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RESEARCH LETTER

Empagliflozin Inhibits Cardiac Late Sodium Current by Ca/Calmodulin-Dependent Kinase II

Julian Mustroph, MD*; Maria J. Baier, PhD*; Steffen Pabel, MD; Thea Stehle, MSc; Maximilian Trum, MD; Zdenek Provaznik, MD; Peter J. Mohler, PhD; Hassan Musa, PhD; Thomas J. Hund, PhD; Samuel Sossalla, MD; Lars S. Maier, MD; Stefan Wagner, MD

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 CaMKII (Ca/calmodulin-dependent kinase II) 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 by 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.

Raw data/analytic methods can be made available for purposes of reproducing results or replicating procedures. Human tissue/proprietary antibodies cannot be made available because of legal constraints. Experiments conform to the Declaration of Helsinki. Human/murine studies were approved by institutional committee. Written informed consent was obtained from patients before tissue donation. Left ventricular samples were obtained from septal resections of 11 patients (8 male/3 female, age 69.3±2.6 years) with AS undergoing valve replacement. Patients had a HF with preserved ejection fraction–like phenotype with hypertrophy and preserved ejection fraction (59.4±1.7%). Murine models of CaMKIIδ knock-out ($CaMKII\delta^{-/-}$),[3] inhibition of CaMKII-dependent $Na_V1.5$ phosphorylation at serine 571 (S571A), and with CaMKII phosphomimetic $Na_V1.5$ 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 (dimethyl sulfoxide). 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-autocamtide-2-related inhibitory peptide (AiP); 2 µmol/L, 30 min). For some experiments, empagliflozin was washed in to ATX-II or H_2O_2 preincubated 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 [A]). ATX-II–dependent (Figure [B]) enhancement of late I_{Na} in

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Correspondence to: Julian Mustroph, MD, University Medical Center Regensburg, Department of Internal Medicine II (Cardiology), Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany. Email julian.mustroph@ukr.de

*J. Mustroph and M.J. Baier contributed equally.

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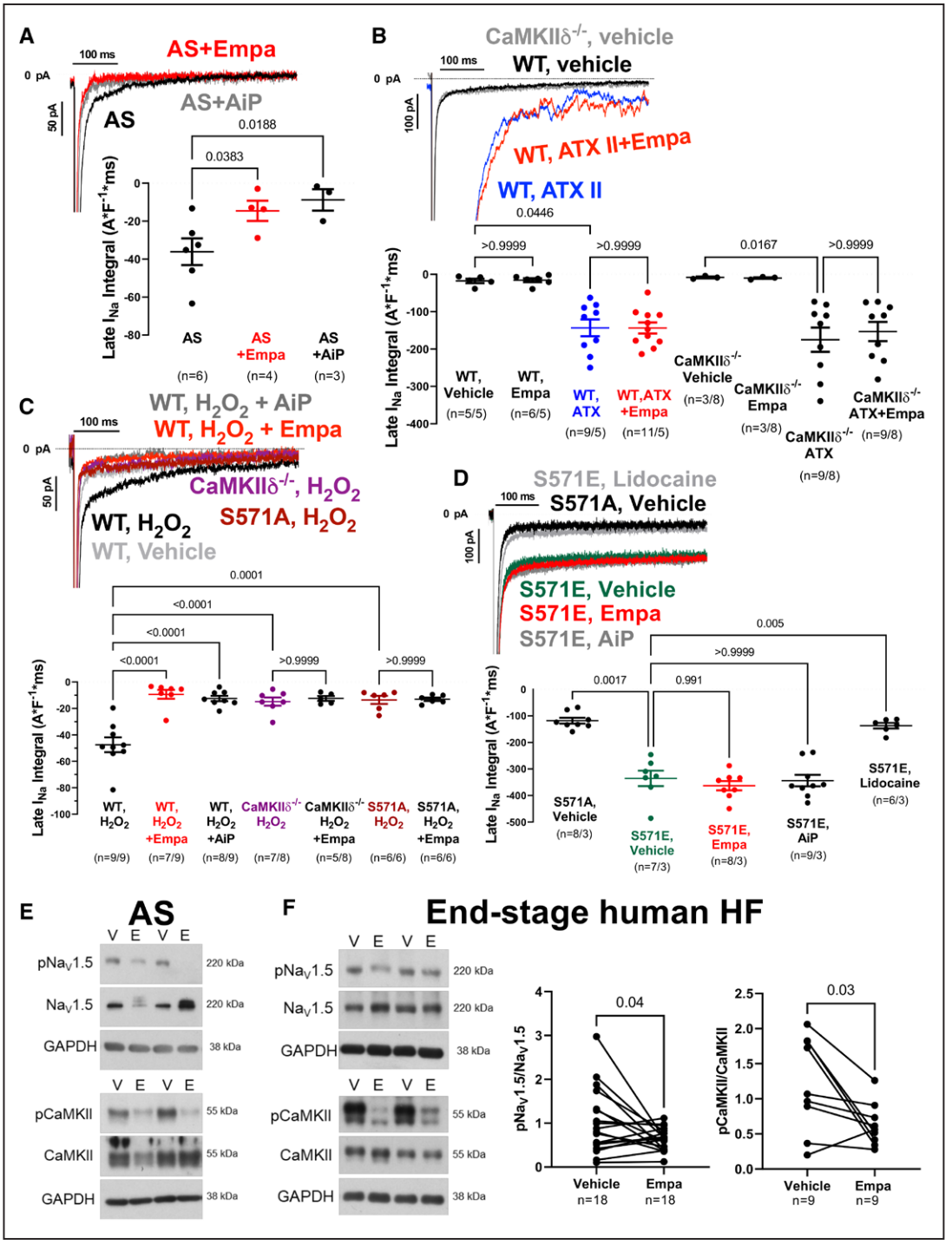

