ABSORPTION OF BUPIVACAINE, ETIDOCaine, LIGNOCaine AND ROPIVACAINE INTO N-HEPTANE, RAT SCIATIC NERVE, AND HUMAN EXTRADURAL AND SUBCUTANEOUS FAT

P. H. ROSENBERG, J. KYTTÄ AND A. ALILA

Partitioning between n-heptane and phosphate buffer (pH 7.4 at room temperature) is often referred to when comparing the lipid solubilities of local anaesthetics (Covino and Vassallo, 1976; Tucker and Mather, 1980). However, for a better understanding of the differences between local anaesthetics in the onset, intensity and spread of blockade, it would be helpful to know also their relative absorptions into non-neural tissues at the site of injection. We investigated the partitioning of four amide local anaesthetics between buffer and: n-heptane, rat sciatic nerve tissue, human extradural fat and human subcutaneous fat. Ropivacaine (AL-381), an experimental amide local anaesthetic (Rosenberg and Heinonen, 1983) was compared with bupivacaine, etidocaine and lignocaine. Ropivacaine and bupivacaine are structurally related: the former is the L form of 1-propyl-2',6'-pipecoloxylidide, and the latter is racemic (DL) 1-butyl-2',6'-pipecoloxylidide.

MATERIALS AND METHODS

Local anaesthetics

Bupivacaine hydrochloride, etidocaine hydrochloride and lignocaine hydrochloride were donated by Astra Pharm. Comp (Södertälje, Sweden). Ropivacaine hydrochloride (AL-381) was obtained from Apothekernes Laboratorium (Oslo, Norway).

SUMMARY

Absorption of four amide local anaesthetics, including a new experimental agent, ropivacaine, in n-heptane, rat sciatic nerve and human extradural and subcutaneous fat was studied in vitro. The relative n-heptane|buffer (37 °C) partitioning of bupivacaine:etidocaine:lignocaine:ropivacaine was 10:39:1:2.9. The absorption of bupivacaine and etidocaine into nerve tissue was identified at steady state, but in extradural and subcutaneous fat etidocaine was taken up significantly more than bupivacaine. The lowest uptake was with lignocaine, and at steady state its mean concentrations were similar in all three tissues. There was a significantly higher concentration of ropivacaine than lignocaine in all tissues. Mean tissue concentrations of bupivacaine in sciatic nerve and subcutaneous fat, on the other hand, were significantly higher than those of ropivacaine. The ratios of relative mean uptake (mg g⁻¹), at steady state, of bupivacaine, etidocaine, lignocaine and ropivacaine were: sciatic nerve 3.3:4:1:1.8, extradural fat 4.1:8.3:1:2.3 and subcutaneous fat 3.8:10.6:1:1.9, all of which were clearly lower than the theoretical n-heptane|buffer partitioning ratios.

Plan of investigation

The buffer used in the studies on absorption contained NaCl 136 mmol litre⁻¹, KCl 5.6 mmol litre⁻¹, CaCl₂ 2.2 mmol litre⁻¹, MgCl₂ 1.3 mmol litre⁻¹, Na₂HPO₄ 1.3 mmol litre⁻¹, d-glucose 10 mmol litre⁻¹ and Tris-HCl 20 mmol litre⁻¹. At 37 °C pH was 7.4.

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For the n-heptane/buffer partitioning studies 1 mg of local anaesthetic was dissolved in 5 ml of buffer, and n-heptane 5 ml (Uvasol grade, Merck Co., Darmstadt, FRG) was added. Incubation was performed with the test tubes in a rotation mixer for 20 min at 37 °C. For the determination of local anaesthetic concentration, samples were taken from the aqueous phase.

Extradural fat (approximately 20–75 mg) was obtained from patients (36–58 yr) undergoing surgery for herniated intervertebral disc. Subcutaneous fat (50–200 mg) was obtained from the same patients, or from other patients in the same age range.

Sciatic nerves were carefully dissected out from rats anaesthetized with halothane. The nerves were immediately immersed in buffer saturated with 95% oxygen in carbon dioxide and desheathed.

