural and intrathecal spaces. The impermeability of the arachnoid mater is required for the confinement of CSF with the intrathecal space. Hence, the apparent permeability coefficients were calculated from the non-ionized drug concentrations as a result of difference in ionization between drug compounds (pK_a 7.60 to 8.26).

Apparent permeability showed a parabolic relationship with the lipophilicity (Fig. 5) with a marked decrease for log

Table II. Epidural Pharmacokinetic Parameters (Mean ± SD) of Homologous Pipecoloxylidide Local Anesthetics After Epidural Administration of an Equimolar Dose (2.2 μM) in Rabbits

	Mepivacaine R-CH_3	R-C_2H_5	Ropivacaine R-C_3H_7	Bupivacaine R-C_4H_9	R-C_5H_{11}	R-C_6H_{13}
C_{max} (μg/ml)	397 ± 130	417 ± 131	367 ± 105	274 ± 89	150 ± 64	69 ± 38
(mM)	1.61 ± 0.53	1.60 ± 0.50	1.34 ± 0.38	0.95 ± 0.31	0.50 ± 0.21	0.22 ± 0.12
AUC (mM · min)	44.7 ± 61.6	39.3 ± 45.3	25.8 ± 14.8	21.5 ± 8.5	12.7 ± 5.1	9.4 ± 0.5
CL_E (ml/min)	0.115 ± 0.077	0.122 ± 0.092	0.122 ± 0.083	0.119 ± 0.049	0.194 ± 0.063	0.235 ± 0.013
CL_I (ml/min)	0.039 ± 0.040	0.045 ± 0.042	0.098 ± 0.087	0.152 ± 0.197	0.568 ± 0.747	1.012 ± 0.631
V_1 (ml)	0.60 ± 0.36	0.70 ± 0.41	0.87 ± 0.62	1.15 ± 1.20	3.59 ± 3.36	3.36 ± 1.73
V_{SS} (ml)	1.02 ± 0.64	1.33 ± 0.69	1.98 ± 0.91	2.96 ± 1.94	6.83 ± 4.88	24.98 ± 19.98
MRT (min)	10.7 ± 8.3	13.3 ± 8.8	20.3 ± 11.1	28.2 ± 22.6	31.6 ± 24.8	81.6 ± 49.4
K_{12} (min^{-1})	0.073 ± 0.077	0.081 ± 0.089	0.125 ± 0.089	0.139 ± 0.081	0.144 ± 0.089	0.284 ± 0.049
K_{21} (min^{-1})	0.091 ± 0.034	0.067 ± 0.031	0.048 ± 0.029	0.038 ± 0.023	0.029 ± 0.019	0.085 ± 0.052
K_{10} (min^{-1})	0.307 ± 0.314	0.262 ± 0.279	0.201 ± 0.172	0.275 ± 0.315	0.181 ± 0.247	0.094 ± 0.061
$T_{1/2\alpha}$ (min)	2.6 ± 1.7	2.8 ± 1.6	2.6 ± 1.3	2.4 ± 1.5	3.0 ± 2.2	1.6 ± 0.2
$T_{1/2\beta}$ (min)	13.6 ± 6.2	18.7 ± 6.3	26.9 ± 11.2	37.8 ± 22.9	43.9 ± 19.2	56.4 ± 34.1

AUC, area under curve; MRT, mean residence time.

