RESEARCH REPORT

Localization of fibroblast growth factor 23 protein in the rat hypothalamus

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Abstract

Fibroblast growth factor 23 (FGF23) is an endocrine growth factor and known to play a pivotal role in phosphate homeostasis. Interestingly, several studies point towards a function of FGF23 in the hypothalamus. FGF23 classically activates the FGF receptor 1 in the presence of the co-receptor αKlotho, of both gene expression in the brain was previously established. However, studies on gene and protein expression of FGF23 in the brain are scarce and have been inconsistent. Therefore, our aim was to localise FGF23 gene and protein expression in the rat brain with focus on the hypothalamus. Also, we investigated the protein expression of αKlotho. Adult rat brains were used to localise and visualise FGF23 and αKlotho protein in the hypothalamus by immunofluorescence labelling. Furthermore, western blots were used for assessing hypothalamic FGF23 protein expression. FGF23 gene expression was investigated by qPCR in punches of the arcuate nucleus, lateral hypothalamus, paraventricular nucleus, choroid plexus, ventrolateral thalamic nucleus and the ventromedial hypothalamus. Immunoreactivity for FGF23 and αKlotho protein was found in the hypothalamus, third ventricle lining and the choroid plexus. Western blot analysis of the hypothalamus

confirmed the presence of FGF23. Gene expression of FGF23 was not detected, suggesting that the observed FGF23 protein is not brain-derived. Several FGF receptors are known to be present in the brain. Therefore, we conclude that the machinery for FGF23 signal transduction is present in several brain areas, indeed suggesting a role for FGF23 in the brain.

KEYWORDS
FGF23, fibroblast growth factor 23, hypothalamus, Klotho, third ventricle

1 | INTRODUCTION

Fibroblast growth factor 23 (FGF23) is the latest of the fibroblast growth factors to be identified and has been shown to play a key role in phosphate homeostasis (Vervloet, 2019; Yamashita et al., 2000). Circulating FGF23 is primarily derived from osteocytes in the bone, but lower levels of fgf23 gene expression are also detected in spleen, heart and liver (Fon Tacer et al., 2010). FGF23 affects target organs, such as the kidney through the FGF receptor 1 (FGFR1) and co-receptor; αKlotho (Vervloet, 2019), however, is also known to have extrarenal involvement and has cardiac, inflammatory and vascular effects (Vervloet, 2019). In addition, FGF23 is suggested to play a role in glucose metabolism (Ursem et al., 2018; Yeung et al., 2020).

FGF23 belongs to the endocrine FGF family, together with FGF19 and FGF21. The brain is an important regulator of glucose homeostasis, and both FGF19 and FGF21 have been shown to be involved in the central regulation of glucose metabolism; currently, their therapeutic potential is being investigated in several trials (Brown et al., 2019; Degirolamo et al., 2016; Izaguirre et al., 2017). In light of endocrine FGFs being involved in the central control of glucose metabolism, it may be that the role of FGF23 in glucose metabolism is also mediated via the brain. For adequate FGF23 signal transduction in the brain, this would require the corresponding receptor (FGFR1) and co-receptor (αKlotho) to be present.

The first study to identify FGF23 in mouse brain found the highest expression of FGF23 mRNA in the ventrolateral thalamic nucleus (VLTN) using in situ hybridization (Yamashita et al., 2000). Subsequent reports, in mice, have been inconsistent in whether there is gene expression of FGF23 in the brain (Fon Tacer et al., 2010; Kaminskas et al., 2019; Laszczyk et al., 2019; Liu et al., 2003). Interestingly, it was shown that FGFR1 and αKlotho mRNA and peptide are present in the brain and more specifically in the hypothalamus, thus enabling action of FGF23 (Clinton et al., 2013; Fon Tacer et al., 2010). Indeed, several studies reported associations between serum FGF23 concentrations and memory deficits, axonal integrity and neural network architecture in humans and animals, pointing to action of FGF23 in the brain (Drew et al., 2014; Hensel et al., 2016; Laszczyk et al., 2019; Liu et al., 2011; Marebwa et al., 2018; McGrath et al., 2019). In addition, preliminary data show an increase in FGF23 protein expression in the murine hypothalamus upon fasting (Morikawa & Komori, 2014). Administration of intracerebroventricular FGF23 resulted in increased FGF23-induced ERK1 expression, co-localising with AgRP immunoreactivity (Morikawa & Komori, 2014).

Together, these findings suggest a role for FGF23 in brain function and the presence of FGF23 in the hypothalamus might point to involvement in energy balance and glucose regulation. It remains, however, to be determined if FGF23 protein is present in the brain, and more specifically hypothalamus, and whether this is produced locally and/or originates from peripheral sites. In humans, FGF23 protein was found in the cerebrospinal fluid (CSF) of adults and children (Kunert et al., 2017; Li et al., 2018). Using the CSF/plasma concentration ratio, the Stokes–Einstein radius and the molecular mass, it was estimated that the majority of measured FGF23 would originate from intrathecal synthesis (Kunert et al., 2017).

Taken together, there is a clear need, as pointed out in various reports, for more studies directed to unravel the presence and origin of central FGF23 and whether this could act in the brain to exert effects on glucose metabolism (Erben, 2016, 2018; Liu & Quarles, 2007; Martin et al., 2012). Therefore, the aim of this study is to localise and visualise FGF23 and its co-receptor, αKlotho, in the rat brain with a special focus on different nuclei of the hypothalamus, important for glucose control.

