Dual effects of mesenteric lymph isolated from rats with burn injury on contractile function in rat ventricular myocytes

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Yatani, Atsuko, Da-Zhong Xu, Keiichi Irie, Kazunori Sano, Anoush Jidarian, Stephen F. Vatner, and Edwin A. Deitch. Dual effects of mesenteric lymph isolated from rats with burn injury on contractile function in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 290: H778–H785, 2006. First published October 7, 2005; doi:10.1152/ajpheart.00808.2005.—Gut-derived factors in intestinal lymph have been shown to trigger myocardial contractile dysfunction. However, the underlying cellular mechanisms remain unclear. We examined the effects of physiologically relevant concentrations of mesenteric lymph collected from rats with 40% burn injury (burn lymph) on excitation-contraction coupling in rat ventricular myocytes. Burn lymph (0.1–5%), but not control mesenteric lymph from sham-burn animals, induced dual positive and negative inotropic effects depending on the concentrations used. At lower concentrations (<0.5%), burn lymph increased the amplitude of myocyte contraction (1.6 ± 0.3-fold; n = 12). At higher concentrations (>0.5%), burn lymph initially enhanced myocyte contraction, which was followed by a block of contraction. These effects were partially reversible on washout. The initial positive inotropic effect was associated with a prolongation of action potential duration (measured at 90% repolarization, 2.5 ± 0.6-fold; n = 10), leading to significant increases in the net Ca2+ influx (1.7 ± 0.1-fold; n = 8). There were no significant changes in the resting membrane potential. The negative inotropic effect was accompanied by a decrease in the action potential plateau (overshoot decrease by 69 ± 10%; n = 4) and membrane depolarization. Voltage-clamp experiments revealed that the positive inotropic effects of burn lymph were due to an inhibition of the transient outward K+ currents that lead to a reduction of action potential plateau. These burn lymph-induced changes in cardiac myocyte Ca2+ handling can contribute to burn-induced contractile dysfunction and ultimately to heart failure.

cardiac myocyte; action potential; calcium currents; potassium currents

BURN INJURY INITIATES A SERIES of pathophysiological changes, including left ventricular (LV) contractile dysfunction (1, 2, 5, 16). Specifically, a progressive fall in LV contractile function despite aggressive fluid resuscitation has been reported in both clinical and experimental studies of burn injury (9, 11). However, the source(s) or the signaling pathways involved in thermal injury-induced myocardial dysfunction remain largely unknown. Recent evidence suggests that burn-induced myocardial contractile dysfunction was due to abnormal cardiac myocyte Ca2+ handling (4, 16, 19, 20, 25, 36). Ventricular myocytes isolated from hearts 24 h after burn injury showed an increase in both systolic and rest Ca2+ concentrations (4, 19, 20, 36). In addition, it has been reported that when animals were treated with inhibitors that regulate cellular Ca2+ cycling, such as diltiazem, a Ca2+ channel blocker; dantrolene, an inhibitor of the sarcoplasmic reticulum (SR) Ca2+ efflux; or ruthenium red, an inhibitor of mitochondrial Ca2+ accumulation, cardiac contractile performance was improved compared with untreated animals (17, 20, 36). However, the mediators or cellular mechanisms responsible for burn-induced contractile dysfunction are not well understood (16).

A series of studies have proposed a role for the gastrointestinal tract in burn sepsis (8, 15, 21). Recently, we (22, 30) have reported that acute lung injury as well as myocardial depression after burn injury is initiated from gut-derived factors transported in mesenteric lymph. In a previous study (30), burned rats exhibited depressed cardiac function, as assessed by LV pressure and LV change in pressure over time, and blunted responses to increases in either preload, coronary blood flow rate, or Ca2+ at 24 h after burn injury. When the main mesenteric lymph duct was ligated to block mesenteric lymph from reaching systemic circulation, burn injury-induced myocardial contractile depression was prevented (30). The data strongly suggest that contractile dysfunction after burn injury could be due to gut-derived factors transported in the mesenteric lymph from burned rats (burn lymph), which mediate abnormal cellular excitation-contraction (E-C) coupling and Ca2+ homeostasis.