Figure. Late I_{Na} inhibition by empagliflozin requires CaMKII.
A, Original recordings and mean data of empagliflozin- or AiP-mediated inhibition of late I_{Na} in human ventricular cardiomyocytes from patients with AS (n=patients). **B**, Original recordings and mean data of late sodium current (late I_{Na}) in murine cardiomyocytes from wild-type (WT) or CaMKIIδ$^{-/-}$ mice (n=cells per 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 CaMKII inhibition (AiP, CaMKII$^{-/-}$), by transgenic inhibition of CaMKII-dependent Na$_V$1.5 phosphorylation (S571A), or in the presence of empagliflozin. **D**, In contrast with local anesthetic lidocaine, neither empagliflozin nor AiP could block enhanced late I_{Na} in mice with phosphomimetic substitution of glutamic acid for serine at 571 (S571E). **E** and **F**, Western blots of cardiomyocytes on empagliflozin show reduced CaMKII-autophosphorylation (T287) and reduced CaMKII-dependent Na$_V$1.5 phosphorylation. For comparison of multiple groups, mixed-effects analysis plus Holm-Sidak (**A**) or linear mixed model plus Sidak were performed. For comparison of 2 groups, paired *t* test was done (**F**). A indicates ampere; AIP, autocamtide-2-related inhibitory peptide; AS, aortic stenosis; ATX II or ATX, Anemonia viridis toxin 2; CaMKII, Ca/calmodulin-dependent kinase II; CaMKIIdelta-/-, CaMKII delta knock out δ; E, Empa; Empa, empagliflozin; F, farad; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HF, heart failure; kDa= kilo Dalton; ms, miliseconds; p, phosphorylated; pA, picoampere; S571A and S571E: Nav1.5 with a phosphomimetic mutation at Ser571 (S571E), or Nav1.5 with the phosphorylation site ablated (S571A); V, vehicle; and WT, wildtype.

Nonstandard Abbreviations and Acronyms

AS	aortic stenosis
CaMKII	Ca/calmodulin-dependent kinase II
HF	heart failure
late I_{Na}	late sodium current

murine wild-type cardiomyocytes was not affected by empagliflozin (not even at 10 and 100 µmol/L), or after wash-in (at 1 µmol/L) to ATX-II preincubated myocytes, which would be expected if empagliflozin were a direct $Na_V1.5$ inhibitor. Moreover, wash-in of empagliflozin (up to 10 µmol/L) also did not inhibit late I_{Na} in myocytes preincubated with a moderate concentration of veratridine (16 nmol/L, experimentally determined as EC_{50} 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 [C]), with maximal efficacy at 6 minutes but not at 2 minutes after onset of exposure (late I_{Na} integral during wash-in: 0 minutes, −50.8±4.3 $A*F^{-1}$ [ampere*farad]*ms; 2 minutes, −39.9±4.6 $A*F^{-1}$*ms, P=0.0934 versus 0 minutes; 4 minutes, −24.2±4.6 $A*F^{-1}$*ms, P=0.0007 versus 0 minutes; 6 minutes, −17.2±4.0 $A*F^{-1}$*ms, P<0.0001 versus 0 minutes, n=6). No additional effect of empagliflozin on late I_{Na} was observed with AiP (not shown) or in myocytes lacking either CaMKIIδ ($CaMKII\delta^{-/-}$) or CaMKII-dependent $Na_V1.5$ phosphorylation at serine 571 (S571A, Figure [C]). Accordingly, the enhanced late I_{Na} in mice with CaMKII phosphomimetic $Na_V1.5$ S571E was blocked by neither empagliflozin nor AiP (Figure [D]). In contrast, lidocaine inhibited late I_{Na} in S571E cells, underscoring that empagliflozin primarily acts by 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 [E and F]).

In conclusion, inhibition of late I_{Na} by empagliflozin is at least in part caused by 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 versus indirect effects on late I_{Na}. We demonstrate that empagliflozin also inhibits late I_{Na} in patients with AS and features of HF with preserved ejection fraction, which may reduce the propensity for arrhythmias and contribute to the positive results of the EMPEROR-Preserved trial (Empagliflozin Outcome Trial in Patients With Chronic Heart Failure With Preserved Ejection Fraction).

ARTICLE INFORMATION

Affiliations

Department of Internal Medicine II (J.M., M.J.B., S.P., T.S., M.T., S.S., L.S.M., S.W.) and Department of Cardiothoracic Surgery (Z.P.), University Medical Center Regensburg, Germany. Davis Heart and Lung Research Institute, Ohio State University, Columbus (P.J.M., H.M., T.J.H.).

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Disclosures

None.

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