2 | MATERIALS AND METHODS

2.1 | Animals

Surplus male Long-Evans rats and male Wistar rats (10–13 weeks old) were used. They were housed in the animal

facility of the Netherlands Institute of Neuroscience in a temperature (21–23°C) and light-controlled room (12:12 h light/dark cycle, 07:00–19:00 lights on). Rats had *ad libitum* access to a standard diet (Teklad global diet 2918; 24% protein, 58% carbohydrate and 18% fat, 3.1 kcal/g, Envigo) and a bottle of tap water. The animal care committee of the Netherlands Institute for Neuroscience approved all experiments according to Dutch legal ethical guidelines.

2.2 | Fluorescent immunocytochemistry

One Wistar rat was used for determining optimal antibody concentrations, three Long-Evans rats were used for subsequent stainings. Rats were injected with i.p. pentobarbital and perfused with cold saline and 10% paraformaldehyde. Brains and kidneys were then collected, postfixed for 24 h in 4% (w/v) paraformaldehyde in phosphate buffered saline (PBS), pH 7.4 and impregnated for several days in a 30% (w/v) sucrose in sterile water cryoprotectant solution. Subsequently, tissues were frozen on dry ice and stored at −80°C. At Bregma −2.16 (Paxinos & Watson, 2007), for each brain, coronal sections were cut on the cryostat (CryoStar NX50, ThermoFisher Scientific [35 μm]) and collected on Superfrost Plus slides (Fisher, Gerhard Menzel GmbH, Germany). Kidney tissue was cut transversally. Kidney sections were used as positive control being an important target organ for FGF23 protein (Vervloet, 2019).

Sections were incubated with a polyclonal Klotho antibody: goat antimouse αKlotho 1/50 (AF1819, R&D Systems, Minneapolis, USA), after being washed in TRIS-buffered saline (TBS; 50-mM Tris-Cl, 150-mM NaCl; pH 7.6). The primary antibody was dissolved in supermix (0.15-M NaCl, 0.05-M Tris, 0.25%w/v gelatin, 0.5% v/v TritonX-100, pH 7.6). Slides were incubated in a humidified chamber for 1 h at room temperature (RT) and overnight at 4°C. Afterwards, sections were washed with TBS, 3 times 5 min, and incubated for 1 h at RT with 1/200 biotinylated horse antigoat IgG (H + L) (BA9500; Vector Laboratories, Burlingham, USA) in supermix. After washing with TBS, sections were incubated with 1/200 Alexa Fluor-488-Streptavidin (s32354, Invitrogen, Carlsbad, USA). After TBS washes, slides were incubated with DAPI (ThermoFisher Scientific) 1/1000, cover slipped with Vectashield (Vector Laboratories, Burlingame, USA) and stored in the dark at 4°C.

A monoclonal FGF23 antibody was used: rat antimouse FGF23 1/25 (MAB26291, R&D Systems, Minneapolis, USA). As this was a rat-on-rat staining, incubation with the primary antibody was preceded by blocking native IgG. To this end, sections were first TBS washed followed by incubation with 0.3% TritonX-100 in PBS (11666789001, Roche, Basel, Switzerland) for 30 min at RT. After TBS washes, endogenous rat IgG was blocked with unconjugated rabbit F (ab′)$_2$ antirat IgG (H + L) 1/10 (6130-01, Southern Biotech, Birmingham, USA) for 2 h at RT. After TBS washes, sections were incubated with the primary antibody as described above. The secondary antibody used was biotinylated goat antirat (H + L) (BA9400; Vector Laboratories, Burlingham, USA). Incubation times and concentrations of the secondary antibody, streptavidin and DAPI and the mounting were identical as described above. Imaging was performed with a confocal microscope: the Leica TCS SP8 X DLS microscope (Leica, Wetzlar, Germany) at the AMC cellular imaging facility.

2.3 | Western blotting

From two male Wistar rats, coronal brain slices were cut with a cryostat from Bregma −0.96 until −3.60. Hypothalamic blocks were cut out using a sharp needle. Also, from one animal, a block of kidney cortex was dissected. Tissue was then homogenised, using the Ultra Turrax homogeniser (IKA, Staufen, Germany) in RIPA lysis buffer (50-mM Tris-HCl (pH 7.6), 150-mM NaCl, 1% TritonX-100, 0.5% sodium deoxycholate, 0.1% SDS, 2-mM EDTA, phosphostop (phospho/STOP, Roche, Basel, Switzerland) and protease inhibitor (Complete Mini-EDTA free, Roche, Basel, Switzerland). Protein concentrations were determined using the Bio-Rad protein assay according to the manufacturer's instructions (Bio-Rad Laboratories, Veenendaal, The Netherlands). Protein (20 μg) was loaded on a 16% SDS-PAGE gel. Proteins were electrotransferred (10–15 min with 350–375 mA) on a nitrocellulose membrane (Millipore, Darmstadt, Germany), and thereafter, membranes were incubated with blocking solution (5% milk in TBS containing 0.2% Tween-20) for 1 h at RT. Subsequently, membranes were incubated overnight at 4°C with primary FGF23 antibody (rabbit antirat FGF23; AB56326, Abcam, Cambridge, UK) diluted 1/200 in supermix. After washing, blots were incubated with horseradish peroxidase (HRP)-linked rabbit specific antibody (DAKO, Glostrup, Denmark), diluted 1/1000 in blocking solution. Protein expression was detected using chemiluminescence with the ECL prime Western Blotting Detection Reagent Kit (GE Healthcare Lifesciences, Little Chalfont, UK). Protein bands were visualised on the ImageQuant LAS4000 (GE Healthcare, Little Chalfont, UK).

