Rostral ventrolateral medulla neuron activity is suppressed by Klotho and stimulated by FGF23 in newborn Wistar rats

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ABSTRACT

Hypertension often occurs in patients with chronic kidney disease (CKD). Considering the decrease in serum Klotho and increase in serum FGF23 levels in such patients, decreased Klotho and increased FGF23 levels were thought to be associated with hypertension. Presympathetic neurons at the rostral ventrolateral medulla (RVLM) contribute to sympathetic activity and regulation of blood pressure. Therefore, we hypothesized that Klotho would reduce the activities of RVLM neurons and FGF23 would stimulate them. Accordingly, this study examined the effects of Klotho and FGF23 on bulbospinal neurons in the RVLM. We used a brainstem-spinal cord preparation to record from RVLM presympathetic neurons and to evaluate the effects of Klotho and FGF23 on firing rate and membrane potentials of these neurons. Our results showed that Klotho-induced RVLM neuron hyperpolarization, while ouabain, a Na+/K+-ATPase inhibitor, suppressed the effects of Klotho on such neurons. Moreover, FGF23 induced RVLM neuron depolarization, while SU5402, an FGF23 receptor (FGFR1) antagonist, induced RVLM neuron hyperpolarization. Histological examinations revealed that Klotho, Na+/K+-ATPase, FGF23, and FGFR1 were present in RVLM neurons and that Klotho was localized in the same neurons as FGFR1. These results suggest that Klotho and FGF23 regulate the activity of RVLM neurons. Klotho may reduce the activity of RVLM neurons via stimulating Na+/K+-ATPase on those neurons while FGF23 may activate those neurons via FGFR1.

1. Introduction

Klotho (1014 amino acids; molecular weight, 130 kDa), which is mainly produced in the distal convoluted tubules of the kidneys, parathyroid glands, and choroid plexus of the brain, is known as an anti-aging and anti-inflammatory protein (Clinton et al., 2013; Kuro-o, 2008; Kurosu et al., 2005). A previous study demonstrated that Klotho supplementation reduces blood pressure (BP) (Takenaka et al., 2019), while another study indicated that Klotho normalizes BP by depressing renin-angiotensin system protein (renin, angiotensin-converting enzyme and angiotensin II type1 receptor) in the kidneys (Zhou et al., 2015). Hypertension often occurs in patients with chronic kidney disease (CKD) (Campese, 1997), among whom serum Klotho levels are significantly decreased (Koh et al., 2001; Nitta et al., 2014). Recently, Klotho was shown to be present in the brain (Degaspari et al., 2015), the role of which is thought to be neuroprotection (Vo et al., 2018). A previous study indicated that silencing brain Klotho through intracerebroventricular injections of adeno-associated virus with KlothoshRNA resulted in earlier BP elevation in rats (Wang and Sun, 2010), while Klotho gene delivery improves hypertension (Wang and Sun, 2009). These studies demonstrated that reduced Klotho in the brain increases brain endothelin1 expression and consequently activates sympathetic nervous system activity (Wang and Sun, 2010). From the aforementioned results, we hypothesized that Klotho would directly affect the central sympathetic nervous system and regulate BP.

The rostral ventrolateral medulla (RVLM) is a key brain region involved in regulating sympathetic nerve activity and BP (Madden and Sved, 2003; Guyenet et al., 2018). RVLM neurons send axons directly to the intermediolateral cell column (IML) neurons connecting with the sympathetic preganglionic neurons (SPNs), which control the
peripheral sympathetic function and BP (Deuchars and Lall, 2015; Deuchars et al., 1995; Oshima et al., 2008; Pilowsky and Goodchild, 2002). Therefore, this study examined the effects of Klotho on RVLM neurons. Given previous reports showing that Klotho increases Na⁺/K⁺-ATPase activity (Sopjani et al., 2011; Tang et al., 2018), we examined whether Na⁺/K⁺-ATPase is related to the Klotho-induced changes in RVLM neuron membrane potentials (MPs).

Klotho is also known to be a component of the FGF23 receptor (FGFR1) (Erben, 2017). FGF23 (251 amino acids; molecular weight, 32 kDa), mainly produced by osteocytes and osteoblasts, is related to the elimination of serum phosphate in the kidneys (Bech et al., 2015) and is reported to cause myocardial hypertrophy, atherosclerosis, and hypertension (Akhabue et al., 2018). Moreover, a previous study shows that the high serum FGF23 levels cause hypertension in patients with CKD (Li et al., 2018). Recently, FGF23 was found to be present in the cerebrospinal fluid (CSF) (Hensel et al., 2016) with studies suggesting increased CSF levels of FGF23 among patients with CKD (Haffner and Leifheit-Nestler, 2017; Marebwa et al., 2018). Based on the aforementioned results, we hypothesized that the high levels of FGF23 among patients with CKD would directly stimulate RVLM neurons. Therefore, the effects of FGF23 on RVLM neurons were also examined.

