Lipid Rescue Reverses the Bupivacaine-induced Block of the Fast Na^+ Current (I_{Na}) in Cardiomyocytes of the Rat Left Ventricle

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ABSTRACT

Background: Cardiovascular resuscitation upon intoxication with lipophilic ion channel–blocking agents has proven most difficult. Recently, favorable results have been reported when lipid rescue therapy is performed, *i.e.*, the infusion of a triglyceride-rich lipid emulsion during resuscitation. However, the mechanism of action is poorly understood.

Methods: The authors investigate the effects of a clinically used lipid emulsion (Lipovenös® MCT 20%; Fresenius Kabi AG, Bad Homburg, Germany) on the block of the fast Na^+ current (I_{Na}) induced by the lipophilic local anesthetic bupivacaine in adult rat left ventricular myocytes by using the whole cell patch clamp technique.

Results: Bupivacaine at 10 μM decreased I_{Na} by 54% (−19.3 ± 1.9 $pApF^{-1}$ *vs.* −42.3 ± 4.3 $pApF^{-1}$; n = 17; $P < 0.001$; V_{Pip} = −40 mV, 1 Hz). Addition of 10% lipid emulsion in the presence of bupivacaine produced a 37% increase in I_{Na} (−26.4 ± 2.8 $pApF^{-1}$; n = 17; $P < 0.001$ *vs.* bupivacaine alone). To test whether these results could be explained by a reduction in the free bupivacaine concentration by the lipid (lipid-sink effect), the authors removed the lipid phase from the bupivacaine–lipid mixture by ultracentrifugation. Also, the resulting water phase led to an increase in I_{Na} (+19%; n = 17; $P < 0.001$ *vs.* bupivacaine), demonstrating that part of the bupivacaine had been removed during ultracentrifugation. The substantially less lipophilic mepivacaine (40 μM) reduced I_{Na} by 27% (n = 24; $P < 0.001$). The mepivacaine–lipid mixture caused a significant increase in I_{Na} (+17%; n = 24; $P < 0.001$). For mepivacaine, only a small lipid-sink effect could be demonstrated (+8%; n = 23; $P < 0.01$), reflecting its poor lipid solubility.

Conclusion: The authors demonstrate lipid rescue on the single-cell level and provide evidence for a lipid-sink mechanism. **(Anesthesiology 2014; 120:724-36)**

ACCIDENTAL systemic toxicity of local anesthetics (LA) is a rare but potentially life-threatening complication of regional anesthesia.[1,2] The symptoms usually involve the central nervous system and the heart, ranging from rather harmless signs such as mild discomfort, nausea, dizziness, or minor electrocardiogram changes up to seizures, coma, ventricular fibrillation, and asystole.[1] Therapy of severe systemic LA intoxication consisted of advanced cardiac live support and has been a challenge because no specific therapy was available. This situation started to change some 15 yr ago, when infusion of a commercially available lipid emulsion was shown to effectively treat intoxications with the lipophilic LA bupivacaine in rats[3] and later in dogs also.[4] Only a few years later, the concept was transferred to the clinic, when patients with LA

What We Already Know about This Topic

- Cardiovascular resuscitation secondary to intoxication with lipophilic ion channel–blocking agents has proven to be a significant therapeutic challenge. Recently, favorable results have been reported when lipid rescue therapy is performed.
- This study determined whether the application of a clinically used lipid emulsion might reverse the bupivacaine-induced changes in the action potential and the fast Na^+ current (I_{Na}) of left ventricular cardiomyocytes and compared the results with those obtained with the hydrophilic local anesthetic mepivacaine

What This Article Tells Us That Is New

- The study demonstrates that a lipid emulsion partially reverses the effects of bupivacaine on the action potential and the fast Na^+ current of left ventricular cardiomyocytes and provides evidence for a lipid-sink mechanism

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intoxication refractory to conventional treatment were successfully treated with infusion of a lipid emulsion.[5,6] Since then, numerous case reports have been published reporting the successful use of lipid emulsion therapy in intoxications with LAs and, more recently, other lipophilic drugs[7] and, moreover, lipid infusion has meanwhile been included in resuscitation guidelines.[8]

To date, the mechanism of action of lipid resuscitation is not well understood. Among different concepts, the so-called lipid-sink hypothesis suggests an accumulation of the lipophilic LA in the lipid phase of the lipid emulsion, thereby decreasing the concentration of the LA in the water phase and hence in the tissue.[9] According to this concept, there are experimental observations that lipid emulsion infusion is effective in the treatment of intoxications with the lipophilic LA bupivacaine, but not with the less lipophilic LAs mepivacaine[10] and ropivacaine.[11,12] Moreover, with increasing concentrations of the lipid, a decrease in the myocardial concentration of bupivacaine was observed.[13] However, lipid resuscitation has also been successfully applied to intoxications with the substantially less lipophilic LA lidocaine,[14] suggesting additional modes of action.

Although the exact mechanism of action is not clearly understood, a common clinical observation reported during the application of the lipid emulsion in patients is a rapid improvement in electrocardiogram alterations, *i.e.*, shortening of the QRS complexes, decrease in QTc-interval, or termination of ventricular arrhythmias.[15,16] This suggests a rather rapid action of the lipid emulsion on the effects of the LA on the cardiac action potential (AP) and hence, the underlying ion channels.

In the current study, we therefore asked whether the application of a clinically used lipid emulsion might reverse the bupivacaine-induced changes in the AP and the fast Na^+ current (I_{Na}) of left ventricular cardiomyocytes and compared the results with those obtained with the hydrophilic LA mepivacaine. We show that a lipid emulsion partially reverses the effects of bupivacaine on the AP and the Na^+ current at the cellular level. Moreover, we provide evidence for a lipid-sink mechanism. Finally, by investigating the effect of lipid emulsion on the LA action on heterologously expressed hKv4.2 + hKChIP2b channels in *Xenopus laevis* oocytes, we show that the effect of the lipid emulsion is not limited to the AP and the Na^+ current in cardiomyocytes, but appears as to be a general mechanism on ion channels.

Materials and Methods

Isolation of Myocytes

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996) and was approved by local government authorities (Government of Middle Franconia, Ansbach, Germany). Myocytes were isolated from the cardiac left ventricular free wall of female Wistar rats (weighing approximately 220 g) as described previously.[17,18] Briefly, after induction of deep anesthesia by intraperitoneally injecting thiopental-sodium (100 mg/kg body mass; Inresa Arzneimittel GmbH, Freiburg, Germany), the heart was quickly excised and placed into cold (4°C) Tyrode solution where it stopped beating immediately. Subsequently, the heart was perfused with modified Tyrode solution containing 4.5 mM Ca^{2+} and 5 mM EGTA (approximately 1 µM free Ca^{2+}) *via* the ascending aorta at 37°C for 5 min. The perfusion was continued for 19 min, recirculating 25 ml of the same solution containing collagenase (CLS type II, 160 U/ml; Biochrom KG, Berlin, Germany) and protease (type XIV, 0.6 U/ml; Sigma-Aldrich Corporation, St. Louis, MO). Finally, the heart was perfused with storage solution[19] containing 100 µM Ca^{2+} for 5 min. Using fine forceps, myocytes were carefully dissected from the subepicardial and the subendocardial layer of the left ventricular free wall and placed in cell culture dishes containing the same solution. Tissue pieces were minced and gently agitated to obtain single cardiomyocytes. After adaption to physiological Ca^{2+} levels, cells were transferred to cell culture dishes containing storage solution supplemented with 100 U/ml penicillin and 0.1 mg/ml streptomycin, stored at 37°C in a water saturated atmosphere containing 5% CO_2 and used for experiments for up to 36 h. Only quiescent single rod-shaped cells with clear cross striations were used for experiments.