The tissue samples were incubated in glass test tubes containing 1 ml of 1-mmol litre⁻¹ local anaesthetic in buffer for every 20 mg of tissue. The incubation medium was stirred constantly, bubbled with oxygen in carbon dioxide and the temperature kept at 37 °C. After incubation for 10, 20, 40 or 60 min, the tissues were blotted on filter paper (MN 640 dE, Macherey, Nagel & Co., Düren, FRG) for exactly 5 s, reweighed and homogenized in a small volume of buffer using a glass piston homogenizer.

The local anaesthetic concentrations were determined by gas chromatography using the method of Mather and Tucker (1974), with some minor modifications. A Varian 1400 gas chromatograph, equipped with a flame ionization detector and a glass column filled with 3% OV-17 on Chromosorb W (100–120 mesh), was used. The carrier gas was nitrogen 30 ml min⁻¹ and one of the other local anaesthetics served as internal standard. The detection limit was 0.01 μg of local anaesthetic when the samples had been homogenized in buffer; the amounts of local anaesthetic assayed were at least 100 times greater. A standard curve was constructed each time. At least four and up to eight studies were performed for each absorption value, representing samples from two to five patients or rats for each value, except with sciatic nerve tissue incubated with bupivacaine for 20 min or etidocaine for 20 min, when studies were undertaken with only three nerves in each case.

Small pieces of extradural and subcutaneous fat from three patients were fixed in buffered formalin for examination by light microscopy.

Student’s t test was used for the evaluation of statistical differences between the mean concentrations of local anaesthetic.

RESULTS

The results of the partitioning of the local anaesthetics in n-heptane are presented in table I and of their absorption in the tissues in figures 1–3.

Increasing the incubation time from 10 min to 40 min resulted in a similar enhancement in the uptake of bupivacaine and etidocaine in sciatic nerve tissue (fig. 1), while that of ropivacaine and

### Table I. Partitioning between n-heptane and buffer (37 °C) of amide local anaesthetics (means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>n-Heptane/Buffer n</th>
<th>μg ml⁻¹</th>
<th>μg ml⁻¹</th>
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<tbody>
<tr>
<td>Bupivacaine</td>
<td>6</td>
<td>20.5 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Etidocaine</td>
<td>6</td>
<td>82.8 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Lignocaine</td>
<td>6</td>
<td>2.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Ropivacaine</td>
<td>7</td>
<td>6.1 ± 0.6</td>
<td></td>
</tr>
</tbody>
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![Fig. 1. Concentrations (mean ± SD) of bupivacaine (●), etidocaine (○), ropivacaine (*) and lignocaine (△) in rat sciatic nerve after incubation at 37 °C in buffer containing 1 mmol litre⁻¹ of the local anaesthetic.](https://academic.oup.com/bja/article-abstract/58/3/310/285865/download)
lignocaine remained essentially unchanged. In extradural fat, initial absorptions of both bupivacaine and etidocaine were similarly rapid, reaching mean maximal or near maximal concentrations within 10 min (fig. 2). An increase in the incubation time to 40 min produced a separation in mean concentrations of the local anaesthetics \( P < 0.05 \) except for bupivacaine and ropivacaine. In subcutaneous fat, there was almost a four-fold increase in the mean uptake of etidocaine, while that of bupivacaine increased only about 1.5 times between 10 and 40 min (fig. 3). Ropivacaine and lignocaine were absorbed to a much smaller degree \( P < 0.01 \), and concentrations of lignocaine in the different tissues were remarkably similar, for example at 40 min the concentration in sciatic nerve tissue was 0.32 mg g\(^{-1}\), in extradural fat 0.33 mg g\(^{-1}\) and in subcutaneous fat 0.32 mg g\(^{-1}\).

Comparison of the greatest mean uptake (etidocaine) with the smallest (lignocaine) in the different tissues after 40 min incubation (assumed steady state) produced the following ratios: sciatic nerve tissue 4, extradural fat 8.3 and subcutaneous fat 10.6.

Microscopic examination showed that the extradural fat samples contained predominantly large fat globules and intermingled capillaries and connective tissue streaks. Connective tissue and small blood vessels were also abundant in subcutaneous fat.