2.4 | qPCR

Six Long-Evans rats were kept under *ad libitum* conditions and decapitated after 33% CO_2/66% O_2 anaesthesia. Brains and spleens were rapidly dissected, frozen on dry

ice and stored at −80°C. Coronal sections were cut on the cryostat (200 μm) to obtain punches, if applicable bilaterally, with a 1-mm diameter blunt needle. Sections were placed in RNAlater (Ambion, Waltham, USA), and once isolated, punches were immediately put on dry ice and stored at −80°C. The following areas were punched: arcuate nucleus (Bregma −1.68 to −3.28), choroid plexus (Bregma −0.48 to −2.08), lateral hypothalamus (Bregma −1.28 to −2.88), paraventricular nucleus (−1.08 to −1.68), VLTN (Bregma −1.68 to −2.88) and ventromedial hypothalamus (−1.68 to −3.08). Spleen was chosen as positive control. FGF23 gene expression is detected at high levels in bone, whereas in spleen, thymus and the heart lower levels are detected (Fon Tacer et al., 2010). We chose to use spleen as a positive control to verify detection at lower expression levels of FGF23.

Tissue was homogenised using the Ultra Turrax homogeniser (IKA, Staufen, Germany) in 300-μl TriReagent. Total RNA was isolated using the Direct-zol RNA Miniprep kit (R2052, Zymo Research, Irvine, USA) with DNAse treatment. The RNA concentration was determined using the DeNovix (DeNovix Inc., Wilmington, USA). RNA quality was analysed with the Bioanalyser (Agilent, Santa Clara, USA). All RIN values were larger than 8.5. cDNA was synthesised with the transcriptor first-strand cDNA synthesis kit with oligod(T) primers (04897030001; Roche Molecular Biochemicals, Mannheim, Germany) using equal RNA input (200 ng for the PVN and 400 ng for the other areas of interest). To check for genomic DNA contamination, several samples were used without reverse transcriptase. One-to-one diluted cDNA was used for all qPCRs. All qPCRs were performed in duplo.

RT-qPCR was performed with the SensiFAST SYBR no-rox kit (Bioline, London, UK) and Lightcycler® 480 (Roche Molecular Biochemicals, Mannheim, Germany). The used rat FGF23 primer had the following sequence: f-TGGCCATGTAGACGGAACAC; r-GGCCCCTATTATCACTACGGAG. The primer was chosen to span intron-exon boundaries to exclude genomic DNA contamination; it was previously validated and has an amplicon length of 91 bp (Feger et al., 2017; Zhang et al., 2016). Spleen cDNA was used as positive control for the FGF23 primer. PCR product size was visualised on DNA agarose gel. As positive control for the qPCR reaction also primers for αKlotho (f-CCATGCCGAGCAAGACTCA; r-CCGTCCAACACGTAGGCTTT), Cyclophilin (f-ATGTGGTCTTTGGGAAGGTG r-GAAGGAATGGTTTGATGGGT) and HPRT (f-GCAGTACAGCCCCAAAATGG r-AACAAAGTCTGGCCTGTATCCAA) were used.

3 | RESULTS

3.1 | Detection of FGF23 and αKlotho in kidney and brain

As there is a high concentration of FGF23 and αKlotho protein in the tubules of the kidney, kidney tissue was used as a positive control for their detection (Vervloet, 2019). The used monoclonal antibody (mAb) for FGF23 was raised against mouse FGF23. Mouse FGF23 shares 95% homology with rat FGF23. The used Ab was previously validated against rat tissue (Spichtig et al., 2014; Sugiura et al., 2018). As this mAb was raised in rat, the secondary Ab (antirat IgG) could bind not only to the Fc-region of the primary Ab but also to native IgG. In order to prevent this, we used a blocking protocol to ensure all native IgG is blocked and can no longer bind to the secondary Ab, as described under methods.

As shown in Figure 1a, the immunoreactivity for FGF23 was observed in kidney tissue, mostly on the basolateral side of the tubules. Figure 1b shows very low background after the blocking protocol. The effectiveness of the blocking can be evidenced by comparison with the incubations without the additional blocking (Figure 1c). Also, immunoreactivity for αKlotho was observed in the tubules with a more diffuse staining pattern.

The same protocol that we established for kidney tissue was used to evaluate brain tissue. Figure 2 depicts the third ventricle and its immunoreactivity for FGF23. Immunoreactivity for FGF23 was observed with increasing intensity towards the ventral side with the strongest signal in the median eminence (Figure 2a). A cytoplasmic staining pattern of FGF23 can be observed along the third ventricle lining (Figure 2b). In the arcuate nucleus and in the median eminence, there was strong diffuse staining (Figure 2c). Several cells (Figure 2d) showed a strong cytoplasmic or perinuclear staining pattern. The VLTN did not show immunoreactivity for FGF23. Adequate blocking of native IgG was also accomplished in brain tissue. No difference in immunoreactivity was found when comparing the Wistar and Long-Evans tissue for all stainings.