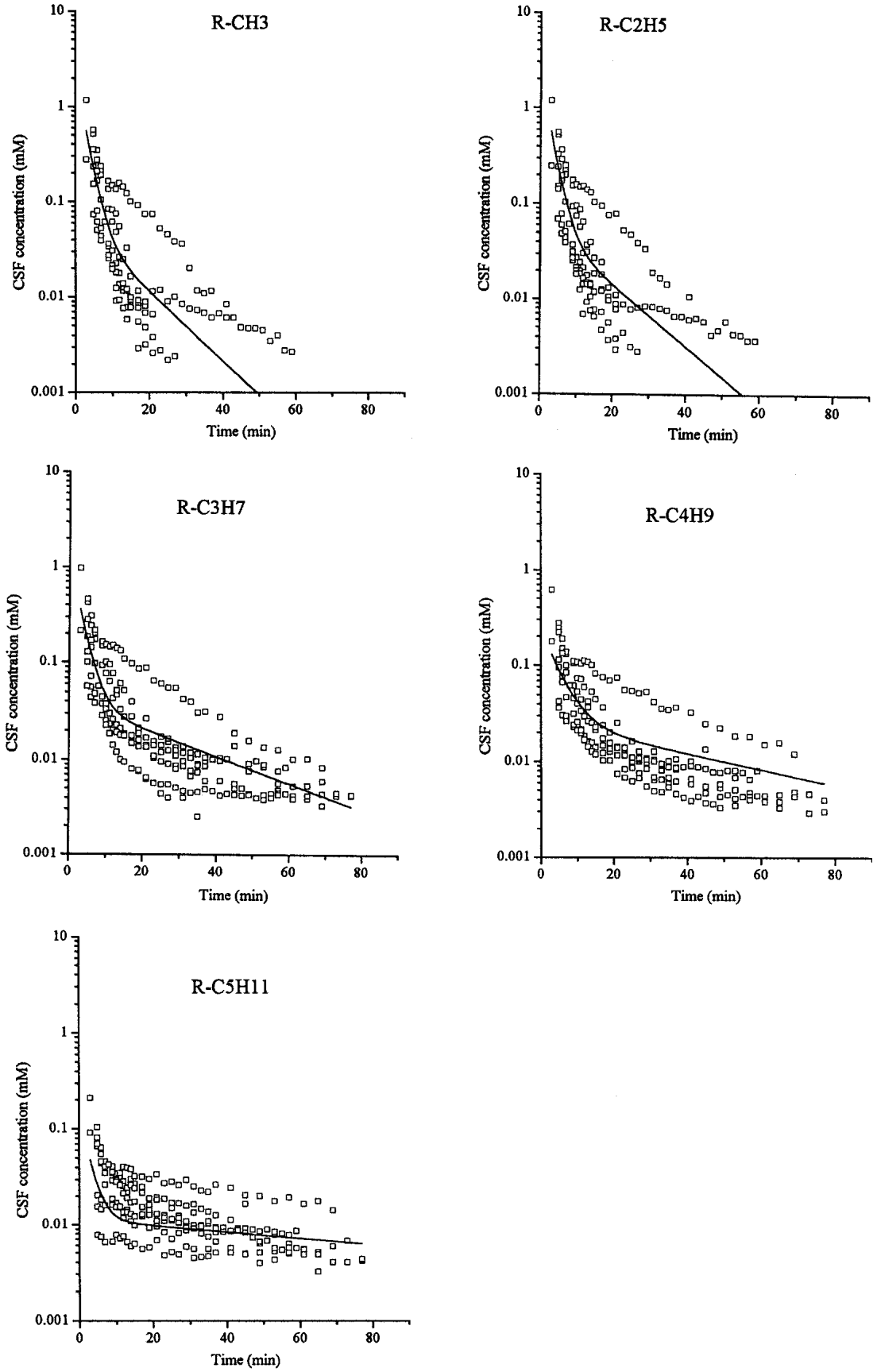

Fig. 3. Measured and population-predicted intrathecal concentrations of homologous pipecoloxylidide local anesthetics after intrathecal administration of an equimolar dose (0.22 μM) in rabbits.

Table III. Intrathecal Pharmacokinetic Parameters (Mean ± SD) of Homologous Pipecoloxylidide Local Anesthetics After Intrathecal Administration of an Equimolar Dose (0.22 μM) in Rabbits

