SPECIAL ARTICLE

The Mechanisms Underlying Lipid Resuscitation Therapy

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Abstract: The experimental use of lipid emulsion for local anesthetic toxicity was originally identified in 1998. It was then translated to clinical practice in 2006 and expanded to drugs other than local anesthetics in 2008. Our understanding of lipid resuscitation therapy has progressed considerably since the previous update from the American Society of Regional Anesthesia and Pain Medicine, and the scientific evidence has coalesced around specific discrete mechanisms. Intravenous lipid emulsion therapy provides a multimodal resuscitation benefit that includes both scavenging (eg, the lipid shuttle) and nonscavenging components. The intravascular lipid compartment scavenges drug from organs susceptible to toxicity and accelerates redistribution to organs where drug (eg, bupivacaine) is stored, detoxified, and later excreted. In addition, lipid exerts nonscavenging effects that include postconditioning (via activation of prosurvival kinases) along with cardiotonic and vasoconstrictive benefits. These effects protect tissue from ischemic damage and increase tissue perfusion during recovery from toxicity. Other mechanisms have diminished in favor based on lack of evidence; these include direct effects on channel currents (eg, calcium) and mass-effect overpowering a block in mitochondrial metabolism. In this narrative review, we discuss these proposed mechanisms and address questions left to answer in the field. Further work is needed, but the field has made considerable strides towards understanding the mechanisms.

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Laboratory experiments by Weinberg et al[1] originally identified lipid resuscitation therapy (LRT) as a potential treatment for local anesthetic toxicity in 1998. Following the clinical translations by Rosenblatt et al[2] and Litz et al,[3] the use of LRT has gained acceptance among the anesthesiology community and professional organizations have advocated for its use.[4–7] In the original work, the authors proposed 4 theories to explain the rapidity of reversal of severe bupivacaine toxicity. These included a metabolic effect (eg, improved fatty-acid oxidation), increased nitric oxide production, a redistributive benefit, and the possibility that the newly created lipid plasma phase functioned as an intravascular lipophilic "sink" into which the offending drug could partition.[1]

During the intervening years, the scientific community has made significant scientific progress on LRT. Beyond confirming the survival benefit of LRT,[8] progress has been made toward understanding the mechanism of lipid-based reversal of local anesthetic toxicity. Evidence from different groups has coalesced around a multimodal mechanism that includes a scavenging effect and a direct effect of the lipid to improve cardiac function and blood pressure. Scavenging describes the action of LRT more accurately than "lipid sink," because it reflects the redistributive benefit, in which lipid functions as a "shuttle" or "subway" in both human and animal models.[9–15] The nonscavenging effects include a cardiovascular benefit and a postconditioning effect. Other proposed mechanisms, including direct channel effects and mass effect on mitochondrial metabolism, have diminished in favor based on lack of evidence or contradictory evidence. Herein, we summarize progress toward understanding a mechanism of LRT and its implications in clinical practice (Fig. 1). We focus on the role that LRT plays in reversal of local anesthetic toxicity, because this is the primary indication for LRT. We also discuss the next steps required to more fully understand the underlying mechanism(s) of LRT.

DISCUSSION

Local Anesthestic Systemic Toxicity

Understanding LRT reversal of local anesthetic systemic toxicity (LAST) requires an appreciation of the targets and effects of local anesthetics that contribute to their clinical toxicity (Fig. 2). This is particularly relevant to the nonscavenging effects of LRT, which are function specific. Because bupivacaine is the canonical local anesthetic studied for both LAST and LRT, we address it specifically. Bupivacaine alters the activities of a number of different cellular targets. The primary therapeutic target of local anesthetics is the voltage-gated sodium channel (NaV channel) where inhibition prevents transmission of sensory and motor signals in axons. Local anesthetics bind to the intracellular domain of the NaV and prevent ion transfer.[16] In addition to the NaV channel,[17] bupivacaine inhibits the voltage-gated Ca^{2+} channel,[18] the K^{+} channel,[19,20] the Na-K ATPase, and other channels and enzymes.[21]

Bupivacaine also uncouples metabotropic signaling across the cell membrane, interfering with downstream activation of kinase signaling both in short time scales (minutes) and long time frames (hours). Immediately, downstream of metabotropic signaling at the membrane, local anesthetics reduce production of secondary messengers such as cyclic adenosine monophosphate.[22] At low concentrations, ropivacaine and lidocaine can reduce phosphorylation (and activation) of protein kinase B (Akt), proto-oncogene tyrosine-protein kinase Src kinase, and other progrowth or cytoprotective kinases.[23,24] During acute cardiovascular toxicity, bupivacaine drives rapid dephosphorylation of Akt and increases phosphorylation of 5′-adenosine monophosphate activated protein kinase (AMPK); these 2 changes are accompanied by integrative signaling changes downstream of mammalian target of rapamycin (mTOR).[25] Activation of AMPK is both salutary and predictable given that bupivacaine reduces oxidative phosphorylation and tissue energy stores. We know the importance of these pathways because activation of AMPK using the AMP analog 5-aminoimidazole-4-carboxamid ribonucleotide (AICAR)

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G.W. is an officer, director, shareholder and paid consultant of ResQ Pharma, Inc. He also created and maintains www.lipidrescue.org, an educational web site.
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The following article discusses an off-label use of lipid emulsion (eg, Intralipid) for treatment of local anesthetic systemic toxicity.

