FGF23 Induces Ventricular Arrhythmias in Mouse Hearts Mediated Through the Phospholipase C Pathway

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ABSTRACT

Background: Fibroblast growth factor 23 (FGF23) is a phosphate regulating peptide hormone released by osteocytes. It becomes markedly elevated in chronic kidney disease (CKD), for which the leading cause of death is cardiovascular disease, particularly sudden cardiac death. Previously, we found that FGF23 increases intracellular calcium in cardiac myocytes and alters cardiac contractility in mouse ventricles ex vivo. We have since observed that FGF23 induces contractile dysrhythmias and hypothesize that these aberrant rhythms are due to ventricular muscle hyperexcitability. Since FGF23 has been shown to induce cardiac effects via fibroblast growth factor receptor 4 (FGFR4) and phospholipase C (PLC), we hypothesize that these arrhythmias are mediated through the PLC signal transduction pathway.

Methods: To test this hypothesis, isolated ventricles from CD1 mouse hearts were perfused ex vivo in a modified Langendorff model and paced at 1.8 Hz. Ventricular contractility and rhythm were analyzed by assessing isometric force during treatment with vehicle or FGF23 with and without pretreatment with a PLC blocker (U73122) or an IP3 receptor antagonist (2-aminoethoxydiphenyl borate, 2-APB). Ectopic contractile events were categorized using criteria from an adjacent on the Lambeth conventions on animal arrhythmias. In order to confirm our mechanistic categorizations, we measured electrocardiographic (ECG) activity and intraventricular pressure via a balloon catheter inserted in the ventricular isolated hearts.

Results: We found that hearts perfused with FGF23 displayed increased mechanical arrhythmias in the form of ventricular premature beats (VPBs), which increased from 0.2/min at baseline to 1.8/min (p < 0.001; n=11). Additionally, FGF23 induced runs of bigeminy in 4 of 11 animals and tachycardia in 6 of 11 animals. Arrhythmias were prevented when hearts were pretreated with a PLC blocker (p<0.05; n=5) or an IP3 receptor antagonist (p<0.05; n=5). ECG recordings of FGF23 treated mouse hearts (n=6) confirmed that the contractile abnormalities corresponded to premature ventricular contractions (PVCs), PVCs in bigeminy, and ventricular tachycardia.

Conclusions: Our results show that in Langendorff perfused mouse hearts, FGF23 induces ventricular arrhythmias that are prevented by blockade of PLC and IP3 receptor signaling. Since ventricular arrhythmias represent a leading cause of morbidity and mortality in CKD patients, this pathway may represent an important therapeutic target.

METHODS

- **Ex vivo contractility**: Hearts were harvested from 12-16 week old C57Bl/6J male mice and artificial coronary perfusion was established using the Langendorff technique. For contractile measurements, atria were removed and hearts were suspended from a force transducer in an oxygenated organ bath. Hearts were paced with bipolar stimulating electrodes at 1.2-1.8 Hz and contractility was measured before and after perfusion of FGF23 [9 ng/mL] for 10 min.

- **Ex vivo ECG**: Hearts were isolated and Langendorff perfused as mentioned above, except atria were kept intact and exhibited spontaneous sinus rhythm. The heart was maintained in a silicone-lined glass dish with oxygenated Ringer’s buffer. ECG was monitored with needle electrodes, flanking the heart in a lead configuration and a balloon catheter was inserted into the left ventricle to monitor corresponding mechanical pressure changes before and after FGF23 [9 ng/mL] perfusion.

- **Contraction and ECG waveforms were measured using Powerlab hardware and LabChart software (ADInstruments). Exotic events were categorized as premature ventricular beats, PVCs in bigeminy and trigeminy, and ventricular tachycardia, using an adjacent on the Lambeth conventions.**

RESULTS

**FGF23 Induces Mechanically Defined Ventricular Dysrhythmias**

- **Figure 1.** Panel A: Average increase in the number of premature ventricular beats from baseline, after FGF23 [9 ng/mL], from 0.2 to 1.8 events/min. Panel B: Runs of bigeminy were induced by FGF23 [9 ng/mL] at an average rate of 0.8 events/min, which did not occur at baseline or with vehicle. Panel C: Runs of ventricular tachycardia were induced by FGF23 [9 ng/mL] at a rate of 0.16 events/min, which did not occur at baseline or with vehicle. Panel D: Baseline contractile pattern. Panel E: Premature ventricular beat, defined as a new contraction before complete relaxation. Panel F: PVC in bigeminy defined as at least four PVCs without an intervening normal contraction. *P<0.05 compared to baseline, using a one way ANOVA with Dunnett’s multiple comparisons test; n=6-8.

- **Figure 2.** Panel A: Premature ventricular beats per minute induced by FGF23 [9 ng/mL] after pretreating with a PLC blocker (U73122 [5 uM]) or IP3 receptor blocker (2-APB [3 uM]). Panel B: PVCs in bigeminy per minute induced by FGF23 [9 ng/mL] after pretreating with a PLC blocker (U73122 [5 uM]) or IP3 receptor blocker (2-APB [3 uM]). * indicates P<0.05 compared to baseline, using a one way ANOVA with Dunnett’s multiple comparisons test; n=4-8. (+) indicates pretreatment with blocker followed by administration of FGF23 [9 ng/mL].

- **Mechanical Dysrhythmias Confirmed with ex vivo ECG**

- **Figure 3.** Panel A: Mechanical categorizations of ventricular dysrhythmias after FGF23 [9 ng/mL] perfusion were confirmed via ex vivo electrocardiography synchronized with intraventricular pressure via left ventricular balloon catheter. Panel B: Premature ventricular beats per minute during 30 minutes of perfusion with vehicle or FGF23. *P<0.05, two-tailed t-test; n=3-5.

CONCLUSIONS and SIGNIFICANCE

- **FGF23** induces ventricular hyperexcitability, which increases intracellular calcium, which induces ventricular arrhythmias.

REFERENCES


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