Patch Clamp Technique

The ruptured-patch whole cell configuration was used as described previously.[18,20] Currents were recorded using an EPC-10 amplifier (HEKA Elektronik Dr. Schulze GmbH, Lambrecht, Germany), controlled by the PULSE-Software (HEKA). Membrane capacitance (C_m) and series resistance (R_s) were calculated using the automated capacitance compensation procedure of the EPC-10 amplifier. During AP measurements, R_s averaged 6.8 ± 2.9 MΩ (mean ± SD, n = 95) and C_m was 130.2 ± 35.0 pF (mean ± SD, n = 95). During I_{Na} measurements, R_s averaged 5.2 ± 1.7 MΩ (mean ± SD, n = 88) and was compensated by 80%. This resulted in an average effective R_s of 1.0 MΩ, leading to an average voltage error of 5.5 mV (average current −5.3 ± 2.4 nA [mean ± SD], n = 88) for the native current, 2.5 mV (average current −2.4 ± 1.0 nA [mean ± SD], n = 41) when blocked with bupivacaine and 4.0 mV (average current −3.8 ± 1.8 nA [mean ± SD], n = 47) when blocked by mepivacaine (see also the section on limitations in the Discussion). C_m averaged 115.8 ± 24.3 pF (mean ± SD, n = 88). Effective R_s and C_m resulted in an average time constant of 120 µs for charging of the membrane capacitance. Together with the relatively slow kinetics of I_{Na} at −40 mV at room temperature, this allowed the peak of the capacitive artifact to be clearly separated from the peak of the Na current. The capacitive artifact

was subtracted using a P/4 leak subtraction protocol. Pipette potentials were corrected for the liquid junction potential of 13 or 9 mV for the solution with reduced Na^+ concentration, respectively. All experiments were performed at room temperature (22°–24°C). For each set of experiments, myocytes obtained from two to four rats were used. Some control experiments were performed on cells from one rat only. All reported potentials are pipette potentials.

Trains of 150 APs were elicited at 1 Hz in cells with a resting membrane potential negative to −80 mV (a V_m positive to −80 mV was considered to be secondary to a leaky electrical access to the myocyte) by depolarizing current pulses of 5 ms duration. After 50 APs, the solution was switched to the solution containing the LA (bupivacaine or mepivacaine). After another 50 APs, the solution was switched to the mixture of lipid emulsion and the LA. The last AP under each condition was evaluated.

To elicit I_{Na}, cells were clamped to −40 mV for 20 ms and then for 80 ms to 0 mV. Holding potential was −90 mV and cycle length was 1 s. I_{Na} was measured at the pulse to −40 mV to assess the Na^+ current during the upstroke of the AP while the pulse to 0 mV served to simulate the plateau phase of the AP. The standard protocol consisted of 200 pulses: 50 under control conditions, 50 with LA, 50 with LA plus lipid emulsion, and another 50 under control conditions. The last current under each condition was evaluated. Leak subtraction was applied using a P/4 protocol, and R_s and C_m were automatically readjusted before each pulse. The extracellular Na^+ concentration was reduced to 20 mM by replacing 118 mM Na^+ with Cs^+. Moreover, this inhibited the inward-rectifying K^+ current,[21] thus rendering the leak subtraction possible. All experiments were conducted under continuous perfusion of 7 ml/min. Current and voltage recordings were low-pass filtered at 5 kHz and sampled at 25 kHz. To compensate for variability in cell size, currents were divided by the cell capacitance and are thus given as current densities in $pApF^{-1}$.

Isolation, Injection, and Maintenance of *X. laevis* Oocytes

Female *X. laevis* were anesthetized by immersing in tap water containing 0.2% MS-222 for 10 min. Ovarian lobes were surgically removed, and oocytes were isolated by enzymatic digestion using collagenase (CLS type II, 260 U/ml; Biochrom KG, Berlin, Germany) in Ca^{2+}-free OR2 solution at 10°C for 3–4 h. We used full-length complimentary DNA transcripts encoding human Kv4.2 (hKv4.2) inserted in pGEM and human KChIP2b (hKChIP2b) included in pGEM-HJ. Linearized plasmids were used as templates for coding RNA synthesis using the mMessage mMachine Transcription Kit T7 (Life Technologies, Grand Island, NY). Defolliculated stage V and VI oocytes were injected with 0.1 ng hKv4.2 + 0.5 ng hKChIP2b coding RNA. Coding RNAs were dissolved in RNAse-free water, and the total volume injected was 50 nl per oocyte. After injection, oocytes were maintained in ND96 solution and were studied 2 days after injection.

Two-electrode Voltage Clamp Experiments

Oocytes were transferred to a perfusion chamber, which is continuously superfused with NaCl-95 solution, and impaled with electrodes (0.1–1.5 MΩ) filled with 3 M KCl. To increase the Lipovenös® (Fresenius Kabi AG, Bad Homburg, Germany) content to more than 10% in the bath solution, a stock of a modified bath solution was designed which was diluted as needed with Lipovenös® or control to assure a constant K^+ concentration of 4 mM and Ca^{2+} and Mg^{2+} concentrations of 1 mM each. Whole cell currents were measured at room temperature (19°–22°C) with the two-electrode voltage clamp technique using an OC-725C amplifier (Warner Instruments LLC, Hamden, CT) controlled by the Pulse-software (HEKA) *via* an LIH-1600 interface (HEKA). An Ag–AgCl pellet placed directly in the bath solution served as a reference electrode for the current injection circuit, whereas an additional Ag–AgCl pellet located close to the oocyte was used to sense the bath potential to minimize series resistance errors. Currents were elicited by voltage steps to +40 mV from a holding potential of −90 mV. Pulsed current data were filtered at 1 kHz and sampled at 5 kHz.

Measurement of LA Concentrations

Bupivacaine and mepivacaine concentrations were measured by gas chromatography–mass spectrometry using an Agilent model 6890plus gas chromatograph and an MSD 5973 in the electron impact selected ion monitoring mode (Agilent Technologies Inc., Santa Clara, CA). Extraction of the samples was adopted from the study by Colin *et al.*[22] Shortly, samples (50 µl) were extracted by fluid–fluid extraction using hexane:ethyl acetate (70:30, 4 ml) plus 0.05% Lipofundin® (20%; B. Braun Melsungen AG, Melsungen, Germany) and glycine buffer at pH 9.0 (250 µl), dried, solved in toluol:methanol (20:1, 250 µl) and injected into the gas chromatograph (0.2 µl, split mode 20:1 at 285°C). Ropivacaine (m/z = 126.1 and retention time = 3.97 min) was used as internal standard for bupivacaine (m/z = 140.1 and retention time = 4.18 min, r^2 = 0.99) and mepivacaine (m/z = 98.1 and retention time = 3.65 min, r^2 = 1.00) quantification. Separation was achieved on a Phenomenex Zebron ZB-1ms column (30 m × 0.25 mM × 0.25 µM; Phenomenex Inc., Aschaffenburg, Germany) at a starting temperature and period of 200°C and 2 min followed by a linear temperature gradient (60 K/min) up to 300°C. Linearity of the detector was given over the whole concentration range with relative recovery rates of 95.7 ± 3.7% (mepivacaine, n = 17) and 100.0 ± 6.3% (bupivacaine, n = 17) and lower limits of quantification of 1.43 µM (mepivacaine) and 1.03 µM (bupivacaine).

