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Resuscitation with Lipid Emulsion: Dose-dependent Recovery from Cardiac Pharmacotoxicity Requires a Cardiotonic Effect

Michael R. Fettiplace, M.S.[1,2,3,*], **Belinda S. Akpa, Ph.D.**[4,*], **Richard Ripper, C.V.T.**[1,2], **Brian Zider, B.S.**[3], **Jason Lang, B.S.**[5], **Israel Rubinstein, M.D.**[2,6], and **Guy Weinberg, M.D.**[1,2]

[1]Department of Anesthesiology, University of Illinois College of Medicine, Chicago, Illinois

[2]Research & Development Service, Jesse Brown Veterans Affairs Medical Center University of Illinois at Chicago

[3]University of Illinois College of Medicine, University of Illinois at Chicago

[4]Department of Chemical Engineering; University of Illinois at Chicago

[5]University of Illinois College of Medicine, Peoria, Illinois

[6]Section of Pulmonary, Critical Care, Sleep and Allergy Medicine, Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois

Abstract

Background—Recent publications have questioned the validity of the "lipid sink" theory of lipid resuscitation while others have identified sink-independent effects and posed alternative mechanisms like hemodilution. To address these issues, we tested the dose-dependent response to intravenous lipid emulsion during reversal of bupivacaine-induced cardiovascular toxicity *in vivo*. Subsequently, we modeled the relative contribution of volume resuscitation, drug sequestration, inotropy and combined drug sequestration and inotropy to this response using an *in silico* model.

Methods—Rats were surgically prepared to monitor cardiovascular metrics and deliver drugs. Following catheterization and instrumentation, animals received a nonlethal dose of bupivacaine to produce transient cardiovascular toxicity, then were randomized to receive one of four treatments: 30% or 20% intravenous lipid emulsion, intravenous saline or no treatment (n = 7 per condition; 28 total animals). Recovery responses were compared to the predictions of a pharmacokinetic-pharmacodynamic model parameterized using previously published laboratory data.

Results—Rats treated with lipid emulsions recovered faster than did rats treated with saline or no treatment. Intravenous lipid emulsion of 30% elicited the fastest hemodynamic recovery followed in order by 20% intravenous lipid emulsion, saline, and no-treatment. An increase in arterial blood

Corresponding Author: Michael Fettiplace, M.S., Department of Anesthesiology M/C515, University of Illinois Hospital & Health Sciences System, 1740 W. Taylor, Chicago, IL 60612, Phone: 410-900-4498, Fax: 312-569-8114, mfetti3@uic.edu.
*Authors contributed equally to this work.

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pressure underlay the recovery in both lipid-emulsion treated groups. Heart rates remained depressed in all four groups throughout the observation period. Model predictions mirrored the experimental recovery and the model that combined volume, sequestration and inotropy predicted *in vivo* results most accurately.

Conclusion—Intravenous lipid emulsion accelerates cardiovascular recovery from bupivacaine toxicity in a dose-dependent manner, driven by a cardiotonic response that complements the previously reported sequestration effect.

Introduction

Intravenous lipid emulsion (ILE) therapy is an unconventional resuscitation tool that reverses cardiac pharmacotoxicity but without a clearly delineated mechanism. The most-widely hypothesized mechanism for the benefit of ILE treatment of local anesthetic systemic toxicity is the colloquially termed “lipid sink.” This posits that after the ILE infusion, the intravascular lipid compartment acts as a sink to sequester the offending drug out of target tissues, thereby reversing toxicity[1–6]. In accordance with the sink theory, some investigators argue against the use of ILE except in the case of the most lipophilic local-anesthetics[7], while others assert the sink cannot fully account for the entire recovery[8–12]. Still others discount recovery as simply a consequence of hemodilution[13]. Alternatively, recent studies indicate important sink-independent effects of ILE such as salutary cardiotonic[14,15] or metabolic[16] effects which may hasten recovery from cardiac pharmacotoxicity. To address these concerns, we tested the hypothesis *in vivo* that ILE produces a dose-dependent rescue from cardiac pharmacotoxicity that follows a distinct physiological recovery of cardiovascular parameters, which is both faster and different from a volume effect or unaided recovery. Further, we evaluated the recovery by applying an *in silico* pharmacokinetic-pharmacodynamic (PK/PD) model to assess mechanistic possibilities. This model was parameterized based on physiologic data for fluid (20% lipid emulsion or 0.9% saline) infusion in rats as previously described by our laboratory[15]. Within the model we generated recovery predictions of cardiovascular parameters as a function of specific proposed mechanisms – viz., volume resuscitation, inotropy, and lipid sequestration. Comparing these *in silico* predictions to *in vivo* observations enabled us to weigh the likelihood of proposed mechanistic possibilities based on the observed rate and patterns of recovery from cardiac pharmacotoxicity.

Materials and Methods

The experiments were conducted under clean surgical conditions at a fixed temperature and humidity in the Veterinary Medical Research Unit of the Jesse Brown VA Medical Center. The protocol was approved by the Animal Care Committee and Biologic Resources Laboratory at the University of Illinois at Chicago and the Institutional Animal Care and Utilization Committee of the Jesse Brown VA Medical Center (Chicago, Illinois).

Animal model

Twenty-eight male Sprague-Dawley rats weighing between 374 and 430g were anesthetized in a bell jar with isoflurane to allow tracheal intubation. All animals were then placed on a

heated stand under a warming lamp and mechanically ventilated with 1.2% isoflurane in 100% oxygen to maintain a constant fraction of minimum alveolar concentration of anesthetic during the experiments. A Harvard rodent ventilator model 680 (Harvard Apparatus, South Natick, MA) was set to deliver a tidal volume of 2.5 mL, at a starting rate of 65 to 70 breaths/min. Catheters were inserted into the left carotid artery and both internal jugular veins. A perivascular Doppler flow meter was placed around the right carotid artery and three subcutaneous needles were inserted to record the electrocardiogram. All animals received an i.v. dose of bupivacaine (10 mg/kg) over 20 s to produce cardiovascular toxicity. We chose this dose because it produces a transient toxicity that does not require chest compressions to recover spontaneous circulation. Ten seconds after the infusion of bupivacaine, animals received one of four treatments: 4mL/kg 30% ILE (30% Intralipid®, Baxter International, Deerfield IL) over 20 s (ILE30, n = 7), 4mL/kg 20% ILE (20% Intralipid®, Baxter International) over 20 s (ILE20, n = 7), 4mL/kg i.v. 0.9% saline over 20 s (saline, n = 7), or no treatment (null; n = 7). The number of animals in each group was based on previous studies conducted in our laboratory and power analysis ($\beta = 0.8$, $\alpha = 0.05$) assuming an effect size of 1.5. Electrocardiogram, carotid flow and carotid pressure were recorded at 1000 Hz with PowerLab Chart 7 (ADInstruments, Colorado Springs, CO) and subsequently output to .mat data files and analyzed in MATLAB (Mathworks, Natick, MA) or manually recorded and evaluated using Prism 4.0b (GraphPad, La Jolla, CA). Following instrumentation and prior to the infusion of bupivacaine, arterial blood gas measurements including pH, pCO_2, pO_2, HCO_3, sO_2, and Lactate (i-STAT1 Analyzer, i-STAT Corporation, NJ) were made to confirm a pH between 7.38 and 7.55 and a serum lactate below 2.0 mmol/L. A second blood gas was taken 10 min after bupivacaine infusion for comparison blood gases.