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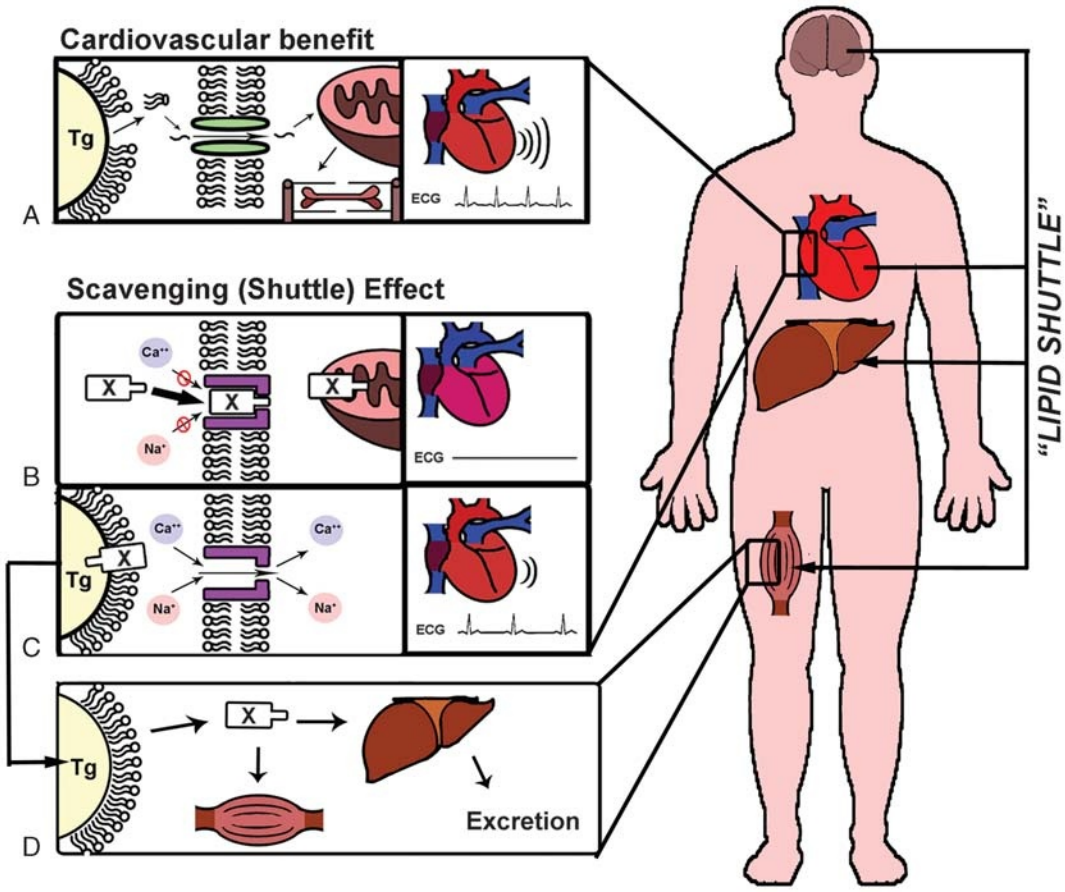

FIGURE 1. Multimodal mechanism of LRT. Lipid emulsion contributes multiple benefits to cardiovascular recovery including a cardiotonic benefit and a scavenging (eg, shuttle) benefit. A, The triglycerides, fatty acids, and/or phospholipids in the lipid emulsion will exert a positive effect on the cardiovascular system, improving blood pressure through direct effects on the heart and/or vasculature. This only occurs after drug concentration drops below channel blocking thresholds. B, Drug X (ie, bupivacaine) can cause toxicity by several mechanisms including blocking of ion channels preventing conduction and depolarization in cardiac and nervous tissue, and/or by interference with mitochondrial energy production. Acute toxicity can result in asystole and cardiovascular collapse. C, Circulating lipid "droplets" from the lipid emulsions can scavenge drug X out of tissue, thereby alleviating the blockage of key inotropic channels and return of cardiac function. D, The lipid droplets facilitate accelerated redistribution of drug X to the skeletal muscle and to the liver where it is conjugated to permit excretion. The movement of drug from high concentration organs to low concentration organs follows traditional pharmacokinetic parameters with skeletal muscle providing the major depot for storage of excess local anesthetic. This redistribution process is referred to as the "lipid shuttle." Figure parts adapted with permission from Ref.[9]

provides additional protection from local anesthetic toxicity in cell models.[26] Whereas acute toxicity drives loss of insulinergic signaling, bathing cells overnight in bupivacaine induces cell death through apoptotic signaling at translational and mitochondrial targets downstream of Akt and mTOR.[27–29]

In addition to loss of growth-related signaling, local anesthetics exert direct independent actions, which appreciably perturb the myocardial contractility apparatus.[30] It is unclear if these effects are the result of altered signaling at the membrane or an independent action.[21] At the sarcoplasmic reticulum, local anesthetics inhibit the ryanodine receptor[31] and reduce the Ca^{2+} sensitivity of myofilaments.[32] At the mitochondria, bupivacaine potently inhibits mitochondrial metabolism[33] and reduces adenosine triphosphate (ATP) production[34] in a nonstereospecific manner.[35] Bupivacaine accumulates in mitochondria[36] of metabolically active tissue[9] and uncouples oxidative phosphorylation, thereby decreasing mitochondrial ATP synthesis and myocardial function.[34,37] The reduction in ATP production results from less fatty-acid oxidation because bupivacaine (and other local anesthetics) block fatty-acid transport into the mitochondria via inhibition of carnitine acylcarnitine translocase (CACT).[38,39] Other studies indicate that bupivacaine inhibits respiratory chain complexes 1 and 3, leading to increased reactive oxygen species (ROS) production.[40] Supplementation of rabbit isolated atria with ATP overcomes the inhibition of contractility produced by bupivacaine, leading some to hypothesize that a reduction in ATP is the primary driver of toxicity in vivo.[41] Loss of ATP interferes with all energy-based activities of the cell including contractility and maintenance of ionic gradients (eg, mitochondrial Ca^{2+} homeostasis). Furthermore, toxicity with bupivacaine activates AMPK (an energy sensing kinase) leading to feedback and sensitization of other signaling pathways (including insulinergic signaling upstream of mTOR), which contributes to spontaneous cardiac recovery.[25] Taken together, these effects reduce ATP production acutely, activate energy-conserving signal pathways over minutes and induce apoptosis in cultured cells over longer time periods (24-hour baths).

