

# Pharmacokinetics of Lidocaine, Bupivacaine, and Levobupivacaine in Plasma and Brain in Awake Rats

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## ABSTRACT

**Background:** We have compared the pharmacokinetics and brain distribution of lidocaine, racemic bupivacaine (bupivacaine), and levobupivacaine in awake, spontaneously breathing rats.

**Methods:** Lidocaine ( $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), bupivacaine ( $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), or levobupivacaine ( $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was continuously administered to rats for 2 h ( $n = 12$ , each anesthetic). Blood samples and cerebral dialysate were collected during infusion and for 2 h after termination of infusion. Concentrations of anesthetics in the cerebral extracellular fluid were measured by microdialysis using the retrodialysis calibration method. Tissue-to-plasma partition coefficients calculated from the total (protein-bound and unbound) and unbound concentrations in plasma and brain as well as pharmacokinetic parameters in plasma and cerebral extracellular fluid were compared among the three anesthetics.

**Results:** There were no differences in plasma total or unbound concentrations between bupivacaine and levobupivacaine. Concentrations of bupivacaine in the cerebral extracellular fluid were significantly higher than levobupivacaine ( $P < 0.001$ ). Despite no differences in the ratio of total brain concentration to total plasma concentration among the three anesthetics, the ratio of cerebral extracellular fluid concentration to plasma unbound fraction of bupivacaine was significantly higher than lidocaine and levobupivacaine ( $0.58 \pm 0.09$ ,  $0.47 \pm 0.18$ , and  $0.40 \pm 0.09$ , respectively;  $P = 0.03$  and  $0.003$ , respectively).

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**Conclusions:** Although the ratio of total brain concentration to total plasma concentrations of lidocaine, bupivacaine, and levobupivacaine was similar, concentration ratio of bupivacaine in the cerebral extracellular fluid to plasma unbound fraction was significantly higher than lidocaine and levobupivacaine.

## What We Already Know about This Topic

- ❖ Although local anesthetics can cause central nervous system toxicity, the distribution of bupivacaine from plasma to brain has been incompletely studied

## What This Article Tells Us That Is New

- ❖ In awake, spontaneously breathing rats receiving intravenous infusion, the ratio of the cerebral extracellular fluid concentration to the plasma unbound fraction was greater for bupivacaine than for levobupivacaine or lidocaine

CENTRAL nervous system (CNS) and cardiovascular toxicities are life-threatening side effects of local anesthetics. Lidocaine and bupivacaine are commonly used for epidural anesthesia/analgesia and nerve blockade. Bupivacaine exerts higher toxicity as well as stronger anesthetic potency compared with lidocaine, and differences exist in the toxicity between its dextro and levorotatory stereoisomers.<sup>1</sup> Although CNS plays an important role in inducing cardiovascular toxicity of bupivacaine<sup>2</sup> and an increase in the cerebral extracellular fluid (ECF) concentrations across the blood–brain barrier secondary to the increase in plasma concentrations may contribute to induce CNS toxicity, relationships between plasma and cerebral extracellular concentrations of bupivacaine are not known. We have examined the relationships of the concentrations of intravenously administered lidocaine, racemic bupivacaine (bupivacaine), and levobupivacaine between plasma and cerebral ECF in awake, spontaneously breathing rats.

## Materials and Methods

### Animal Preparation

All the study protocols were approved from the Institutional Animal Care and Use Committee (Osaka, Japan). In the first experiment, 36 male Sprague–Dawley rats aged 8–10 weeks

and weighing 350–400 g (CLEA Japan, Inc., Tokyo, Japan) were included. The guide cannulas for microdialysis (GI-12; Eicom, Kyoto, Japan) were inserted so that those tips were placed into the nucleus accumbens 2–3 days before experiments following the previously reported method.<sup>3</sup> On the day of experiments, the carotid artery and the cervical vein were cannulated with polyethylene catheters for measuring blood pressure, heart rate, blood sampling, and infusing drugs, besides the microdialysis probe (A-UI-12-02; Eicom), which was inserted through the guide cannula and was perfused with the artificial cerebrospinal fluid containing internal standards.

### Experimental Protocol

After full recovery from anesthesia, animals were randomly divided into three groups receiving lidocaine, bupivacaine, and levobupivacaine ( $n = 12$ , each group). After confirming the stable rate of loss of the internal standards in the cerebral dialysate and baseline hemodynamic measurement, continuous infusion of lidocaine ( $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), bupivacaine ( $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), or levobupivacaine ( $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) *via* the intravenous catheter was started, which lasted for 2 h. Infusion rates of local anesthetics were determined based on our previous reports, and preliminary studies not to induce hemodynamic changes or the CNS toxicity such as excitation, hyperventilation, or convulsions.<sup>4,5</sup> Mean arterial blood pressure and heart rate were continuously monitored, and arterial blood samples (0.5 ml) were drawn before infusion of drugs (baseline), 15, 30, 45, 60, 90, 120, 135, 150, 165, 180, 210, and 240 min after starting intravenous infusion for measuring plasma concentrations, with the same volume of drug-free blood obtained from another rat being transfused to prevent blood dilution and hemodynamic changes. Blood gas was measured at baseline, at the end of the infusion of anesthetics (120 min), and at the end of experiments (240 min).