Solutions and Drugs

Modified Tyrode solution was used for cell isolation and as bath solution and contained NaCl, 138 mM; KCl, 4 mM;

$MgCl_2$, 1 mM; NaH_2PO_4, 0.33 mM; $CaCl_2$, 2 mM; glucose, 10 mM; and HEPES, 10 mM (pH 7.30 with NaOH). The Na^+ concentration was reduced to 20 mM by replacing 118 mM Na^+ with Cs^+ for measuring I_{Na}. Myocyte storage solution contained NaCl, 130 mM; NaH_2PO_4, 0.4 mM; $NaHCO_3$, 5.8 mM; $MgCl_2$, 0.5 mM; $CaCl_2$, 1 mM; KCl, 5.4 mM; glucose, 22 mM; and HEPES, 25 mM (pH 7.40 with NaOH in the presence of 5% CO_2) and supplemented with 1 mg/ml bovine serum albumin (albumin fraction V; Merck KGaA, Darmstadt, Germany), 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin. The pipette solution contained glutamic acid, 120 mM; KCl, 10 mM; $MgCl_2$, 4 mM; EGTA, 10 mM; HEPES, 10 mM; and Na_2ATP, 2 mM (pH 7.20 with KOH). ND96 solution contained NaCl, 96 mM; KCl, 2 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1.8 mM; and HEPES, 5 mM (pH 7.40 with NaOH), supplemented with 100 U/ml of penicillin and 0.1 mg/ml of streptomycin (Sigma-Aldrich Corporation). NaCl-95 solution contained NaCl, 95 mM; KCl, 4 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1 mM; and HEPES, 10 mM (pH 7.40 with Tris). The ×5 stock of the modified bath solution contained NaCl, 60 mM; KCl, 18.25 mM; $MgCl_2$, 5 mM; $CaCl_2$, 5 mM; and HEPES, 20 mM (pH 7.4 with NaOH).

Bupivacaine and mepivacaine were obtained from Sigma and were freshly dissolved in the bath solution on each day of experiments. Bupivacaine was used at a concentration of 10 μM in most experiments, a plasma concentration that can be reached in patients.[16] Mepivacaine was used at a concentration of 40 μM, a concentration that was reported to cause similar cardiotoxicity as 10 μM bupivacaine.[10] Experiments in which the lipid emulsion (Lipovenös® MCT 20%; Fresenius Kabi AG) was applied were started with a bath solution containing 10% of a control solution adapted to the Na^+ content (approximately 2 mM) and osmolarity (290 mOsm, adapted with mannitol in the control solution) of Lipovenös®. Eventually, the solution was exchanged to the same solution containing 10% of the lipid emulsion instead of control. Ultracentrifugation was performed at 40,000 rpm (110,000*g*) in an Optima L60 ultracentrifuge (Beckman Coulter Inc., Brea, CA) using a Ti70.1 rotor for 2 h at 4°C.

Data Evaluation and Statistical Analysis

Data were analyzed using the PULSE-FIT software (HEKA), IGOR Pro (WaveMetrics Inc., Lake Oswego, OR), and Microsoft Excel (Microsoft Corporation, Redmond, WA) as described previously.[18] To indicate the precision of the mean values, average data are given together with the SEM (mean ± SEM) and the number of experiments if not stated otherwise. To report the variability of the data, means together with their respective SDs are given in the table, Supplemental Digital Content 1, http://links.lww.com/ALN/A968, and 95% CIs are detailed in the table, Supplemental Digital Content 2, http://links.lww.com/ALN/A969.[23] To estimate the reduction in the bupivacaine concentration by the lipid emulsion, concentration–response analysis was performed in the relevant range of concentrations assuming the previously described complete block of I_{Na} at high concentrations of bupivacaine.[24] Concentration–response curves of Kv channels were calculated at +40 mV from charge rather than current amplitude because bupivacaine and mepivacaine are open-channel blockers of Kv4 channels.[25,26] In both cases, a Hill function with variable slope was fitted to the data. Statistical significance was evaluated by paired or unpaired Student *t* test or paired or unpaired one-way ANOVA followed by a Newman–Keuls multiple comparison test when more than two groups were compared using Prism 5 (GraphPad Software Inc., La Jolla, CA). A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Lipid Emulsion Reverses the Effects of Bupivacaine on the Upstroke of the AP

Action potentials were recorded to assess the overall effect of lipid rescue on the cardiac cellular electrophysiology. Because of the heterogeneity of AP duration (APD) within the rat left ventricular free wall,[27] myocytes from epicardial and endocardial regions were analyzed separately. Figure 1 depicts the effect of lipid rescue on the epicardial AP; 10 μM of the lipophilic bupivacaine, a concentration reportedly reached during intoxication,[16] significantly reduced the overshoot (fig. 1D; $P < 0.001$) and maximal upstroke velocity (by 56%; fig. 1E; $P < 0.001$) of the AP (table 1). Lipid rescue with 10% Lipovenös® partially reversed the effect on the overshoot ($P < 0.001$) and increased maximal upstroke velocity by 31% ($P < 0.001$; table 1). Similar effects were observed in endocardial myocytes (table 1). Bupivacaine also significantly increased APD to 50% (APD_{50}) and 90% (APD_{90}) repolarization, which was completely reversed by lipid rescue (fig. 1, F and G; table 1) in epicardial myocytes. In endocardial myocytes, AP prolongation by bupivacaine was less profound and, surprisingly, lipid rescue shortened APD_{90} even below its duration measured under control conditions (table 1). Because the hydrophilic LA mepivacaine is reportedly less cardiotoxic than bupivacaine,[10] a fourfold higher dose was used. Similar to bupivacaine, 40 μM mepivacaine significantly reduced AP overshoot and upstroke velocity ($P < 0.001$ each; fig. 2, A–E; table 2). However, it did not significantly alter repolarization (fig. 2, F and G; table 2). As expected for a relatively hydrophilic LA, lipid rescue did not reverse the effects on overshoot and upstroke velocity. In fact, upstroke velocity was even slightly but significantly reduced by Lipovenös® (fig. 2, A–E; table 2). Surprisingly, Lipovenös® somewhat prolonged APD_{50} ($P < 0.001$) and APD_{90} ($P < 0.05$) in the presence of mepivacaine (fig. 2, F and G; table 1). Both LAs tended to slightly hyperpolarize the resting membrane potential. This effect was significant in some of the experiments (table 1). Lipovenös® did not have a consistent effect on the resting membrane potential. The tendency of Lipovenös® to prolong epicardial and to shorten endocardial APD was tested in a series of control experiments. Lipovenös® alone marginally but significantly prolonged APD_{90} in epicardial myocytes

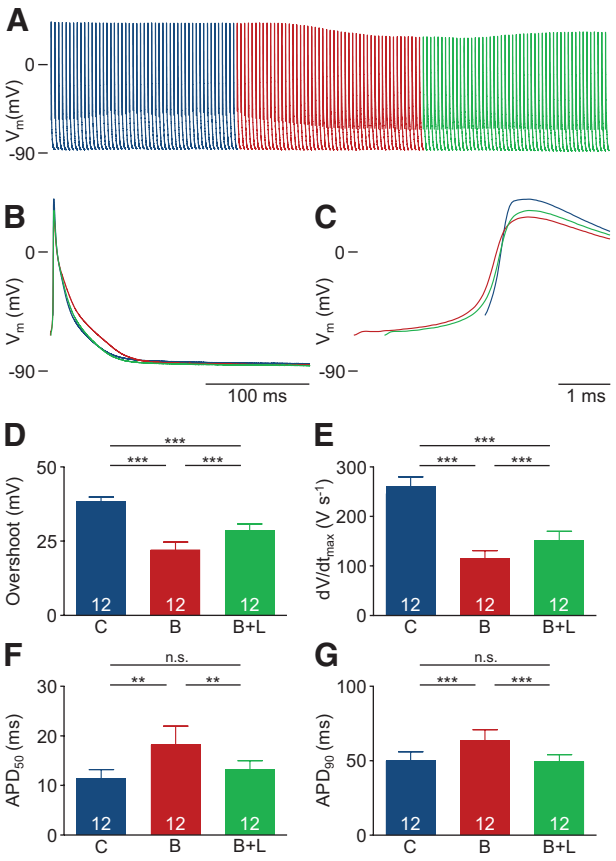

Fig. 1. Lipid rescue of the epicardial action potential (AP): bupivacaine. (*A*) A representative train of 150 APs recorded in a left ventricular epicardial cardiomyocyte at 1 Hz under control conditions (*blue*), with 10 μM bupivacaine (*red*), and with 10 μM bupivacaine and 10% Lipovenös® (*green*). (*B*) The last APs recorded under each condition are superimposed. (*C*) The upstroke of the APs shown in *B*. (*D*) Mean overshoot of the AP. (*E*) Maximal upstroke velocity (dV/dt_{max}) of the AP. (*F*) AP duration at 50% repolarization (APD_{50}). (*G*) AP duration at 90% repolarization (APD_{90}). The numbers in the bars give the number of myocytes investigated. Data are given as mean ± SEM. $^{**}P < 0.01$. $^{***}P < 0.001$. B = 10 μM bupivacaine; B+L = 10 μM bupivacaine + 10% Lipovenös®; C = control; dV/dt_{max} = maximal upstroke velocity of the AP; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); n.s. = not significant; overshoot = overshoot of the AP; V_m = membrane potential.