Bupivacaine exerts a complicated set of adverse effects on ion channel function, kinase signaling, and energy production in cells. Because of the complexity of signaling, bupivacaine may produce toxicity by actions at a number of different targets. As

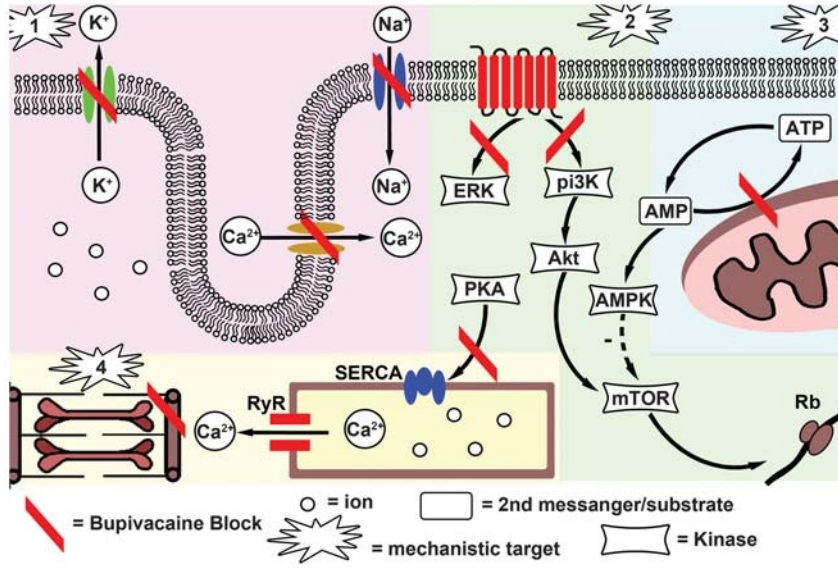

FIGURE 2. Cellular targets contributing to local anesthetic toxicity. Local anesthetic molecules will drive toxicity at a number of sites. (1) Local anesthetics will block channel function; canonical function of local anesthetics is via blockage of the NaV channel, but local anesthetics will also block potassium channel and calcium channel currents. (2) Local anesthetics will block signaling at metabotropic receptors in the membrane and reduce downstream kinase activation including prosurvival kinases in the reperfusion-injury-salvage kinase pathways. Targets include ERK, PKA, pi3K, Akt and downstream targets including glycogen synthase kinase (not pictured), tuberous sclerosis complex (not pictured), mTOR, ultimately modifying translation at the Rb. (3) Toxicity will interfere with high-energy phosphate production in the mitochondria with a reduction in ATP levels, activation of AMPK and reduction of cyclic AMP levels. Activation of AMPK from will inhibit mTOR further diminishing downstream signaling. (4) Local anesthetics will finally interfere with the contractility apparatus in the myocardium at both the endoplasmic reticulum (perturbing calcium handling) and at the sarcomere, interfering with contractility. pi3K, phosphoinositol-3-kinase; PKA, protein kinase α; Rb, ribosome.

such, pharmacological agonists/antagonists at the same targets could theoretically exacerbate or alleviate toxicity from bupivacaine. Consistent with this hypothesis, supplementation with individual agents including AICAR,[26] ATP,[41] and Akt inhibitors including rapamycin and LY294002[25] modify toxicity with bupivacaine in a synergistic or antagonistic fashion. Furthermore, metabolic conditions including diabetes[42,43] and/or deficiency of specific enzymes or cofactors including carnitine[44,45] increase susceptibility to local anesthetic toxicity. Controlling for these effects adds a layer of complexity to the necessary scientific studies, requiring controls for both local anesthetic and for lipid emulsion. In studies attempting to dissect the various mechanisms of LRT, experimentalists must use appropriate controls to differentiate exacerbation of bupivacaine toxicity with blockade of prosurvival signaling that lipid resuscitation may activate. We will address this for specific studies below.

Clinical Presentation

Clinically, toxicity presents with central nervous system and/or cardiovascular symptoms. Blockage of NaV and other channels in the central nervous system can produce excitation or depression leading to an array of symptoms including altered mental status (agitated or obtunded), prodromal symptoms (eg, tinnitus, dysesthesias, paresthesias), seizures, and/or coma. In the cardiovascular system, blockage of channels and other signaling proteins can inhibit the conduction system, alter contractility, and increase (low concentration) or decrease (high concentration) systemic vascular resistance. Signs of mild or early cardiovascular toxicity include hypertension and tachycardia, whereas more severe toxicity produces arrhythmias, bradycardia, conduction defects, and depression of cardiac output. Early conduction disturbances present in the electrocardiogram as PR interval and QTc lengthening or QRS widening and can progress to bundle branch block, complete heart block, or asystole. Direct effects on the contractile apparatus and ATP depletion further depress cardiac output leading to progressive hypotension and cardiogenic shock. Effects on energy production reduce contractility and automaticity, leading to asystole. In the vasculature, loss of ionotropic and metabotropic signaling also produce vasodilation, resulting in hypotension and contributing to shock.

Scavenging Effect (eg, "Shuttle")

Although previously regarded as a static "sink," the scavenging benefit provided by intravenous lipid emulsion (ILE) is more appropriately considered a dynamic or "shuttle" effect. Lipid resuscitation therapy is delivered as a large intravascular bolus followed by an infusion, which creates a large, or bulk-phase, lipid-soluble compartment in the blood. The lipid compartment provides a medium for bupivacaine to partition in and out of, thus yielding a theoretical "shuttle" or "subway" to transfer bupivacaine from drug sensitive organs with high blood flow, such as the heart, brain, and kidney, to organs that can store (eg, muscle, adipose) and detoxify (eg, liver) the drug (Fig. 2). The shuttle benefit is reproducible, with replication of the resulting pharmacokinetic effects in multiple animal models and 3 human experimental trials.[9–14] Two components underlie this effect specifically: (1) the binding properties of the lipid to the drug and (2) the redistributive effects of the lipid.

Binding

The lipid "droplets" from phospholipid-emulsified triglycerides exist primarily as unilamellar shells of phospholipid and phytosterols filled with a hydrophobic triglyceride core (Fig. 2) with smaller group of pure-phospholipid micelles.[46,47] The binding

properties of these lipid emulsions have made them useful as drug carriers for delivery of lipid-soluble drugs like propofol, and the binding properties make them putative drug receivers as well.[48] A number of lines of evidence support the existence of a partitioning effect based on ex vivo, in vivo, and in vitro studies. Cotreating bupivacaine blocked channels with lipid emulsion reestablishes currents in NaV channels[49,50] and voltage sensitive proton channels.[51] Furthermore, cotreatment with unesterified linolenic acid and stearic acid antagonizes bupivacaine-induced sodium channel blockade.[52] Ex vivo studies demonstrate that lipid emulsion increases bupivacaine efflux from isolated, perfused hearts and reduces cardiac bupivacaine concentrations in serial minibiopsies.[53] In intact rats, ILE treatment given after bupivacaine-induced cardiac arrest reduces cardiac bupivacaine concentrations compared with those treated with a vasopressor.[54] In vitro, lipid binds local anesthetics with a concentration-dependent effect,[55] and levels of lipid emulsion as low as 1 mg/mL can reduce plasma concentrations of bupivacaine by 88%.[56] The binding effect of lipid is nonunitary as lipid partitions more drug at higher drug concentrations, both in vitro[55] and in vivo for rats[9] and humans.[11] Analogous results occur in vivo, confirming a concentration-dependent binding.[9] The specific properties that determine uptake of drug by ILE are not clear, but studies indicate that uptake and redistribution follow traditional thermodynamic parameters.[11,55] A mixture of effects, including lipophilicity, ionization of weak base/acid, and other physiochemical characteristics, likely govern drug binding. In particular, the positive charge on local anesthetic molecules at physiologic pH may be just as important to binding as lipophilicity (below and Fig. 3).