In the second experiment, total contents of local anesthetics in the whole brain tissue were measured in 15 male Sprague–Dawley rats. Catheters were inserted into the cervical vein under general anesthesia with sevoflurane. After recovery from anesthesia, they received the same solution of lidocaine, bupivacaine, or levobupivacaine at the same infusion rate as in the first experiment ( $n = 5$  for each anesthetic). They were euthanized with 100 mg/kg thiopental 120 min after starting the infusion and killed by decapitation. After immediate removal of the brain, surface blood vessels were cleaned and dissected on moist filter paper and kept at  $-70^\circ\text{C}$  until analysis.

### In Vitro and In Vivo Microdialysis Probe Calibration Study

For quantitative measurement of the extracellular concentration of local anesthetics in the brain, we used a retrodialysis technique based on the principle that relative loss (RL) of an internal standard is related to the relative recovery of the solutions of interest. The dialysis probe was calibrated *in*

*vitro* and *in vivo* using 3-hydroxybupivacaine ( $0.5 \text{ } \mu\text{g/ml}$ ) for measuring lidocaine or ropivacaine ( $0.2 \text{ } \mu\text{g/ml}$ ) for measuring bupivacaine and levobupivacaine as internal standards following a previously reported method.<sup>3</sup> The *in vivo*  $K$  factor, defined as the ratio of RL of 3-hydroxybupivacaine ( $\text{RL}_{\text{OHBU}}$ ) to RL of lidocaine ( $\text{RL}_{\text{LID}}$ ) was  $1.05 \pm 0.01$ , and the ratios of RL of ropivacaine ( $\text{RL}_{\text{ROP}}$ ) to RL of bupivacaine ( $\text{RL}_{\text{BU}}$ ) and to RL of levobupivacaine ( $\text{RL}_{\text{LEVO}}$ ) were  $1.11 \pm 0.05$  and  $1.09 \pm 0.04$ , respectively ( $n = 5$ ), and the mean values were used for calculation. Concentrations of lidocaine in the cerebral ECF ( $\text{LID}_{\text{ex}}$ ) were calculated as  $\text{LID}_{\text{dialysate}} \times K/\text{RL}_{\text{OHBU}}$ , where  $\text{LID}_{\text{dialysate}}$  is the concentration of lidocaine in the dialysate.<sup>6</sup> Concentrations of bupivacaine and levobupivacaine in the cerebral ECF were also calculated using the same equation with their concentrations in the dialysate,  $K$  factor, and  $\text{RL}_{\text{ROP}}$ . Relative recovery of lidocaine and  $\text{RL}_{\text{OHBU}}$ , bupivacaine and  $\text{RL}_{\text{ROP}}$ , and levobupivacaine and  $\text{RL}_{\text{ROP}}$  were almost equal and not affected by the extracellular concentrations of the local anesthetics of interest.

### Analysis of Plasma and Intracerebral Local Anesthetic Concentrations

Concentrations of total (protein bound and unbound) and unbound lidocaine in plasma as well as total lidocaine in the brain were determined by liquid chromatography–mass spectrometry (4000QTRAP, Applied Biosystems, Foster City, CA), according to previously reported methods.<sup>5</sup> For measuring bupivacaine and levobupivacaine, midazolam ( $3 \text{ } \mu\text{M}$ ,  $100 \text{ } \mu\text{l}$ ) was added to the samples as an internal standard and measured by mass spectrometry at  $m/z$  288.8, 288.8, and 326.7, respectively. Unbound fraction in plasma was measured after ultrafiltration using a membrane (YM-30; Millipore Corporation, Billerica, MA). Concentrations of unbound anesthetics in the cerebral dialysate were measured by a previously reported method with small modifications.<sup>3</sup> Briefly, indwelling microdialysis probes were perfused with the cerebrospinal fluid at a rate of  $2 \text{ } \mu\text{l/min}$ , and the dialysate was collected for every 5 min. Dialysates were kept at  $4^\circ\text{C}$  and injected onto the high-performance liquid chromatograph equipped with electrochemical detector (HTEC-500; Eicom) on the same day of experiments. Calibration curves for lidocaine were constructed for each run over the range of  $0.02$ – $20 \text{ } \mu\text{g/ml}$ , and calibration curves for both bupivacaine and levobupivacaine were  $0.02$ – $2.0 \text{ } \mu\text{g/ml}$ . The values of  $r^2$  were greater than 0.999 for all anesthetics, and the limit of detection was  $0.01 \text{ } \mu\text{g/ml}$ . Within-day and day-to-day coefficients of variation of lidocaine, bupivacaine, and levobupivacaine were less than 6%. We have examined the concordance of the concentrations of local anesthetics measured by electrochemical detection and mass spectrometry; the details are described in the appendix.