**DISCUSSION**

The relative lipid solubility of bupivacaine, etidocaine and lignocaine obtained in the present n-heptane/buffer partitioning studies was 20.5:83.8:2.1 (10:39:1), which is similar to figures based on partitioning between n-heptane and phosphate buffer at room temperature, 27:128:3 (9:44:1) or 28:141:3 (10:49:1) (Covino and Vassallo, 1976; Mather and Tucker, 1978). Comparing our results with those of others indicates that etidocaine partitions more in a
Ringer-type buffer than in the usual phosphate buffer. Also, a change in temperature from 37 °C to room temperature (23–24 °C) may markedly increase the solubility of local anaesthetics and result in temperature-related variability in partitioning between two chemically different solvents.

Increasing the incubation time markedly enhanced the uptake of the most lipophilic agents, etidocaine and bupivacaine, in subcutaneous fat, in contrast to that in extradural fat in which the maximal concentration seemed to be reached at an early stage of incubation. This may be, in part, a reflection of the size of the tissue samples, the subcutaneous fat pieces being up to 10 times larger (by weight) than the pieces of extradural fat. However, saturation was apparently achieved by 40 min in all tissues.

The large standard deviations of the local anaesthetic concentrations, particularly in the case of the most lipophilic agents, were probably related to a great interindividually variation in both the macroscopic and microscopic appearance of the fatty tissues (Ramsey, 1959).

Up to 20 min of incubation, there were quite small differences between the mean absorptions of the four local anaesthetics in sciatic nerve tissue. At 40 min the agents had separated into two groups, the mean concentrations of etidocaine and bupivacaine being about twice those of ropivacaine and lignocaine. In comparison with the uptake in fatty tissues or n-heptane there seemed to be a better correlation between the uptake in sciatic nerve tissue and in vitro protein-binding capacity, which is almost identical for etidocaine and bupivacaine, and about 30% lower for lignocaine (Covino and Vassallo, 1976).

The fact that etidocaine and bupivacaine, incubated at identical concentrations, behaved similarly in their partitioning in nerve tissue, whereas etidocaine was taken up significantly more in the fat tissues, supports the well-known clinical picture of differences between these two agents. For instance, in order to produce satisfactory regional nerve blockade etidocaine has to be administered in solutions of twice the concentration of bupivacaine (Bridenbaugh et al., 1973; Tammisto, Rosenberg and Tigerstedt, 1975). Twelve hours after extradural administration of etidocaine to sheep, extradural fat contained considerable concentrations of etidocaine (Tucker and Mather, 1980).

It was interesting to observe that, after reaching equilibrium, the concentrations of lignocaine were similar in the three tissues. This may be related to the properties of the lignocaine molecule and is supported by the recent finding that there is no difference in the diffusion of lignocaine into myelinated and unmyelinated nerve fibres (Fink and Cairns, 1984). With the more lipid soluble local anaesthetics there may be a difference in penetration related to the high lipid content of myelin (Guidotti, 1972).

The molecular similarity of bupivacaine and ropivacaine suggests similarities in their physical and nerve blocking characteristics. However, ropivacaine is clearly less lipid soluble although, on the other hand, it has been shown to block myelinated A-delta nerve fibres more readily than bupivacaine (Rosenberg and Heinonen, 1983). Since the pKa values of both agents are almost identical (ropivacaine 8.0, bupivacaine 8.1) other characteristics which promote membrane penetration may be involved. It is possible that, in comparison with bupivacaine, the smaller molecular size with less lipid solubility of ropivacaine would assist in the penetration all the way to the sodium channels. In contrast, probably as a result of higher tissue binding (protein and lipid) experimental nerve blockade with bupivacaine has been shown to be slightly longer than that with racemic 1-propyl-2',6'-pipecoloxylide (Åberg, Dhuner and Sydnes, 1978).

In conclusion, solubility in a pure organic solvent may not be a good predictive test of clinical differences between local anaesthetic agents. There was much less variation in the absorption of amide local anaesthetics into fatty tissues than in n-heptane. Ropivacaine, an amide local anaesthetic of the pipecoloxylide type, was found to be intermediate to bupivacaine and lignocaine in uptake into n-heptane and fatty tissues.

The high affinity for biological fat of the lipophilic local anaesthetics can prevent a significant portion of an injected dose from reaching its site of action. In patients, interindividually variability in the amount and structure of extraneural fatty tissue may be a major reason for varying spread and intensity of regional anaesthesia with bupivacaine and etidocaine.

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REFERENCES