We (41) previously found that burn lymph at low concentrations (0.1–0.5%) increases Ca2+ influx and twitch contraction in rat LV myocytes, suggesting that the gut-derived factors, which are transported in the mesenteric lymph from burned rats, directly alter E-C coupling. The concentrations of burn lymph used in these previous studies were, however, below the potential peak concentrations that might be observed in vivo. In the current study, to determine the role of burn lymph on burn injury-induced ventricular dysfunction, we further examined the physiologically relevant concentrations of burn lymph (0.1–5%) on E-C coupling in rat ventricular myocytes isolated from healthy rats.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (250–350 g) were used in this study. The New Jersey Medical School Animal Care Committee approved all experiments. A total of 24 rats were used, and at least four to eight rats were examined for each protocol; n represents the number of myocytes examined per protocol.

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Burn injury model and mesenteric lymph collection. The procedures used to induce burn injury were similar to those described by Walker and Mason (34). Briefly, rats were deeply anesthetized (with 50 mg/kg of pentobarbital sodium), and a scald burn on 40% of the total body surface area was induced by immersing the back of the animal through a template into boiling water (100°C) for 10 s, followed by an abdominal burn induced by immersion for 5 s. All rats were resuscitated with 5 ml ip saline to prevent damage to the underlying abdominal organs. These conditions produce a uniform third-degree burn (12). The sham-burned rats were anesthetized, placed in the plastic template, and immersed in room temperature water.

Mesenteric lymph was collected as previously described (31, 33, 41). Briefly, the main mesenteric lymph duct was identified and cannulated with silastic tubing. The catheter was secured, and the mesenteric lymph was collected hourly (1–6 h) from both burn-injured and sham-injured rats. During lymph fluid collection, animals were kept under anesthesia, i.e., a combination of morphine (2.5 mg/kg sc) and pentobarbital sodium (17 mg/kg ip). Because the highest level of biological activity, as examined by endothelial cell death assay, was observed in the samples collected 2 and 3 h after burn injury (10), we used these lymph samples in the present studies. The collected lymph was centrifuged at 400 g for 15 min to remove all cellular elements and flash frozen at −80°C. Burn lymph was dialyzed with experimental solution with the use of MINI dialysis units (Pierce) for 2 h at a concentration between 0.1% and 5% vol/vol to maintain a vehicle concentration of <5%.

Because the experiments were done on the basis of the volume of lymph, we measured total protein concentration using a Bio-Rad protein assay and followed the manufacturer's standard protocol for microtiter plates. Total protein concentrations were almost identical between control and burn lymph samples. Average protein concentrations (in mg/ml) for control sham burn lymph (n = 6) and burn lymph (n = 6) collected at 2 and 3 h after sham burn or burn injury were 31.5 ± 3 and 37.8 ± 1 for sham burn and 35.0 ± 7 and 37.6 ± 8 for burn lymph, respectively.

Analysis of mechanical function and Ca²⁺ transients. LV myocytes were isolated from normal rats as previously described (39). Myocyte twitch contraction and Ca²⁺ transients were measured as previously described (40, 41). Briefly, isolated LV myocytes were perfused with Tyrode solution (in mmol/l): 120 NaCl, 2.6 KCl, 1.0 CaCl₂, 1.0 MgCl₂, 11 glucose, and 5 HEPES (pH 7.3) at 32°C and field stimulated at 1.0 Hz. Myocyte contractile and relaxation function was measured with the use of a video motion edge detector. For the Ca²⁺ transient measurements, cells were loaded with 5 μM fura-2 AM at room temperature for 30 min. Intracellular free Ca²⁺ was monitored as the ratio of 340 to 380 nm fluorescence of fura-2 with the use of the Photocan dual-beam spectrofluorophotometer (Photon Technology). The changes in Ca²⁺ transients were evaluated by direct reading of the fluorescence intensity.

Electrophysiological recordings. Action potentials (APs) were recorded with the use of the perforated patch technique (41). Whole cell patch-clamp studies were performed as described previously (23, 24). Cell capacitance was measured by using voltage ramps of 0.8 V/s from a holding potential of −50 mV. All experiments were performed at room temperature (20°C–22°C). The experimental chamber (0.2 ml) was placed on a microscope stage, and external solution changes were made rapidly by using a modified Y-tube technique (37).