	Mepivacaine R-CH_3	R-C_2H_5	Ropivacaine R-C_3H_7	Bupivacaine R-C_4H_9	R-C_5H_{11}
C_{max} (μg/ml)	97 ± 90	101 ± 97	87 ± 85	59 ± 57	22 ± 21
(mM)	0.39 ± 0.37	0.39 ± 0.37	0.32 ± 0.31	0.20 ± 0.20	0.07 ± 0.07
AUC (mM · min)	7.0 ± 4.1	7.8 ± 4.5	5.4 ± 3.7	3.6 ± 2.9	2.3 ± 1.4
CL_E (ml/min)	0.042 ± 0.023	0.041 ± 0.028	0.056 ± 0.029	0.097 ± 0.060	0.124 ± 0.055
CL_I (ml/min)	0.003 ± 0.003	0.004 ± 0.004	0.019 ± 0.015	0.096 ± 0.129	0.301 ± 0.362
V_1 (ml)	0.10 ± 0.07	0.10 ± 0.08	0.19 ± 0.12	0.93 ± 0.99	1.51 ± 1.74
V_{SS} (ml)	0.14 ± 0.09	0.17 ± 0.14	0.77 ± 0.66	3.22 ± 3.67	8.59 ± 9.02
K_{12} (min^{-1})	0.045 ± 0.053	0.052 ± 0.053	0.107 ± 0.076	0.102 ± 0.068	0.319 ± 0.275
K_{21} (min^{-1})	0.094 ± 0.024	0.086 ± 0.023	0.046 ± 0.022	0.042 ± 0.015	0.041 ± 0.004
K_{10} (min^{-1}	0.481 ± 0.140	0.502 ± 0.205	0.344 ± 0.122	0.219 ± 0.157	0.199 ± 0.151
$T_{1/2\alpha}$ (min)	1.4 ± 0.5	1.4 ± 0.6	1.7 ± 0.7	2.5 ± 1.1	2.2 ± 1.7
$T_{1/2\beta}$ (min)	9.1 ± 4.5	10.1 ± 4.9	25.3 ± 14.0	33.0 ± 14.8	55.2 ± 33.0

AUC, area under curve.

P above 3. Parabolic relationships have been described between the monkey transmeningeal permeability and lipophilicity in an heterogeneous series of drugs (21). This was also described for nonmeningeal tissues such as human transdermal permeability of an homologous series of nonsteroidal anti-inflammatory compounds (29), and for rabbit cornea permeability of an homologous series of steroids (30). One explanation for such a biphasic relationship between permeability and lipophilicity may be related to the dual nature of the membrane with hydrophilic and hydrophobic domains. Indeed, the drug must first be inserted into the polar head group region of the membrane, a process being controlled by its lipophilicity Then, the drug is transferred to the apolar region of the membrane, a process controlled by its hydrophilicity. Hence, the overall partitioning process depending on these two steps can be rate-limiting for the transfer from epidural to intrathecal space.

The "rule of five" proposed by Lipinsky *et al.* (25), validated for intestinal permeation, should not apply to meningeal permeation. This rule indicates that if two or more of the descriptors exceed a limit (number of H-bond donors >5, number of H-bond acceptors >10, MW >500, and log P >5), the drug will have poor absorption or permeability. In the current series of compounds, the hexyl pipecoloxylidide derivative did not cross the meninges. However, this compound only reached the limit for one descriptor (i.e., log P = 4.7). Hence, another rule has to be set up for meningeal permeability, the current work suggesting a decrease in permeability above log P of 3 and a lack of permeability above around 5.

Diffusion across the spinal meninges can be considered as the main route by which local anesthetics move from the epidural space to their sites of action in the spinal cord and in the spinal nerves. *Ex vivo* models on isolated meninges allow an evaluation of the intrinsic permeation properties of drugs. However, result from *ex vivo* permeability experiments should be considered with caution for the prediction of intrathecal bioavailability following epidural administration as several confounding factors may interfere *in vivo*: e.g., the gradient of pressure between epidural and intrathecal spaces, the meningeal surface area in contact with the solution epidurally injected, and the drug trafficking in the epidural space. Indeed, the epidural space is a fluid-free space containing mainly epidural fat and blood vessels, and several competitive processes are driving the epidural disposition of drugs: (i) uptake into the CSF after diffusion through the spinal meninges, (ii) uptake into the systemic circulation after diffusion through capillary vessel walls, and (iii) distribution into epidural fat. Hence, such a complexity requires an in-depth investigation of the epidural drug trafficking.