Computational modeling

A physiologically based PK/PD model of this experimental scheme was used to assess the likelihood of proposed mechanisms underlying the observed dose-response behavior. The model is based on a previously reported pharmacokinetic model[12] but with a number of modifications. First, the model is changed to represent a 350 g Sprague-Dawley rat with appropriate organ volumes and flows[17] as well as drug binding and elimination parameters[18–20]. Plasma-tissue partition coefficients are estimated using a mechanistic model that considered tissue composition[21]. Metabolic elimination of bupivacaine is captured using an intrinsic unbound clearance estimated from the hepatic extraction ratio[19] using the same approach described in Kuo *et al*[12]. Second, a pharmacodynamic model is introduced that represents bupivacaine cardiotoxicity as a decrease in cardiac output. Cardiovascular function is represented as being depressed as a function of total bupivacaine concentration in heart tissue using a maximal effect model of the Hill form[22], *viz* Equation 1. The parameters of this dose-response model, Hill constants β and γ, and half-maximal effective concentration (EC_{50}), were estimated based on the observations reported by Weinberg *et al.*[2]

$$E_{bupivacaine} = \frac{C^{\beta}_{bupivaciane,tissue}}{EC^{\beta}_{50} + C^{\beta}_{bupivacaine,tissue}} \quad [1]$$

Lipid emulsion pharmacokinetics is explicitly modeled as an administration to the venous compartment with subsequent delivery to organs via circulatory flows. Lipid metabolism is represented by a kinetic expression of the Michaelis-Menten form.[23] The inotropic function of lipid[15] is captured within the pharmacodynamic model as an increase in cardiac output by relating vascular lipid concentration to an increase in flow *via* an E_{max} relationship of the form given in Equation 2, where $E_{max,lip}$, EC_{50}, and γ are fitting parameters.

$$E_{lipid}=\frac{E_{max,lip}C_{lipid,plasma}^{\gamma}}{EC_{50}^{\gamma}+C_{lipid,plasma}^{\gamma}} \quad [2]$$

The flow-promoting effect of fluid infusion is represented as an increase in cardiac flow proportional to the fractional increase in venous return (Equation 3).

$$E_{volume}=\mathrm{K}_{volume}\left(\frac{Q_{venous\ return}}{Q_{baseline}}-1\right) \quad [3]$$

Homeostatic responses (mechanoreceptor-mediated autonomic control) are modeled as negative feedback control dependent on the upward departure of cardiac output from baseline. This is implemented via an auxiliary variable, U, whose magnitude evolves with the changing cardiac output as per Equation 4. Only proportional control is implemented, with control constant k_p.

$$\frac{dU}{dt}=\begin{cases} k_p[Q_{cardiac\ output}-Q_{baseline}] & for\ Q_{cardiac\ output}>Q_{baseline} \\ -k_pU & for\ Q_{cardiac\ output}\leq Q_{baseline} \end{cases} \quad [4]$$

Thus, the cardiac output at any given time is evaluated as:

$$Q_{cardiac\ output}=Q_{baseline}(1-E_{bupivacaine})(1+b_L E_{lipid})(1+b_v E_{volume})(1-\alpha U) \quad [5]$$

The constant α allows for further tuning of the control response. The model parameters were estimated from the *in vivo* data presented in Fettiplace *et al.*[15] using the parameter estimation facility of the Systems Biology Toolbox[24]. The four treatments presented in the experimental component of this work were simulated: (i) ILE30; (ii) ILE20; (iii) 0.9% Saline (Volume effect only); and (iv) no fluid intervention. Interrogation of specific mechanisms for the ILE30 and ILE20 conditions is achieved by changing the value of a binary coefficient for each effect variable in Equation 5. When set to one or zero, the coefficient switches the contribution of the corresponding mechanism on or off as desired so that the impact of volume resuscitation (binary variable: b_V) or positive inotropy (binary variable: b_L) can be assessed. The presence or absence of the sequestration/sink effect is toggled by changing the binding capacity of lipid from a value of 2,130 μM to zero as described in previous work[12]. In this work, the five mechanisms examined were (i) no treatment effect (null), (ii) volume only (same as saline treatment), (iii) volume & inotropy, (iv) volume & sink and (v) volume, inotropy & sink.

Data and statistical analyses

Cardiovascular metrics including carotid flow, mean arterial pressure (MAP), and heart rate (HR) were analyzed in MATLAB (Mathworks). Carotid resistance (CR) was calculated as MAP divided by carotid blood flow; due to differences in absolute flow and MAP levels, CR calculations were normalized to a baseline period of 30 s preceding bupivacaine-infusion and analysis was conducted on the relative CR level s. Rate-pressure-product (RPP) was calculated as MAP*HR. Samples from the same experimental-assignment were aligned based on 'key events' entered during experiments, specifically infusion of bupivacaine, and groups were compared to their baseline level (t = 0) using continuous Mann-Whitney U-test at 1Hz. For intergroup comparisons, a continuous Kruskal-Wallis test (nonparametric ANOVA) was used and individual group differences were confirmed with a Dunn's multiple comparisons posttest for nonparametric data. Blood gases were analyzed in the same manner, but were first grouped into ILE-treated conditions and null/saline treated conditions to increase analysis power. Comparisons were conducted across time (baseline, 10 min) and between conditions (ILE-treated, null/saline). To determine 50% recovery time-point, data were down-sampled to 1 Hz and checked for the first 20 consecutive seconds where data exceeded 0.5. The flow probe fell off the carotid artery in one animal from the ILE30 group and one animal in the saline group, so these animals were excluded from flow-based calculations (including peripheral-resistance calculations). To characterize recovery from 50 to 100%, once the recovery to 50% time-point was identified, experimental traces were re-aligned in MatLab to the 50% time-point and a continuous Kruskal-Wallis test was implemented with Bonferroni posttests to assess time differences during recovery among groups. Differences from CR at 50% were assessed using a continuous Mann-Whitney U-test. For illustrative purposes, only the 90% confidence interval is depicted in images of recovery from 50–100%. Confidence intervals (CI) were calculated by bootstrapping the data up to 1000 data-points and calculating CI based on median-values (bootci function in MatLab). To assess time-within the 95% CI, model outputs at (1.33 hz) were checked for fit within the experimentally-derived CI from bupivacaine infusion (t = 0) until 8 min after infusion.