To the best of our knowledge, the effects of Klotho and FGF23 on bulbospinal RVLM neurons have not been previously investigated. Accordingly, the present study examined the direct effects of Klotho and FGF23 on bulbospinal RVLM neurons using brainstem–spinal cord preparations (Oshima et al., 2015) and employed histological analyses to examine the presence of Klotho, Na⁺/K⁺-ATPase, FGF23, and FGF23R1 in RVLM neurons.

2. Experimental procedures

2.1. General preparations

Experiments were performed using brainstem–spinal cord preparations (Fig. 1A) collected from 0- to 5-day-old Wistar rats as previously described (Oshima et al., 2015, 2018). The experiment protocols were approved by the Institutional Review Board of the National Defense Medical College and were in accordance with the National Guidelines for the Conduct of Animal Experiments. The preparations were continuously superfused with a solution containing (in mmol/L) 124 NaCl, 5.0 KCl, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃, and 30 glucose maintained at 25 °C–26 °C (artificial cerebrospinal fluid [aCSF]). The pH (7.4) and oxygenation were maintained by bubbling 95% O₂–5% CO₂ through the solution.

2.2. Patch-clamp electrodes

Electrodes were pulled in one stage from thin-wall borosilicate filament capillaries (GC100TF-10, outer diameter 1.0 mm; Clark Electromed, Reading, UK) using a vertical puller. The electrodes had a tip diameter of 1.8–2.0 μm and a resistance of 4–6 MΩ. The electrode solution for whole-cell recordings consisted of (in mmol/L) 130 potassium gluconate, 10 HEPES, 10 EGTA, 1 CaCl₂, and 1 MgCl₂, with the pH adjusted to 7.2–7.3 using KOH. The electrode tips were filled with 0.2% Lucifer Yellow (Sigma, St. Louis, MO, USA).

2.3. Recording procedure

We used a patch-clamp amplifier (AxoPatch, ID; Axon Instruments, Sunnyvale, CA, USA) to record the MPs. RVLM neurons were obtained from the ventral side of the medulla. To confirm whether the recorded RVLM neuron was a bulbospinal neuron, the existence of antidromic action potentials (APs) was examined by electrical stimulation (5–30 V, 0.1 ms, single pulse) of the IML at the Th₂ level using a tungsten electrode (30-μm tip diameter; Unique Medical, Tokyo, Japan) (Oshima et al., 2015) (Fig. 1B). All data were recorded and analyzed using PowerLab (AD Instruments, Colorado Springs, CO, USA). During the whole-cell recordings, neurons were labeled with 0.2% Lucifer Yellow (lithium salt; Sigma) through either spontaneous diffusion or iontophoresis.

Fig. 1. Brainstem-spinal cord preparation and antidromic action potentials (APs).
A. Brainstem-spinal cord preparation of a neonatal Wistar rat. IX, X, XI, and XII are the numbers of cranial nerves. Electrical stimulation was applied to the IML at the Th₂ level via an electrode. B. Antidromic APs in an RVLM neuron that was evoked by electrical stimulation (†) to the IML at the Th₂ level. Four sweeps induced by different stimuli are superimposed. Membrane trajectories APs evoked by 10-, 20- and 30-V are overlapping with constant latency, whereas AP was not evoked by 5-V stimulus (horizontal line).

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2.4. Experimental protocols

2.4.1. Protocol (1)
During MP recording of the bulbospinal RVLM neurons, the preparations were superfused with Klotho (50–200 pmol/L; Sigma) or ouabain (a Na\(^+\)/K\(^+\)-ATPase inhibitor, 3 μmol/L; Sigma) dissolved in aCSF. Ouabain was used to examine whether Na\(^+\)/K\(^+\)-ATPase was related to the Klotho-induced changes in RVLM neuron MPs. Each drug superfusion lasted 3–10 min. Changes in the MPs were determined 3–5 min after the start of each drug superfusion. Bulbospinal RVLM neurons were superfused with a tetrodotoxin (TTX) solution (0.5 mmol/L; FUJIFILM Wako, Tokyo, Japan) for 10 min to block synaptic transmissions from other neurons to the recorded bulbospinal RVLM neurons. Thereafter, the neurons were superfused with Klotho (200 pmol/L) dissolved in a TTX solution followed by MP recording.

2.4.2. Protocol (2)
During MP recording of the bulbospinal RVLM neurons, the preparations were superfused with FGF23 (20–80 pmol/L; FUJIFILM Wako) or SU5402 (a FGFR1 antagonist, 4 μmol/L; Sigma) dissolved in aCSF. After 10 min of superfusion with TTX, the recorded neurons were superfused with FGF23 (80 pmol/L) dissolved in a TTX solution followed by MP recording.

