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Possible Mechanism of Irreversible Nerve Injury Caused by Local Anesthetics

Detergent Properties of Local Anesthetics and Membrane Disruption

Norihito Kitagawa, M.D., Mayuko Oda, M.D.,† Tadahide Totoki, M.D.‡*

Background: **Irreversible nerve injury may result from neural membrane lysis due to the detergent properties of local anesthetics. This study aimed to investigate whether local anesthetics display the same properties as detergents and whether they disrupt the model membrane at high concentrations.**

Methods: **Concentrations at which dodecyltrimethylammonium chloride and four local anesthetic (dibucaine, tetracaine, lidocaine, and procaine) molecules exhibit self-aggregation in aqueous solutions were measured using an anesthetic cation-sensitive electrode. Light-scattering measurements in a model membrane solution were also performed at increasing drug concentrations. The concentration at which drugs caused membrane disruption was determined as the point at which scattering intensity decreased. Osmotic pressures of anesthetic agents at these concentrations were also determined.**

Results: **Concentrations of dodecyltrimethylammonium chloride, dibucaine, tetracaine, lidocaine, and procaine at which aggregation occurred were 0.15, 0.6, 1.1, 5.3, and 7.6%, respectively. Drug concentrations causing membrane disruption were 0.09% (dodecyltrimethylammonium chloride), 0.5% (dibucaine), 1.0% (tetracaine), 5.0% (lidocaine), 10.2% (procaine), and 20% (glucose), and osmotic pressures at these concentrations were 278, 293, 329, 581, 728, and 1,868 mOsm/kg H_2O, respectively.**

Conclusions: **These results show that all four local anesthetics form molecular aggregations in the same manner as dodecyltrimethylammonium chloride, a common surfactant. At osmotic pressures insufficient to affect the membrane, local anesthetics caused membrane disruption at the same concentrations at which molecular aggregation occurred. This shows that disruption of the model membrane results from the detergent nature of local anesthetics. Nerve membrane solubilization by highly concentrated local anesthetics may cause irreversible neural injury.**

SINCE the description by Rigler *et al.*[1] of cauda equina syndrome occurring after continuous spinal anesthesia with 5% lidocaine, most anesthesiologists have become concerned about irreversible neural injury caused by high concentrations of local anesthetics. To clarify the mechanisms of such neurotoxicity, numerous studies have been performed in the fields of animal behavior, electrophysiology, and histopathology.[2–4]

Neural injury after spinal anesthesia initially seemed to result from the specific effects of lidocaine because most reported cases of cauda equina syndrome occurred in patients who had received spinal anesthesia using this agent.[1,5–8] However, intrathecal administration of highly concentrated tetracaine was also shown to cause irreversible neural injury.[1,9] Therefore, any local anesthetic agent administered in high concentration was suspected to have the potential for neurotoxic effects.

We speculate that irreversible nerve injury results from neural membrane lysis due to the detergent properties of local anesthetics. In fact, at high concentrations in aqueous solution, dibucaine and tetracaine molecules form self-aggregations, a phenomenon known as *micellar formation*. This represents an essential characteristic of detergents.[10–12] However, whether lidocaine or procaine form similar molecular aggregations is unclear.[10]

At or above the concentrations required for molecular aggregation, surfactants dissolve hydrophobic substances into the hydrophobic core of the molecular aggregate, disrupting the hydrophobic structure. This property of detergents, solubilization, sees wide application in the extraction of proteins from biologic membranes.[13,14]

The current study first determined critical micellar concentrations of four local anesthetics in aqueous solution using a local anesthetic cation-sensitive electrode and then elucidated the concentrations at which these anesthetics disrupted a model phospholipid membrane. By performing the same investigations for dodecyltrimethylammonium chloride (DoTMACl), a typical surfactant, and comparing the results between local anesthetics and DoTMACl, we have demonstrated that solubilization, a general property of detergents, might cause disruption of nerve cell membranes.