(46.8 ± 4.5 ms, n = 10 *vs.* 43.8 ± 4.3 ms, n = 10; $P < 0.05$) and tended to shorten the endocardial APD_{90} (87.5 ± 15.4 ms, n = 5 *vs.* 105.9 ± 23.1 ms, n = 5, not significant). Resting membrane potential, maximal upstroke velocity, and overshoot were not significantly altered (not shown).

The results on the upstroke velocity and overshoot of the AP demonstrate that Lipovenös® reversed the block of cardiac I_{Na} induced by bupivacaine but not or to a substantially lesser degree by mepivacaine. The AP prolongation induced by bupivacaine is consistent with an additional block of repolarizing K^+ currents by this LA[25] which was also reversed by Lipovenös®. To further characterize the effect of lipid rescue therapy on the level of ionic currents, we decided to focus on I_{Na} because it is the primary target of LAs in the heart. Moreover, we will also detail some findings on heterologously expressed repolarizing K^+ channels.

Lipid Rescue Reverses the Inhibition of I_{Na} by Bupivacaine

Figure 3, A and B, depicts representative Na^+ currents from left ventricular cardiomyocyte, and figure 3C depicts average normalized I_{Na} amplitudes. The inset of figure 3A shows the pulse protocol. Bupivacaine at 10 μM reduced I_{Na} by 54% (−19.3 ± 1.9 $pApF^{-1}$ *vs.* −42.3 ± 4.3 $pApF^{-1}$; n = 17; $P < 0.001$). Lipovenös® of 10% increased I_{Na} by 37% in the presence of bupivacaine (−26.4 ± 2.8 $pApF^{-1}$; n = 17; $P < 0.001$). Washout with control solution returned I_{Na} to control values.

A "lipid sink," *i.e.*, the reduction in the free LA concentration by absorption of the LA by the lipid, has been proposed as a possible mechanism of lipid rescue therapy.[28] To directly assess the contribution of the lipid sink, we removed the lipid phase from the solution containing both 10 μM bupivacaine and 10% Lipovenös® by ultracentrifugation. If a lipid sink contributes to the effects of Lipovenös®, the water phase of the centrifuged solution should, due to the reduced bupivacaine concentration, have a similar effect as the whole solution. Figure 3, D–F, illustrates the effects of the water phase: I_{Na} was increased by 17% (−27.3 ± 2.0 $pApF^{-1}$ *vs.* −23.0 ± 1.7 $pApF^{-1}$; n = 17; $P < 0.001$, control: −50.1 ± 4.0 $pApF^{-1}$), demonstrating the contribution of the lipid-sink effect. However, the total effect of Lipovenös® seems to be larger than the effect of the water phase alone, indicating that part of the effect is dependent on the presence of the lipid ("direct lipid effect").

According to the lipid-sink hypothesis, the effect of Lipovenös® on the mepivacaine-induced block should be markedly smaller than in the case of bupivacaine. Lipovenös® increased I_{Na} blocked by 40 μM mepivacaine by 17% (−35.1 ± 3.9 $pApF^{-1}$ *vs.* −30.0 ± 3.5 $pApF^{-1}$; n = 24; $P < 0.001$, control: −44.3 ± 5.4 $pApF^{-1}$, fig. 4, A–C). Surprisingly, also the water phase of the solution containing Lipovenös® and mepivacaine increased I_{Na} by 8% (−37.9 ± 3.6 $pApF^{-1}$ *vs.* −35.1 ± 3.2 $pApF^{-1}$; n = 23; $P < 0.01$, control: −48.2 ± 4.4 $pApF^{-1}$) compared with mepivacaine alone (fig. 4, D–F).

The total effect of Lipovenös® on the LA blocked I_{Na} was significantly ($P < 0.05$) larger for bupivacaine than for mepivacaine (fig. 5A). As expected from the higher degree of lipophilicity of bupivacaine, this was due to a significantly ($P < 0.01$) higher lipid-sink effect (fig. 5B), whereas the direct lipid effect was similar for both LAs (fig. 5C). For this comparison, the direct lipid effect was assessed by dividing the effect of the complete Lipovenös® solution in each experiment by the mean effect of the water phase and normalized to the first pulse after perfusion with the Lipovenös® solution. Consistent with a direct lipid effect, Lipovenös® also significantly ($P < 0.01$) increased I_{Na} in the absence of LA (fig. 5D).

Table 1. Lipid Rescue of the Action Potential: Bupivacaine

		Control	10 μM Bupivacaine	10 μM Bupivacaine + 10% Lipovenös®
V_m (mV)	Epicardial	−87.8 ± 0.7	−88.3 ± 0.6*	−88.2 ± 0.6*
	Endocardial	−86.4 ± 0.8	−86.8 ± 0.9	−87.1 ± 0.7
Overshoot (mV)	Epicardial	38.1 ± 1.6	21.8 ± 2.8***	28.5 ± 2.1***†††
	Endocardial	41.2 ± 2.5	26.6 ± 5.3***	33.6 ± 3.8 **††
dV/dt_{max} (Vs^{-1})	Epicardial	258.9 ± 19.7	114.9 ± 15.0***	150.4 ± 18.5***†††
	Endocardial	278.1 ± 47.9	141.9 ± 34.2***	181.1 ± 38.9***††
$APD_{0\ mV}$ (ms)	Epicardial	4.5 ± 0.6	5.4 ± 1.3	4.8 ± 0.8
	Endocardial	25.3 ± 6.7	28.2 ± 8.2	20.3 ± 4.7†
APD_{20} (ms)	Epicardial	2.7 ± 0.2	6.1 ± 1.7*	3.4 ± 0.3†
	Endocardial	7.6 ± 1.3	20.3 ± 3.6***	10.8 ± 1.7†††
APD_{50} (ms)	Epicardial	11.3 ± 1.9	18.2 ± 3.8**	13.1 ± 1.9††
	Endocardial	48.3 ± 11.9	61.3 ± 12.7*	40.5 ± 7.6††
APD_{90} (ms)	Epicardial	50.0 ± 6.1	63.6 ± 7.3***	49.6 ± 4.5†††
	Endocardial	119.4 ± 21.3	132.0 ± 20.9	93.6 ± 13.1**†††
n	Epicardial	12	12	12
	Endocardial	7	7	7

Data are given as mean ± SEM.

*P < 0.05. **P < 0.01. ***P < 0.001 *vs.* control. †P < 0.05. ††P < 0.01. †††P < 0.001 *vs.* 10 μM bupivacaine.

$APD_{0\ mV}$ = action potential duration at 0 mV; APD_{20} = action potential duration at 20% repolarization; APD_{50} = action potential duration at 50% repolarization; APD_{90} = action potential duration at 90% repolarization; dV/dt_{max} = maximal upstroke velocity of the action potential; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); n = number of myocytes investigated; overshoot = overshoot of the action potential; V_m = resting membrane potential.