Following epidural administration, the elimination clearance, accounting for the elimination in both CSF and blood, was higher than the intercompartmental distribution clearance for the least lipophilic compound whereas the reverse was true for the most lipophilic one. Such a feature revealed different epidural disposition pattern as a function of drug lipophilicity. Indeed, the relative weight of the distribution process compared to the elimination process increased with the lipophilicity: the CL_I / CL_E ratio ranging from 0.3 to 4. This variation in the relative weight of distribution and elimination processes resulted in difference in the apparent elimination from the epidural space illustrated by the elimination half-life and mean residence time. Such information could be of interest in the design of new chemical entities for which a defined epidural pharmacokinetic profile is required.

If the elimination clearance was minimally increased with drug lipophilicity, the intercompartmental distribution clearance displayed a dramatic increase for the two most lipophilic drugs. Such influence of lipophilicity on drug distribution in the epidural space is not unlikely given the fact that epidural space is filled with fat tissue. Indeed, distribution in epidural fat can be assumed to be a simple partitioning process. A higher partitioning into epidural fat of bupivacaine compared to lidocaine, a less lipophilic local anesthetic not belonging to the pipecoloxylidide series, has previously been reported (31).

The influence of drug characteristics on the epidural clearance is not easy to delineate because the elimination from the epidural space is the sum of the CSF and blood elimination pathways whose relative magnitude cannot be evaluated. Moreover, diffusion pathways either through spinal meninges or through capillary wall vessels are not clearly known, being usually assumed to result from a passive process. However, previous investigations with different anesthetic and analgesic drugs suggest that the blood elimination pathway should be predominant over the CSF elimination pathway. Indeed, intrathecal bioavailability of epidurally in-