Materials and Methods

Dibucaine HCl, tetracaine HCl, lidocaine HCl, procaine HCl, and dimyristylphosphatidylglycerol were purchased from Sigma (St. Louis, MO). Carboxylated polyvinyl chloride (−COOH 1.8% wt/wt), tetrahydrofuran, dioctylphthalate, and DoTMACl were obtained from Wako Chemical (Tokyo, Japan). All other chemicals used were of the highest available grade. Water used was purified by a Milli-Q system (Millipore, Bedford, MA), and specific conductivity was maintained at less than $1.0 \times 10^{-7}\ \Omega^{-1} \cdot cm^{-1}$.

* Assistant Professor, † Research Associate, ‡ Professor and Chairman.

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Address reprint requests to Dr. Kitagawa: Department of Anesthesiology, Saga Medical School, Nabeshima, Saga, 849-8501, Japan. Address electronic mail to: kitagawa@mail.anes.saga-med.ac.jp.

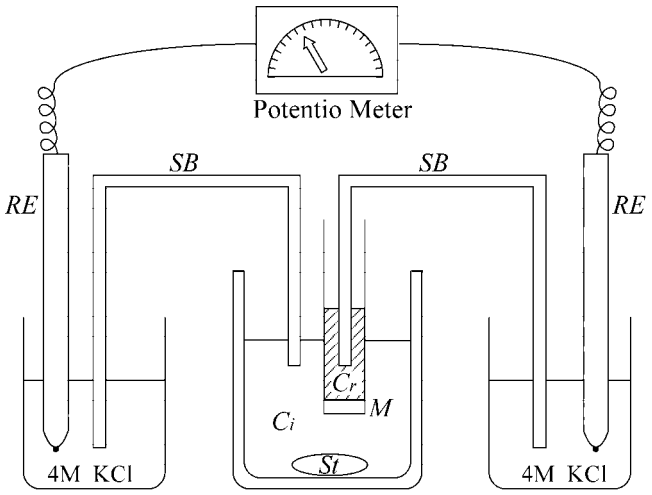

Fig. 1. Schematic diagram of carboxylated polyvinyl chloride membrane electrode. *Ci* = drug concentration to be titrated; *Cr* = reference drug concentration (constant); *M* = electrode membrane; *RE* = Ag–AgCl reference electrode; *SB* = salt bridge of 4 M KCl agar; *St* = magnetic stirrer.

Electrode Preparation and Measurement of Critical Micellar Concentration

The electrode membrane was prepared by mixing 0.9 g carboxylated polyvinyl chloride, 2.1 g dioctylphthalate, and 9 ml pure tetrahydrofuran. The mixture was heated to 60°C, after which the resultant clear viscous material was spread onto a glass plate, and the tetrahydrofuran was evaporated off for 24 h at room temperature. After gradual evaporation of solvent, the gel membrane (approximately 0.2 mm thick) was cut out and placed over one end of a polyvinyl chloride tube (diameter, 9 mm; length, 10 cm), using the carboxylated polyvinyl chloride and tetrahydrofuran viscous mixture as an adhesive.[15]

This electrode was used in the concentration cell system as shown in figure 1. *Cr* and *Ci* denote local anesthetic concentrations in the reference and sample solutions, respectively, and *M* indicates the electrode membrane. The electromotive force of the cell was measured using a model 614 electrometer (Kethley, Cleveland, OH) with impedance greater than 1×10^6 Ω. A calomel electrode (Corning, Medfield, MA) was used for reference. According to Nernstian principles, for the electrode to provide ideal responses to local anesthetic or surfactant cations, the relation between electromotive force and logarithm of drug concentration must be linear, with a slope of −59 mV/decade at 25°C. Our previous report indicated that these electrodes work ideally on responses to local anesthetic cations at concentrations greater than 10^{-5} mM for each of the local anesthetics used in the current study.[15] Deviation from Nernstian linear responses occurs when molecular self-aggregation occurs, as established by Newbery *et al.*[16] (see appendix).