### Pharmacokinetic Analysis

Pharmacokinetic parameters of lidocaine, bupivacaine, and levobupivacaine in plasma and in the cerebral ECF were

calculated by noncompartmental analysis based on the data obtained from the end of infusion until the last sampling time using WinNonlin Professional 5.1 (Pharsight Corporation, Mountain View, CA) as reported previously.<sup>3</sup> We estimated the three different tissue-to-plasma partition coefficients: the ratio of the total brain concentration to total plasma concentration ( $K_p$ ), the ratio of the total brain concentration to unbound plasma concentration ( $K_{p,u}$ ), and the ratio of the unbound brain concentration to unbound plasma concentration ( $K_{p,uu}$ ) as reported by Gupta *et al.*<sup>7</sup> using the following equations:

$$K_p = \text{AUC}_{\text{tot,br}}/\text{AUC}_{\text{tot,pl}} = (\text{AUC}_{\text{u,brECF}}/f_{\text{u,brECF}})/\text{AUC}_{\text{tot,pl}} \quad (1)$$

$$K_{p,u} = \text{AUC}_{\text{tot,br}}/\text{AUC}_{\text{u,pl}} = (\text{AUC}_{\text{u,brECF}}/f_{\text{u,brECF}})/\text{AUC}_{\text{u,pl}} \quad (2)$$

$$K_{p,uu} = \text{AUC}_{\text{u,brECF}}/\text{AUC}_{\text{u,pl}} \quad (3)$$

where  $\text{AUC}_{\text{tot,br}}$  is area under the concentration–time curve (AUC) from time 0 to infinity of total lidocaine, bupivacaine, or levobupivacaine in the whole brain;  $\text{AUC}_{\text{tot,pl}}$  and  $\text{AUC}_{\text{u,pl}}$  are AUC of the total and unbound concentrations in plasma, respectively.  $\text{AUC}_{\text{u,brECF}}$  denotes the AUC of the unbound concentrations in the cerebral ECF obtained in the microdialysis study.  $\text{AUC}_{\text{tot,br}}$  is not able to be directly measured *in vivo* and expressed as  $\text{AUC}_{\text{u,brECF}}/f_{\text{u,brECF}}$ ,<sup>7</sup> where  $f_{\text{u,brECF}}$  is the ratio of unbound concentrations in cerebral ECF to the total amount per gram of brain tissue and is also expressed as  $1/V_{\text{u,br}}$ .<sup>7</sup>  $V_{\text{u,br}}$  stands for the unbound volume of distribution in the brain and is calculated using the following equation<sup>8</sup>:

$$V_{\text{u,br}} = (A_{\text{br}} - V_{\text{bl}} \cdot C_{\text{bl}})/C_{\text{u,brECF}} \quad (4)$$

where  $A_{\text{br}}$  is the total amount of the local anesthetics per gram of the brain, which was measured in the second experiment.  $V_{\text{bl}}$  is the volume of blood per gram of brain, which was assumed to be 15  $\mu\text{l/g}$  brain.<sup>9</sup>  $C_{\text{bl}}$  and  $C_{\text{u,brECF}}$  are total concentrations in plasma and unbound concentration in the cerebral ECF, respectively, measured at the end of infusion (120 min) in the first experiment. Calculated  $V_{\text{u,br}}$  and  $f_{\text{u,brECF}}$  were assumed to be constant with time (0–240 min).<sup>7</sup> The fraction of local anesthetics bound to erythrocytes was ignored because the content of anesthetics in the blood vessels in the brain expressed as the volume of blood in the brain ( $V_{\text{bl}}$ ) multiplied by total plasma concentrations ( $C_{\text{bl}}$ ) is much smaller than the total amount in the brain ( $A_{\text{br}}$ ).

### Statistical Analysis

The number of animals in each group was determined based on our preliminary study ( $n = 6$ ) in which AUC of levobupivacaine was  $5.0 \pm 0.9 \text{ min} \cdot \mu\text{g} \cdot \text{ml}^{-1}$ . On the basis of the formula for normal theory and assuming a type I error protection of 0.05 and a power of 0.80 allowing us to detect a 25% change in the cerebral extracellular concentrations, 12 animals were required for each group. All values are expressed as means  $\pm$  SD.

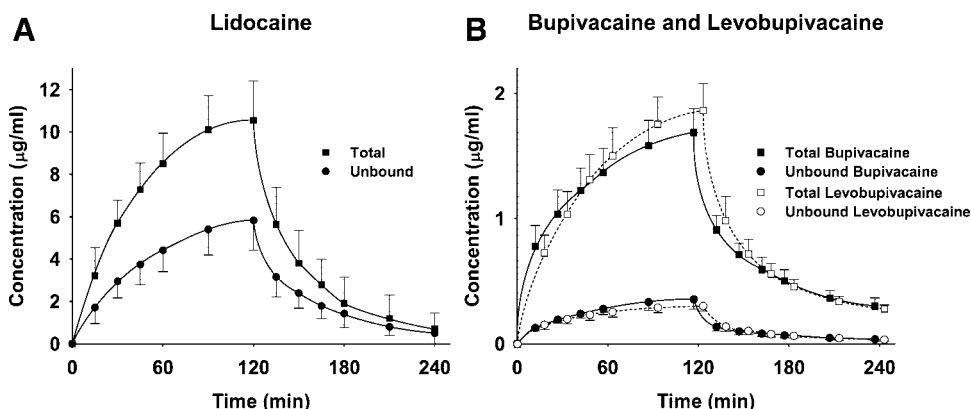
Statistical analysis was performed using SigmaStat 3.0 (Systat Software, Inc., Richmond, CA). Differences in mean arterial blood pressure, heart rate, hemoglobin content, and blood gas data during the whole experiments were examined by two-factor repeated-measure ANOVA using the agents and measurement times as factors, followed by Student–Newman–Keuls test for multiple comparisons. Differences in the concentrations of bupivacaine and levobupivacaine in plasma and in the cerebral ECF during the whole experiments were examined by one-factor repeated-measure ANOVA. Pharmacokinetic parameters of anesthetics in plasma, cerebral ECF, tissue-to-plasma partition coefficients ( $K_p$ ,  $K_{p,u}$ , and  $K_{p,uu}$ ), and the unbound volume of distribution in the brain ( $V_{\text{u,br}}$ ) were compared with one-factor ANOVA, followed by Student–Newman–Keuls test for multiple comparisons. Values of  $P$  less than 0.05 were considered significant.