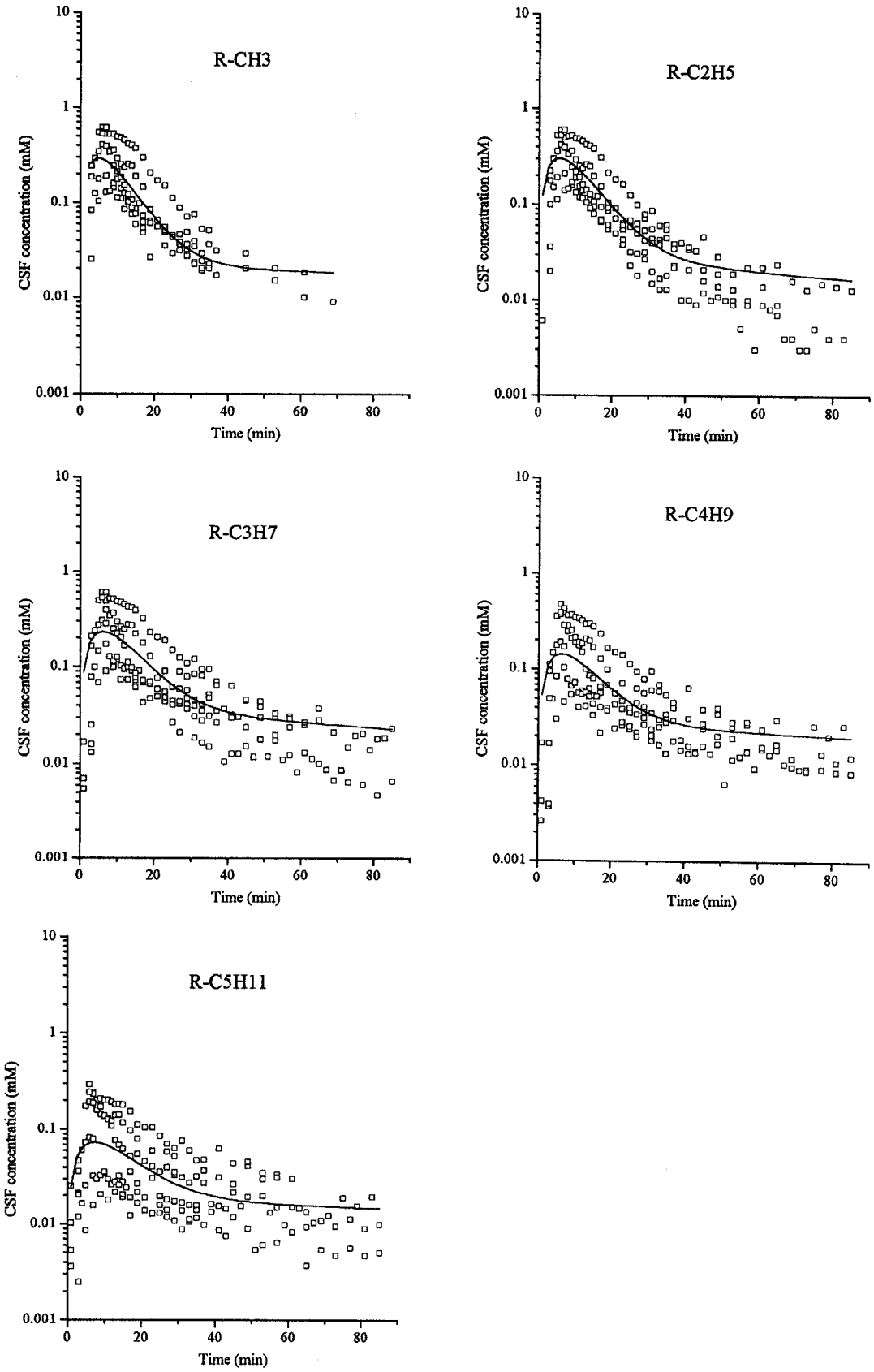

Fig. 4. Measured and population-predicted intrathecal concentrations of homologous pipecoloxylidide local anesthetics after epidural administration of an equimolar dose (2.2 μM) in rabbits.

Table IV. Intrathecal Biopharmaceutic Parameters (Mean ± SD) and Mean CSF Bioavailability (%) of Homologous Pipecoloxylidide Local Anesthetics After Epidural Administration of an Equimolar Dose (2.2 μM) in Rabbits

	Mepivacaine R-CH_3	R-C_2H_5	Ropivacaine R-C_3H_7	Bupivacaine R-C_4H_9	R-C_5H_{11}
C_{max} (μg/ml)	102 ± 51	108 ± 50	104 ± 59	78 ± 51	46 ± 36
(mM)	0.42 ± 0.21	0.42 ± 0.19	0.38 ± 0.22	0.27 ± 0.18	0.15 ± 0.12
T_{max} (min)	6.8 ± 1.2	6.8 ± 1.2	6.8 ± 1.2	6.8 ± 1.2	8.0 ± 2.9
AUC (mM · min)	5.0 ± 2.6	5.9 ± 1.9	6.3 ± 2.8	4.7 ± 2.2	3.6 ± 1.9
K_a (min^{-1})	0.171 ± 0.049	0.159 ± 0.059	0.152 ± 0.060	0.144 ± 0.054	0.125 ± 0.068
F-csf (%)	7.2	7.5	11.6	13.1	15.9

AUC, area under curve.

jected drugs is low (<20%), indicating that drugs injected in the epidural space are mainly eliminated via blood epidural vessels (12,15,18,19). Besides epidural trafficking, intrathecal disposition has to be considered *per se,* that is after intrathecal administration. Indeed, because epidural and intrathecal compartments are linked, drug disposition in the epidural space influences intrathecal drug disposition following epidural administration.

Several competitive processes are also involved in the intrathecal disposition of drugs: (i) uptake into the blood vessels of the pia and arachnoid mater, (ii) diffusion through the pia mater into the most superficial portions of the spinal cord, (iii) diffusion through the arachnoid and dura mater into the epidural space, and (iv) diffusion to the deeper areas of the spinal cord through the spaces of Virchow–Robin. The magnitude of the intrathecal elimination clearance was 1.5- to 3-fold lower compared to the epidural elimination clearance. Such a difference should result from a smaller local blood flow compared to the epidural site. Moreover, the difference between intrathecal and epidural elimination clearance decreased with drug lipophilicity. Although the physiological mechanisms responsible for drug removal from the intrathecal space are in part different from those from the epidural space, the same general trend was observed with regard to the relative weight of distribution and elimination processes. The CL_I/CL_E ratio ranged from 0.07 to 2.5 from methyl to pentyl derivative, the hexyl derivative being not detectable in the CSF dialysates. Hence, taken together our data show that epidural and intrathecal disposition are sensitive in a similar manner to the drug lipophilicity.