To estimate the bupivacaine concentration in the water phase of the centrifuged solution, the relative increase in I_{Na} was plotted to a concentration–response curve obtained for the relevant range of concentrations (fig. 5E). Interpolation using a Hill function indicated that the increase in the blocked I_{Na} by the lipid-sink effect corresponded to a reduction in the bupivacaine concentration in the water phase to approximately 5.9 μM. Notably, the total increase in the blocked I_{Na} after the application of bupivacaine + Lipovenös® (total lipid effect) was as large as if the bupivacaine concentration had been reduced to approximately 3.9 μM.

Lipid Rescue Reverses the Inhibition of hKv4.2 + hKChIP2b Currents by Bupivacaine

To investigate whether lipid rescue of ionic currents is a general mechanism, additional experiments on hKv4.2 + hKChIP2b currents in *X. laevis* oocytes were performed, thereby investigating the lipid effect on a different ion channel in a different experimental system. Kv4.2 and the β subunit KChIP2 are ion channel subunits that underlie the repolarizing transient outward K^+ current (I_{to}) in cardiomyocytes. Figure 6, A and B, depicts representative hKv4.2 + hKChIP2b currents measured at +40 mV from an oocyte exposed to 0, 100, and 300 μM concentrations of bupivacaine (fig. 6A) and to the same concentrations of bupivacaine in the presence of 10% Lipovenös® (fig. 6B). The lipid emulsion significantly increased the IC_{50} of bupivacaine (calculated from the charge at +40 mV) from 126.6 ± 17.9 μM (n = 14) to 218.2 ± 29.4 μM (n = 15; P < 0.05; fig. 6C). The hillslope was unaffected (1.1 ± 0.1, n = 14 *vs.* 1.3 ± 0.1, n = 15, n.s.). The IC_{50} for mepivacaine was considerably higher (1,688 ± 235 μM, n = 13) and tended to be lower rather than higher in the presence of the lipid (1,203 ± 146 μM, n = 19; P = 0.07; fig. 6D). Also for mepivacaine, the hillslope was unaffected (1.0 ± 0.0, n = 13 *vs.* 0.9 ± 0.0, n.s., n = 19).

In a separate series of experiments, using a highly concentrated stock of the bath solution, we were able to record the concentration–response of hKv4.2 + hKChIP2b currents blocked by 300 μM bupivacaine to increased Lipovenös® concentrations. The relative charge increased quite linearly up to 40% of Lipovenös® concentration and was nearly twice as high at 80% lipid emulsion as at 10% (82.5 ± 1.2% *vs.* 42.9 ± 2.3%, n = 13; P < 0.001; fig. 6E).

The contribution of a lipid-sink mechanism was also evaluated in this series of experiments (fig. 6F); after ultracentrifugation, the water phase of the lipid and 100 μM bupivacaine-containing solution was as effective as the whole mixture in reversing the bupivacaine-induced block (whole solution: +34.4 ± 3.9%, n = 10, P < 0.001; water phase: +39.2 ± 3.6%, n = 10; P < 0.001; effect whole solution *vs.* effect water phase: n.s.). Together with the mepivacaine data, this indicates that at 10%, Lipovenös® did not exert a direct lipid effect on hKv4.2 + KChIP2b channels.

A Part of the Effect of Lipid Rescue Can Be Attributed to a "Lipid-sink" Mechanism

The data presented indicate the presence of a lipid-sink effect. To directly assess this effect, LA concentrations were measured by gas chromatography followed by mass spectrometry (table 3). After ultracentrifugation of the solution

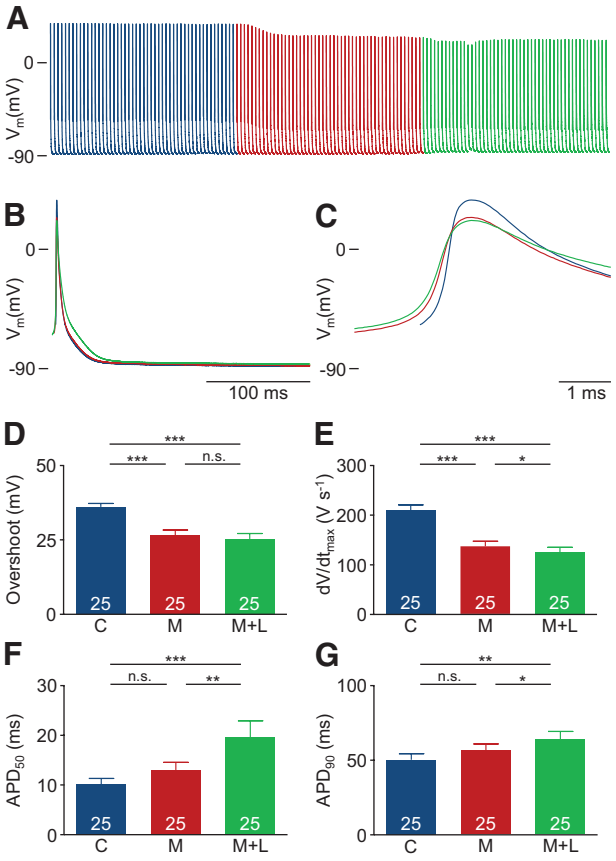

Fig. 2. Lipid rescue of the epicardial action potential (AP): mepivacaine. (*A*) A representative train of 150 APs recorded in a left ventricular epicardial cardiomyocyte at 1 Hz under control conditions (*blue*), with 40 μM mepivacaine (*red*), and with 40 μM mepivacaine and 10% Lipovenös® (*green*). (*B*) The last APs under each condition are superimposed. (*C*) The upstroke of the APs shown in B. (*D*) Mean overshoot of the AP. (*E*) Maximal upstroke velocity (dV/dt_{max}) of the AP. (*F*) AP duration at 50% repolarization (APD_{50}). (*G*) AP duration at 90% repolarization (APD_{90}). The numbers in the bars give the number of myocytes investigated. Data are given as mean ± SEM. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. C = control; dV/dt_{max} = maximal upstroke velocity of the AP; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); M = 40 μM mepivacaine; M+L = 40 μM mepivacaine + 10% Lipovenös®; n.s. = not significant; overshoot = overshoot of the AP; V_m = membrane potential.

containing bupivacaine and 10% Lipovenös®, the concentration of bupivacaine (original concentration: 10 μM and 100 μM) was significantly reduced in the water phase by approximately 30% ($P < 0.001$; table 3), demonstrating the lipid-sink effect. The concentration of bupivacaine in the water phase was remarkably close to the concentration estimated by concentration–response analysis. Concomitantly, the concentration in the lipid-enriched phase was increased. Also for mepivacaine, we noted a small decrease in the concentration in the water phase, which was significant for 10 μM only. The decrease in LA concentration was larger for bupivacaine than for mepivacaine (at 10 μM: $P = 0.05$ and at 100 μM: $P < 0.001$). These data confirm our interpretation of the electrophysiological experiments.

Discussion

We demonstrate lipid rescue of the cardiac AP and I_{Na} after intoxicating cardiomyocytes with bupivacaine. Lipid rescue did not reverse the effects of mepivacaine on the AP, although it increased I_{Na}. Part of the lipid effect was attributable to a lipid-sink mechanism. Lipid rescue was also effective on hKv4.2 + KChIP2b currents in *X. laevis* oocytes resulting in a rightward shift of the concentration–response curve for bupivacaine, but not for mepivacaine.

Effects of the LA on AP Upstroke and I_{Na}

Local anesthetics block inactivated Na^+ channels;[24,29] therefore, the block exhibits use-dependence and depends on the membrane potential.[1,30] We concentrated on the clinically relevant use-dependent block using APs and a voltage protocol mimicking the physiological resting membrane potential and AP shape of the cardiomyocyte. The effects of bupivacaine on AP upstroke and I_{Na} are in good agreement with previous findings where the IC_{50} of bupivacaine for cardiac Na^+ currents was between 3 and 8.6 μM, depending on model and enantiomer.[24,29,31] For mepivacaine, only limited information on cardiac I_{Na} is available.[1] In a study conducted on closely related[32] tetrodoxin-resistant neuronal Na^+ currents,[33] the IC_{50} of use-dependent block was 13–15 μM for bupivacaine and 70–90 μM for mepivacaine.