Several avenues of research suggest a correlation between the octanol-water partition coefficient of a drug in the neutral form (LogP) and the likelihood of successful reversal of an overdose by LRT. This also applies to the octanol-water partition coefficient that is pH dependent (taking into account charged molecules), formally known as logD. In vitro evidence confirms that lipid emulsion binds lipophilic drugs more effectively,[55] but one must ask, "Does this translate to clinical practice"? Indeed, retrospective evidence by French et al[57] demonstrated that the LogP provides a good predictor of the efficacy of lipid emulsion when applied to xenobiotic overdose. Others have replicated these findings in retrospective studies (with some data overlap).[58] In animal models and isolated heart studies, ILE more effectively reverses toxicity caused by the more lipophilic drugs while less effectively reversing toxicity caused by the less lipophilic ones. In regard to local anesthetics, bupivacaine is highly lipophilic (LogP, 3.6; LogD, 2.7 at pH 7.4) and the associated toxicity is responsive to LRT, whereas less lipophilic local anesthetics like ropivacaine (LogP, 2.9) and mepivacaine (LogP, 2.0) are also clinically responsive but LRT may not produce as robust a recovery in experimental models of overdose.[59,60] Meta-analysis confirms this discrepancy as ILE produced a robust and homogenous benefit for bupivacaine toxicity, but ILE for mepivacaine was an outlier in 1 study based on funnel-plot analysis.[8] The logP-dependent benefit extends to animal models of β-blocker toxicity, where overdose with the most lipophilic β-blocker, propranolol (LogP, 3.1), responds to treatment with lipid emulsion[61] whereas overdoses of less lipophilic β-blockers metoprolol[62] (LogP, 1.79) and atenolol[63] (LogP, 0.1) are less responsive.

Factors aside from LogP also contribute to the partitioning effects. Most drugs used in medicine are weak acids/bases with a specific pKa/pKb, so the ability to take on/give up charge suggests that the magnitude of any partitioning effect is pH sensitive.[64] A number of clinical reports confirm the complexity of this effect, by demonstrating that ILE can combat drug overdoses with low (even negative) LogD. Examples include lamotrigine[65–67] (LogD at physiological pH −0.19) and baclofen[68] (LogD at physiological pH −1.72). Others have observed the same in animal models where lipid emulsion significantly reduces toxicity and mortality from *N*-methylamphetamine (LogD at physiological pH −0.57).[69] In addition, the typical formulation used for lipid resuscitation (Intralipid) is a mixture of triglycerides (primarily long chain) emulsified with egg phospholipid. The resultant lipid droplets contain a nonpolar core of triglycerides surrounded by a

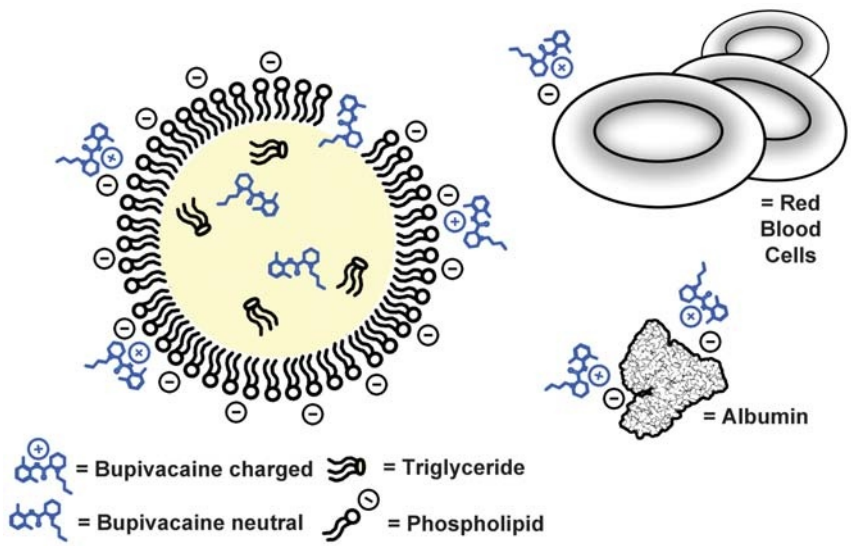

FIGURE 3. Intravascular binding of bupivacaine by lipid emulsion. In blood at physiological pH (7.4), bupivacaine will exist as a mixture of neutral and cationic (positively-charged) species. The positively-charged species will associate with plasma proteins (eg, albumin) and red blood cells, based on their negative surface charges due to electrostatic interactions. The addition of lipid will provide a third compartment (eg, lipid along with plasma and red cell) for bupivacaine to bind into. The lipid "droplets" are composed of a unilamellar shell of phospholipid (and some phytosterols) with hydrophobic triglyceride cores. Based on lipophilic partitioning, uncharged bupivacaine to insert into the membrane or move into the hydrophobic core. Additionally, the positively charged molecules will associate with the negatively charged phospholipids on the surface of the droplets based on electrostatic principles.

phospholipid/triglyceride shell.[47] The charged phospholipids produce a strongly negative zeta-potential of −40 mV.[46] This surface charge exerts strong electrostatic attractive force on drugs possessing a positive charge at physiological pH (including bupivacaine). Clearly, both electrostatic and lipophilic interactions contribute to the scavenging efficacy of LRT, but more research is needed to understand the full details of this interfacial interaction (Fig. 3).