### Results

During experiments, symptoms of CNS toxicity such as excitation, depression, or convulsions were not observed while all animals were awake at the end of experiments. There were no significant differences in baseline, end-infusion, and end-experiment values for mean arterial blood pressure, heart rate, hemoglobin content, blood gas data, or between-drug groups for these parameters (data not shown).

Plasma concentrations of total and unbound lidocaine, bupivacaine, and levobupivacaine reached the highest levels at the end of infusion (120 min; figs. 1A and B). Peak plasma concentration divided by dose ( $C_{\text{max}}/\text{dose}$ ) of both total and unbound lidocaine was significantly higher than total and unbound bupivacaine and levobupivacaine, respectively ( $P < 0.05$  for both). There were no differences in the elimination half-time ( $t_{1/2}$ ) or mean residence time among total lidocaine, bupivacaine, and levobupivacaine or among their unbound fractions in plasma (table 1). There were no differences in clearance or volume of distribution at steady state ( $V_{\text{d,ss}}$ ) among total lidocaine, bupivacaine, and levobupivacaine; however, both these parameters of unbound lidocaine were significantly lower than those of bupivacaine and levobupivacaine ( $P < 0.001$  for all). Plasma concentrations of total bupivacaine and levobupivacaine and of unbound bupivacaine and levobupivacaine were comparable, and there were no differences in the pharmacokinetic parameters between these two anesthetics (fig. 1B and table 1). Protein-binding ratios of lidocaine, bupivacaine, and levobupivacaine in plasma calculated as  $(\text{AUC}_{\text{tot,pl}} - \text{AUC}_{\text{u,pl}})/\text{AUC}_{\text{tot,pl}}$  were  $41 \pm 14\%$ ,  $86 \pm 3\%$ , and  $86 \pm 4\%$ , respectively ( $n = 12$ ).

Concentrations of lidocaine, bupivacaine, and levobupivacaine in the cerebral ECF were also highest at the end of infusion (fig. 2).  $C_{\text{max}}/\text{dose}$  of lidocaine in the cerebral ECF was significantly higher than those of bupivacaine and levobupivacaine ( $P < 0.001$  for both). In contrast to plasma,  $C_{\text{max}}/\text{dose}$ ,  $\text{AUC}_{0-\infty}/\text{dose}$ , and the concentrations of bupivacaine in the cerebral ECF throughout the experiments were significantly higher than levobupi-



**Fig. 1.** Plasma concentration–time profiles of total and unbound lidocaine, bupivacaine, and levobupivacaine. (A) Plasma concentrations of total (protein bound and unbound) and unbound lidocaine. (B) Plasma concentrations of total and unbound racemic bupivacaine (bupivacaine) and levobupivacaine. Data are expressed as the mean  $\pm$  SD of 12 experiments. There were no differences between total bupivacaine and levobupivacaine or between unbound bupivacaine and levobupivacaine concentrations.

vacaine ( $P = 0.006$ ,  $P = 0.04$ , and  $P < 0.001$ , respectively; table 2 and fig. 2B). Apparent clearance (clearance divided by bioavailability,  $Cl/F$ ) and apparent volume of distribution (volume of distribution divided by bioavailability,  $Vd/F$ ) of lidocaine were significantly smaller relative to those of both bupivacaine and levobupivacaine ( $P < 0.001$  for all). Both  $Cl/F$  and  $Vd/F$  of bupivacaine were significantly smaller compared with those of levobupivacaine ( $P < 0.001$  and  $P = 0.04$ , respectively). There were no differences in  $t_{1/2}$  or mean residence time among lidocaine, bupivacaine, and levobupivacaine.

There were no differences in  $K_p$  among the three anesthetics ( $P = 0.9$ , fig. 3). On the other hand,  $K_{p,u}$  of both bupivacaine and levobupivacaine was significantly higher than lidocaine ( $P < 0.001$  for both), with no differences being observed between bupivacaine and levobupivacaine.  $K_{p,uu}$  of bupivacaine was also significantly higher than that of lidocaine and levobupivacaine ( $P = 0.03$  and  $0.003$ , respectively). Contents of lidocaine, bupivacaine, and levobupivacaine in the whole brain at 120 min after starting infusion ( $A_{br}$ ) measured in our second experiment were  $23.1 \pm 2.6$ ,  $6.8 \pm 1.5$ , and  $6.6 \pm 1.8$   $\mu\text{g/g}$  brain, respectively.  $V_{u,br}$  of levobupivacaine was signifi-

cantly larger than lidocaine and bupivacaine ( $P < 0.001$  for both).

### Discussion

In this study, we measured the concentrations of lidocaine, bupivacaine, and levobupivacaine in plasma, brain, and cerebral ECF. Although there were no differences between the concentrations of bupivacaine and levobupivacaine in plasma, levels of unbound bupivacaine in the cerebral ECF were significantly higher than levobupivacaine. Pharmacokinetic analysis has also revealed differences between bupivacaine and levobupivacaine only in the cerebral ECF and not in plasma. For elucidating these pharmacokinetic differences between plasma and cerebral ECF, we have calculated the tissue-to-plasma partition coefficients.