Immunoreactivity of αKlotho in the third ventricle is depicted in Figure 3a. The staining was observed along the lining of the third ventricle (Figure 3b). At the bottom of the third ventricle, in the arcuate nucleus and the median eminence, a stronger and more diffuse staining pattern was observed, as well as in projections of presumably β-tanycytes (Figure 3c). Klotho was observed in both cytoplasmic, perinuclear and nuclear cellular compartments.
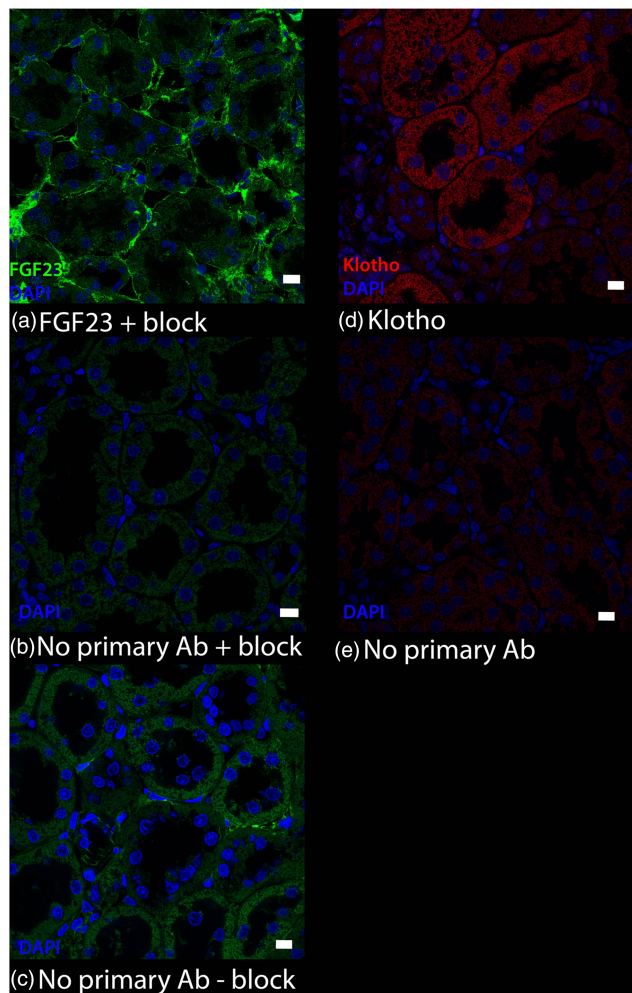
FIGURE 1 Kidney tissue (a) blocked with IgG Ab and incubated with FGF23 mAb (green); (b) negative control: blocked but not incubated with primary Ab; (c) not blocked and without primary Ab; (d) incubated with αKlotho Ab (red); (e) negative control (n = 4; 63x magnification; DAPI is blue; scale bar length is 10 μm)

The choroid plexus showed immunoreactivity for both FGF23 and αKlotho (Figures 4 and 5, respectively). FGF23 was observed in the cytoplasm of cells in the choroid plexus and on the basolateral side of the cells as well as in vascular endothelial cells. For αKlotho, immunoreactivity was seen in cytoplasm throughout the whole choroid plexus.

3.2 | Evaluation of the FGF23 protein size in hypothalamus

To further verify the presence of FGF23 protein in the hypothalamus, western blotting was used on whole hypothalamic blocks. Intact FGF23 has a molecular mass of 32 kDa (Erben, 2018). A band was observed around 32 kDa, corresponding to the molecular mass of intact FGF23 (Figure 6). The band around 60 kDa could be caused by dimer formation, a common phenomenon in western blots, caused by formation either during the procedure of protein denaturation or due to natural forming dimers that are not dissociated by the sample buffer.

3.3 | Analysis of the gene expression of FGF23 in hypothalamus and choroid plexus

As described above, FGF23 protein expression was found in the third ventricle lining, the choroid plexus, the hypothalamus and more specifically, the arcuate nucleus. In the next experiment, qPCR was used to assess whether FGF23 mRNA was present in the brain in punches of several brain regions of interest. As FGF23 mRNA is known to be expressed in the spleen (and not in kidney), spleen tissue served as a positive control (Fon Tacer et al., 2010; Nakashima et al., 2016). We could detect significant mRNA expression of FGF23 in the spleen (Ct = 25). In none of the brain regions, FGF23 mRNA was observed even when using high RNA input for cDNA synthesis, concentrated cDNA (1:1) and in duplo measurements ($n = 6$). PCR products were put on agarose gel, depicted in Figure 7, in order to confirm results. Accordingly, only the spleen showed a band at the expected amplicon length, whereas no bands were observed for the brain regions (i.e., arcuate nucleus, lateral hypothalamus, paraventricular nucleus, choroid plexus, VLTN and the ventromedial hypothalamus).