Redistribution

Although the binding of drug into a static reservoir (or "sink") contained in the plasma presents a simple (and thus attractive) explanation, this process likely cannot remove enough drug to reverse toxicity. In essence, the 20 mL of lipid introduced from a 100 mL bolus of 20% ILE is small in comparison with the 5000 mL of circulating blood and 35 kg of muscle mass in a typical 70 kg patient. Thus, the key to LRT is active redistribution of drug from toxin-susceptible organs to toxicity-neutral (nontoxicity target) organs. Lipid facilitates dynamic scavenging whereby the drug partitions in to and out of lipid droplets (in the circulation) based on passive electrostatic and thermodynamic (entropic) parameters.[9,70] Complementary results from a number of different laboratories underlie this shift in thinking about the role of ILE and the redefinition of LRT from a sink to a shuttle. In 2013, Shi et al[10] demonstrated that treatment with ILE could acutely reduce concentrations in heart and brain, while increasing concentrations in liver, and effects were consistent with accelerated redistribution. They further elaborated that ILE treatment modified pharmacokinetic parameters, both increasing the alpha or redistribution half-life of bupivacaine in whole blood and shortening the β or elimination half-life. The authors found more bupivacaine in whole blood acutely as lipid accelerated removal of bupivacaine from heart and other organs. They further speculated that the "lipid sink" drove the redistribution along with other potential factors like "reticulo-endothelial uptake" of the lipid that might underlie the increased liver concentrations. Litonius et al[14] also found modifications in pharmacokinetic parameters in volunteers treated with ILE after a small dose of bupivacaine. They observed no difference in nonlipid bound (eg, clear) plasma bupivacaine fraction in lipid versus controls but did see an approximately 44% reduction of context sensitive half-life of total plasma bupivacaine (from 45 to 25 min). They interpreted this result as accelerated redistribution of bupivacaine after ILE treatment. Another volunteer study using ILE pretreatment before an infusion of lidocaine found similar effects of ILE on local anesthetic pharmacokinetics with reductions in unentrapped lidocaine levels in the plasma during lipid treatment.[12] The same group extended these findings by demonstrating that ILE could effectively sequester amiodarone[71] and amitriptyline[72] in pig models. Finally, Dureau et al[11] reported similar redistributive results in a human clinical trial and found that ILE pretreatment reduced peak serum concentrations of levobupivacaine and ropivacaine (C_{max}) by 30% and 26%, respectively. Computational models demonstrated that benefit arose from an increased volume of distribution and increased clearance of drug. Further computer simulations indicated that lipid would produce a more pronounced effect at higher concentrations of drug, predicting a maximal benefit when drug concentrations were rising.[11]

Although these studies suggested that lipid infusion accelerates redistribution of local anesthetic, the authors offered conflicting interpretations of the results. Experiments by Fettiplace et al[9] reconciled these interpretations by providing firm evidence that ILE acts both to partition bupivacaine and drive redistribution. In an intact rat model, the authors measured bupivacaine at various time points in the whole (lipid-laden) blood, the centrifuged or lipid-free (clear) plasma, the lipid blood fraction, and key organs (eg, heart, brain, liver, skeletal muscle). The study demonstrated that ILE partitions bupivacaine in an intact animal model, by showing increased whole blood drug concentration at analogous cardiac bupivacaine concentrations after ILE but with no such effect in clear plasma.[9] These findings confirmed that the altered partitioning of bupivacaine between heart and blood was entirely lipid-dependent. As such, bupivacaine concentration in whole blood increased (consistent with Shi et al[10]) whereas it decreased in clear plasma and ILE treatment shortened redistribution half-life in lipid-free plasma (consistent with Litonius et al[14]). The work provided robust evidence that bupivacaine partitions into the blood-lipid compartment and that lipid infusion accelerates redistribution via higher concentrations of drug in lipid-laden whole blood; this lipid compartment then facilitates redistribution of drug towards the more slowly perfused liver and skeletal muscle. Finally, the authors paired the experimental models with an in silico model that confirmed that no special mechanisms (like reticulo-endothelial scavenging) were needed to explain this redistribution and increased liver uptake. The net effect of partitioning causes cardiac and brain bupivacaine concentrations to decline more rapidly in ILE-treated animals than in controls. The steep partition curves of bupivacaine in lipid predict greatest benefit of ILE when blood bupivacaine concentration is highest (early in toxicity) consistent with the predictions of Dureau et al.[11] Moreover, the contemporaneous rapid decline of bupivacaine in the lipid fraction and increase in liver and skeletal muscle bupivacaine comports with a model where lipid carries bupivacaine from target organs to reservoir organs. The entire process occurs in a matter of a few minutes.

Furthermore, the extended data presented in the computational models and animal models address previous concerns about the "lipid sink" theory. A few authors criticize scavenging as an acceptable "single theory" primarily based on studies demonstrating the lack of statistically elevated drug concentration in venous samples of animals[72,73] or humans[12,14] treated with LRT. In addition, in a rabbit model of clomipramine overdose, lipid increased survival but no additional drug was removed with extracorporeal venovenous hemofiltration in the lipid treated group.[74] These criticisms assume that a scavenging effect would durably increase intravascular concentration of drug. It is evident from many publications that capture is concentration dependent[9,11,55] and recent animal studies show that the effect is very rapid and short-lived; that is, by the time cardiovascular parameters recover, the difference in blood-drug concentration disappears. However, we can identify the accelerated redistribution based on changes of standard pharmacokinetic parameters as seen in the 3 human studies.[11,12,14] Another shortcoming of such experimental models is the measurement of mixed blood in venous samples from large veins. Based on pharmacokinetic models of LRT, we would expect the highest concentration of drug in small veins carrying the efflux of drug-sensitive organs (or the aorta for left ventricular efflux), but this effect would disappear in mixed-blood.[70] Therefore, the lack of difference from patients' venous samples provides little information (positive or negative) about a capture effect.