There were no differences in  $K_p$ , the ratio of AUC of total brain concentrations to AUC of total plasma concentrations, among the three anesthetics.  $K_p$  of these anesthetics was approximately 2–3, being comparable with the ratio of total concentrations in the brain homogenates against the concentrations in plasma in our previous studies,<sup>4,5</sup> suggesting that

**Table 1.** Pharmacokinetic Parameters of Lidocaine, Bupivacaine, and Levobupivacaine in Plasma

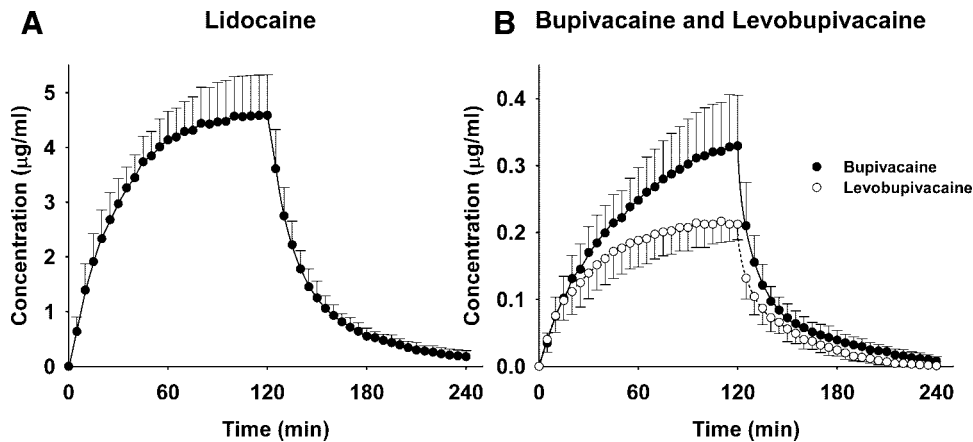
|  | Total         |                |                      | Unbound       |                        |                         |
|--|---------------|----------------|----------------------|---------------|------------------------|-------------------------|
|  | Lidocaine     | Bupivacaine    | Levobupivacaine      | Lidocaine     | Bupivacaine            | Levobupivacaine         |
| $C_{max}/\text{dose}$ (mg/ml)                                    | $174 \pm 30$  | $141 \pm 16^*$ | $155 \pm 18^\dagger$ | $95 \pm 23$   | $30 \pm 6^\ddagger$    | $25 \pm 3^\ddagger$     |
| $t_{1/2}$ (min)  | $43 \pm 12$   | $50 \pm 6$     | $49 \pm 3$           | $45 \pm 15$   | $45 \pm 5$             | $41 \pm 6$              |
| $AUC_{0-\infty}/\text{dose}$ (min $\cdot$ g $\cdot$ ml $^{-1}$ ) | $8.1 \pm 2.9$ | $7.8 \pm 1.1$  | $7.7 \pm 0.8$        | $4.6 \pm 1.4$ | $1.1 \pm 0.3^\ddagger$ | $1.0 \pm 0.3^\ddagger$  |
| Clearance (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ )           | $154 \pm 117$ | $129 \pm 16$   | $131 \pm 13$         | $236 \pm 114$ | $955 \pm 226^\ddagger$ | $1032 \pm 310^\ddagger$ |
| MRT (min)  | $64 \pm 20$   | $77 \pm 8$     | $73 \pm 5$           | $68 \pm 22$   | $66 \pm 8$             | $60 \pm 11$             |
| $Vd_{ss}$ ( $\times 10^3$ ml/kg)                                 | $2 \pm 1$     | $2 \pm 1$      | $2 \pm 1$            | $3 \pm 1$     | $13 \pm 6^\ddagger$    | $11 \pm 4^\ddagger$     |

Values are the mean  $\pm$  SD of 12 experiments.

\*  $P < 0.01$ ,  $^\dagger P < 0.05$ , and  $^\ddagger P < 0.001$  compared with lidocaine.

$AUC_{0-\infty}$  = area under the plasma concentration versus time curve from zero to infinity;  $C_{max}$  = peak plasma concentration; dose = total dose administered during experiments; MRT = mean residence time;  $t_{1/2}$  = elimination half-time;  $Vd_{ss}$  = volume of distribution at steady state.





**Fig. 2.** Cerebral extracellular fluid concentration–time profiles of lidocaine, bupivacaine, and levobupivacaine. (A) Cerebral extracellular fluid concentrations of lidocaine. (B) Cerebral extracellular fluid concentrations of bupivacaine and levobupivacaine. Data are expressed as the mean  $\pm$  SD of 12 experiments. Overall concentrations of bupivacaine were significantly higher than levobupivacaine ( $P < 0.001$ ).

the total content of these anesthetics in the brain; that is, sum of the protein-bound and unbound fractions in the cerebral ECF and fractions bound to brain parenchyma was higher than those in plasma.  $K_{p,u}$ , the ratio of AUC of total brain concentrations to AUC of unbound plasma concentrations accounts for the differences in binding to blood components.  $K_{p,u}$  of both bupivacaine and levobupivacaine was significantly higher than lidocaine, reflecting the lower unbound plasma concentrations resulting from higher protein binding ratio compared with lidocaine.  $K_{p,uu}$  denotes the ratio of AUC of unbound concentrations in the cerebral ECF to AUC of unbound plasma concentrations.  $K_{p,uu}$  of bupivacaine was significantly higher than levobupivacaine, resulting from higher concentrations in the cerebral ECF than levobupivacaine despite similar plasma concentrations.