Effects of the LA on Repolarization

The APD prolongation by bupivacaine indicates a block of repolarizing K^+ channels. Cardiac repolarization in rat is achieved by the transient outward K^+ current (I_{to}), slowly or noninactivating currents, and the inward-rectifying K^+ current I_{K1}.[27] For I_{to} charge block, Castle[25] reported an IC_{50} of 23 μM for bupivacaine and 790 μM for mepivacaine. I_{K1} was not affected by bupivacaine and blocked approximately 50% by 3 mM of mepivacaine. These data explain why bupivacaine but not mepivacaine prolonged APD in our study. Our IC_{50} for hKv4.2 + KChIP2b channels expressed in *X. laevis* oocytes is somewhat higher than that reported for I_{to}.[25] This is consistent with findings from our group showing greater IC_{50} values for the aminoquinolines primaquine and chloroquine in oocytes expressing hKv4.2 + hKChIP2b compared with native I_{to}.[34]

Effects of Lipid Rescue on Cellular Electrophysiology

We examined the effects of a clinically used lipid emulsion on single cardiomyocytes at a concentration that can be reached in lipid resuscitation in patients.[16] Bupivacaine concentrations and the experimental design were chosen to replay the situation in the patient and to provide insight into the underlying mechanisms. Experiments, in which the lipid phase of the solution containing Lipovenös® and bupivacaine

Table 2. Lipid Rescue of the Action Potential: Mepivacaine

		Control	40 μM Mepivacaine	40 μM Mepivacaine + 10% Lipovenös®
V_m (mV)	Epicardial	−87.9 ± 0.5	−88.1 ± 0.4	−87.6 ± 0.4*††
	Endocardial	−88.2 ± 0.4	−88.4 ± 0.4	−88.0 ± 0.4†
Overshoot (mV)	Epicardial	35.9 ± 1.1	26.3 ± 1.8***	25.1 ± 1.9***
	Endocardial	41.5 ± 0.7	35.6 ± 1.2***	35.6 ± 1.2***
dV/dt_{max} ($V\ s^{-1}$)	Epicardial	208.8 ± 10.5	136.1 ± 10.0***	124.1 ± 9.6***†
	Endocardial	294.5 ± 10.9	198.8 ± 10.0***	191.6 ± 9.9***
$APD_{0\ mV}$ (ms)	Epicardial	3.4 ± 0.2	3.3 ± 0.2	3.9 ± 0.3***†††
	Endocardial	28.4 ± 5.6	28.4 ± 5.5	23.2 ± 4.4*††
APD_{20} (ms)	Epicardial	2.3 ± 0.1	3.0 ± 0.2	4.1 ± 0.5***††
	Endocardial	10.0 ± 2.5	15.1 ± 3.7***	12.0 ± 2.5†
APD_{50} (ms)	Epicardial	10.1 ± 1.1	13.0 ± 1.5	19.5 ± 3.3***††
	Endocardial	56.9 ± 9.7	59.7 ± 9.9	48.7 ± 8.5**†††
APD_{90} (ms)	Epicardial	50.0 ± 4.1	56.2 ± 4.5	63.5 ± 5.5**†
	Endocardial	125.2 ± 15.4	125.2 ± 14.9	102.2 ± 13.1***†††
n	Epicardial	25	25	25
	Endocardial	34	34	34

Data are given as mean ± SEM.

*P < 0.05. **P < 0.01. ***P < 0.001 *vs.* control. †P < 0.05. ††P < 0.01. †††P < 0.001 *vs.* 10 μM bupivacaine.

APD_{0mV} = action potential duration at 0 mV; APD_{20} = action potential duration at 20% repolarization; APD_{50} = action potential duration at 50% repolarization; APD_{90} = action potential duration at 90% repolarization; dV/dt_{max} = maximal upstroke velocity of the action potential; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); n = number of myocytes investigated; overshoot = overshoot of the action potential; V_m = resting membrane potential.

was removed by ultracentrifugation, indicated that a part of the effect of Lipovenös® on AP and I_{Na} was independent of the presence of the lipid. This is an experimental evidence for the contribution of a lipid-sink effect to the effects of Lipovenös® in our model. However, another part of the effect was dependent on the presence of the lipid. This effect was also present in the absence of the LA and was largely responsible for lipid rescue of I_{Na} after block by mepivacaine. We could not demonstrate lipid rescue from mepivacaine of the AP. This was probably due to a combination of the small-sized effect on I_{Na} together with a slight depolarization of the resting membrane potential by the lipid emulsion which likely led to an increased inactivation of the Na^+ channels, sensitizing I_{Na} for mepivacaine. This depolarization was not present in the AP experiments with bupivacaine.

Lipid rescue was also assessed for hKv4.2 + KChIP2b channels. Concentration–response experiments demonstrated an increased IC_{50} of bupivacaine but not of mepivacaine in the presence of the lipid emulsion. Moreover, we also demonstrated a concentration dependence of the effect of the lipid emulsion. At 10%, the lipid itself did not influence the Kv currents.

Limitations of the Patch Clamp Technique

The cardiac I_{Na} is a large current with rapid activation and inactivation kinetics, potentially driving the whole cell patch clamp technique to its limits. To minimize voltage errors, we lowered the extracellular Na^+ concentration to reduce I_{Na} and used low-resistance pipettes and R_s compensation to reduce the effective R_s. This resulted in an average time constant of 120 μs for the capacitive artifact and an average voltage error between 5.5 (control conditions) and 2.5 mV (bupivacaine block). This voltage error was small but nonetheless different between the control and blocked conditions and could possibly affect our results. To assess its consequences, we evaluated the degree of block induced by bupivacaine and mepivacaine separately for the 25% of cells with the largest control currents (median bupivacaine: −8.2 nA, mepivacaine: −8.9 nA) and the 25% with the smallest control currents (median bupivacaine: −3.1 nA, mepivacaine: −2.4 nA). Block was similar in these groups both for bupivacaine (large I_{Na}: 55 ± 1%, n = 10, small I_{Na}: 53 ± 2%, n = 11, n.s.) and for mepivacaine (large I_{Na}: 30 ± 2%, n = 11, small I_{Na}: 28 ± 2%, n = 12, n.s.). Moreover, our data on I_{Na} correlate well with the AP upstroke velocity, which is largely unaffected by R_s. Taken together, a distortion of our results by the voltage error is unlikely.

Clinical Implications

Lipid resuscitation has been effectively used to treat intoxications with lipophilic LAs and other lipophilic drugs. Moreover, there are reports in which lipid rescue has also successfully been used in intoxications with less lipophilic drugs such as mepivacaine.[15] In the current study, the effectiveness of lipid rescue of the cardiac AP correlated with the lipophilicity of the LA, and we could demonstrate that a large part of this effect was due to a lipid-sink mechanism. However, the slight reversal of the mepivacaine-induced block of I_{Na} might provide encouragement to further investigate lipid rescue of less lipophilic compounds also.