Based on these accumulated data, our perspective on the scavenging effect has therefore shifted. Instead of a static "lipid sink" trapping bupivacaine, the emulsion provides a dynamic compartment, acting as a "lipid shuttle" that moves drug around the circulatory system, accelerating redistribution of local anesthetics.[12,14] This shuttle accelerates the movement of drug out of toxin-susceptible organs, through the plasma lipid phase and toward reservoir or receiver organs (liver and skeletal muscle) that embody the new "sinks" for bupivacaine in this process. This advanced understanding of the scavenging effect has implications for clinical treatment of toxicity especially when we consider oral drug overdoses where significant amounts of drug remain outside

the circulatory system in the gastrointestinal tract (typically involving drugs other that local anesthetics).[75]

Nonscavenging Benefits

Additional factors beyond scavenging, originally postulated and more recently confirmed experimentally, contribute to the reversal of toxicity by lipid. Because bupivacaine exerts toxicity via metabolic modifications,[38,41] and a metabolic substrate (fatty acids) antagonizes this toxicity, members of the community speculated that a metabolic benefit might underlie LRT. In addition, before the advent of lipid resuscitation, Van de Velde et al[76–78] demonstrated that lipid reduces myocardial ischemia-reperfusion (IR) injury in a series of papers from 1996 to 2000. Despite interest in possible nonscavenging effects of LRT, scavenging effects paradoxically proved an important confounder and delayed experimental confirmation of such effects. Given that lipid exerts a binding effect, simple partitioning-based removal of bupivacaine could explain many experimental results. This changed with the introduction of in silico modeling by Kuo and Akpa.[70] They demonstrated that a scavenging effect alone was likely not sufficient to explain the robust recovery of cardiovascular parameters. Subsequently, the original model was extended and paired with in vivo and ex vivo experiments to control for the scavenging component. The controlled computational model confirmed additional effects including a volume effect (dilution and preload)[79] and a cardiotonic effect.[9,79,80]

Beyond the volume and cardiotonic effects, lipid provides an additional benefit through the postconditioning effect first reported by Van de Velde et al.[76–78] However, bupivacaine toxicity is sensitive to kinase inhibition, so controls were needed to differentiate exacerbation of toxicity from inhibition of lipid-based signaling systems. Controls were developed using a spontaneously recoverable model of bupivacaine toxicity[81] with a known lethal dose[1] and adjusted based on pharmacokinetic profiles of cardiac bupivacaine concentrations.[9] These experimental modifications simultaneously controlled for a scavenging-based effect and identified bupivacaine-specific and ILE-specific effects.[25] That is, bupivacaine and ILE exert independent actions on biochemical signaling, which both contribute to recovery.[25] Based on these targeted models, the results indicate that nonscavenging effects include volume, direct cardiovascular benefit, and postconditioning through targets of canonical insulin signaling (Fig. 4).

Cardiovascular Effects

A cardiovascular benefit of LRT results from effects on both the heart and the vasculature. A number of models demonstrate that Intralipid supports blood pressure in human and animal models, both during and in the absence of local anesthetic toxicity. Lipid emulsion and elevated free-fatty acids drive vasoconstriction[82,83] possibly by interfering with nitric oxide signaling[84,85] or by modifying adrenergic sensitivity.[86–89] This comports with clinical evidence demonstrating that Intralipid in particular increases blood pressure when used for extended periods of time as total parenteral nutrition.[90–92] Intralipid alone, without contemporaneous drug toxicity, also increases blood pressure in animal models[80,91,92] and in vitro models.[83] When given in the context of drug toxicity, Intralipid[79,94,95] and SMOFlipid[96] increase arterial blood pressures during the recovery phase. It is unclear if improvements in the pump (cardiac function) or changes in the vasculature mediate these improved blood pressures because experimental evidence favors both. However, it is clear that cardiovascular function does not improve until cardiac concentration of local anesthetic drops below sodium-channel blocking thresholds (Fig. 5).

A number of studies indicate that a combination of volume (preload) and direct effects of the lipid on the heart mediate the positive cardiovascular effect.[9,79] Practitioners treating LAST deliver LRT intravenously as a substantial bolus (1.5 mL/kg) followed by an infusion of 0.25 mL/kg per minute with up to a total of 10 to 12 mL/kg of lipid delivered over 30 minutes. In isolated heart studies[80,97] and in vivo hearts,[93] lipid emulsion given in the absence of drug toxicity exerts both inotropic and lusitropic effects. The heart uses fatty acids as the preferred fuel under aerobic conditions and cycles them from the intracellular triglyceride pool into mitochondria

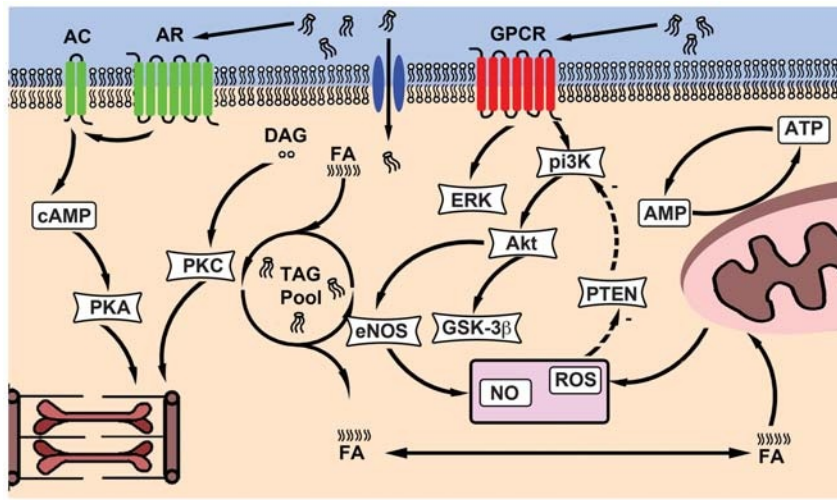

FIGURE 4. Cellular targets of lipid emulsion that may improve contractility and protect against IR injury following a local anesthetic insult. At the extracellular site, triglycerides from lipid emulsion may sensitize the cell to adrenergic signaling leading to an increase in cAMP. It may also directly target GPCRs. Downstream of GPCRs (eg, insulin receptor) there is activation of RISK targets including Akt, ERK, GSK, and eNOS. Further, FAs from the lipid emulsion will be incorporated in to the intracellular TAG pool from which fatty acids are used for β-oxidation to produce high-energy phosphates (eg, ATP) and ROS. Reactive oxygen species bursts may inactivate PTEN upstream up Akt. In the longer term, DAG from breakdown of lipid emulsions will activate PKC, which can effect downstream contractility changes at the sarcomere. cAMP, cyclic adenosine monophosphate; DAG, diacyl glycerol; eNOS, endothelial nitric oxide synthase; FAs, fatty acids; GPCRs, g-protein coupled receptors; PTEN, phosphatase and tensin homolog; TAG, triglyceride.