**Table 2.** Pharmacokinetic Parameters of Lidocaine, Bupivacaine, and Levobupivacaine in the Cerebral Extracellular Fluid

|   | Lidocaine     | Bupivacaine       | Levobupivacaine  |
|---|---------------|-------------------|------------------|
| $C_{max}/\text{dose}$<br>(mg/ml)                                    | 76 $\pm$ 12   | 27 $\pm$ 6*†      | 18 $\pm$ 2*      |
| $t_{1/2}$ (min)   | 27 $\pm$ 5    | 28 $\pm$ 5        | 22 $\pm$ 8       |
| $AUC_{0-\infty}/\text{dose}$<br>(min $\cdot$ g $\cdot$ ml $^{-1}$ ) | 2.1 $\pm$ 0.4 | 0.64 $\pm$ 0.16*‡ | 0.41 $\pm$ 0.10* |
| Cl/F (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ )                   | 491 $\pm$ 82  | 1656 $\pm$ 381*†  | 2661 $\pm$ 1027* |
| MRT (min)   | 37 $\pm$ 7    | 37 $\pm$ 7        | 33 $\pm$ 6       |
| Vd/F ( $\times 10^3$ ml/kg)   | 19 $\pm$ 6    | 67 $\pm$ 16*‡     | 79 $\pm$ 17*     |

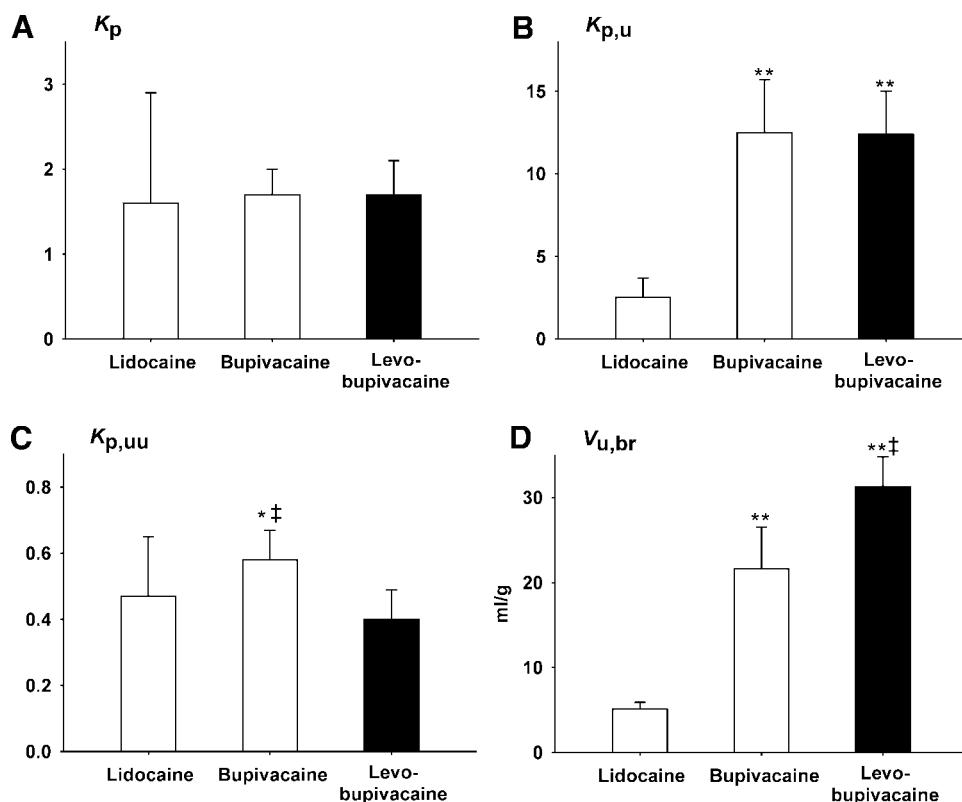
Values are the mean  $\pm$  SD of 12 experiments.

\*  $P < 0.001$  compared with lidocaine, †  $P < 0.01$  and ‡  $P < 0.05$  compared with levobupivacaine.

$AUC_{0-\infty}$  = area under the concentration vs. time curve from zero to infinity; Cl/F = apparent cerebral extracellular fluid clearance;  $C_{max}$  = peak concentration in the cerebral extracellular fluid; dose = total dose administered during experiments; MRT = mean residence time;  $t_{1/2}$  = elimination half-time; Vd/F = apparent volume of distribution, where F is the bioavailability.

