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Empagliflozin inhibits cardiac late sodium current via Ca/Calmodulin-dependent kinase II

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Recently, it was published in Circulation that empagliflozin inhibits H_2O_2-induced cardiac late sodium current (late I_{Na}).[1] Using computational modeling and point mutagenic approaches, Philippaert et al. suggested a possible site of empagliflozin-binding within $Na_V1.5$ similar to that of local anesthetics, supportive of direct drug binding to $Na_V1.5$, although this remains to be determined conclusively and alternative mechanisms may exist.[1] We have previously shown that Ca/calmodulin-dependent kinase II (CaMKII) binds to $Na_V1.5$, stimulates late I_{Na} and affects its H_2O_2-dependent regulation.[2,3] We also demonstrated that empagliflozin inhibits CaMKII in failing human and murine cardiomyocytes.[4]

Here we show that inhibition of H_2O_2-induced late I_{Na} by empagliflozin cannot solely be mediated via direct drug binding but depends on CaMKII-dependent phosphorylation of $Na_V1.5$ at serine 571. We demonstrate that empagliflozin inhibits late I_{Na} in patients with aortic stenosis (AS) and phenotypic features of heart failure (HF) with preserved ejection fraction (HFpEF).

Raw data/analytic methods can be made available for purposes of reproducing results or replicating procedures. Human tissue/proprietary antibodies cannot be made available for legal constraints. Experiments conform to the Declaration of Helsinki. Human/murine

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JM, MJB, SW designed experiments, interpreted data, wrote the manuscript, and are responsible for the integrity of the article. JM, MJB, TS, MT, TJH, HM, SP acquired data and revised the manuscript. SS, PJM, LSM revised the manuscript for critical intellectual content.

Conflict of Interest Disclosures

None.

studies were approved by institutional committee. Written informed consent was obtained from patients prior to tissue donation. LV samples were obtained from septal resections of 11 patients (8 male/ 3 female, aged 69.3±2.6) with AS undergoing valve replacement. Patients had a HFpEF-like phenotype with hypertrophy and preserved EF (59.4±1.7%). Murine models of CaMKIIδ knock-out (CaMKIIδ$^{-/-}$)[3], inhibition of CaMKII-dependent $Na_V1.5$ phosphorylation at serine 571 (S571A), and with CaMKII phospho-mimetic $Na_V1.5$ S571E mutation were tested for involvement of CaMKII-$Na_V1.5$ phosphorylation. Isolated ventricular myocytes were incubated (30 min) with empagliflozin (1 μmol/l) or control (DMSO). Some cardiomyocytes were incubated with inhibitors of open-state Na channel inactivation (ATX-II or veratridine) or lidocaine (100 μmol/l, 30 min) for direct Na channel inhibition. H_2O_2 (100 μmol/l, 5 min) was used to induce reactive oxygen species, which stimulate late I_{Na} in HF via CaMKII[3] (tested with CaMKII-inhibitor myristoylated-AiP; 2 μmol/l, 30 min). For some experiments, empagliflozin was washed-in to ATX-II or H_2O_2 pre-incubated myocytes.

Late I_{Na} was measured as described previously[2,3]. Resting membrane potential was held at -120 mV and I_{Na} elicited by depolarizing to -20 mV for 1000 ms, quantified by integrating from 100 to 500 ms of the start of depolarization (normalized to membrane capacitance). Western blots used human ventricular tissue exposed to empagliflozin/vehicle (30 min)[4]. Data were analyzed using mixed-effects analysis with Holm-Sidak, linear mixed model with random factor ‘individual’ and Sidak correction, or paired t-test (GraphPad Prism 9).

