The effects of fibroblast growth factor 23 on SHSY-5Y neuronal cells

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INTRODUCTION

- Chronic kidney disease (CKD) is associated with increased levels of fibroblast growth factor (FGF23) (1). It has recently been shown that FGF23 has a role in the CNS, as impaired memory formation is noted in CKD patients with high FG23 serum concentration (1).
- FGF23, Klotho, and FGF receptors are expressed in the CNS, and the low molecular weight of FGF 23 allows the transportation of FGF23 through the blood–cerebrospinal fluid (CSF) barrier (1).
- Mice overexpressing FGF23 show impairment in hippocampal LTP and spatial memory deficits.
- Long Term Potentiation (LTP) and Long Term Depression (LTD) are calcium driven processes that strengthen and weaken memory formation (2).
- We used the well-established neuroblastoma cell line, SHSY-5Y, to test the impact of acute and long term application of FGF23 on ryanodine receptor calcium release (2).
- Our objective was to determine whether FGF23 could directly induce calcium release in SHSY-5Y cells, and if incubation of SHSY-5Y cells in FGF23 alters SHSY-5Y ryanodine receptor induced calcium release.

METHODS

- SH-SY5Y neuroblastoma cells were grown in a T-150 cell culture using SH-SY5Y media (10% fetal bovine serum (PAA, Piscataway, NJ), 1:200 penicillin/ streptomycin, 50% Ham's F-12 media, 50% eagle minimum essential media where cells were maintained in a maintained in a 37ºC, 5%CO2, 95% O2 incubator
- Cells were plated at a density of 5,000 – 10,000 cells/cm2 on 35 mm plastic tissue culture dishes (TPP), and allowed 1-5 days of growth
- On the day of imaging, media was removed and cells were washed in extracellular solution buffer three times, then incubated in 2µM fura-2 AM (Invitrogen) in ECS for 30 minutes.
- Cells were washed an additional two times and allowed an additional 15 minutes for de-esterification of Fura-2 AM
- Caffeine (30mM; selective ryanodine receptor activator) and KCl (120mM) were perfused using VC-8T perfusion system (Warner Instruments, Hamden, CT) and driven by P720/66 high flow peristaltic pumps (Instech, Plymout Meeting, PA).
- Perfusion protocol for acute application of FGF23: 2minutes ECS, 4 minutes FGF23 (19000 pg/ml), 3 minutes washout (ECS), 1 minute KCl, 2 minute washout (ECS), 2minute caffeine, and 1 minute washout. ECS buffer consisted of 137mM NaCl, 5mM KCl, 1mM Na2HPO4, 1mM MgSO4, 10mM HEPES, 22mM D-(+)-glucose, 1.8mM CaCl2, pH 7.35.

RESULTS

Changes in calcium responses after incubation of SHSY-5Y cells with FGF23

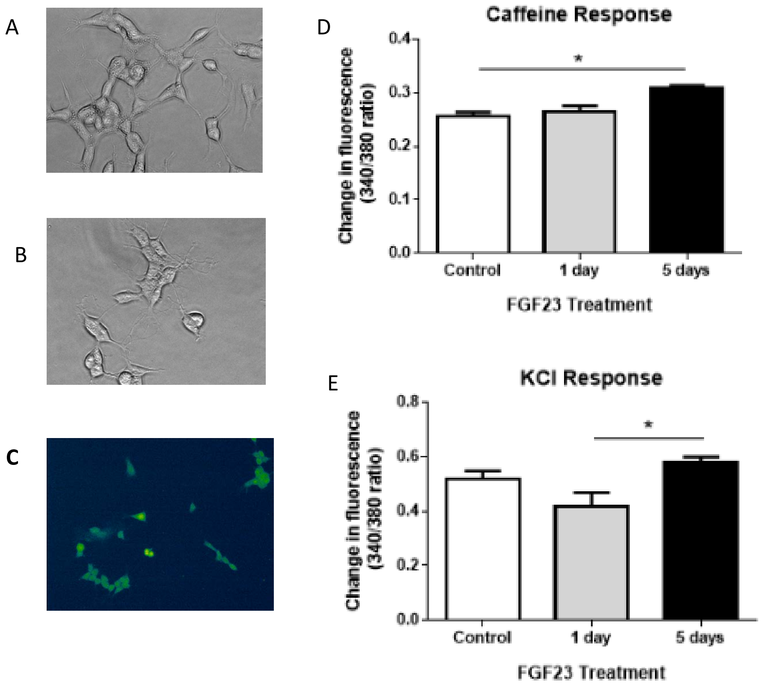

Figure 1. A & B) Representative images of the isolated and cultured SHSY-5Y neurons used for calcium imaging during the experiment. Cells were incubated for 1 or 5 days in 19,000 pg/ml FGF23 prior to calcium imaging. C) Image of the Fura-2 calcium indicator dye loaded cells. D) Summary data of the change in intracellular calcium (340/380 ratio) after caffeine stimulation. A significant increase in caffeine response was observed in the 5 day FGF23 incubation compared to vehicle (n=3; $P<0.05$). E. Summary data of the change in intracellular calcium after KCl treatment. A significant different in response was observed between the 1 and 5 day treatments with FGF23 (n=3; $P<0.05$).

SUMMARY

- FGF23 did not directly alter calcium levels in SHSY-5Y neurons
- Incubation of SHSY-5Y cells in FGF23 for 5 days increased caffeine-induced ryanodine calcium release
- FGF23 Incubation of SHSY-5Y cells increased KCl induced calcium release in 5 day compared to 1 day incubation

CONCLUSION

- Elevated FGF23 levels in blood can travel into the CSF of patients with CKD.
- Neuronal calcium release has long been associated with memory formation through LTP and LTD generation.
- Results of our experiment show for the first time that chronic incubation of FGF23 in a neuronal cell line alters calcium release dynamics through an unidentified mechanism in ryanodine receptor calcium release dynamics.
- FGF23 may also alter the mechanism of membrane depolarization induced calcium release as the response to KCl depolarization were different.
- We hypothesize that the chronic elevation in FGF23 associated with CKD leads to alterations in calcium release dynamics in neurons which may play a role in altered memory formation.

REFERENCES

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