Ca²⁺ current (ICa) was recorded in external solution containing (in mmol/l) 2 CaCl₂, 1 MgCl₂, 135 tetraethylammonium chloride, 5 4-aminopyridine, 10 glucose, and 5 HEPES (pH 7.3). The pipette solution contained (in mmol/l) 100 Cs-aspartate, 20 CsCl, 1 MgCl₂, 2 MgATP, 0.5 GTP, 10 BAPTA, and 5 HEPES (pH 7.3). For AP and K⁺ current recordings, myocytes were bathed in a Tyrode solution containing (in mmol/l) 135 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 KCl, 10 glucose, and 10 HEPES (pH 7.3). The pipette solution for AP recordings contained amphoterocin B (200 μg/ml) and (in mmol/l) 140 KCl, 2 MgCl₂, 10 NaCl, 2 ATP, and 5 HEPES (pH 7.3). To record K⁺ currents, nifedipine (10 μM) was added to the Tyrode solution to block Ica. In some experiments, to block Na⁺ currents, tetrodotoxin (10–20 μM) was added to the Tyrode solution. The patch pipette solution contained (in mmol/l) 110 K aspartate, 20 KCl, 2 MgCl₂, 2 ATP, 0.5 GTP, EGTA, and 5 HEPES (pH 7.3).

Statistics. Data are reported as means ± SE. Comparisons between conditions were evaluated with the Student’s t-test, with significance imparted at the P < 0.05 level.

RESULTS

Effects on cardiomyocyte contractions and Ca²⁺ transients. When we studied the effects of burn lymph on contractility using physiologically relevant concentrations (i.e., a concentration range that might be observed in vivo), we found that burn lymph induces dual positive and negative inotropic effects on myocyte contraction. Figure 1A shows a representative example of the effects of burn lymph (1%) on the amplitude of ventricular myocyte contraction triggered by field stimulation. Consistent with our previous study (41) conducted with lower concentrations of burn lymph (<0.5%), the application of burn lymph (0.5–2%) increased the amplitude of contraction (1.6 ± 0.3-fold; n = 10 from 5 rats). The positive inotropic effects occurred rapidly, within 10–30 s after the application, and reached a steady level within 2–3 min.

The force-frequency relation is often used to describe the contractile state and can be altered by inotropic interventions that change SR Ca²⁺ content (6). We therefore examined the effects of burn lymph at 0.5- to 4-Hz stimulation frequencies (Fig. 2). The amplitude of cell shortening decreased slightly at higher frequencies (<2 Hz), consistent with a negative staircase reported in rat ventricular trabeculae (6, 7). As in the 1-Hz data, myocyte shortening was increased in the presence of burn lymph at all frequencies examined, but the slightly negative staircase was similar to control. The results suggest that the positive inotropic effects of burn lymph do not greatly affect SR Ca²⁺ uptake and release rates.

The positive inotropic effects were followed by a reduced contractile response to field stimulation and resulted in a complete block of contractility after 6–10 min. This dual effect of burn lymph was concentration dependent, and the contractile block occurred rapidly with higher concentrations (>2%). Both positive and negative inotropic effects were reversible on washout of burn lymph. After functional recovery of the twitching (1–2 min after washout), however, the peak amplitude remained higher than control levels for ~10–15 min before returning to control levels. The results suggest that these differing effects of burn lymph are due to different signaling pathways involved in E-C coupling (positive and negative inotropy). It is noteworthy that these effects were achieved with the use of burn lymph dialyzed with experimental solution to exclude any effects due to altered ionic composition, especially extracellular Ca²⁺ concentrations. Consistent with previous observations with 0.1–0.5% control mesenteric lymph from rats after sham burn injury, higher concentrations (1–5%; n = 6 from 4 rats) had no significant effect on contraction. Figure 1B shows an example typical of contractility observed during the application of control lymph (5%).