Results

Blood gases

Pre-treatment blood-gas parameters were not different ($P > 0.05$ for all intergroup comparisons). At 10 min the null/saline group had an elevated lactate level compared to baseline value (<2 mmol/L; $p < 0.01$) and compared to the 10-min lactate levels for ILE30 and ILE20 treatment groups (fig. 1, $p < 0.01$). Additionally, the 10-min HCO_3 level was decreased in the null/saline treated condition from 28.2 +/− 0.6 mmol/L (standard error measurement: SEM) to 24.8 +/− 0.9 mmol/L (SEM) ($p < 0.05$), and the 10-min pH was decreased from 7.48 +/− 0.01 (SEM) (at baseline to 7.41 +/− 0.02 (SEM) at 10-min ($p < 0.05$). No differences were observed in pCO2, pO2 or sO2.

Overall group survival & time to 50% recovery

All animals in all groups survived the entire experiment without any intervention except mechanical ventilation, and all animals returned to 50% RPP by the end of recording. The

order of median recovery times was as follows: ILE30 recovered the fastest, followed by ILE20, then saline and finally the null group (fig. 2). Neighboring groups did not have statistically different recovery times, but all non-neighboring groups were statistically different (table 1). Heart rate recovered to 50% of baseline faster than other cardiovascular parameters ($p < 0.01$).

Continuous recording analysis

Using a fine-grained analysis (1 Hz), we performed continuous tests for difference from baseline in all groups. The order of recovery to baseline cardiovascular parameters was as follows: ILE30 recovered fastest, followed by ILE20 treated group, then saline treated group with the null group experiencing the slowest recovery. Following recovery, both ILE30 and ILE20 groups experienced an increase of MAP above baseline levels (fig. 3A and B). All groups had sustained decreases in heart rate across the entire sampling period (fig. 3C and D). Both ILE-treated groups recovered to baseline RPP, but neither the saline nor the null group recovered to baseline RPP during the period of analysis (fig. 3E and F). All groups recovered to baseline flow levels (fig. 3G and H).

Characterization of Recovery

In all groups, flow and RPP recovered in parallel (fig. 4A; Null and ILE20 not pictured to reduce clutter) with a strong correlation between RPP and flow ($r^2 = 0.89 +/- 0.01$ [SEM]) and between MAP and flow ($r^2 = 0.89 +/- 0.014$ [SEM]); the correlation of flow with heart rate was weaker that the other correlations ($r^2 = 0.70 +/- 0.04$ [SEM]; see heart rate and flow graphs in figure 3C and G for representative curves). All groups experienced vasodilation as they recovered and passed 50% flow ($p < 0.05$) with a relative increase in CR at 50% but a return to baseline CR levels as they approached 100% flow. Saline experienced a more significant vasodilation then either ILE30 ($p < 0.05$) or ILE20 ($p < 0.05$) (fig. 4B, ILE20 not pictured to reduce clutter). While recovering beyond 50% flow to 100% flow, both ILE20 and ILE30 recovered to 100% faster than null ($p < 0.05$, fig. 4C, ILE20 not pictured to reduce clutter) while saline recovered flow to a similar extent as both ILE20 and ILE30. During recovery from 50% to 100% MAP, both ILE30 and ILE20 recovered faster than null, and overshot the baseline level of MAP (fig. 4D, ILE20 not pictured to reduce clutter) as confirmed in the continuous sampling analysis. In contrast, saline-treated animals recovered to 100% MAP but did not overshoot baseline. There were no major differences in the characterization of recovery of ILE30 and ILE20 so ILE 20 has been omitted from the graphs in figure 4.

Pharmacokinetic-Pharmacodynamic model

The PK/PD model was used to simulate specific treatment conditions (fig. 5A) and specific mechanisms (fig. 5B). The model predicted the same order of recovery seen in the experiments with ILE30 recovering first, followed by ILE20, then saline and finally null. Altering the simulated mechanism of action in the ILE30 treatment provided a mechanism-dependent model and demonstrated the following order of predicted recovery from slowest to fastest: null, volume resuscitation alone, volume & sink, volume & inotropy, volume & inotropy & sink. Computational data were checked for best fit to the experimentally

observed 95%CI (fig. 6A); the combined volume/inotropy/sink model provided the best agreement with experimental data for ILE30 simulations (fig. 6B) remaining within the 95%CI from experimental data for 86 percent of the time. Volume & inotropy provided the second best fit, remaining within the 95%CI generated from experimental data for 76 percent of the time, while volume & sink provided the third best fit (37%), and volume only (2%), and null (2%) provided the worst fits. Simulations of ILE20 were similar to ILE30, with the combination of volume/inotropy/sink providing the best fit of 88 percent, followed by volume/inotropy (69%), volume/sink (44%), volume only (5%) and null (3%) providing worse fits. The saline (volume-only) model fit best within the 95%CI of saline-treated animals (fig. 6C and D, 75 percent of time within 95%CI), and the null model fit best to the responses of the animals in the null experimental group (51 percent of the time within 95% CI).

Discussion

In this study, we demonstrated a dose-dependent recovery from bupivacaine-induced cardiotoxicity with the higher dose of ILE producing a faster recovery. Animals treated with ILE30 experienced the fastest recovery of cardiovascular parameters followed in order by ILE20, saline and null. Furthermore, we confirmed that ILE induces a recovery of cardiovascular parameters that is faster and distinct from a volume-based resuscitation[13]. The recovery of RPP and flow in both lipid treatment groups was driven by increases in MAP, a finding that is consistent with previous studies of ILE-induced reversal of bupivacaine-induced cardiovascular toxicity[14] and with studies of the inotropic effect of ILE in the absence of systemic toxicity[15]. In contrast to the recovery in ILE-treated rats, the recovery of flow in the saline group was associated with vasodilation below baseline peripheral resistance; this response was not observed in the ILE or null groups. This result agrees with other groups research, detailing the vasoconstrictive effects of ILE during recovery from local-anesthetic toxicity[25,26].

Intravenous lipid emulsion improved cardiovascular recovery by several parameters tested in our experimental model: 1) reduced time required to return to 50% of baseline RPP, MAP and flow; 2) improved end-point cardiovascular parameters; and 3) improved statistical recovery in a fine-grain analysis. The observed dose-dependent response suggests that higher concentrations of intravenous lipid formulations would provide additional clinical benefit. While ILE20 was used for the original clinical resuscitations[27,28] and is now considered standard of care, the original animal experiments used a 30% formulation to demonstrate effectiveness[5]. Formulations with concentrations higher than 30% may provide even more clinical benefit but their use is impractical because of poor stability[29]. Despite the experimental benefit of ILE30, more studies are needed to define potential adverse effects of acute infusion. The adverse effect profiles of lower percentage formulas are well established,[30] but less is known about 30% formulations due to the limited availability of 30% lipid emilsions at clinical pharmacies and because 30% formulations are usually diluted prior to infusion in order to reduce final lipid concentration. However, in the limited number of clinical studies, 30% Intralipid® infusion exhibits a better safety profile with less side effects than infusion of either 20% or 10% lipid emulsions[31–33] possibly owing to the lower concentrations of phospholipids[30].