Recently, the contribution of ion channel block to cardiac LA toxicity and the clinical importance of the lipid-sink

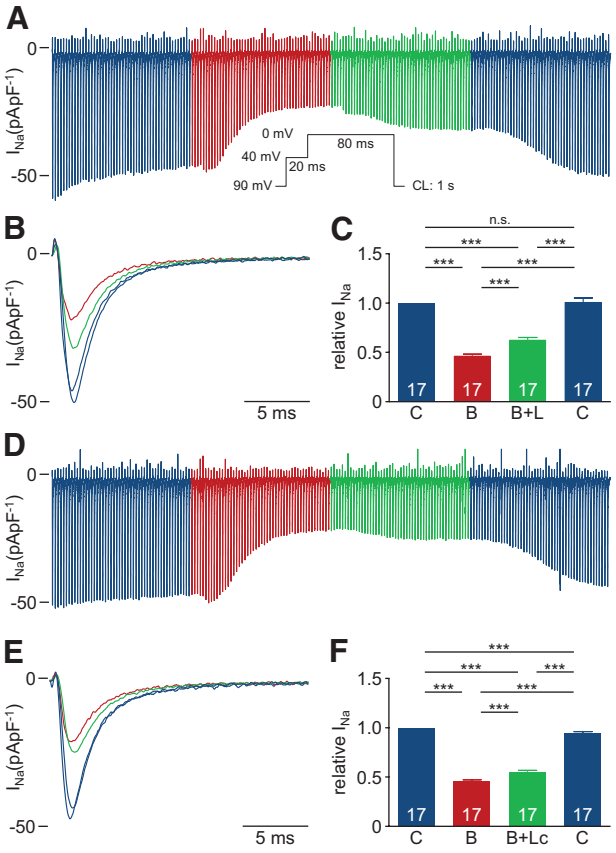

Fig. 3. Lipid rescue of I_{Na}: bupivacaine. (*A*) A representative train of 200 Na^+ currents obtained from a left ventricular cardiomyocyte at 1 Hz under control conditions (*blue*), with 10 μM bupivacaine (*red*), and with 10 μM bupivacaine and 10% Lipovenös® (*green*). I_{Na} was elicited by a 20 ms voltage step from −90 to −40 mV followed by a 80 ms step to 0 mV to simulate the effects of the action potential on Na^+ channel block (see *inset*). Cycle length was 1 s. (*B*) The last I_{Na} under each condition superimposed. (*C*) Average normalized I_{Na} under each condition. (*D–F*) I_{Na} recorded under similar conditions as before; however, the lipid phase was removed from the bupivacaine–lipid mixture by ultracentrifugation to demonstrate the lipid-sink effect. The numbers in the bars give the number of myocytes investigated. Data are given as mean ± SEM. ***P < 0.001. B = 10 μM bupivacaine; B+L = 10 μM bupivacaine + 10% Lipovenös®; B+Lc = the water phase of 10 μM bupivacaine + 10% Lipovenös® after ultracentrifugation; C = control; CL = cycle length; I_{Na} = cardiac fast Na^+ current; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); n.s. = not significant.

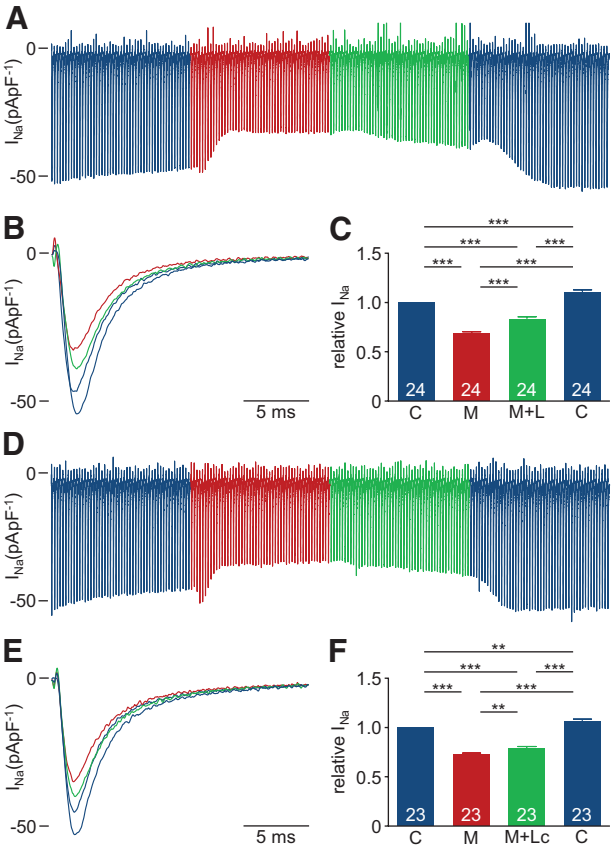

Fig. 4. Lipid rescue of I_{Na}: mepivacaine. (*A*) A representative train of 200 Na^+ currents obtained from a left ventricular cardiomyocyte at 1 Hz under control conditions (*blue*), with 40 μM mepivacaine (*red*), and with 40 μM mepivacaine and 10% Lipovenös® (*green*). (*B*) The last I_{Na} under each condition superimposed. (*C*) Average normalized I_{Na} under each condition. (*D–F*) I_{Na} recorded under similar conditions as before; however, the lipid phase was removed from the mepivacaine–lipid mixture by ultracentrifugation to demonstrate the lipid-sink effect. The numbers in the bars give the number of myocytes investigated. Data are given as mean ± SEM. **P < 0.01. ***P <0.001. C = control; I_{Na} = cardiac fast Na^+ current; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); M = 40 μM mepivacaine; M+L = 40 μM mepivacaine + 10% Lipovenös®; M+Lc = the water phase of 40 μM mepivacaine + 10% Lipovenös® after ultracentrifugation; n.s. = not significant.

effect have been questioned, and alternative explanations have been proposed.[28] In this context, it is helpful consider the specific models and symptoms examined. In most experimental studies, bupivacaine was applied at a dose that resulted in asystole. However, many case reports imply that arrhythmias, including bradycardia, supraventricular, and ventricular tachycardia, are more common in the clinical situation than primary asystole.[15] In the electrocardiogram, the typical early sign of bupivacaine intoxication is broadening and deformation of the QRS complex, indicating a delayed conduction of cardiac excitation consistent with Na^+ channel block.[35–37] The QTc time is mildly prolonged[36,37] due to K^+ channel block.[2,26,38–41] The main reason for ventricular tachycardia and consecutive ventricular fibrillation in these patients is likely a slowing of cardiac conduction by Na^+ channel block.[2] At higher concentrations, bupivacaine additionally induces a reduction of contractility and asystole, attributable to complete Na^+ channel block and interference with pacemaking, Ca^{2+} handling, and mitochondrial function.[1,2] Consequently, the partial reversal of the bupivacaine effects on I_{Na} and the

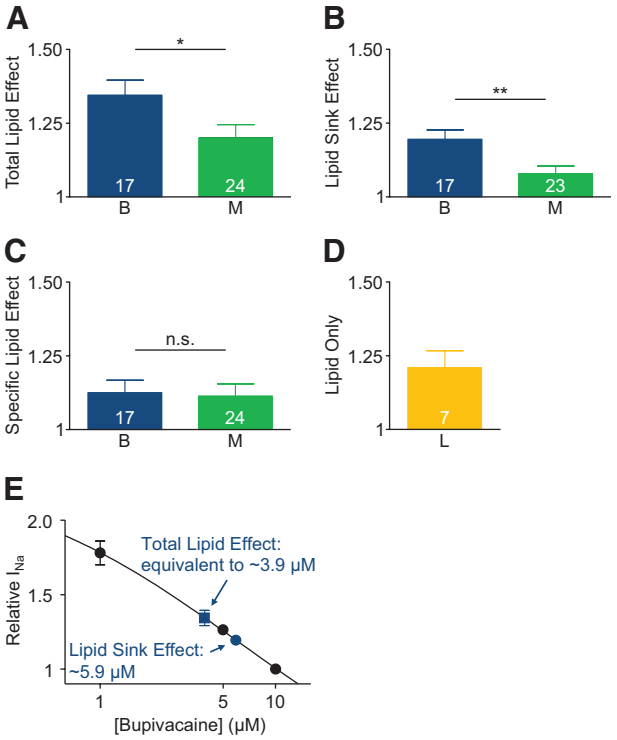

Fig. 5. Dissection of the mechanism of lipid rescue: evidence for a lipid-sink effect. (*A*) Average total effect of Lipovenös® on I_{Na} blocked by bupivacaine and mepivacaine. (*B*) Average lipid-sink effect of Lipovenös® on I_{Na} blocked by bupivacaine and mepivacaine. The lipid-sink effect is the effect of the centrifuged mixture of lipid and LA. (*C*) The part of the effect of Lipovenös® that cannot be attributed to the lipid-sink effect. (*D*) Effect of Lipovenös® on I_{Na}. (*E*) Concentration–response analysis (*black circles*) of I_{Na} normalized to I_{Na} measured at 10 µM bupivacaine (n = 7) used to estimate the effective bupivacaine concentration in the water phase of the centrifuged bupivacaine + Lipovenös® (*blue circle*, lipid-sink effect, data from *B*). The *blue box* indicates the seemingly larger reduction of the bupivacaine concentration caused by the total lipid effect (data from *A*). Please note the logarithmic scaling of the x-axis. The numbers in the bars give the number of myocytes investigated. Data are given as mean ± SEM. * $P < 0.05$. ** $P < 0.01$. B = 10 µM bupivacaine; I_{Na} = cardiac fast Na^+ current; L = 10% Lipovenös® (Lipovenös® MCT 20%; Fresenius Kabi AG, Bad Homburg, Germany) only; M = 40 µM mepivacaine; n = number of myocytes investigated; n.s. = not significant.