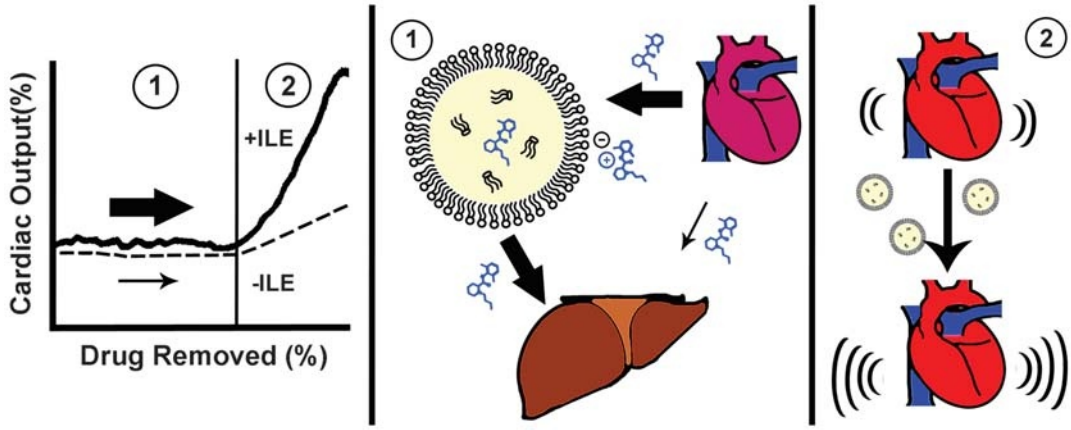

FIGURE 5. Concentration dependent recovery of cardiovascular function. The mechanism of benefit is dependent on drug-tissue concentration as seen in the graph. Cardiovascular function will not improve until bupivacaine concentrations drop below channel blocking thresholds (adapted with permission from Ref.[9]). Recovery from toxicity is contingent on redistribution of drug from heart to muscle and liver. Addition of lipid will move drug out of cardiac tissue more rapidly to move to the second stage. At these concentrations, the primary benefit is derived from the scavenging and redistribution effect (1). Once concentrations fall, lipid boosts cardiovascular function through a cardiovascular benefit dependent on volume, cardiac effects, vascular effects, and postconditioning (2).

for β-oxidation and production of high-energy phosphates.[98,99] Magnetic resonance studies show that supplementation of oleic acid (one of the primary fatty acids in Intralipid) preferentially improves contractility (as measured by dP/dt) and relaxation (−dP/dt) in models of heart failure while having no effect on rate-pressure product or left ventricular developed pressure. In rat models of LAST, lipid increases carotid flows at equal cardiac drug concentrations during recovery.[9] What moderates this improvement in contractility is unclear, but some studies have asserted a direct ion channel effect of lipid emulsion.[50,52] More recent work indicates rapid activation of insulin-related kinase signaling both during toxicity and in absence of toxicity.[25]

In contrast to the cardiac effects, other studies confirm an additional circulatory benefit with primarily noncardiac mechanisms. Three pig studies indicated that, whereas ILE increases blood pressure during LAST, it does not alter cardiac index or ejection fraction.[94–96] These authors instead report that increased vascular resistance moderates the improvements in pressure. Interpreting these studies warrants a few caveats. Pigs suffer a hypersensitivity reaction to ILE causing generalized cutaneous discoloration and hemodynamic effects including severe pulmonary hypertension,[100] whereas in rat models Intralipid protects against monocrotaline-induced pulmonary hypertension and right heart failure.[101] Local anesthetics also either vasoconstrict or vasodilate in a concentration-dependent manner. At lower concentrations, local anesthetics will increase vascular tone, whereas at higher concentrations they induce vasorelaxation by interacting with a number of protein kinases and second messengers (protein kinase C [PKC], mitogen activated protein kinase [MAPK], extracellular signal-regulated kinase [ERK], c-Jun N-terminal kinase [JNK], Ca^{2+}, endothelial nitric oxide synthase).[102–106] In addition, local anesthetics block pharmacological agent–induced vasoreactivity of a number of compounds (phenylephrine, acetylcholine, KCl).[107–109] This dualistic concentration effect creates ambiguities for experimental models using lipid emulsion. It is important to differentiate lipid-induced vascular effects from local anesthetic concentration-dependent transition from vasodilatation to vasoconstriction. For instance, as ILE sequesters bupivacaine, the endothelial concentration may shift from a vasodilatory (ie, higher) concentration to a vasoconstictive (ie, lower) concentration giving the false appearance that ILE is causing vasoconstriction when it may just reflect a vasoconstrictive concentration of bupivacaine. In isolated vasculature models, lipid emulsion provides partitioning effects, which will undoubtedly complicate the picture.[110,111] Further work is needed to determine the responses to ILE in humans both during and in the absence of toxicity.

Postconditioning Benefit

Lipid provides beneficial signaling that reduces IR injury (Fig. 4). Van de Velde et al[76–78] demonstrated this in a series of papers studying models of reperfusion after coronary ligation. Rahman et al[112] reinvigorated interest in the topic by identifying that members of the reperfusion injury salvage kinase (RISK) pathway participate in this protective effect. Recent work demonstrates that IR and local anesthetic toxicity share similar pathways and mechanisms. For instance, bupivacaine activates proapoptotic signaling pathways and inhibits members of the RISK pathway.[113] Acute bupivacaine toxicity activates AMPK signaling and reduces Akt signaling with loss of signaling downstream of mTORC1.[25] At high concentrations, local anesthetics drive proapoptotic signaling by blocking ERK along with Akt, resulting in cytotoxicity in mice myoblasts.[28] Local anesthetic challenge also diminishes signaling downstream of mTORC1 with resulting dephosphorylation of ribosomal protein s6[27] and interference with autophagosome clearance.[114] At sufficiently high concentrations, bupivacaine drives opening of the mitochondrial permeability transition pore (mPTP), which leads to calcium leak, release of cytochrome C, activation of apoptosis, and death of myocytes.[115]

In models of bupivacaine toxicity, adding lipid reduces the time to opening of the mPTP[115] and activates RISK signaling.[25] The restoration of signaling is complex, because sensitization at insulin receptor substrate 1 (IRS-1) by loss of downstream (eg, s6 kinase) activation leads to loss of feedback inhibition and secondary hyperactivation of Akt and inhibitory phosphorylation of glycogen synthase kinase (GSK-3β) during spontaneous cardiac recovery from a bupivacaine challenge. Lipid emulsion itself activates Akt with downstream phosphorylation of GSK-3β and cotreatment with both ILE and bupivacaine sequentially activates both AMPK and Akt pathways.[24] The activation of Akt and inhibition of GSK-3β contribute to prevention of opening of the mPTP; this effect is consistent with studies showing that ILE attenuates

IR injury.[112,116,117] Lou et al[118] proposed a mechanism of protection by ILE in IR injury based on accumulation of palmitoylcarnitine, which slightly uncouples mitochondria at complex IV and releases a burst of ROS, which signal RISK activation. Reactive oxygen species can oxidize and inactivate phosphatase and tensin homolog, which leads to hyperphosphorylation of prosurvival kinases,[119] making this a plausible but incompletely tested hypothesis.

