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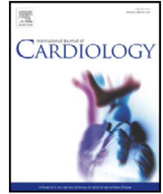

Letter to the Editor

Chemokine ligand 9 modulates cardiac repolarization via Cxcr3 receptor binding

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Inflammation influences various disease etiologies and evidence is accumulating that it may also affect risk for arrhythmia and sudden cardiac death [1–3]. However, knowledge concerning the underlying mechanisms is scarce, and while a direct effect of inflammatory mediators on cardiomyocyte electrophysiological properties is plausible [4,5], this is as yet unexplored. The possibility of a direct effect of inflammatory mediators on cardiomyocte electrical properties draws support from previous findings in neurons, where cytokines and chemokines have been recognized as modulators of neuronal excitability [6–8]. Here, we studied the direct functional effects of chemokine (C-X-C motif) ligand 9 (Cxcl9), a small type-II interferon inducible chemotactic factor that signals via the G-protein coupled receptor Cxcr3 [9], on the electrophysiological properties of mouse ventricular myocytes.

Left ventricular myocytes were isolated by enzymatic dissociation [10] from male FVB/NJ mice (Charles River Laboratories) unless otherwise mentioned. All animals received humane care and the study protocols complied with institutional guidelines. Action potentials (APs) and sarcolemmal ion currents were recorded at 36 ± 0.2 °C using the amphotericin-B perforated patch-clamp and ruptured patch-clamp techniques, respectively. Action potentials were measured using Tyrode's solution containing (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NaOH); pipette solution contained (in mM): K-gluconate 125, KCl 20, NaCl 10, amphotericin-B 0.22, HEPES 10; pH 7.2 (KOH). Extracellular solution for K^+ current measurements was Tyrode's solution with 0.25 mM $CdCl_2$, which strongly inhibits Na^+ currents and blocks Ca^{2+} currents, and thereby also prevents activation of the transient outward Ca^{2+}-activated Cl^- current. Pipette solution for K^+ current measurements contained (in mM): K-gluconate 125, KCl 20, NaCl 10, MgATP 5.0, EGTA 10 HEPES 10; pH 7.2 (KOH). Extracellular solution for $I_{Ca,L}$ measurements contained (in mM): TEA-Cl 145, CsCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.5, HEPES 5.0; pH 7.4 (NMDG-OH); pipette solution contained (in mM): CsCl 145, K_2-ATP 5.0, EGTA 10, HEPES 10; pH 7.2 (NMDG-OH). $I_{Ca,L}$ was measured in the presence of 0.2 mM 4,4′ diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS; Sigma-Aldrich, MO, USA) to block the Ca^{2+}-activated Cl^- current. Voltage control, data acquisition, and analysis were performed using custom software. The effects of mouse recombinant Cxcl9 (500 ng/mL rmCxcl9; Cat#:492-MM-010/CF, R&D systems, MN, USA) were measured in paired experiments, i.e., in the absence (control, CTRL) and presence of rmCxcl9 in the same myocyte. Data are mean ± SEM. Group comparisons were made using the *t*-test or Two-Way Repeated Measures ANOVA followed by pairwise comparison using the Student–Newman–Keuls test. $p < 0.05$ defined statistical significance.

APs were measured at 6 Hz and analyzed as described previously [10]. Fig. 1A shows representative APs measured in CTRL, in the presence of rmCxcl9, and after washout. We observed a significant, and reversible, AP shortening after application of rmCxcl9. On average, rmCxcl9 caused a 13% reduction in AP duration at 90% repolarization (APD_{90}; Fig. 1B). AP shortening may be due to decreased L-type Ca^{2+} current ($I_{Ca,L}$), increased K^+ currents, or a combination of both. First, K^+ currents were measured during 5 s hyper- and depolarizing voltage clamp steps from a holding potential of −80 mV (see Fig. 1D, inset, for protocol) and analyzed as described previously [11]. Neither the fast and slow transient K^+ currents ($I_{to,f}$ and $I_{K,slow}$, respectively) nor the steady-state K^+ currents (I_{ss} and I_{K1}), were affected by rmCxcl9 (Fig. 1, C and D). Next, $I_{Ca,L}$ was measured using a two-step protocol