We demonstrate that late I_{Na} can be reduced by empagliflozin in ventricular myocytes from patients with AS similar to CaMKII-inhibitor AiP (figure 1A). ATX-II-dependent (figure 1B) enhancement of late I_{Na} in murine wildtype cardiomyocytes was not affected by empagliflozin (not even at 10 and 100 μmol/l), nor after wash-in (at 1 μM) to ATX-II pre-incubated myocytes, which would be expected if empagliflozin were a direct $Na_V1.5$ inhibitor. Moreover, wash-in of empagliflozin (up to 10 μM) also did not inhibit late I_{Na} in myocytes preincubated with a moderate concentration of veratridine (16 nM, experimentally determined as EC50 by dose-response, data not shown). In sharp contrast, both veratridine and ATX-II-enhanced late I_{Na} were blocked by lidocaine (not shown). Empagliflozin robustly inhibited H_2O_2-induced late I_{Na}, (figure 1C), with maximal efficacy at 6 min but not already at 2 min after onset of exposure (late I_{Na} integral during wash-in: 0 min −50.8±4.3 A*F^{-1}*ms, 2 min: −39.9±4.6 A*F^{-1}*ms, p=0.0934 vs. 0 min; 4 min: −24.2±4.6 A*F^{-1}*ms, p=0.0007 vs. 0 min; 6 min: −17.2±4.0 A*F^{-1}*ms, p<0.0001 vs. 0 min). No additional effect of empagliflozin on late I_{Na} was observed upon AiP (not shown), or in myocytes lacking either CaMKIIδ (CaMKIIδ$^{-/-}$) or CaMKII-dependent $Na_V1.5$ phosphorylation at serine 571 (S571A, figure 1C). Accordingly, the enhanced late I_{Na} in mice with CaMKII phospho-mimetic $Na_V1.5$ S571E was neither blocked by empagliflozin nor AiP (figure 1D). In contrast, lidocaine inhibited late I_{Na} in S571E cells, underscoring that empagliflozin primarily acts via CaMKII-$Na_V1.5$ phosphorylation. Empagliflozin dose-response revealed an IC_{50} for inhibition of H_2O_2-dependent late I_{Na} of 0.086 μmol/l in murine myocytes (not shown). Empagliflozin inhibited CaMKII-autophosphorylation and CaMKII-dependent phosphorylation of $Na_V1.5$ in AS and HF (figure 1E+F).

In conclusion, inhibition of late I_{Na} by empagliflozin is at least in part due to inhibition of CaMKII-dependent regulation of $Na_V1.5$[2,4]. If cardiac Na channels were solely directly inhibited, empagliflozin, like local anesthetics, should have blocked ATX-II/veratridine-stimulated late I_{Na}, but it did not. Nevertheless, the target of empagliflozin in the heart remains unclear[5] and further research is needed to better understand direct vs. indirect effects on late I_{Na}. We demonstrate that empagliflozin also inhibits late I_{Na} in patients with AS and features of HFpEF, which may reduce the propensity for arrhythmias and contribute to the positive results of the EMPEROR-Preserved trial.