The effects of burn lymph on the results of Ca²⁺ transient agree with myocyte contraction results. Figure 3 shows an example of burn lymph (1%)-induced effects on Ca²⁺ tran-
sients. Burn lymph induced dual positive and negative effects on the peak amplitude of Ca²⁺ transients but did not change diastolic Ca²⁺ concentration. In pooled data, burn lymph (1%) increased the peak amplitude of Ca²⁺ transients (1.7 ± 0.2-fold). The average increase in Ca²⁺ transients in seven myocytes from four isolations was from 0.17 ± 0.02 to 0.26 ± 0.03.

The initial positive effects were followed by a standstill of the myocytes. As shown by the continuous recording in Fig. 3B, beating resumed after a 1- to 2-min washout period, and the functional recovery was associated with an increase in the amplitude of Ca²⁺ transients. Consistent with the results observed in myocyte contraction, control mesenteric lymph at higher concentrations (1–5%) had no detectable effects on Ca²⁺ transients (n = 4 from 2 different rats).

Because the rate of Ca²⁺ decline during twitch reflects primarily Ca²⁺ removal via the SR Ca²⁺ uptake, we compared the time course of Ca²⁺ transients. With burn lymph, the magnitude of the steady-state, field-stimulated Ca²⁺ transient was increased, whereas no significant change was observed in the time course of Ca²⁺ decline (Fig. 3A). This is demonstrated by an analysis of the time for 50% decay (T50%) of Ca²⁺ transient. The numbers of T50% in the absence and presence of 1% burn lymph and after washout were 284 ± 15, 263 ± 11, and 258 ± 10 ms, respectively.

Taken together with the results observed in the effects on the force-frequency relation, these data indicate that burn lymph increases contractility with little or no effects on SR Ca²⁺ uptake rate.

Effects of burn lymph on APs. We next examined the cellular mechanisms responsible for dual (positive and negative) inotropic effects of burn lymph. Figure 4 shows a typical example of APs recorded before, during, and after burn lymph application. Burn lymph (1%) caused a marked increase in the AP duration (APD). APD measured at 90% repolarization was increased by 2.5 ± 0.6-fold (n = 10). No changes in resting
membrane potential were observed with the initial prolongation of APD. The prolongation of APD was then followed by a decrease in AP overshoot (69 ± 10%; n = 4). After a 5- to 10-min application of burn lymph, the plateau potential remained reduced, whereas APD was significantly prolonged (Fig. 4, 1- and 3-min applications). As can be seen in the 5- and 7-min application APD in Fig. 4, the myocytes did not completely repolarize, and the membrane potential remained at depolarized levels. These effects, however, were partially reversed on washout (3–5 min) of burn lymph (Fig. 4).

These results indicate that the initial positive inotropic effect was associated with a prolongation of APD and the negative inotropic effect was accompanied by a reduction of AP plateau and membrane depolarization. Effects on $K^{+}$ channel currents. We have recently shown that burn lymph (0.1–0.5%) inhibits transient outward $K^{+}$ currents ($I_{to}$) in rat ventricular myocytes. By reducing $I_{to}$, burn lymph prolonged the AP, which secondarily increased Ca$^{2+}$ influx (41). To further examine the cellular mechanisms that underlie burn lymph-induced changes in the AP profile, we examined the effects of burn lymph on $I_{to}$. Consistent with previous studies (41), the application of burn lymph (1%) markedly reduced peak amplitude of $I_{to}$ (Fig. 5, A and B). However, the current waveform or current-voltage ($I$-$V$) relationships demonstrate that the voltage-dependent kinetics of $I_{to}$ was not significantly altered (Fig. 5C). Figure 5D shows the dose-response relationship for the blocking effect of peak $I_{to}$, indicating a half-maximal burn lymph response at ~1%. Increasing concentrations of burn lymph up to 5% did not further reduce $I_{to}$.

Blocking of $I_{to}$ occurred immediately and reached a steady-state level within 2 min after the application, similar to responses observed in myocyte contraction experiments. Interestingly, further increases in burn lymph did not markedly affect $I_{to}$, and complete block of the current was not achieved, even at the highest concentration used in the present study (5%). Moreover, the block of $I_{to}$ was not readily reversible; the current did not reverse after a 5- to 10-min wash period.