Among the three parameters,  $K_p$  and  $K_{p,u}$  were calculated based on the AUC of total concentrations in the brain ( $AUC_{tot,br}$ , Eqs. 1 and 2), which was not able to be directly measured and calculated from the AUC of the unbound concentrations in the ECF ( $AUC_{u,brECF}$ ) and volume of distribution of the unbound fraction in the brain ( $V_{u,br}$ ).<sup>8</sup>  $V_{u,br}$  was calculated from the differences in the contents of anesthetics between the whole brain and blood vessels in the brain, divided by concentrations of unbound fraction in the cerebral ECF (Eq. 4). The mean  $V_{u,br}$  values of lidocaine, bupivacaine, and levobupivacaine were 5.1, 21.6, and 31.3 ml/g brain, respectively, and approximately 25- to 150-fold larger than the volume of the brain extracellular space, which is reported to be 0.12–0.20 ml/g brain.<sup>10,11</sup> These results suggest that all these anesthetics are extensively distributed intracellularly or bind to proteins in the ECF.

There are several studies examining the intracerebral concentration of anesthetic agents in awake animals<sup>3,12–14</sup>; unfortunately, however, tissue-to-plasma partition coefficients have not been examined in those studies. Regarding local anesthetics, previous studies<sup>13,14</sup> have been predominantly focused on epidural or intrathecal pharmacokinetics after epidural administration, whereas few studies examined the concentrations in the cerebral ECF,<sup>3</sup> diffused across the blood–brain barrier after intravenous administration. In this study, we have measured the concentrations in the brain followed by calculation of the tissue-to-plasma partition coefficients. Because intracerebral pharmacokinetics is susceptible to cerebral blood flow, we administered local anesthetics at rates too slow to induce CNS effects such as excitation and depression, thereby successfully maintaining blood gas levels during the whole experiments. Although concentrations of bupivacaine in the cerebral ECF were significantly higher than levobupivacaine, the total amount (tissue-bound and unbound) of bupivacaine in the brain was similar to levobupivacaine, suggesting that a higher ECF concentration



**Fig. 3.** Tissue-to-plasma partition coefficients and intracerebral volume of distribution of lidocaine, bupivacaine, and levobupivacaine. (A–C) Tissue-to-plasma partition coefficients ( $K_p$ ,  $K_{p,u}$ , and  $K_{p,uu}$ ) and (D) volume of distribution ( $V_{u,br}$ ) of the unbound fraction of lidocaine, bupivacaine, and levobupivacaine in the brain. Data are expressed as the mean  $\pm$  SD of 12 experiments. \*  $P < 0.05$ , \*\*  $P < 0.01$  compared with lidocaine, †  $P < 0.01$  compared with bupivacaine or levobupivacaine.

is not directly related to the higher CNS toxicity of bupivacaine than that of levobupivacaine reported previously.<sup>1,4</sup>

This study has several limitations. First of all, concentrations of anesthetics in the cerebral ECF were measured by high-performance liquid chromatograph equipped with an electrochemical detector. Despite precise quantitation of local anesthetics by high-performance liquid chromatography in our previous study as well as in other reports,<sup>3,13,14</sup> and good concordance with the results measured by mass spectrometry, a similar quantitation method with mass spectrometry for measuring the cerebral ECF concentrations in the place of electrochemical detection would be more preferable.<sup>7</sup> Second, concentrations of anesthetics in plasma and in the cerebral ECF were measured only for 2 h after termination of the infusion, which may influence calculation of the pharmacokinetic parameters based on the terminal elimination phase.

Third, we determined the content of anesthetics in the whole brain ( $A_{br}$ ) in another group of animals; however, they were not subjected to the microdialysis study in which the pharmacokinetic parameters in the cerebral ECF were measured as reported previously.<sup>7,8</sup> We tried to calculate and compare the pharmacokinetic parameters of anesthetics both in plasma and cerebral ECF, but sampling of blood and cerebral dialysate was required until their concentrations decreased to very low levels. In fact,

anesthetics in both plasma and cerebral ECF were undetectable in one animal receiving bupivacaine at the end of experiments, suggesting that their levels in the whole brain would also be low, and such a low level eventually made it difficult to perform precise measurement. Despite these limitations, differences of  $A_{br}$  existing between rats in the first and second experiments would not influence the relationships of tissue-to-plasma partition coefficients among the three anesthetics, because  $A_{br}$  was much larger than the amount of anesthetics in the cerebral blood vessels ( $V_{bl} \times C_{bl}$ ) and  $V_{u,br}$ , a determinant of  $K_p$  and  $K_{p,u}$ , is predominantly dependent on  $A_{br}$  and the concentration of anesthetics in the cerebral ECF (Eq. 4).

In summary, we have shown that concentrations of bupivacaine in the cerebral ECF are higher than levobupivacaine despite similar concentrations in plasma and in the whole brain. Further studies are warranted to elucidate the mechanism responsible for differences in the toxicity of the stereoisomers.