Future Directions

A number of complicated questions remain in regard to other possible interactions between ILE and bupivacaine through toxicity and recovery. Local anesthetics modify signaling via other kinases and second messengers including p38 MAPK,[120,121] AMPK,[29] ROS,[122] calcium sensitization,[123] protein kinase α,[124] and PKC.[124,125] Lipid also modifies signaling in many pathways while functioning as a metabolic fuel. Lipid activates Akt, Erk1/2, and GSK-3β after IR injury,[112,116–118] as well as AMPK in peripheral tissues,[126] and can drive cardiac hypertrophy.[127] Long-term lipid treatment activates PKCθ and PKCδ via diacylglycerol to promote insulin resistance.[128–131] Other pathways including adrenergic signaling,[132] fatty-acid processing,[115] and opioid signaling[133] are implicated as participants in LRT (Fig. 4). It is clear that ILE and local anesthetics have antagonistic (or potentially synergistic) effects on intracellular signaling during LRT. Future studies are needed to control for the independent and combinatorial roles that ILE and local anesthetics play during toxicity and its reversal.

Unsupported Mechanisms

Through the years, members of the scientific community have proposed a number of possible mechanisms to explain the benefit of LRT. However, a number of these theories have propagated without robust experimental evidence or comprehensive readings of the literature. These unsupported mechanisms include direct channel-based effects, lipid modification of Ca^{2+} entry to cardiomyocytes and a theorized mass effect of lipid overcoming bupivacaine's block of the CACT.

Channel-Based Effects

It is appealing to think that lipid emulsions could exert direct actions at ion channels blocked by local anesthetics, but studies of lipids' effect on channels contradict the theory that ILE directly drives increased channel currents. Intralipid and Lipofundin reduce NaV1.5 activity in a use dependent fashion,[50] and fatty acids reduce currents at sodium, calcium, and other channels.[52,134–139] Only a single study by Wagner et al[49] found that Lipovenös infusion increases NaV currents both during toxicity and in the absence of toxicity. All the studies except Wagner et al[49] failed to account for lipid's scavenging-based effects and thus cannot preclude that scavenging may contribute the predominant rescue effect (see discussion above in nonscavenging benefits). Finally, Mottram et al[52] demonstrated that both stearic and linolenic acid inhibit NaV currents and reduce bupivacaines' NaV block, potentially by competing at the binding site.[140] As such, triglycerides or fatty acids may moderate channel currents, but they do not act as simple agonists.

Calcium Signaling

Beyond effects at ion channels in general, the idea that lipid emulsion leads to increased myocardial contractility by improving calcium influx was widely reported without substantive supporting evidence. Gueret et al[141] proposed this idea in 2007 as a response to 2 articles on successful reversal of calcium channel blocker overdose in animal models.[142,143] Subsequently, the community[144,145] has propagated this hypothesis without critically considering the lack of evidence to support it. Only a single study supports the claim that fatty acids increase calcium currents[146] whereas a host of more contemporary studies from the same group and others indicates that fatty acids and triglycerides inhibit the L-type calcium channel.[134–137] Most recently, a well designed, controlled study by Kryshtal et al[147] evaluated the use of ILE as rescue agent for verapamil toxicity. The group observed a scavenging-based effect of ILE on verapamil but no direct effect on calcium currents. Thus, the calcium hypothesis lacks experimental support.

Lipid Overcoming the Mitochondrial Block

Many authors have postulated that the increased intracellular concentration of fatty acids after ILE could overcome bupivacaine's block of CACT by mass action, but this hypothesis also lacks supporting evidence. Cocaine, cocaethylene,[39] and bupivacaine (unpublished data from the same set of experiments as the prior citation) all inhibit CACT in an uncompetitive manner, and thus, increasing the fatty-acid substrate concentration cannot completely overcome the inhibition of mitochondrial uptake. In an intact rat model of bupivacaine toxicity, cardiac output does not recover until myocardial drug concentrations fall below channel-blocking (and CACT blocking) thresholds[9] (Fig. 5). Therefore, outcompeting the CACT block by mass action is highly unlikely as an early contributor to recovery. Finally, Heinonen et al[94] confirmed that, during recovery from bupivacaine overdose in intact pigs, no CACT block was observed. This lack of mass effect also applies to lipid-based competition with at a local-anesthetic binding site as postulated by Mottram et al.[52] Competition may occur, but cardiovascular recovery does not occur until drug concentrations drop below channel blocking thresholds.

CONCLUSIONS

In summary, the understanding of lipid emulsion reversal of LAST has progressed substantially in the past 20 years. Lipid works through a multimodal mechanism comprising (1) a lipid shuttle that moves drug out of cardiac tissue and brain to muscle where it is stored and liver where its metabolism leads to detoxification; (2) improving myocardial contractility, cardiac output, blood flow and blood pressure through actions on the vasculature and heart; and (3) activating cardio-protective pathways and providing a postconditioning benefit.

More work is needed to better understand the molecular basis of these mechanisms. First, the nature of the lipid-drug binding is not completely elucidated. Molecular models and magnetic resonance studies of the bupivacaine-lipid binding could provide additional information on the interfacial dynamics. The exact nature of the cardiotonic, vasoconstrictive and postconditioning effects are not fully understood. More study is required to deduce the mechanisms underlying these effects but experimental designs must control for simple partitioning and differentiate between effects secondary to bupivacaine toxicity versus inhibition of lipid-based signaling. Furthermore, studies must account for the concentration-dependent vasodilatory vs vasoconstrictive effects of local anesthetics. We are hopeful for the future of the field as research over the past 20 years has yielded great progress and consensus around the mechanism. We are glad that blanket statements like "The mechanism is not understood" can be advanced to say, "We know how it works and are refining the details."

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