2.4.3. Protocol (3)
To determine whether the changes in RVLM neuron activities caused by klotho or FGF23 superfusion were transmitted to the IML neurons, IML neuron MPs were recorded and RVLM areas were micro superfused (10–30 μL) with Klotho (200 pmol/L) or FGF23 (80 pmol/L) (Oshima et al., 2015, 2018). In order to successfully micro superfuse over RVLM areas, the tip of micro superfusion pipette was placed within 1 mm above the ventral surface of RVLM areas.

2.5. Immunofluorescence staining
To histologically determine the presence of Klotho, Na\(^+\)/K\(^+\)-ATPase, FGF23, and FGFR1, immunofluorescence staining was performed. After the aforementioned experiments, the preparations were fixed with 4% paraformaldehyde in 0.1 M PBS for 1 h at 4 °C, immersed in 18% sucrose–PBS overnight, embedded in optimal cutting temperature compound (Sakura Finetek, Japan), frozen on dry ice, and cut into 20-μm-thick transverse sections followed by immunofluorescence staining. Images were obtained using a conventional fluorescence microscope (LSM510; Carl Zeiss Co., Oberkochen, Germany).

2.6. Protocols for immunofluorescence staining
The Lucifer Yellow (Sigma, St. Louis, MO, USA)-stained RVLM neurons that responded to Klotho were stained for Klotho and tyrosine hydroxylase (TH). The presence of TH-positive RVLM neurons was determined to confirm whether the examined area was a C1 area. Thereafter, immunofluorescence staining for Klotho and Na\(^+\)/K\(^+\)-ATPase was performed to determine whether both were present in the same RVLM neurons. Staining was performed before Klotho superfusion.

The Lucifer Yellow (Sigma, St. Louis, MO, USA)-stained RVLM neurons that responded to FGF23 were stained for FGFR1 and Klotho. The presence of Klotho in the FGFR1-positive RVLM neurons was determined to confirm whether Klotho was a component of FGFR1. To determine whether FGF23 and FGFR1 were present in the same RVLM neurons, immunofluorescence staining for FGF and FGFR1 was performed. Staining was performed before FGF23 superfusion.

The following primary antibodies (1:400 dilution) were used for immunofluorescence: mouse anti-TH antibody (Sigma, St. Louis, MO, USA), rabbit anti-Klotho antibody (Sigma, St. Louis, MO, USA), mouse anti-Na\(^+\)/K\(^+\)-ATPase antibody (Funakoshi, Tokyo, Japan), rabbit anti-FGF23 antibody (Funakoshi, Tokyo, Japan), and mouse anti-FGFR1 antibody (Sigma, St. Louis, MO, USA). Secondary antibodies for fluorescence staining (1:1000 dilution) included Alexa Fluor 488 donkey anti-mouse IgG (Molecular Probes/Invitrogen, Eugene, OR, USA), Alexa Fluor 546 donkey anti-rabbit IgG (Molecular Probes/Invitrogen, Eugene, OR, USA), and Alexa Fluor 633 goat anti-mouse IgG (Molecular Probes/Invitrogen, Eugene, OR, USA).

2.7. Statistics
Data are expressed as means ± standard deviation (SD). A paired t-test for paired observations was used to compare MPs recorded before and during (or after) drug superfusion. Statistical significance was set at P < 0.05. All data were analyzed using JMP Pro 12 statistical software (SAS Inc., Cary, NC, USA).

3. Results
A total of 98 bulbospinal RVLM neurons were examined. The average ± SD of MP and frequency of action potential (FAP) of such
neurons under aCSF were $-44.1 \pm 3.8$ mV (Range, from $-36.4$ mV to $-54.7$ mV) and $0.5 \pm 0.6$ Hz (from $0.0$ Hz to $3.8$ Hz) respectively.