Furthermore, by extending a previously-described pharmacokinetic model[12] and adding a pharmacodynamic component, we found that the rapid recovery of cardiovascular parameters in ILE-treated rats most likely requires a cardiotonic effect[15]. Taken together, these data confirm that several mechanisms beyond the "lipid sink"[2] underlie the rapid resuscitation from local anesthetic systemic toxicity. With several mechanisms at work, our observations explain how ILE might provide benefit in the treatment of less-lipophilic drugs that also provoke cardiovascular toxicity such as baclofen[34] (LogD at pH 7.4 = −1.72) and lamotrigine[35–37] (LogD at pH 7.4 = −0.19). Extending this logic, the data also suggest that synthetic phospholipid dispersions designed to sequester drugs[38–40] may not reverse cardiotoxicity as effectively as ILE if they lack an inotropic effect. The mechanism that causes the inotropy is a matter of speculation, but additional mitochondrial processing of fatty-acids[16] increased energetic intermediates[41], modulation of intrinsic signaling systems[42,43] or fatty-acid modification of Ca+ currents[16,44] could all play a role.

The potential to treat more than just local anesthetic or lipophilic drug overdoses could have a large impact on medicine beyond the operating room. Adverse cardiovascular events account for almost 17% of drug overdose admissions[45] to hospitals, and contribute to the more than 30,000 drug-related deaths each year in the United States[46]. Certain cardiotoxic agents have specific treatments (*e.g.*, monoclonal antibodies for digitalis toxicity), but many drug overdoses affecting the cardiovascular system lack mechanistically unique treatments. Generic treatments such as sodium bicarbonate and euglycemic hyperinsulemia are used in some of these cases to increase cardiac output in the face of toxicity with only modest success[47]. In the past few years, practitioners have increasingly considered ILE as another generic treatment for cardiac pharmacotoxicity resulting from overdose of local anesthetics and a variety of other drugs. Despite a number of published clinical reports[37,48–52] showing a beneficial effect of ILE in combating toxicity from various categories of drugs (*i.e.*, calcium channel blockers, β blockers, tricyclic antidepressants and atypical antipsychotics), the lack of a fully explanatory mechanism has led some to assert that the benefit is overstated[7–11,13,53]. Moreover, there is the continuing question of what drugs are appropriate to treat with ILE. Further studies are needed but a predominant inotropic effect indicates that lipid resuscitation has therapeutic potential beyond overdoses involving lipophilic drugs.

There are a number of limitations to our study. We used a lower dose of bupivacaine even though this dose may not replicate clinical situations of full cardiac arrest. Our goal was to produce a transient cardiovascular toxicity to avoid the use of concomitant chest compressions that previous ILE resuscitation studies have relied on[16,54–60]. Further, since ILE has reported cardioprotective effects during ischemia-reperfusion[42,61,62] we wanted to avoid systemic hypoperfusion that high dose bupivacaine elicits. We considered pharmacological interventions to probe the mechanisms but instead used an *in silico* model (details described in the next paragraph) to assess the system because of the complexity of the interplay between bupivacaine, ILE and the cardiovascular system. Local-anesthetics can modulate several cellular pathways simultaneously, such as but not limited to blocking the sodium channel[63], uncoupling oxidative phosphorylation in the mitochondria[64,65], inhibiting acylcarnitine exchange in the mitochondria[66], and interfering with intracellular signaling

cascades[67,68]. Pharmacological interventions, such as inhibitors, used to mediate specific mechanisms may in fact, exacerbate bupivacaine toxicity by interfering with one of these many pathways. Detangling this augmentation of toxicity from inhibition of lipid resuscitation becomes a challenge. Additionally, these specific inhibitors may be subject to precisely the same sequestration effect thought to be a main feature of lipid action[2]. If ILE sequesters the pharmaceutical inhibitor of interest, then it prevents an unambiguous assessment of the inhibitor's effects on resuscitation.

In comparison, other researchers are increasingly using *in silico* models to assess and predict complex physiological events in the context of critical care.[69] Our adoption of a computational approach offers the possibility of explicitly representing proposed mechanisms. This is achieved by the use of dynamic semi-empirical models to represent phenomena that are expected to impact cardiac performance. Use of a physiological compartment model allows the effects of bupivacaine and infused fluids to be related directly to anesthetic concentrations and excess fluid volumes at their respective sites of action. In addition to capturing the flow-promoting effect of increased venous return, the model explicitly accounts for local hemodilution *via* dynamic evaluation of erythrocyte and plasma protein concentrations. An explicit model of ILE distribution and elimination permits the inotropic and sequestering impact of ILE to be related to instantaneous values of lipid concentration in the heart. Further, the rapidly acting response of baroreceptor-mediated homeostasis has been incorporated to prevent nonphysiological dynamics (*e.g.*, prolonged elevation of cardiac output).

Unlike in the experimental study, elements of the PK/PD model are easily activated or deactivated (as described in the last paragraph of the *Computational Modeling* section of the Methods), making it possible to probe the potential role of individual mechanisms. However, as mechanisms within the model are fully coupled, predictions should be interpreted with care. The overall impact of the sink and inotropic mechanisms on predicted recovery are not, for example, purely additive. Rather, they interact *via* highly nonlinear relationships. Furthermore, the effect models and feedback control model we employed make no attempt to separately address the impact of bupivacaine toxicity and fluid interventions on specific cardiovascular variables (HR, MAP, peripheral resistance, *etc*.). Assessing the role of the model in light of these limitations is the subject of ongoing research.

Conclusion

We have found a dose-dependent response to ILE in recovery from a non-lethal challenge of bupivacaine with 30% intravenous lipid emulsion producing a faster recovery than 20% intravenous lipid emulsion. The higher percentage lipid formulation accelerated recovery that was not driven by a volume-only effect. Additionally, a PK/PD model suggests that a cardiotonic effect predominates over the 'lipid sink' in providing a rapid cardiovascular recovery from bupivacaine-induced cardiovascular toxicity. Additional studies using other cardiotoxic drugs and animal models are warranted to further assess these observations.