Measurement of Membrane Disruption Concentration

Dimyristylphosphatidylglycerol was completely dissolved into chloroform, and then the solvent was removed under reduced pressure overnight. After dispersing dimyristylphosphatidylglycerol into 150 mM aqueous NaCl solution and sonicating for 1 h at 30°C, phospholipid bilayers were obtained as a suspension of single-walled vesicles (0.1 mg/ml) and maintained at 25°C in a thermocontrolled box. Numerous studies have indicated that phospholipid bilayer membranes can be used as a simple model of biologic membranes with suitable stability.[17–19] The relation between light-scattering intensity of the model membrane solution and drug concentration was then measured. If disruption of the model membrane occurs, the intensity of light scattering decreases, because this parameter is correlated to membrane size.[20,21] In model membrane suspension mixed with DoTMACl, local anesthetics, or glucose, 90° light scattering was monitored at a wavelength of 600 nm using an RF-5000 spectrophotofluorometer (Shimazu, Kyoto, Japan). Osmotic pressures of these substances at each concentration causing membrane disruption were also measured using an Osmotic Pressure Auto & Stat OM-6030 (Kyoto Daiichi Kagaku, Kyoto, Japan).

The concentration of NaCl was 150 mM in all solutions studied. The pH of sample solutions was monitored using a Radiometer Ion 85 Analyzer (Copenhagen, Denmark) and Orion combination glass electrode (Boston, MA) during potentiometric and light-scattering titrations. We ensured that all measurements were made at 25.0°C by using a temperature-controlled water jacket. In both electrode and light-scattering studies, the pH values of all solutions tested ranged from 4.5 to 5.7. Critical micellar concentrations and membrane disruption concentrations were measured three times, and averages of these readings were obtained.

Results

Critical Micellar Concentration

Figure 2 shows the relation between drug concentration and electromotive force. All drugs tested exhibited straight-line responses, with slope values of approximately −59 mv/decade at low concentrations. That is, Nernstian responses were observed, indicating that the electrode displays ideal responses to DoTMA and all anesthetic cations tested. For all drugs, deviations from Nernstian responses were noted at high concentrations, with dibucaine and tetracaine exhibiting considerable deviations, whereas lidocaine and procaine displayed relatively small deviations. In any case, the results indicate that DoTMA and molecules of local anesthetic exhibit self-aggregation. Concentrations at which this phenomenon occurred are listed in table 1.