4 | DISCUSSION

The main objective of this study was to analyse the localization of FGF23 protein and determine whether FGF23 mRNA is expressed in the brain, especially in hypothalamic areas important for glucose metabolism. Our results clearly show FGF23 protein expression in the cellular lining of the third ventricle, which consists of ependymocytes and tanycytes. Furthermore, FGF23 immunoreactivity was most apparent at the bottom of the third ventricle, in the median eminence and diffusely in the hypothalamus with a high signal in the arcuate nucleus. The presence of FGF23 protein in the hypothalamus was further confirmed by western blotting. In addition, FGF23 protein was also observed in the choroid plexus. Whereas FGF23 protein was evidently present in these brain areas, FGF23 gene expression was not. Taken together, our data suggest that the FGF23 protein present in the hypothalamus is from peripheral origin.
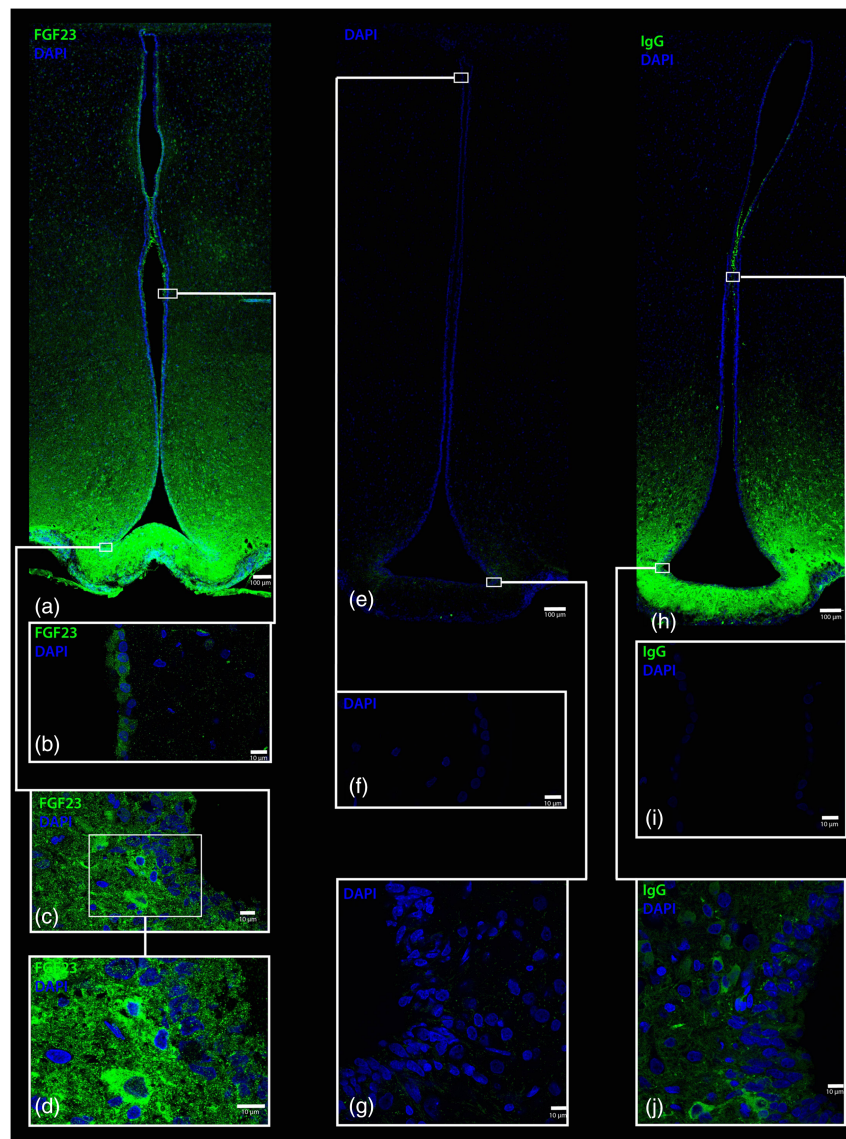
FIGURE 2 Third ventricle (3V) (a) blocked with IgG Ab and incubated with FGF23 mAb (green); (b) detail 3V lining; (c) detail arcuate nucleus; (d) negative control: blocked but not incubated with primary Ab (e) detail 3V lining; (f) detail arcuate nucleus; (g) not blocked and without primary Ab; (h) detail 3V lining; (j) detail arcuate nucleus (n = 4; 10x magnification and 63x magnification for details; DAPI is blue; scale bar length of panels a, d and g is 100 μm, others 10 μm)

In earlier studies identifying FGF23 mRNA expression using in situ hybridization, it was shown that FGF23 mRNA was expressed in the ventrolateral thalamic nucleus of the brain (Liu et al., 2003; Yamashita et al., 2000). Subsequent studies, however, did not find FGF23 gene expression in various other brain regions, including the hypothalamus and ventrolateral thalamic nucleus, which is in agreement with our results (Fon Tacer et al., 2010; Hensel et al., 2016; Kaminskas et al., 2019). Another study in newborn Wistar rats, studying neurons in the rostral ventrolateral medulla, also found FGF23 immunoreactivity in these neurons (Oshima et al., 2020). A study on hippocampal function and structural defects in FGF23-deficient mice, however, was unable to detect FGF23 immunoreactivity in the brain in their wild-type group (Laszczyk et al., 2019).