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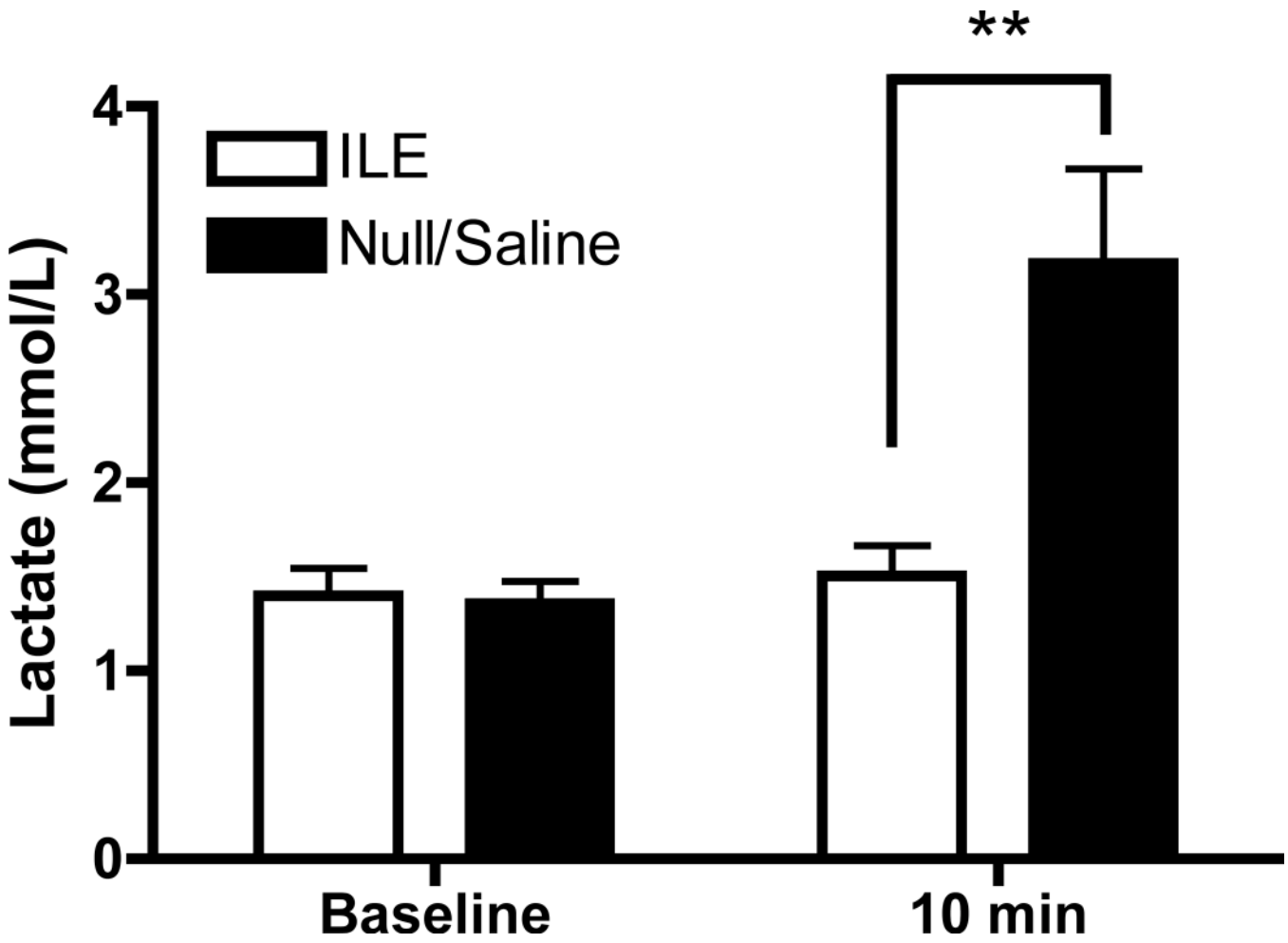

Figure 1.

Baseline and 10-min lactate levels for the combined treatment conditions (ILE = combined 30% intravenous lipid emulsion & 20% intravenous lipid emulsion). At 10 min, Saline\Null was elevated relative to baseline ($p < 0.01$) and relative to the 10-min lactate levels in the combined ILE group (** $p < 0.01$). Ten-minute ILE levels were no different from baseline levels.

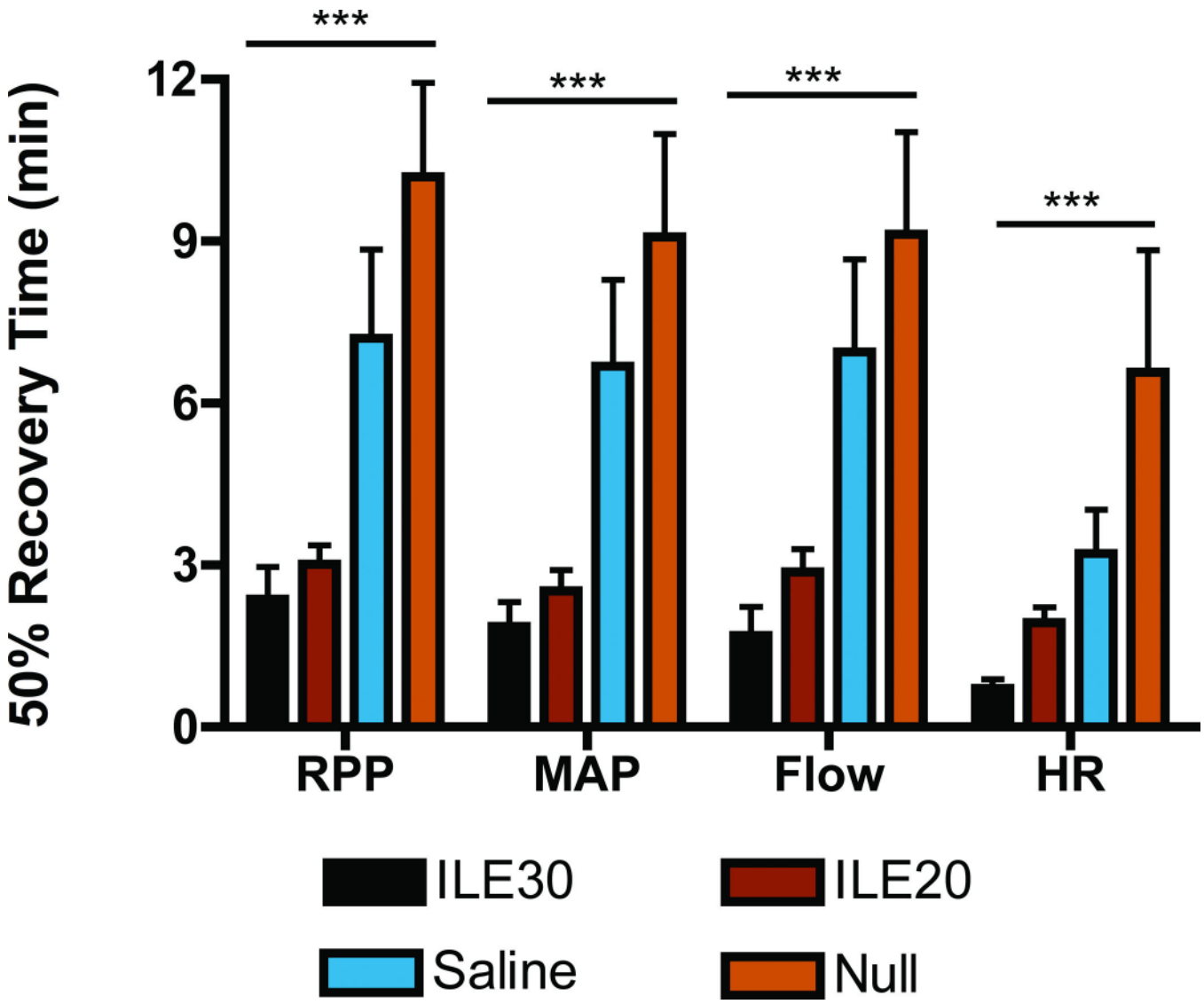

Figure 2.