In ventricular myocytes, $\alpha_{1}$-adrenergic receptor (AR) agonists (e.g., phenylephrine) inhibit $I_{to}$ via a PKC-mediated

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**Fig. 3.** Effects of burn lymph on Ca$^{2+}$ transients. A: Ca$^{2+}$ transients recorded before (control), in the presence of burn lymph, and after washout are shown on expanded timescale. B: continuous recording of Ca$^{2+}$ transients. Average increase in Ca$^{2+}$ transient amplitude with burn lymph (1%) was from 0.17 ± 0.02 to 0.26 ± 0.3 (n = 4).

**Fig. 4.** Effects of burn lymph (1%) on action potential recorded in rat ventricular myocytes at different time points: control (before burn lymph application), during application (between 1 and 7 min), and after washout.
signaling pathway (3, 27, 35). We therefore tested the effects of burn lymph in the presence of prazosin, a nonspecific \(\alpha_1\)-AR antagonist. Prazosin \((10^{-6}\text{M})\) did not interfere with the inhibitory effects of burn lymph on \(I_{\text{to}}\). There were no differences in the response of \(I_{\text{to}}\) to burn lymph \((0.5–1\%)\) between control and prazosin-treated myocytes.

The inward rectifier \(K^+\) currents \((I_{\text{K1}})\) are important in maintaining the resting membrane potential and abbreviate excitability by accelerating the terminal repolarization phase of APs in ventricular myocytes (26, 32). We thus examined the effects of burn lymph \((1\%)\) on \(I_{\text{K1}}\) (Fig. 6). To measure \(I_{\text{K1}}\), hyperpolarizing pulses were applied from a holding potential of \(-40\text{ mV}\) to test potentials between \(-50\) and \(-100\text{ mV}\) (Fig. 6, A and B). The currents measured at the end of the test pulse before and after the application of burn lymph were plotted in Fig. 6C. The data show that burn lymph suppressed \(I_{\text{K1}}\) at \(-100\text{ mV}\) by an average of \(2\%\) \((n = 4)\). These results suggest that block of \(I_{\text{K1}}\) by burn lymph may contribute to spontaneous depolarization from the resting membrane potential observed in AP experiments. Mesenteric lymph from sham-burned rats \((0.1–5\%)\) had no significant effects on either \(I_{\text{to}}\) or \(I_{\text{K1}}\).

Effects of lymph on \(I_{\text{Ca}}\). One of the main determinants of myocyte contraction is \(Ca^{2+}\) influx. To determine whether a decrease in \(Ca^{2+}\) influx could provide an explanation for markedly decreased contraction associated with depressed AP plateau, we examined the effects of burn lymph \((0.5–2\%)\) on \(I_{\text{Ca}}\). To minimize \(Ca^{2+}\)-dependent inactivation and rundown, myocytes were dialyzed with BAPTA, a fast \(Ca^{2+}\) chelator (28, 29). The traces in Fig. 7 show a typical example of the effects of burn lymph \((2\%)\) on \(I_{\text{Ca}}\). Burn lymph reduced \(I_{\text{Ca}}\) without changing the \(I-V\) relationships. The blocking effects occurred slowly \((\sim 1\text{ min after the application})\) and were usually complete within 3–5 min. There was no evidence of any stimulatory effect. Burn lymph produced a concentration-dependent decrease in \(I_{\text{Ca}}\) (Fig. 8A). For each concentration of burn lymph, \(I-V\) relationships before and after application were obtained by using the same protocol shown in Fig. 7. Percent reductions of peak \(I_{\text{Ca}}\) obtained with various concentrations of burn lymph are summarized in Fig. 8B. Burn lymph produced inhibition of \(I_{\text{Ca}}\) at concentrations higher than \(1\%\), and a half-maximal \(I_{\text{Ca}}\) block \((IC50)\) occurred at \(-2\%\).