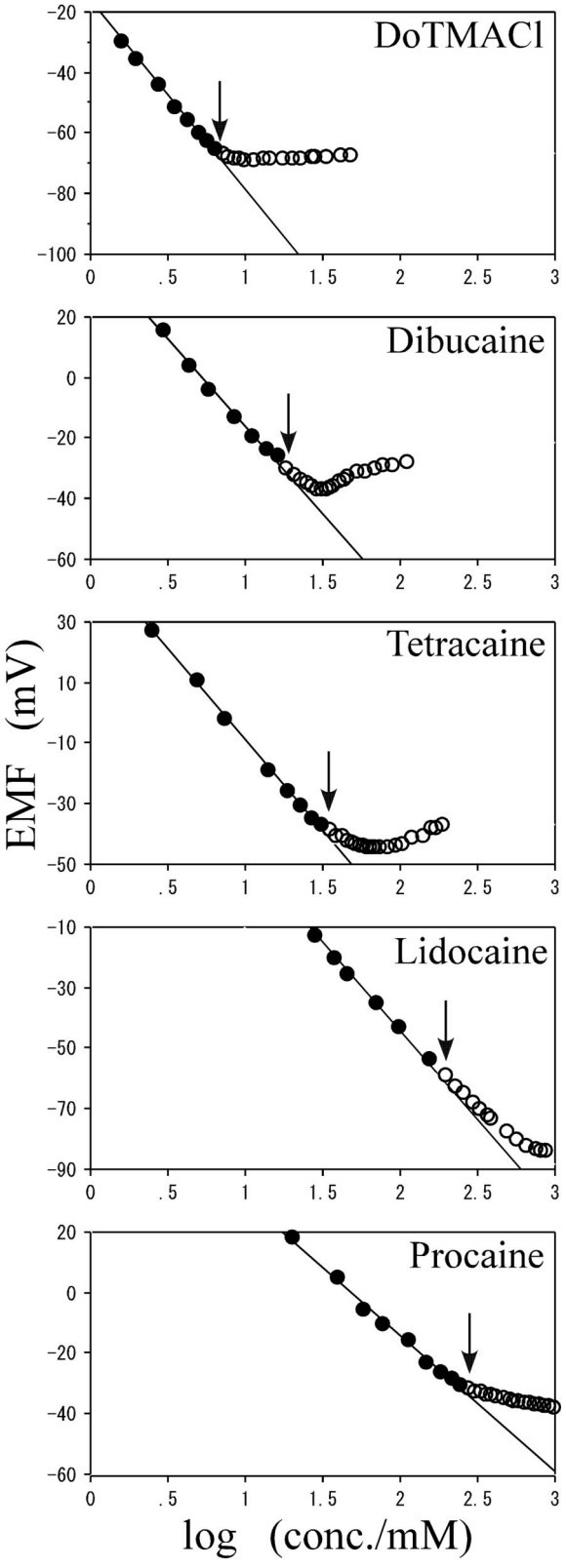

Fig. 2. Relation between electromotive force (EMF) and logarithm of concentrations in dodecyltrimethylammonium chloride (DoTMACl) and four local anesthetics. Slope values of all drugs at low concentrations are almost −59 mV/decade, Nernstian response (*filled circles*). Deviations from Nernstian straight lines are observed for all drugs (*open circles*). *Arrows* indicate initial point at which drug molecules aggregate.

Membrane Disruption Concentration

Figure 3 shows the relation between drug concentration and intensity of light scattering in the model membrane solution. The scattering intensity of membrane solution mixed with DoTMACl, local anesthetics, or glucose increased at low concentrations and decreased at high concentrations. A sudden decrease in light scattering indicated solubilization and disruption of the model membrane. Concentrations and osmotic pressures at which membrane disruption occurred are listed in table 1.

Discussion

Because the pH values of all solutions tested were at least 2 units less than the pKa (the pH at which 50% of the local anesthetic is in the charged and 50% is in the uncharged form) of each local anesthetic, almost 100% of anesthetic molecules existed in monovalent cationic form.[22] Most DoTMA molecules are also cationic species in this pH range.

Critical Micellar Concentration of Surfactant and Local Anesthetics

Generally, surfactants are amphiphilic, displaying both hydrophilic and hydrophobic parts to the molecular structure. When concentrations reach critical levels, surfactant molecules aggregate by aligning the hydrophobic moieties toward the core, with the hydrophilic portions aligned toward the aqueous environment. This occurs because molecular aggregation is driven by hydrophobic interactions, the force by which water molecules repulse the hydrophobic moiety of surfactant.[23] All local anesthetics in clinical use can be seen as a type of surfactant because they basically comprise both hydrophilic amine and hydrophobic aromatic moieties.