From a kinetic point of view, the evaluation of drug transfer rate through spinal meninges, from the epidural space to the intrathecal space, is an important feature in the selection of a new anesthetic or analgesic chemical entity designed for epidural administration as this parameter governs the speed of access of the drug to the CSF and hence to the sites of action in the spinal cord.

The *in vivo* drug transfer rate through spinal meninges showed a linear decrease in apparent absorption rate with increasing lipophilicity (Fig. 6, $r^2 = 0.9584$) contrasting with the parabolic relationship observed between the *ex vivo* apparent permeability and lipophilicity (Fig. 5). Theoretically, *in vivo* absorption rate and *ex vivo* permeability should display a strong correlation. However, the relation between these two parameters was hindered by the least lipophilic compound (methyl derivative) that displayed a low *ex vivo* permeability while having the highest K_a. Based on these results, extrapolations of spinal absorption rate from *ex vivo* investigations of apparent permeability of drugs through spinal meninges should be made with caution. Nevertheless, *ex vivo* studies on isolated meninges should be of interest to investigate the way to enhance meninges permeability in order to increase the CSF bioavailability of epidurally administered drugs.

Besides the kinetic aspects, the magnitude of the transfer

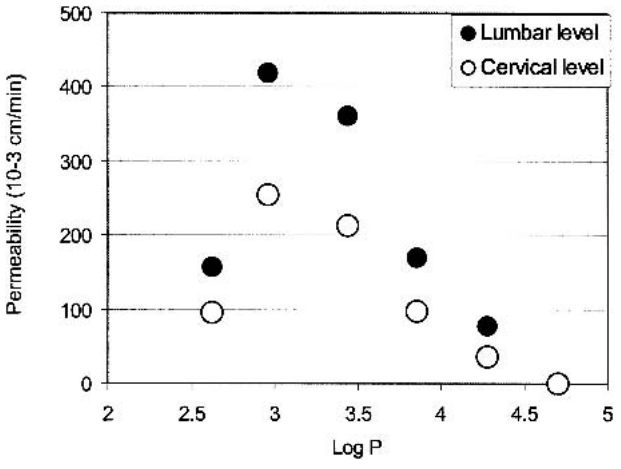

Fig. 5. Relationship between the *ex vivo* apparent permeability coefficient through cervical and lumbar spinal meninges in rabbits and log P of homologous pipecoloxylidide local anesthetics.

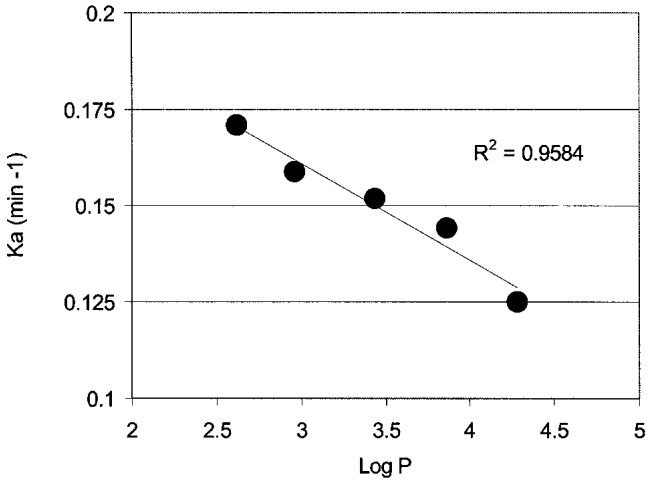

Fig. 6. Relationship between the *in vivo* absorption rate constant (K_a, min^{-1}) through spinal meninges in rabbits and log P of homologous pipecoloxylidide local anesthetics.