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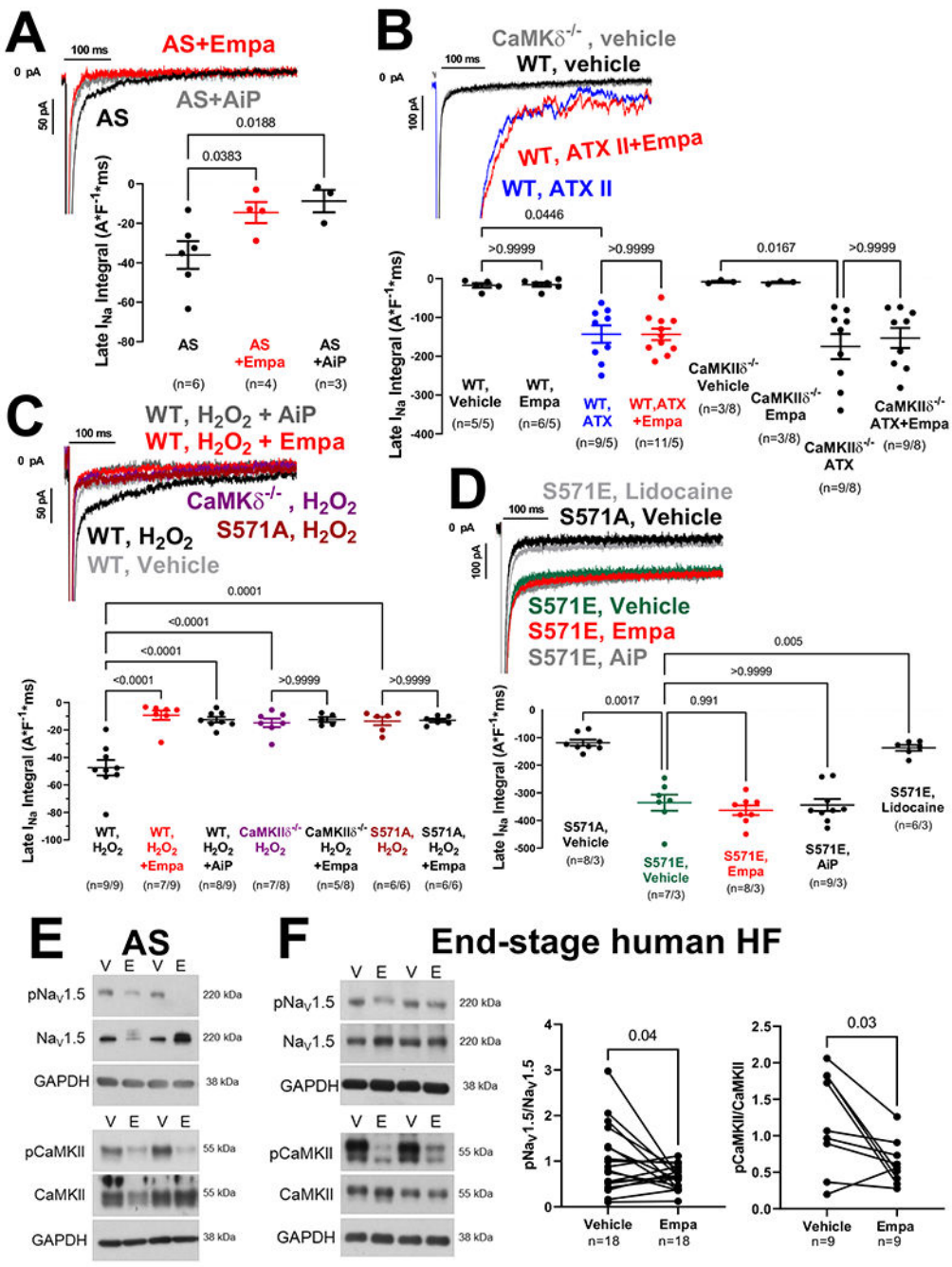

Figure 1:

A) Original recordings and mean data of empagliflozin- or AiP-mediated inhibition of late I_{Na} in human ventricular cardiomyocytes from patients with AS (n=patients). **B)** Original recordings and mean data of late I_{Na} in murine cardiomyocytes from WT or CaMKIIδ$^{-/-}$ mice (n=cells/mice). The ATX-dependent enhancement of late I_{Na} could not be blocked by empagliflozin. **C)** In contrast, the H_2O_2-dependent stimulation of late I_{Na} was blocked by either CaMKII inhibition (AiP, CaMKII$^{-/-}$), transgenic inhibition of CaMKII-dependent $Na_V1.5$ phosphorylation (S571A) or in the presence of empagliflozin. **D**) In contrast to local

anesthetic lidocaine, neither empagliflozin nor AiP could block enhanced late I_{Na} in mice with phosphomimetic substitution of glutamic acid for serine at 571 (S571E). **E+F)** Western blots of cardiomyocytes upon empagliflozin shows reduced CaMKII-autophosphorylation (T287) and reduced CaMKII-dependent $Na_V1.5$ phosphorylation.

For comparison of multiple groups, mixed-effects analysis+Holm-Sidak (1A) or linear mixed model+Sidak were performed. For comparison of two groups, paired t-test was done (1F).