Graphic representation of time to 50% recovery for rate-pressure-product (RPP), mean arterial pressure (MAP), carotid flow (Flow) and heart rate (HR) in response to treatment with 30% intravenous lipid emulsion (ILE30), 20% intravenous lipid emulsion (ILE20), Saline and nothing (Null). Accompanying recovery data are depicted in table 1.(*** $p <$ 0.001)

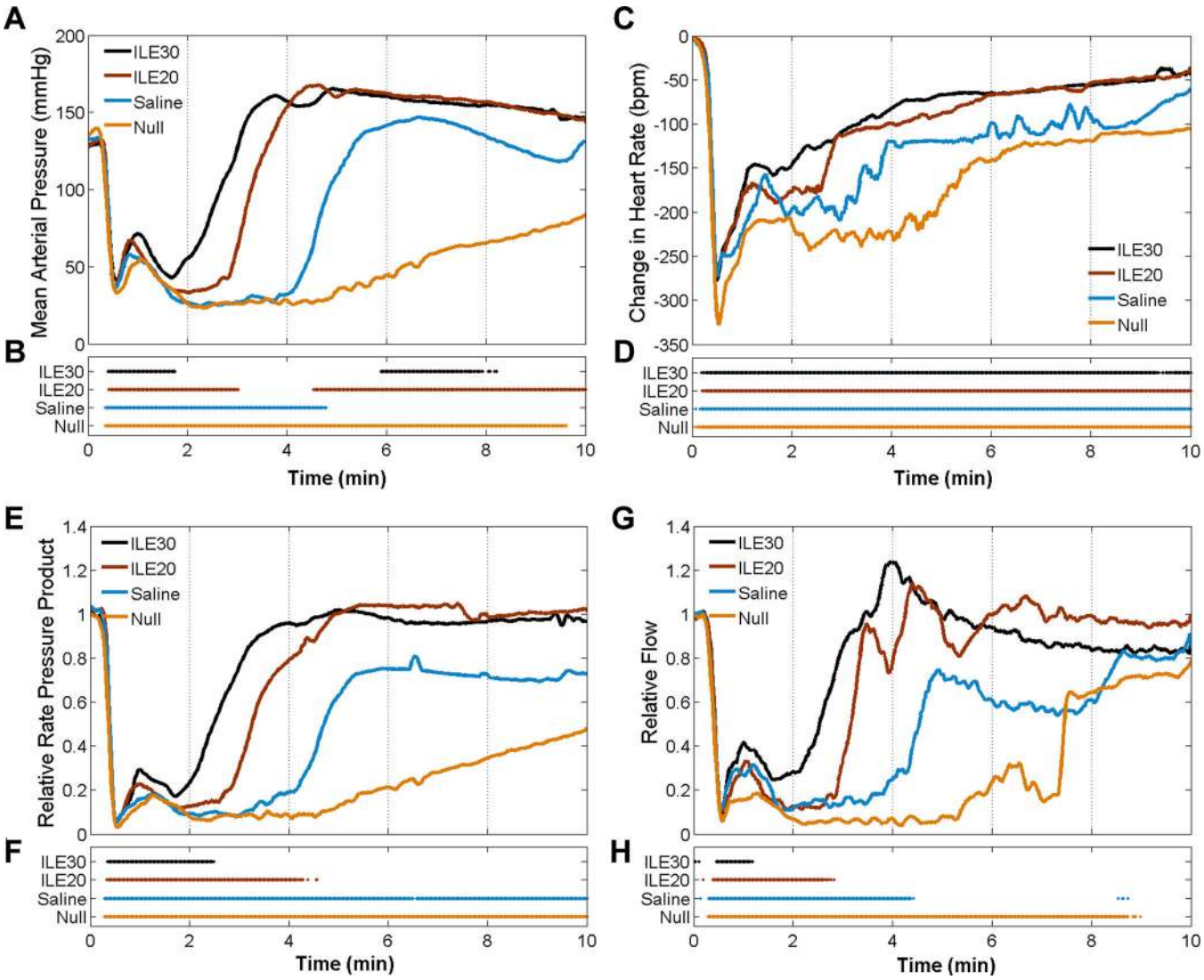

Figure 3.

(A) Median plot of mean arterial pressure normalized to baseline (average over 20-s prior to bupivacaine infusion; 10 mg/kg bupivacaine infusion at t = 0). Response to four different intravenous infusions starting at t = 30 s: 4mL/kg 30% intravenous lipid emulsion (ILE30), 4mL/kg 20% intravenous lipid emulsion (ILE20), 4mL/kg 0.9% saline (Saline), and no treatment (Null). **(B)** Plot of time points when rate-pressure-product is different from baseline ($p < 0.05$) based on paired Mann-Whitney U-test with 1-s time-scale for 30% intravenous lipid emulsion, 20% intravenous lipid emulsion, saline, and null. **(C,D)** Same as A & B, but for median heart rate. **(E,F)** Same as A & B but for median rate-pressure-product **(G,H)** Same as A & B, but for carotid blood flow.