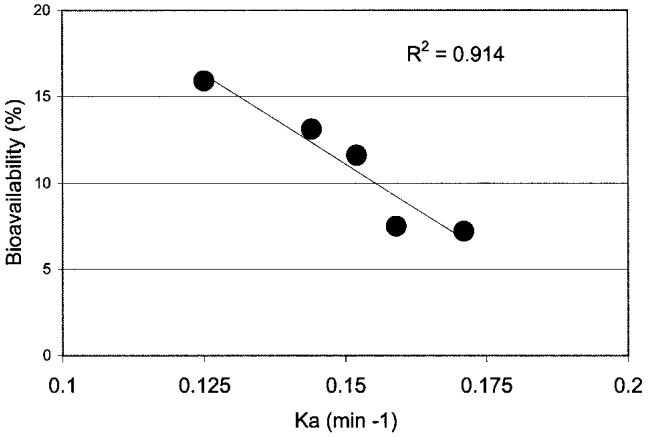

Fig. 7. Relationship between intrathecal bioavailability (%) and *in vivo* absorption rate constant (K_a, min^{-1}) through cervical spinal meninges of homologous pipecoloxylidide local anesthetics in rabbits.

through the spinal meninges following epidural administration has to be considered, as it governs the magnitude of the pharmacological effect. The intrathecal bioavailability of five pipecoloxylidide derivatives (methyl to pentyl derivatives) was low (7.2 to 15.9%) in accordance with previous published data (12,15–21). Moreover, it should be mentioned that the hexyl derivative was not detected in the intrathecal space, whereas it was measured in the epidural space, in agreement with our *ex vivo* experiment that displayed its lack of meningeal permeability.

A striking aspect of spinal disposition is that the transfer rate decreased with lipophilicity whereas the intrathecal bioavailability increased with lipophilicity, displaying a strong linear relationship with log P (r^2 = 0. 9698, data not shown). Such a relation was unexpected because, intuitively, the compounds displaying the more rapid transfer were thought to have the highest intrathecal bioavailability. The inverse linear relationship (Fig. 7, r^2 = 0.914) between intrathecal bioavailability and rate of transfer suggests some confounding factor. Such apparent discrepancy should find an explanation by considering the relative complexity of the epidural disposition of drugs that involves competitive processes of elimination in CSF and in blood and distribution in epidural fat. We have shown *in vivo* that the rate of transfer through the spinal meninges decreased with lipophilicity. Although the rate of transfer through the epidural capillary vessel walls was not investigated, it can reasonably be considered that it should also decrease with lipophilicity given the fact the basic mechanisms of membrane permeation are similar (i.e., passive diffusion through phospholipid bilayers). Furthermore, drug uptake into the blood circulation after epidural administration predominates over drug uptake in CSF given the low intrathecal bioavailability . Hence, it can be assumed that the more lipophilic compounds—that have a lower permeability—are proportionally less eliminated in the blood circulation—the main epidural clearance process—and are thus more available for a transfer into CSF through the meninges. Moreover, the lipophilic compounds have a higher epidural residence time that could favor the transfer in CSF. These findings support previous work from our laboratory showing that bupivacaine had a lower intrathecal bioavailability than lidocaine although its absorption rate was higher (12). However, it should be mentioned that these two local anesthetics are not structurally related unlike those studied in the current work. This assumption cannot be challenged by data in the literature because this is the first comparative study of intrathecal bioavailability of a series of homologous compounds. However, it should be mentioned that a recent report dealing with opioids concluded the opposite, that is, that less hydrophobic drugs should have a greater intrathecal bioavailability (14). However, this assumption was inferred from the intrathecal AUC after epidural administration which is not *per se* an index of bioavailability but has to be considered in connection with the AUC following intrathecal administration, which was not investigated in their study. Indeed, considering only the intrathecal AUC after epidural administration in our work would have led to an erroneous conclusion (see Table IV).

In conclusion, the current work has shown, on an *ex vivo* model of isolated spinal meninges, a parabolic relationship between apparent permeability and lipophilicity in a homologous series of six compounds including three marketed local anesthetic drugs. The *in vivo* investigation has shown different epidural disposition patterns as a function of drug lipophilicity; the relative weight of the distribution process compared to the elimination process increasing with the lipophilicity. Moreover, the absorption rate constant through spinal meninges from epidural to intrathecal space displayed a linear decrease with increasing lipophilicity. The most striking finding was the fact that the intrathecal bioavailability, which was low, increased when the absorption rate decreased as a result of specific competitive clearance and distribution processes in the epidural space. Such findings may be of interest for the design of new chemical entities acting on the spinal cord that are intended to be administered by the epidural route.

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