Critical aggregation concentration is usually determined from the relation between drug activity and concentration,[24] with the activity of a drug reflecting the solvation state of the ionic solute. A local anesthetic cation-sensitive electrode measures monomeric activity of anesthetic cations as an electrical potential, instead of concentration. The electrode method used in this investigation thus offers a more precise means of determining molecular aggregation than conventional measurements such as light scattering, vapor pressure osmometry, surface tension, or conductivity testing, all of which indirectly measure drug activity.[10,12,24] In a less hydrophobic surfactant, aggregations usually comprise fewer molecules, so that the change in relation between drug concentration and activity is not significant even if molecular aggregation occurs. Molecular aggregation of lidocaine or procaine seems difficult to detect, because aggregations comprise fewer molecules, due in turn to the reduced hydrophobicity of these agents compared with dibucaine or tetracaine. In fact, molecular aggrega-

Table 1. Drug Concentrations at Which Molecular Aggregation and Model Membrane Disruption Occurred, and Osmotic Pressures at Which Membrane Disruption Occurred

	Critical Molecular Aggregation	Model Membrane Disruption	
	Concentration, % wt/vol (mM)	Concentration, % wt/vol (mM)	Osmotic Pressure, mOsm/kg
DoTMACl	0.15 ± 0.02 (6.1)	0.09 ± 0.01 (4.1)	278
Dibucaine	0.6 ± 0.1 (18.4)	0.5 ± 0.1 (15.3)	293
Tetracaine	1.1 ± 0.1 (38.0)	1.0 ± 0.1 (34.5)	329
Lidocaine	5.3 ± 0.4 (195)	5.0 ± 0.7 (184)	581
Procaine	7.6 ± 0.4 (280)	10.2 ± 1.5 (368)	728
Glucose	—	20 ± 3.0 (1,222)	1,868

The concentration of NaCl was 150 mM in all solutions studied.

Data are expressed as mean ± SD. Data in parentheses represent mean values in millimolars.

tion of lidocaine and procaine has not yet been clearly shown by conventional indirect measurements of drug activity. However, the current study revealed small deviations in the drug–activity relation by means of the cation-sensitive electrode, indicating molecular aggregation of the drugs.

The critical micellar concentration of DoTMACl, a typical surfactant, was 0.15%, consistent with values reported in the literature.[25] Critical micellar concentrations reported for dibucaine, tetracaine, and procaine are 1.1, 1.7, and 2.2%, respectively,[11,12] with the former two values nearly twice those obtained in the current study (table 1). However, reported critical micellar concentrations of procaine are approximately four times smaller than that identified in this study (7.6%). Basically, molecular aggregation of surfactant is correlated with hydrophobic strength, as previously mentioned. Conversely, as is well known, anesthetic potency also depends on hydrophobicity, because more hydrophobic drugs attach more tightly to action sites on the membrane.[26] Anesthetic potency and the force responsible for molecular aggregation must therefore be correlated. According to this theory, the critical micellar concentration of procaine should be approximately 16-fold larger than that of dibucaine because the anesthetic potency of procaine is 16-fold weaker than that of dibucaine.[26,27]