DISCUSSION

It has been proposed that cardiac depressant factors, present in the systemic circulation after burn injury, play an important role in burn-induced myocardial depression (5, 14–16, 30). Recently, on the basis of the observation that burn-induced myocardial dysfunction is due to factors contained in intestinal lymph (30), we investigated the direct effects of burn lymph on E-C coupling. An unexpected finding was that burn lymph increased the magnitude of contraction and \(Ca^{2+}\) transients in rat ventricular myocytes. The enhanced myocyte function resulted from a distinct blocking effect on \(I_{\text{to}}\). By reducing \(I_{\text{to}}\), burn lymph prolonged APD, which, in turn, increased \(Ca^{2+}\) influx via L-type \(Ca^{2+}\) channels (41). However, it was not clear how to reconcile our previous in vitro findings of burn lymph-induced increases in myocyte contraction (41) with clinical and...
experimental in vivo observations of myocardial contractile dysfunction after burn injury (14).

In previous studies (10), the concentrations of burn lymph (0.1–0.5%) used were below the potential peak concentrations (3–5%) that might be observed in vivo on the basis of the volume of intestinal lymph produced and the blood volume of the rat. Because changes in ionic concentrations, including K⁺, Na⁺, and Ca²⁺, significantly alter cellular E-C coupling, the lower concentration was used to exclude any possible effects due to altered electrolytes. Thus it was possible that burn lymph exhibits a positive inotropic effect as well as a negative inotropic effect on myocyte function and that this dual effect of burn lymph is concentration dependent. In the present study, to test this hypothesis and to reconcile discrepancies observed previously, we further studied concentration-dependent effects of physiologically relevant concentrations (0.1–5%) of burn lymph on E-C coupling. It is important to note that burn lymph, as well as control lymph, was dialyzed with experimental solution to exclude any effects due to altered ionic compositions.

Dual effect (positive and negative) of burn lymph on myocyte function. In the present study, we found that burn lymph causes both positive and negative inotropic effects on cardiac myocyte. This dual effect of burn lymph was dependent on the concentrations used. At lower concentrations (0.5–1%), burn lymph initially had a positive inotropic effect, but this was then followed by a decrease in contraction. At higher concentrations

Fig. 6. Effects of burn lymph (1%) on inward rectifier K⁺ currents (Iₖᵢ). A family of currents elicited from a holding potential of −40 mV by voltage steps from −50 to −100 mV in 10-mV increments before control (A) and after 5-min application of burn lymph (B). C: I-V relationships of Iₖᵢ before and during application of burn lymph (○, control; ●, after burn lymph application). Inward current amplitudes at 300 ms were normalized to cell capacitance to give current densities (pA/pF). Data points are means ± SE of 4 cells.

Fig. 7. Effects of burn lymph on Ca²⁺ current (I_{Ca}). Original current traces before (A) and 3–5 min after application of burn lymph (B). Currents were elicited from a holding potential of −50 mV to indicated test potentials at 0.1 Hz. I-V relationships for peak I_{Ca} (C). I_{Ca} was normalized to cell capacitance to give current densities (pA/pF).

Fig. 8. Concentration-dependent block of I_{Ca} by burn lymph. A: current traces in the absence and presence of burn lymph. I_{Ca} was elicited by test pulses to +10 mV from a holding potential of −50 mV at 0.1 Hz. B: pooled data for effects of burn lymph on I_{Ca}. Data points are means ± SE of 5 to 9 myocytes.
burn lymph rapidly blocked contraction. These inotropic effects were reversible on washout. The data suggest that burn lymph contains stimulatory and inhibitory inotropic components. A quantitative or temporal correlation between the depressed LV function in vivo and changes in intrinsic contractile function in vitro is difficult to interpret, although these results are consistent with data of decreased contractile function previously obtained at the organ level (14, 30). The results also support the hypothesis that burn lymph-induced changes in contractile function, which are associated with abnormal Ca\(^{2+}\) handling at the cellular level, are important initiating events that significantly contribute to cardiac deterioration of postburn hearts.

Although the exact chemical composition of the active components in gut-derived factors in intestinal lymph has not yet been evaluated (18), the effects we observed in cellular experiments may be attributed to the existence of two distinct (opposite) inotropic active components in burn lymph. An alternative possibility would be that a single active component mediates two effects, stimulatory and inhibitory, depending on the concentration.