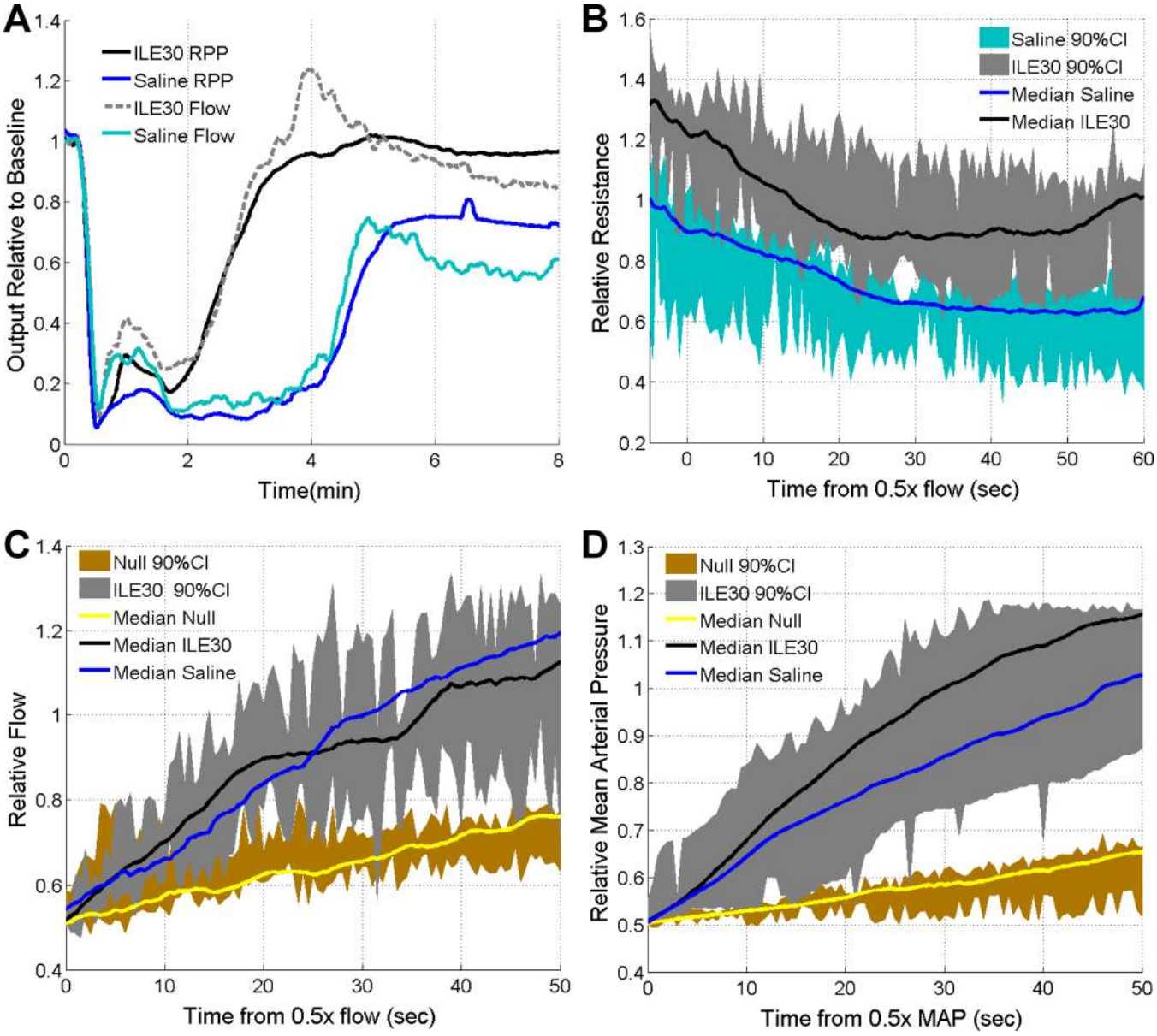

Figure 4.

(A) Plot of both median rate-pressure-product (RPP) and median carotid flow (Flow) for 30% intravenous lipid emulsion (ILE30) and saline treatments. **(B)** Plot of median carotid resistance relative to prebupivacaine baseline with accompanying 90% confidence intervals (90%CI) aligned to the recovery of 50% flow (0.5× flow); alignment was conducted on individual animals and aggregated post-alignment. **(C)** Plot of median carotid flow (Relative Flow) relative to prebupivacaine baseline with accompanying 90% confidence intervals (90%CI) aligned to the recovery of 50% flow (0.5×). **(D)** Plot of median mean-arterial-pressure relative to prebupivacaine baseline with accompanying 90% confidence intervals (90%CI) aligned to the recovery of 50% mean arterial pressure (0.5× MAP).

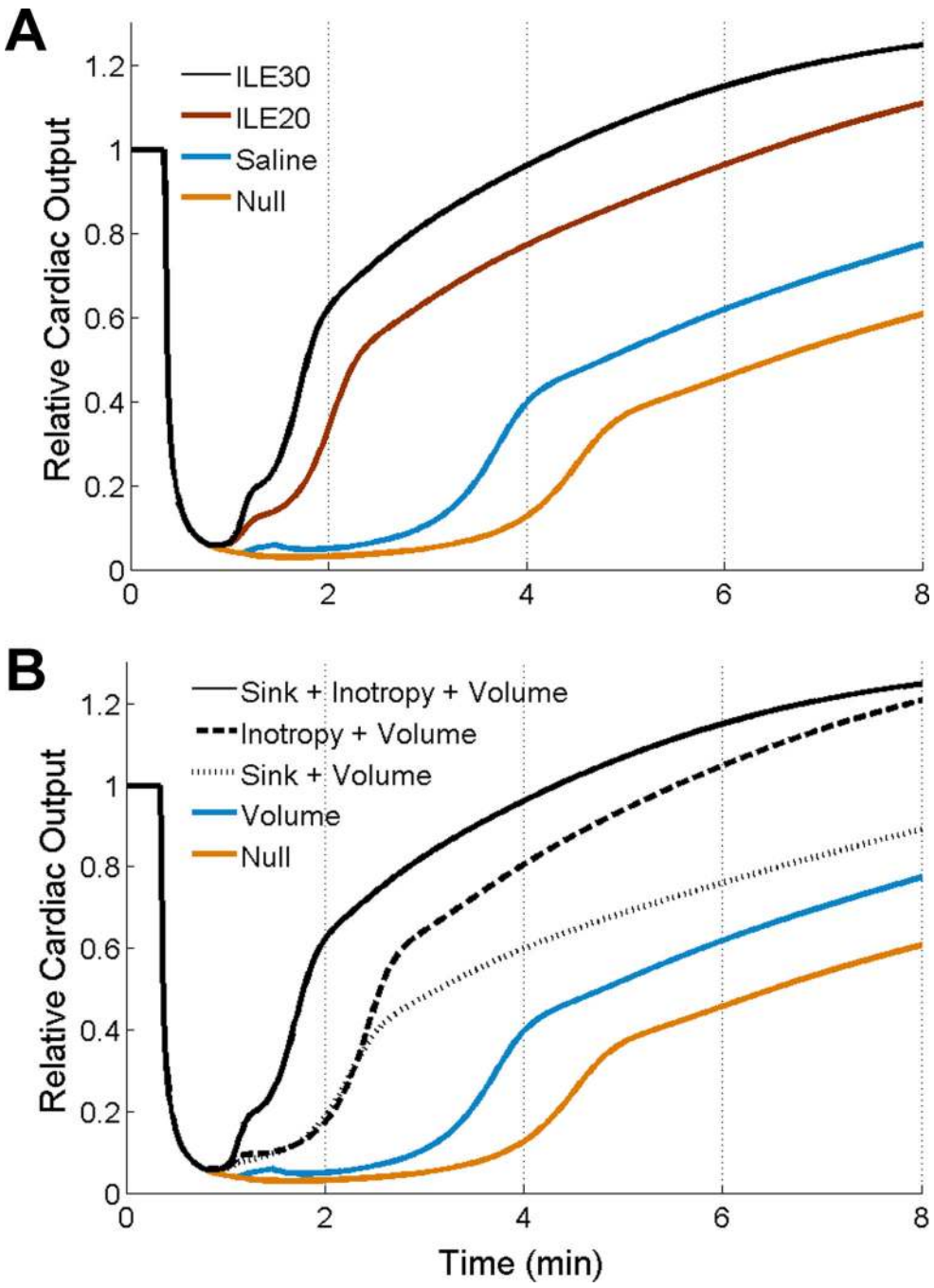

Figure 5.