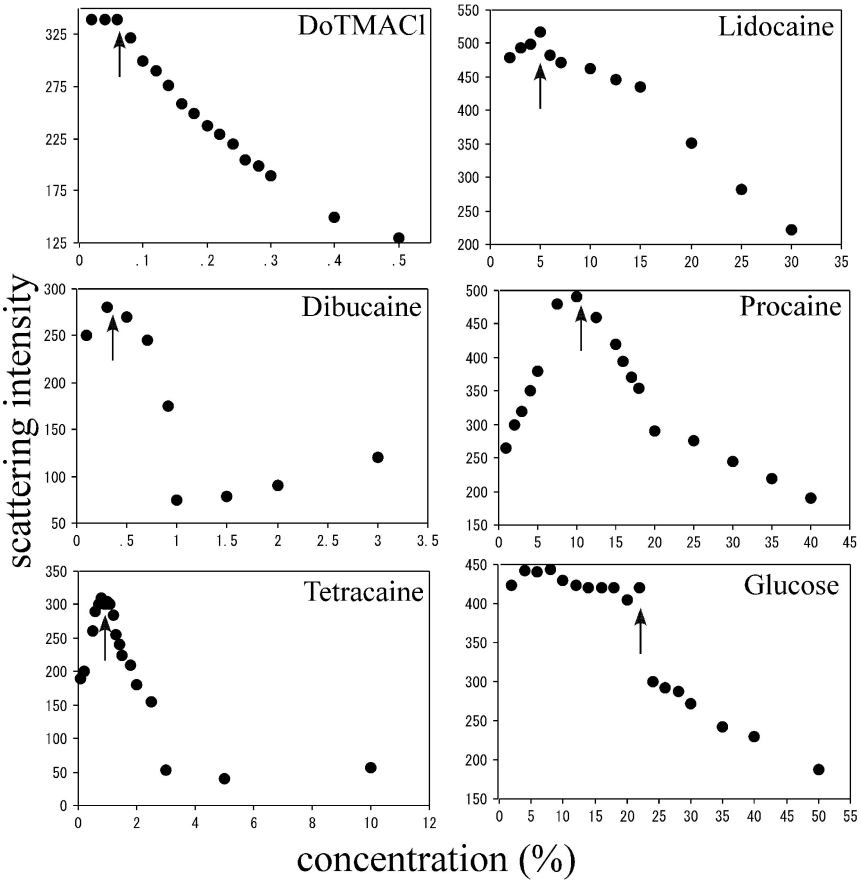

Fig. 3. Relation between scattering intensity in the model membrane suspension and drug concentration. Scattering intensity increased for all agents tested, particularly local anesthetics, at low concentrations. Increasing drug concentration resulted in sudden decreases of scattering intensity at high concentrations. Scattering changes of local anesthetic solution only at 600 nm can be ignored because they are very small compared with those of dimyristylphosphatidylglycerol membrane solution. Accordingly, these changes originate from lipid membranes and show that model membranes were disrupted by all agents, including glucose. *Arrows* indicate initial point of membrane disruption. DoTMACl = dodecyltrimethylammonium chloride.

The critical micellar concentration of procaine obtained in our study was approximately 13-fold larger than that of dibucaine (0.6 *vs.* 7.6%), a value approaching those suggested by the above theory. The data obtained by the cation-sensitive electrode in the current study therefore seem reliable compared with previous reports of local anesthetic aggregation. Differences between the results of previous investigations and those of this study probably result from the methods used to measure drug activity (indirect *vs.* direct).

The critical micellar concentration of lidocaine has not previously been reported, with the current study offering the first report of molecular aggregation for lidocaine. The critical concentration of lidocaine is ninefold less than that of dibucaine (0.6 *vs.* 5.3%), almost equal to the difference in anesthetic potency between the two drugs (a sevenfold difference).[27] The concentrations at which amphiphilic drugs form molecular aggregations are those at which the detergent effect occurs. This concentration is specific to the individual drug and is extremely important in drug activity and toxicity. Because this property is applied to protein separation from biologic membranes,[13,14] a surfactant disrupts membrane structure at concentrations equal to or greater than the critical micellar concentration. This is the same principle by which a detergent dissolves an oily stain into the molecular aggregate, allowing the stain to be washed away.

Concentration of Model Membrane Disruption

The model membrane disruption concentrations of DoTMACl, dibucaine, tetracaine, lidocaine, and procaine were almost equal to their respective critical micellar concentrations (table 1). Local anesthetics therefore seem likely to disrupt the cell membrane by solubilization in the manner of the typical surfactant DoTMACl. Another possible mechanism of membrane disruption is the effect of osmotic pressure. Despite lacking any surfactant activity, glucose disrupts the model membrane at concentrations of 20% or greater, where osmolality is 1,868 mOsm/kg or above (table 1). This osmotic pressure is much higher than those at which local anesthetics cause membrane disruption, clearly showing that the mechanism of membrane disruption by concentrated local anesthetics does not involve hyperosmolality. All local anesthetics therefore seem extremely likely to display surfactant activity and cause nonspecific membrane disruption at high concentrations.