**Burn lymph alters myocyte contraction through changes in APD: ionic mechanisms of AP changes.** In current-clamp recordings, we found that burn lymph-induced concentration-dependent inotropic effects were associated with changes in AP configuration. The initial positive inotropic effect was associated with a prolongation of APD, leading to significant increases in the net Ca\(^{2+}\) influx. The increase in \(I_{\text{Ca}}\) was not directly responsible for the observation because burn lymph had no effect on \(I_{\text{Ca}}\), which is consistent with data previously obtained at lower concentrations of burn lymph (<0.5%) (41). Instead, burn lymph inhibited \(I_{\text{o}}\), resulting in the prolongation of the AP. When peak \(I_{\text{o}}\) was measured as a maximum outward current, burn lymph blocked \(I_{\text{o}}\) in a concentration-dependent manner. The threshold potential for \(I_{\text{o}}\) activation, as well as the waveform during the voltage-clamp step, remained unchanged. The reduction of \(I_{\text{o}}\) began at concentrations as low as 0.1%, and the effects occurred immediately, reaching a steady-state level within 2 min after the application. However, a complete block of the current was not achieved, even at the highest concentration used in the present study (5%). The effects were not readily reversible on washout.

In previous studies (41), to examine the effects of prolonged APD caused by burn lymph on net Ca\(^{2+}\) influx, we examined the integral of \(I_{\text{Ca}}\) by using an AP voltage clamp. The studies demonstrated that myocytes stimulated with a prolonged AP waveform derived from burn lymph treatment showed significantly increased net inward Ca\(^{2+}\) influx (1.7 ± 0.1-fold). The results support the idea that AP prolongation by burn lymph causes a large increase in Ca\(^{2+}\) entry per beat via \(I_{\text{Ca}}\). At lower concentrations, the effects of burn lymph on E-C coupling involve a change in Ca\(^{2+}\) transients without direct effects on Ca\(^{2+}\) channels. These effects are similar to those observed with acute effects of \(\alpha_1\)-AR stimulation. However, burn lymph-induced \(I_{\text{o}}\) block was not influenced by prazosin, suggesting that burn lymph effects were not mediated by \(\alpha_1\)-AR.

In the present study, we found that at higher concentrations of burn lymph, AP prolongation was followed by a reduction of the plateau potential and membrane depolarization. These changes in the AP configuration were associated with a decrease in \(I_{\text{Ca}}\). Burn lymph produced a concentration-dependent decrease in \(I_{\text{Ca}}\) without changing the I-V relationships; the threshold and peak potentials of \(I_{\text{Ca}}\) remained unchanged. Reduction of \(I_{\text{Ca}}\) began at concentrations of ~1%. The recovery was partial, which may be partially due to the fact that Ca\(^{2+}\) channel currents run down with time (38).

Thus it is possible that the block of \(I_{\text{Ca}}\) counterbalanced the block of \(I_{\text{o}}\), leading to a decrease in the net Ca\(^{2+}\) influx. These effects may be directly responsible for the negative inotropic effects observed with higher concentrations of burn lymph. In addition to \(I_{\text{Ca}}\), \(I_{\text{K1}}\) was also influenced by higher concentrations of burn lymph. An inhibition of \(I_{\text{K1}}\) may be responsible for the inability of the cell to repolarize completely (13, 32). Our voltage-clamp data suggest that inhibition of \(I_{\text{o}}\) was directly responsible for the prolongation of the APD, which in turn increased Ca\(^{2+}\) influx and the positive inotropic effects associated with lower concentrations of burn lymph. The higher concentrations of burn lymph, on the other hand, led to a decrease in Ca\(^{2+}\) influx and negative inotropic effects. These decreases occurred because of the blocking of \(I_{\text{Ca}}\) and \(I_{\text{K1}}\), which then resulted in a decreased AP overshoot and membrane depolarization.

In conclusion, we have shown that burn lymph can alter myocyte contraction either positively or negatively, depending on the concentrations used, presumably by a direct effect on ionic channels including K\(^+\) and Ca\(^{2+}\). Thus the present data provide strong support for the hypothesis that burn lymph is involved in contractile alterations in postburn hearts.

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