We did observe FGF23 protein in the brain, and it was previously shown that FGF23 protein was present in CSF (Kunert et al., 2017; Li et al., 2018). Thus, together with the lack of mRNA expression of FGF23, this suggests that FGF23 found in the brain is not brain-derived but originates from the periphery. Transport of FGF23 to the brain could either be over the blood–brain barrier (BBB) or over the blood-CSF barrier (BCSFB). The latter possibility is supported by the positive immunoreactivity for FGF23 in the cytoplasm of cells in the choroid plexus and in the surrounding vascular endothelial cells. However, transport over the BBB is also possible, given the immunoreactivity in the median eminence. In line with this notion, FGF21 is not expressed in the brain but has been shown to enter the brain from the circulation via the BBB (Bookout et al., 2013; Hsuchou et al., 2007). Also, it was described that FGF19 can enter the brain from the blood compartment (Hsuchou et al., 2013). Thus, it might well be that like the other endocrine growth factors that FGF23 can cross the BBB.
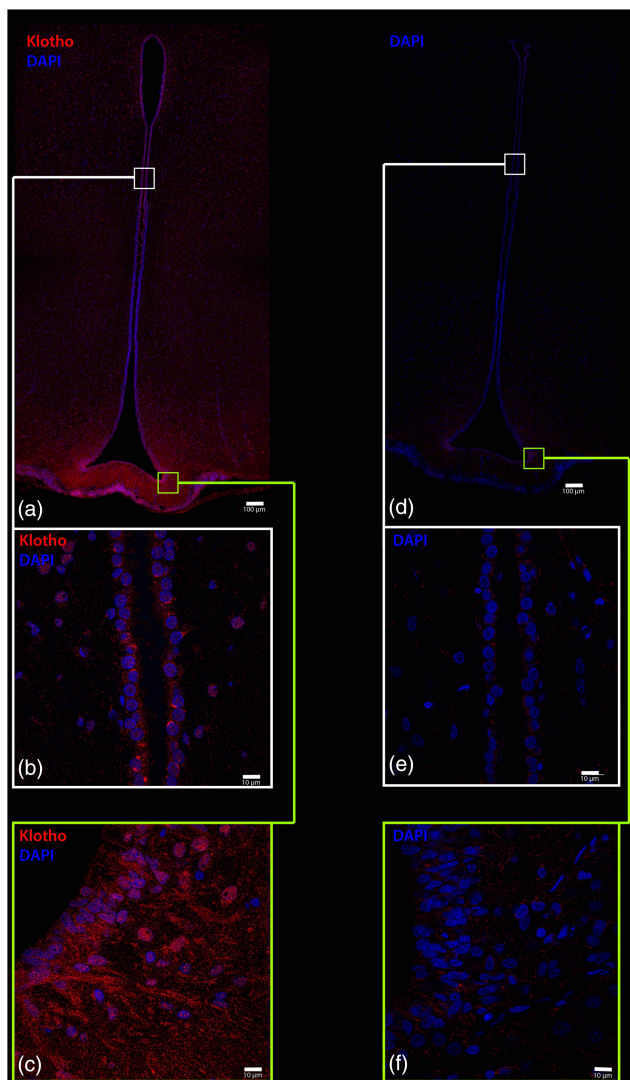
FIGURE 3 Third ventricle (3V) (a) incubated with αKlotho Ab (red); (b) detail 3V lining; (c) detail arcuate nucleus; (d) negative control: not incubated with primary Ab (e) detail 3V lining; (f) detail arcuate nucleus (n = 4; 10x magnification and 63x magnification for details; DAPI is blue; scale bar length of panels a and d is 100 μm, others 10 μm)

Classically, FGF23 acts on cells expressing the FGFR1. More specifically, FGF23 is known to activate the c-isoform, FGFR1c, after binding to the co-receptor αKlotho (Vervloet, 2019). FGFR1c is known to be broadly expressed in the brain, including in the hypothalamus and third ventricle lining of the brain in rats, mice and humans (Belluardo et al., 1997; Bookout et al., 2013; Fon Tacer et al., 2010; Jiao et al., 2011; Weickert et al., 2005). Beyond the classical FGFR1 target, it has also been shown that FGF23 can bind to the other receptors, that is, FGFR2, FGFR3 and FGFR4 (Vervloet, 2019). The binding to these receptors is αKlotho independent and results in unique downstream signalling pathways, altering gene transcription (Vervloet, 2019). FGFR2 and FGFR3 are also expressed in the brain, including the hypothalamus (Fon Tacer et al., 2010). The receptor FGFR1 is not solely the receptor for FGF23 and can be activated by other FGFs; therefore, the presence of this receptor would not indicate, per se, the presence of FGF23 signalling in the hypothalamus (Izaguirre et al., 2017).

In order for FGF23 to activate the FGFR1c receptor, the presence of the co-receptor αKlotho is required. This co-presence induces a 20-fold increase in affinity for FGF23 (Erben, 2018). Through alternative splicing, αKlotho can either be membrane-bound or soluble. Debate continues on whether soluble αKlotho can function as a co-receptor. αKlotho gene and protein expression have been confirmed throughout the brain, including the hypothalamus (Clinton et al., 2013; Olauson et al., 2017). The highest expression is present in the choroid plexus (Clinton et al., 2013). In the current study, we also observed protein expression of αKlotho in the choroid plexus, hypothalamus and third ventricle lining.

With multiple potential receptors and the co-receptor αKlotho in place, the machinery for FGF23 signal transduction is present in the brain (Clinton et al., 2013; Fon Tacer et al., 2010). Furthermore, we found FGF23 protein in the choroid plexus, the third ventricle lining and the hypothalamus. These results support the hypothesis that FGF23 has a function in the brain. Although there are several associations found for FGF23 and brain function, the physiological significance of FGF23 in the brain remains to be determined and we can only speculate about its function. The best studied function of FGF23 is regulation of serum phosphate concentration; hence, it could be that it is also involved in central regulation of phosphate in both CSF and brain parenchyma. In addition, several studies point towards a link between the high FGF23 concentrations seen in patients receiving haemodialysis and their cognitive abilities (Drew et al., 2014; Liu et al., 2011; Marebwa et al., 2018). This link is supported by the finding that FGF23 has a direct effect on hippocampal cells in vitro, reducing neuronal ramification and altering synaptogenesis (Hensel et al., 2016).