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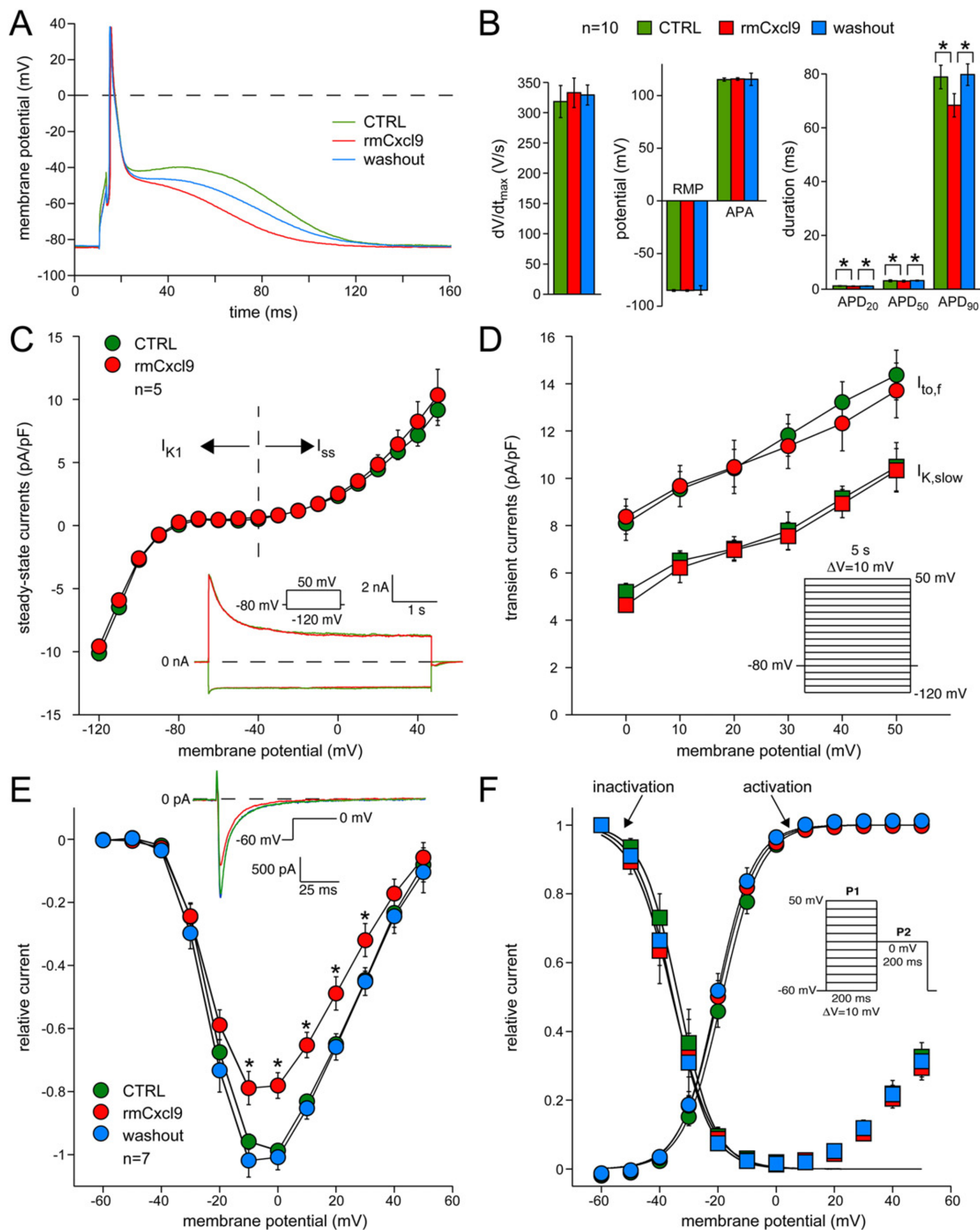

Fig. 1. Recombinant mouse Cxcl9 (rmCxcl9) shortens AP duration and reduces the L-type Ca^{2+} current ($I_{Ca,L}$). A. Representative examples of APs measured in cardiomyocytes from wild-type male FVB/NJ mice at 6 Hz under control conditions (CTRL), in the presence of recombinant mouse cxcl9 (rmCxcl9), and upon washout. B. Average AP properties in CTRL, RmCxcl9 and washout condition. RMP = resting membrane potential, dV/dt_{max} = maximal upstroke velocity, APA = AP amplitude, and APD_{20}, APD_{50}, and APD_{90} = AP duration at 20, 50 and 90% repolarization, respectively. C. Average current–voltage (I–V) relationships of steady-state K^+ currents, I_{K1} and I_{ss}, under CTRL and in the presence of rmCxcl9. Inset, representative recordings upon hyperpolarizing pulses to −120 mV and depolarizing pulses to 50 mV. D. Average I–V relationships of $I_{to,f}$ and $I_{K,slow}$. Inset, Voltage clamp protocol used. E. Average I–V relationships of $I_{Ca,L}$ under CTRL, in the presence of rmCxcl9, and upon washout. Inset. Representative $I_{Ca,L}$ recordings upon depolarizing pulses to 0 mV. Please note that the currents are normalized to the largest $I_{Ca,L}$ under CTRL conditions. F. $I_{Ca,L}$ voltage-dependence of (in)activation. Inset. Two-step protocol used for determining $I_{Ca,L}$ properties. $^*p < 0.05$.

(see Fig. 1F, inset) and analyzed as described in detail previously [12]. Fig. 1E, inset, shows representative $I_{Ca,L}$ recordings upon depolarizing pulses to 0 mV in CTRL, in the presence of rmCxcl9, and after washout. Application of rmCxcl9 reversibly decreased $I_{Ca,L}$ (by 21% at 0 mV peak; Fig. 1E). Current inactivation time constants (data not shown) and voltage-dependency of (in)activation were unaffected by application of rmCxcl9 (Fig. 1F). These findings demonstrate a direct effect of rmCxcl9 on cardiac APs via reduction of $I_{Ca,L}$.

Since Cxcl9 is known to affect intracellular responses by binding to the G-alpha$_{(i)}$-coupled receptor Cxcr3 [9], we hypothesized that the rmCxcl9-induced AP shortening is mediated via Cxcr3 receptor binding and G-protein signaling. Firstly, we assessed the involvement of G-alpha$_{(i)}$-coupled receptors by pretreating cardiomyocytes with 2 μg/ml pertussis toxin (PTX; Calbiochem, EMD Chemicals, Inc. San Diego, Ca, USA) for 3 h at 37 °C. No AP shortening was observed upon application of rmCxcl9 to PTX-pretreated cardiomyocytes (Fig. 2A),

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