Pharmacokinetic-pharmacodynamic model of cardiac output relative to baseline (1.0) following bupivacaine-induced cardiac depression and subsequent recovery with different treatments. **(A)** Treatment-specific curves matched to experimental conditions for 30% intravenous lipid emulsion (ILE30), 20% intravenous lipid emulsion (ILE20), 0.9% Saline (Saline) and no treatment (Null). For ILE30 and ILE20, the modeled response includes mathematical contributions from a volume effect, a sink effect and an inotropic effect. **(B)** Mechanism-specific contributions to recovery from bupivacaine cardiac depression

following an infusion of 30% intravenous lipid emulsion; mechanisms represented are no effect (Null), volume effect only (Volume; equivalent to 0.9% Saline model), sink & volume, inotropy & volume, and a combination of sink, inotropy & volume. Specific mechanisms and combinations were probed by turning on and off individual mathematical components as described in the Computational Modeling section of the methods.

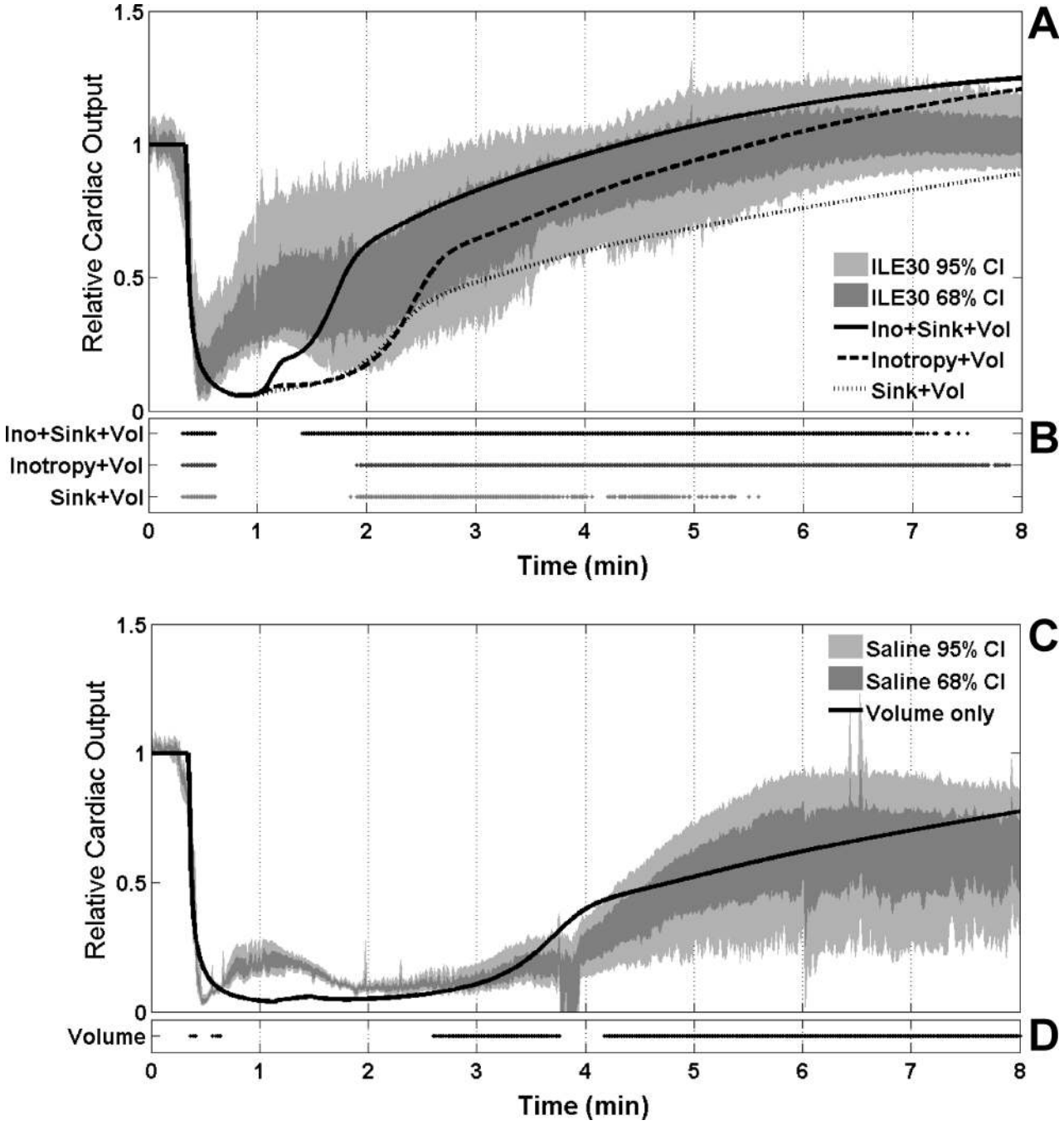

Figure 6.

(A) 95% confidence-interval (95%CI) and 68% confidence interval (68%CI) generated with experimental rate-pressure-product data for 30% intravenous lipid emulsion (ILE30) experimental treatment overlaid with pharmacokinetic-pharmacodynamic model predictions. **(B)** Continuous plot of time when the pharmacokinetic-pharmacodynamic curves for volume & sequestration (sink + vol), volume & inotropy (inotropy + vol), and volume/inotropy/sequestration (ino + sink + vol) remain within the experimental confidence interval. **(C)** 95% confidence-interval and 68% confidence interval (68%CI) generated with experimental

rate-pressure-product data for saline treatment overlaid with pharmacokinetic-pharmacodynamic prediction for the volume resuscitation mechanism. **(D)** Continuous plot of time when volume curve remains within the experimental confidence interval.

Table 1

Time to 50% Recovery of Cardiovascular Parameters

Parameter	ILE30	ILE20	Saline	Null
RPP (*ks*: *p*=0.0003)	**131s (53, 230s)** $^{\Delta,**}$	**176s (129, 233s)** *	**263s (192, 673s)** ¶	**580s (356, 868s)** $^{¶¶, §}$
MAP: (*ks*: *p*=0.0002)	**110s (46, 178s)** $^{\Delta,**}$	**164s (97, 207s)** *	**253s (167, 635s)** ¶	**466s (265, 825s)** $^{¶¶, §}$
Flow: (*ks*: *p*=0.0004)	**134s (36, 201s)** $^{\Delta,***}$	**170s (111, 234s)** *	**359s (151, 682s)** ¶	**427s (271, 826s)** $^{¶¶¶, §}$
HR: (*ks*: *p*=0.0003)	**38s (18, 68s)** $^{\Delta,***}$	**143s (74, 158s)**	**162s (75, 312)** ¶	**280s (63, 727s)** ¶¶¶

All values expressed as median + 95% confidence interval (lower limit, upper limit).

Flow = carotid blood flow; HR = heart rate; ILE20 = 20% intravenous lipid emulsion; ILE30 = 30% intravenous lipid emulsion; *ks* = Kruskal-Wallis value; MAP = mean arterial pressure; RPP = rate pressure product.

*Different from Null $p < 0.05$;

***vs.* Null $p < 0.01$;

****vs.* Null $p < 0.001$

$^{\Delta}$Different from Saline $p < 0.05$

§Different from ILE20 $p < 0.05$

¶Different from ILE30 $p < 0.05$;

¶¶*vs.* ILE30 $p < 0.01$;

¶¶¶*vs.* ILE30 $p < 0.001$