Lastly, several reports show an association between FGF23 and glucose metabolism (Fernández-Real et al., 2013; Ursem et al., 2018; Wojcik et al., 2012; Yeung et al., 2020). Preliminary evidence suggests that injection of FGF23 in the ventricles increases hypothalamic expression of NPY and AgRP, which stimulates food intake and affects glucose homeostasis (Morikawa & Komori, 2014). We showed that FGF23 protein is present in the median eminence and third ventricle lining,
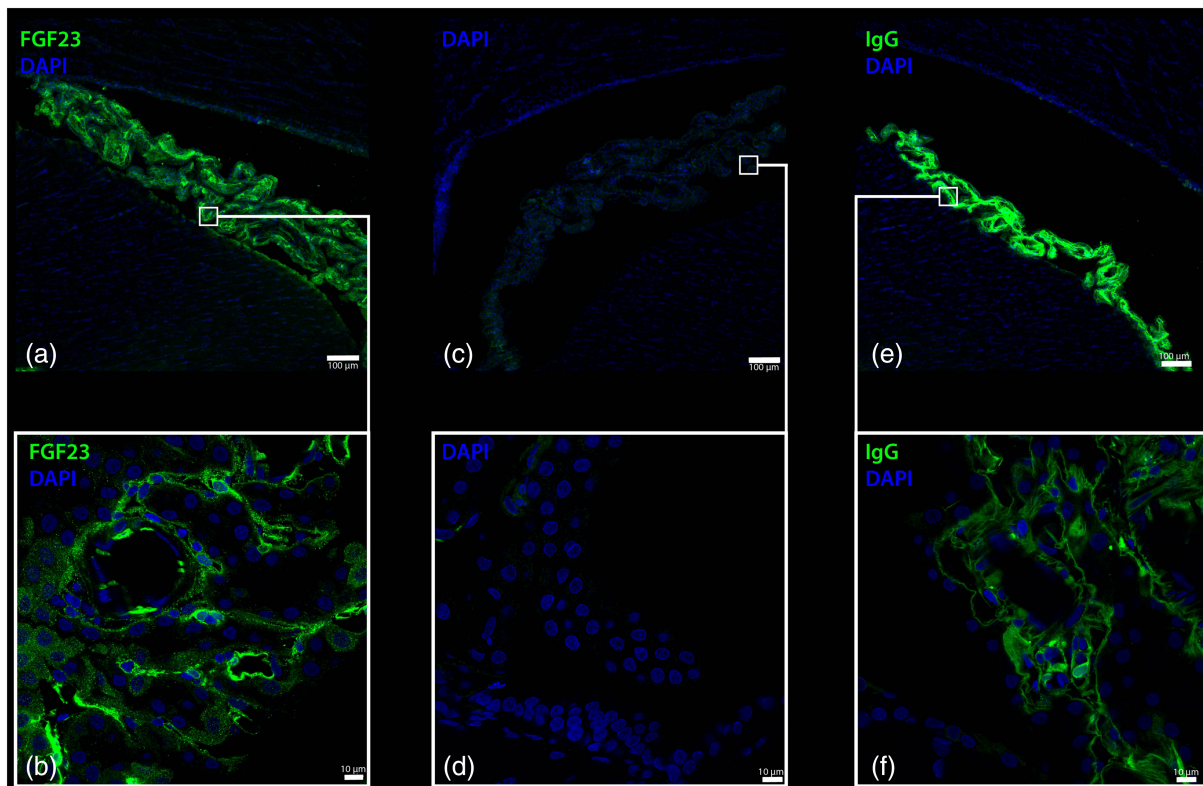
FIGURE 4 Choroid plexus (CP) (a) blocked with IgG Ab and incubated with FGF23 mAb (green); (b) detail CP; (c) negative control: blocked but not incubated with primary Ab (d) detail CP; (e) not blocked and without primary Ab; (f) detail CP (n = 4; 10x magnification and 63x magnification for details; DAPI is blue; scale bar length of panels a, c and e is 100 μm, others 10 μm)
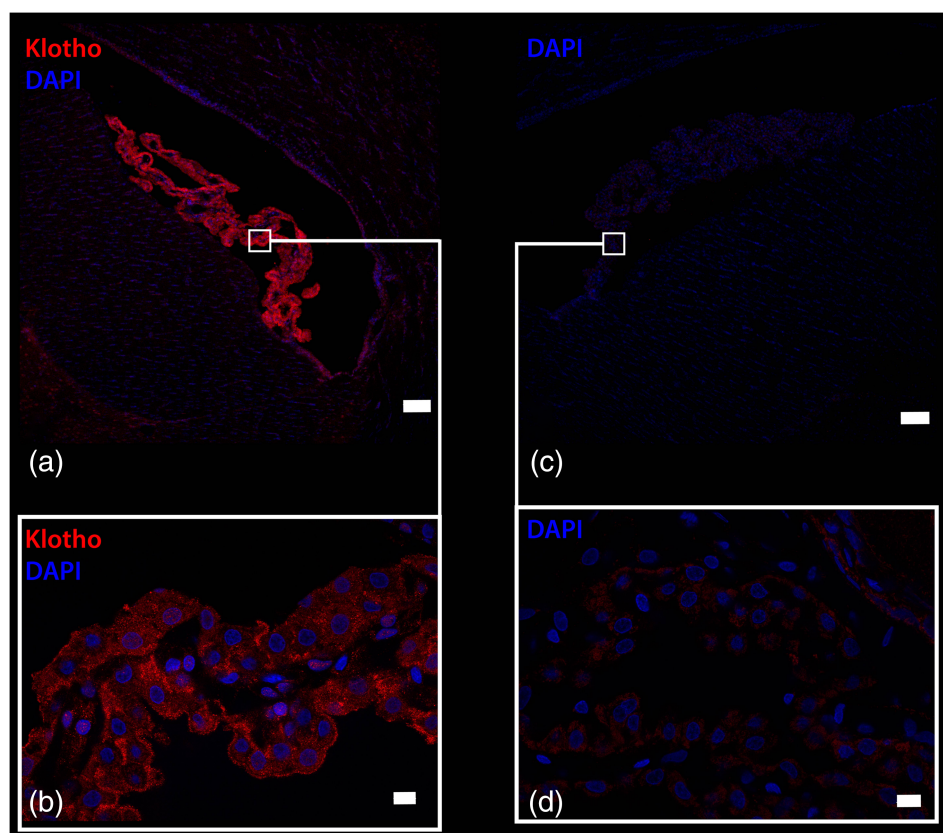