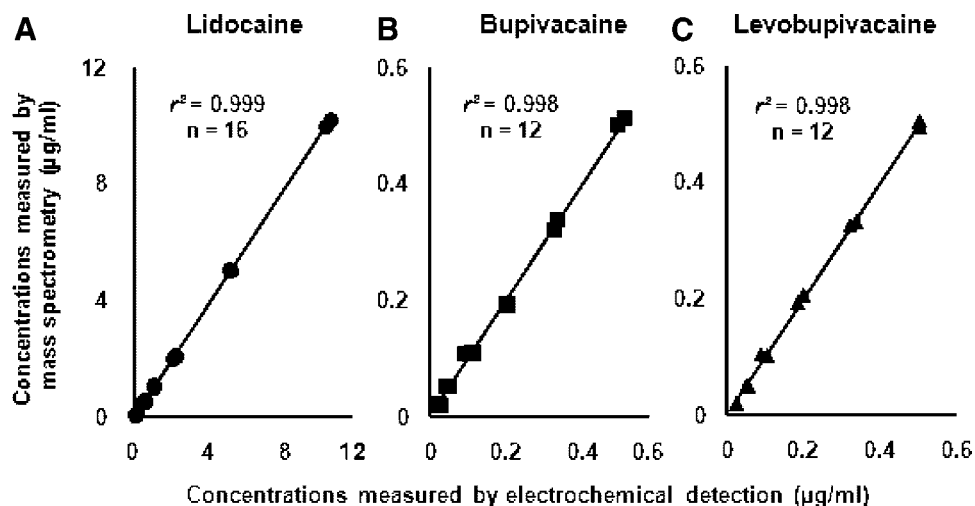
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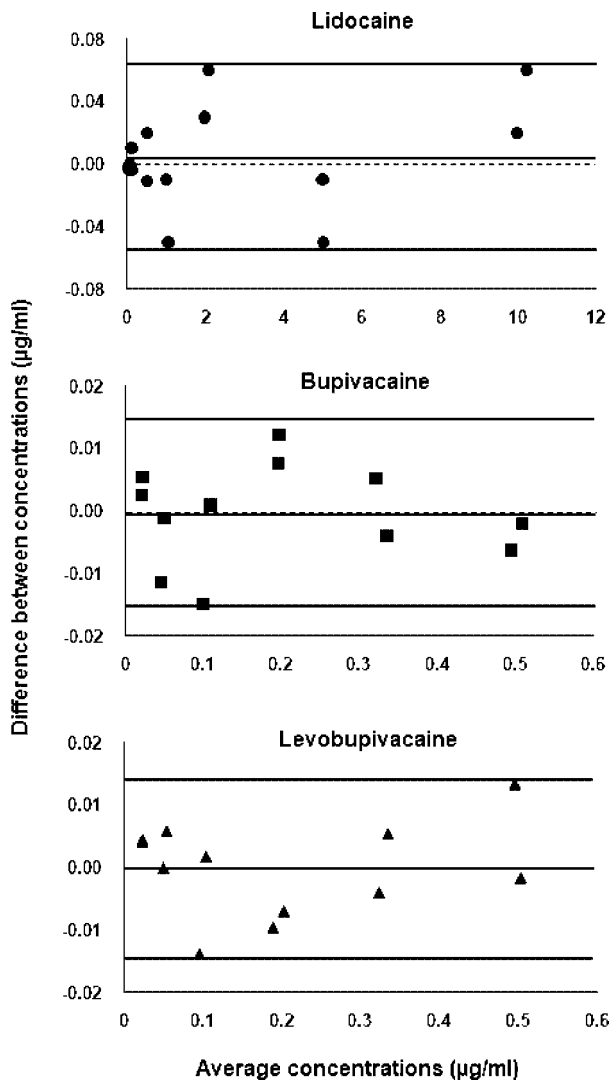
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### Appendix: Concordance of the Concentrations of Local Anesthetics Measured By Electrochemical Detection and Mass Spectrometry

For examining the correlations between methods for determining local anesthetics concentrations, we have prepared standard solutions of lidocaine (0.02–10.0  $\mu\text{g/ml}$ ), bupivacaine, and levobupivacaine (0.02–0.5  $\mu\text{g/ml}$ ) in the cerebrospinal fluid, measured their concentrations by electrochemical detection and mass spectrometry, and compared their results using a Spearman rank order test ( $r^2$ ). Agreement was determined according to the method described by Bland–Altman plots. We calculated mean difference (bias), SD of the difference (precision), and limit of agreement (95% confidence interval) for the values measured by electrochemical detector and mass spectrometry. The correlation coefficients ( $r^2$ ) between methods were more than 0.99 for lidocaine, bupivacaine, and levobupivacaine ( $P < 0.01$  for all, fig. 4). Mean (bias)  $\pm$  SD of the difference (precision) were  $0.00 \pm 0.03$ ,  $0.00 \pm 0.01$ , and  $0.00 \pm 0.01$   $\mu\text{g/ml}$  for lidocaine, bupivacaine, and levobupivacaine, respectively. Limit of agreement ranged from  $-0.06$  to  $0.07$ ,  $-0.02$  to  $0.01$ , and  $-0.01$  to  $0.01$   $\mu\text{g/ml}$  for lidocaine, bupivacaine, and levobupivacaine, respectively (fig. 5).



**Fig. 4.** Linear regression plots of the concentrations measured by electrochemical detection and mass spectrometry. Each plot for (A) lidocaine ( $n = 16$ ), (B) racemic bupivacaine (bupivacaine) ( $n = 12$ ), and (C) levobupivacaine ( $n = 12$ ). Solid lines represent the regression lines.  $P < 0.001$  for significance of the Spearman rank order correlation for lidocaine, bupivacaine, and levobupivacaine.



**Fig. 5.** Bland-Altman plots for describing average and difference of the measured concentrations by electrochemical detection and mass spectrometry. *Solid lines* represent the bias, the upper 95% confidential interval (+1.96 SD) and the lower 95% confidential interval (-1.96 SD) for lidocaine (n = 16), racemic bupivacaine (n = 12), and levobupivacaine (n = 12).