This result seems contrary to the accepted action of local anesthetics in stabilizing biologic membranes. However, the protective action of local anesthetics on membranes occurs at much lower concentrations than those at which membrane solubilization resulted in the current study.[28] Seeman[29] demonstrated that, although local anesthetics produce membrane stabilization in erythrocytes at low anesthetic concentrations, high concentrations identical to those obtained in our study result in hemolysis. He also speculated that hemolysis resulted from the detergent properties of local anesthetics.

The concentrations at which local anesthetics cause irreversible neural injury after spinal anesthesia have been reported as 5% for lidocaine,[1,5–8] 0.5–1.2% for tetracaine,[1,9] and 10% for procaine.[30,31] These concentrations are basically identical to those at which the detergent effect of local anesthetics was noted and disruption of the model membrane occurred in the current study. Considering Seeman's reports[28,29] and the results of the current study, the irreversible neural injury induced by concentrated local anesthetics may result from the disruption of neural membranes by the solubilizing action of anesthetics. If so, because this is a nonspecific effect of local anesthetics as detergents, local anesthetics should not be used clinically at the concentrations at which the detergent properties emerge or at which anesthetic molecular aggregation appears. Although neurologic sequelae seldom occur after intrathecal administration of highly concentrated anesthetics, because of the dilution of anesthetic in cerebrospinal fluid, the risk of exposing nerve roots to high concentrations of local anesthetic remains when a concentrated local anesthetic, such as 5% lidocaine, is used.

The anesthetic concentration producing membrane disruption in our study was determined under conditions in which the anesthetic existed in cationic form. However, most local anesthetics in clinical use are present in both cationic and neutral forms *in vivo*. The concentrations of local anesthetic at which membrane disruption occurs *in vivo* would therefore seem likely to be lower than those obtained by the current study because the neutral forms of local anesthetic are more hydrophobic than the cationic forms, and the primary factor behind micellar formation is molecular hydrophobicity. Accordingly, irreversible neurologic injury *in vivo* may occur at concentrations lower than those suggested in the current study. Further *in vitro* and *in vivo* studies are needed to confirm our hypothesis in regard to neurologic sequelae produced by high concentrations of local anesthetic.

In conclusion, local anesthetics used clinically can form molecular aggregations at high concentrations, resulting in the appearance of detergent properties in these agents. Membrane disruption can thus result from the resultant solubilization. Concentrations of local anesthetic at which these properties emerged in our study were in accordance with those at which cauda equina syndrome has reportedly occurred after spinal anesthesia. The mechanisms of irreversible neurologic injury induced by high concentrated local anesthetic seem likely to result from the detergent nature of local anesthetics.

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Appendix

When an ion-sensitive electrode responds in an ideal manner to a target monovalent cation, the electromotive force, E, can be determined according to the following Nernstian equation:

$$E = \frac{RT}{F} \log \frac{\gamma rCr}{\gamma iCi},$$

where R is the gas constant; F is the Faraday constant; T is the absolute temperature (25°C); Ci and Cr are concentrations of the objective ion in sample and reference solutions, respectively; and γi and γr are the activity coefficients of ion in the sample and reference solutions, respectively. Under experimental conditions, Cr is kept constant and small enough to consider γr as approaching unity. Accordingly, the equation can be converted to the following:

$$E = -59.2 \log(\gamma iCi) + \text{constant}.$$

Therefore, in the relation between $\log(\gamma i\ Ci)$ and E, a straight line with a slope value of approximately −59 mV/decade indicates an ideal response of the electrode to the objective ion. That is, the activity coefficient of the ion (γi) in sample solution approximates unity, with each ion in the solution existing in a loose network, without influencing with its neighbors. If ionic concentration increases above a certain level, deviation from the Nernstian straight line occurs, as seen in our study. This indicates that the activity coefficient of the ion (γi) has decreased because of changes in ionic solvation state from sparse to dense, at which point some ion molecules aggregate.[16]

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