FIGURE 5 Choroid plexus (CP) (a) incubated with αKlotho Ab (red); (b) detail CP; (c) negative control: not incubated with primary Ab (d) detail CP (n = 4; 10x magnification and 63x magnification for details; DAPI is blue; scale bar length of panels a and c is 100 μm, others 10 μm)
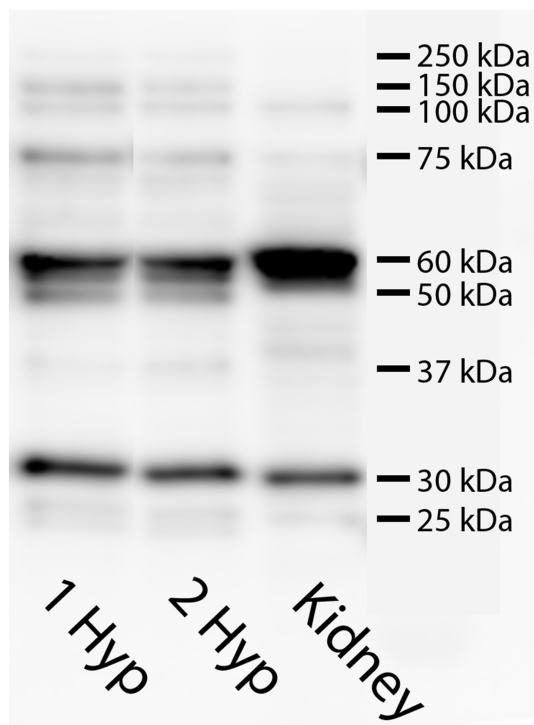
FIGURE 6 Western blot for FGF23 protein (∼32 kDa) expression with tissue from hypothalamus (Hyp) and kidney (n = 2)
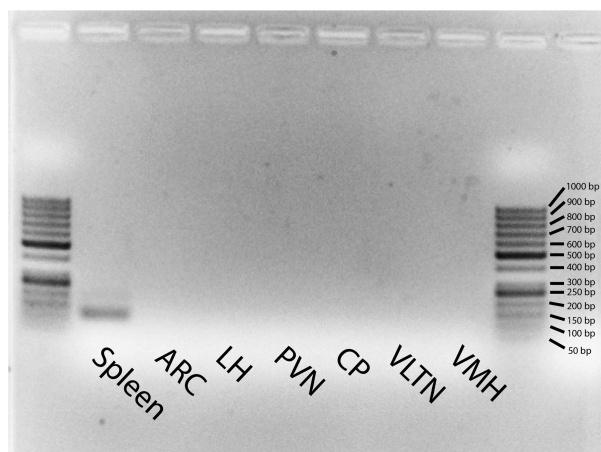

FIGURE 7 DNA gel with FGF23 PCR product of samples taken from the following: spleen, arcuate nucleus (ARC), lateral hypothalamus (LH), paraventricular nucleus (PVN), choroid plexus (CP), ventrolateral thalamic nucleus (VLTN) and ventromedial hypothalamus (VMH) (n = 6)

including areas where tanycytes are located. These cells are known to have processes extending into the hypothalamus and have been shown to play a role in food intake regulation and glucose homeostasis (Langlet, 2019). Hypothetically, FGF23 signalling in the brain may be involved in appetite regulation and circulating glucose levels. This corroborates the previous notion that plasma-derived FGFs may have a central function (Kaminskas et al., 2019).

A few limitations in this study need to be acknowledged. The study was set up to localise FGF23 in the brain and is exploratory in nature. Hence, we cannot draw firm conclusions on which effect FGF23 may have on the brain. Also, based on morphology, we identified areas with positive immunoreactivity but did not yet specify the distinct cell types, which should be the subject for future studies. However, this is the first study to visualise and localise FGF23 protein in the hypothalamus.

Our study reveals that FGF23 protein is present in the choroid plexus, third ventricle lining and the hypothalamus. We did not, however, detect FGF23 mRNA expression in these areas. This suggests that FGF23 in the brain is from peripheral origin. Immunoreactivity for αKlotho was also observed in areas positive for FGF23. In literature it is described that several FGFRs are expressed in the mentioned regions (Belluardo et al., 1997; Bookout et al., 2013; Fon Tacer et al., 2010; Jiao et al., 2011; Weickert et al., 2005). Taken together, the machinery for FGF23 signal transduction is present in different brain areas. With this, exciting questions arise on the potential functions of FGF23 in the brain. Further investigation into the central role of FGF23 in phosphate homeostasis, glucose homeostasis and cognitive impairments is strongly recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

SU, SlF, AH and CD conceived the experimental ideas and design; SU and VB collected the data with help of UA, LE and CMM. SU analysed the data and wrote the manuscript. SlF, AH, CMM and CD reviewed and edited the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15375.

DATA AVAILABILITY STATEMENT

The original data files are available by contacting the first or last author.

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