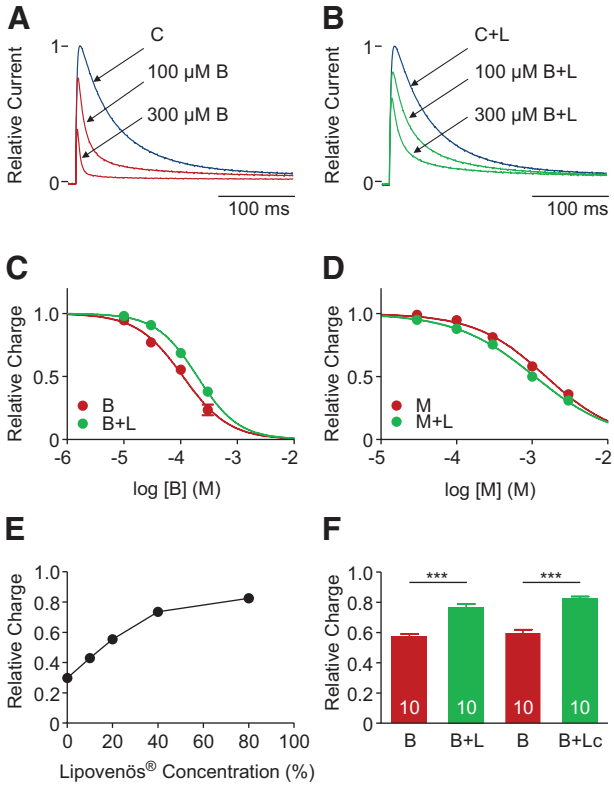

Fig. 6. Lipid rescue of Kv4.2 + KChIP2b currents in *Xenopus laevis* oocytes. (*A*) Kv4.2 + KChIP2b currents elicited at +40 mV under control conditions (*blue*) and with 100 µM and 300 µM bupivacaine in the bath (*red*). (*B*) Kv4.2 + KChIP2b currents elicited at +40 mV under control conditions (*blue*) and with 100 µM and 300 M bupivacaine plus 10% Lipovenös® in the bath (*green*). (*C* and *D*) Concentration–response curves for bupivacaine (*C*) and mepivacaine (*D*) under control conditions (*red*) and with 10% Lipovenös® in the bath (*green*). (*B*) n = 14; B+L, n = 15; M, n = 13; M+L, n = 19. (*E*) Concentration dependence of the effect of Lipovenös® on the block of Kv4.2 + KChIP2b currents by 300 µM bupivacaine (n = 13). (*F*) Comparison of the effects of 10% Lipovenös® and of the water phase of the bupivacaine–lipid mixture on the block of Kv4.2 + KChIP currents by 100 µM bupivacaine. The numbers in the bars give the number of oocytes investigated. Data are given as mean ± SEM. *** $P < 0.001$. B = 10 µM bupivacaine; B+L = 10 µM bupivacaine + 10% Lipovenös®; B+Lc = the water phase of 10 µM bupivacaine + 10% Lipovenös® after ultracentrifugation; C = control; C+L = control + 10% Lipovenös®; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); M = 40 µM mepivacaine; M+L = 40 µM mepivacaine + 10% Lipovenös®; M+Lc = the water phase of 40 µM mepivacaine + 10% Lipovenös® after ultracentrifugation; n = number of myocytes investigated; n.s. = not significant.

AP may well contribute the clinical effectiveness of lipid resuscitation.

We present evidence for a dual mode of action of the lipid emulsion, the lipid-sink effect and a direct lipid effect. The lipid-sink effect will be most important at plasma concentrations of near IC_{50} (*i.e.*, at the concentrations used in the current study), where the concentration–response relation is steepest. In fact, plasma concentrations of approximately 10 µM of bupivacaine have been reported in successful lipid resuscitation.[16] The direct lipid effect is unlikely to depend on the bupivacaine concentration because it is also present in the absence of LA. Considering the physiology of the microcirculation, both effects may act sequentially; the lipid emulsion consists of artificial chylomicrons of approximately 500 nM diameter,[42] which cannot easily leave the blood vessels (pore size approximately <30 nM),[43,44] and are broken down by lipoprotein lipase to free fatty acids which then leave the capillary by

Table 3. LA Concentrations

	LA	LA + 10% Lipovenös®	LA + 10% Lipovenös® Water Phase	LA + 10% Lipovenös® Lipid-enriched Phase
Bupivacaine (μM)				
10 μM		8.8 ± 0.3	6.5 ± 0.2***	45.4 ± 5.0
n		6	6	6
100 μM	91.8 ± 1.0	93.7 ± 1.2	64.7 ± 3.0***	445.2 ± 54.5
n	7	7	7	7
Mepivacaine (μM)				
10 μM		11.5 ± 0.6	10.1 ± 0.9*	13.8 ± 0.9
n		6	6	6
100 μM	102.8 ± 1.0	105.8 ± 3.2	103.9 ± 3.5 (P = 0.08)	115.9 ± 3.0
n	7	7	7	7

Concentrations of LA dissolved in Tyrode solution and measured by gas chromatography–mass spectrometry. Because the lipid-enriched phase still contained a high amount of water, the concentrations for the lipid-enriched phase do not truly reflect the concentrations of the LA in the lipid itself. Data are given as mean ± SEM.

*P < 0.05. ***P < 0.001 *vs.* LA + 10% Lipovenös®.

LA = local anesthetic; lipid-enriched phase = the lipid-enriched phase of the solution after ultracentrifugation; Lipovenös® = Lipovenös® MCT 20% (Fresenius Kabi AG, Bad Homburg, Germany); n = number of separate experiments; water phase = the water phase of the solution after ultracentrifugation.

diffusion.[42,45] The first action of lipid rescue *in vivo* may be a reduction of the free bupivacaine concentration in the plasma and consequently in the interstitial fluid (the lipid-sink effect), before a sufficient concentration of fatty acids reach the myocytes for an additional direct lipid effect. Here also a direct effect of fatty acids on bupivacaine binding by the Na^+ channel may be important.[46] Although the current data and results from others[9,11,47] give clear evidence for a lipid sink *in vitro* and *in vivo*, it has been difficult to demonstrate the lipid-sink effect in other experiments *in vivo* and in the clinical setting.[48,49] For example, Litonius *et al.*[48] observed a relevant reduction in the free bupivacaine concentration (from >20 μg/l to <13 μg/l) after lipid application in human, which was statistically not significant but nevertheless is consistent with a lipid-sink effect and warrants further examination. Our results exclude neither other beneficial effects of the lipid emulsion that improve metabolic function or contractility nor effects present only at the whole body level such as accelerated redistribution of the LA.[28]

Taken together, we demonstrate lipid rescue of cardiac electrophysiology from bupivacaine intoxication at the cellular level and identify a lipid-sink effect and a direct lipid effect as underlying mechanisms.

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Competing Interests

The authors declare no competing interests.

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