3.1. Hyperpolarizing effects of Klotho on bulbospinal RVLM neurons

To examine the effects of Klotho on bulbospinal RVLM neurons, the same neurons were superfused with Klotho. Accordingly, Klotho-hyperpolarized and decreased FAP in the RVLM neurons at each of the examined concentrations [for 50 pmol/L, $n = 12$: before, $-44.5 \pm 3.4$ mV (from $-39.1$ mV to $-48.6$ mV); during, $-46.6 \pm 3.7$ mV (from $-39.1$ mV to $-52.0$ mV); $P < 0.01$; FAP: before, $1.0 \pm 1.1$ Hz (from $0.0$ Hz to $3.8$ Hz); during, $0.5 \pm 0.6$ Hz (from $0.0$ Hz to $1.6$ Hz); $P < 0.05$], [for 100 pmol/L, $n = 12$: before, $-44.6 \pm 4.1$ mV (from $-40.1$ mV to $-52.2$ mV); during, $-47.7 \pm 5.3$ mV (from $-40.9$ mV to $-59.4$ mV); $P < 0.01$; FAP: before, $0.5 \pm 0.3$ Hz (from $0.1$ Hz to $1.1$ Hz); during, $0.1 \pm 0.1$ Hz (from $0.0$ Hz to $0.3$ Hz); $P < 0.01$], [for 200 pmol/L, $n = 8$: the results are shown in Fig. 2A–C]. Furthermore, to determine whether Klotho by
itself inactivated the RVLM neurons, the same neurons were superfused with Klotho (200 pmol/L) dissolved in a TTX solution. Accordingly, Klotho (200 pmol/L) was added to the same neurons. Accordingly, the addition of Klotho did not significantly depolarize such neurons (n = 5) (Fig. 3G–I). Values are presented as means ± SD. **P < 0.01 vs. before superfusion with FGF23 dissolved in a TTX solution.

3.2. Depolarizing Effects of ouabain on RVLM neurons

To determine whether the effect of Klotho on the RVLM neurons was caused by Na⁺/K⁺-ATPase, bulbospinal RVLM neurons were superfused with ouabain. Accordingly, ouabain (3 μmol/L) depolarized the bulbospinal RVLM neurons (n = 6) (Fig. 2A–C). Furthermore, to determine whether Klotho inactivated the ouabain-depolarized RVLM neurons, Klotho (200 pmol/L) was added to the same neurons. Accordingly, the addition of Klotho did not significantly hyperpolarize such neurons (n = 5) (Fig. 3D–F). To determine whether ouabain activated the Klotho-hyperpolarized RVLM neurons, ouabain (3 μmol/L) was added to the same neurons. Accordingly, the addition of ouabain significantly depolarized such neurons (n = 5) (Fig. 3G–I). These results suggest that the hyperpolarizing effect of Klotho on RVLM neurons were caused by the activation of Na⁺/K⁺-ATPase. Thus, Na⁺/K⁺-ATPase may be present downstream of the Klotho signaling pathway in RVLM neurons.

3.3. Depolarizing effects of FGF23 on RVLM neurons

To determine whether the effect of Klotho on the RVLM neurons was caused by Na⁺/K⁺-ATPase, bulbospinal RVLM neurons were superfused with ouabain. Accordingly, Klotho (200 pmol/L) dissolved in a TTX solution depolarized the RVLM neurons (n = 6). Values are presented as means ± SD. **P < 0.05 vs. before superfusion with FGF23 dissolved in a TTX solution.

To determine the effects of FGF23, RVLM neurons were superfused with FGF23. Accordingly, FGF23 depolarized and increased the FAP in the RVLM neurons at each of the examined concentrations (for 20 pmol/L, n = 8: before, −45.1 ± 4.5 mV (from −41.0 mV to −54.7 mV); during, −43.1 ± 4.6 mV (from −37.7 mV to −51.3 mV); P < 0.05; FAP: before, 0.1 ± 0.2 Hz (from 0.1 Hz to 0.5 Hz); during, 0.4 ± 0.4 Hz (from 0.1 Hz to −1.1 Hz)], [for 40 pmol/L, n = 7: the results are shown in Fig. 4A–C], [for 80 pmol/L, n = 7: before, −43.0 ± 3.0 mV (from −40.1 mV to −49.1 mV); during, −39.9 ± 3.8 mV (from −35.8 mV to −47.0 mV); P < 0.01; FAP: before, 0.2 ± 0.4 Hz (from 0.1 Hz to 1.0 Hz); during, 0.5 ± 0.8 Hz (from 0.1 Hz to 2.0 Hz)]. Furthermore, to determine whether FGF23 by itself activated the RVLM neurons, the same neurons were superfused with FGF23 (80 pmol/L) dissolved in a TTX solution. Accordingly, the recorded bulbospinal RVLM neurons showed depolarization during and after superfusion with FGF23 dissolved in a TTX solution (n = 6) (Fig. 4D, E). These results may show that FGF23 by itself depolarized the bulbospinal RVLM neurons.

To determine whether the effect of FGF23 on the RVLM neurons was mediated by FGFR1, bulbospinal RVLM neurons were superfused with SU5402. Accordingly, SU5402 (4 μmol/L) hyperpolarized the bulbospinal RVLM neurons (n = 8) (Fig. 5A–C), suggesting the presence of FGF23 in the RVLM. Furthermore, to determine whether SU5402 suppressed the effect of FGF23 on RVLM neurons, SU5402 (4 μmol/L) was added to FGF23-depolarized bulbospinal RVLM neurons. Accordingly, the addition of SU5402 hyperpolarized the FGF23-depolarized RVLM neurons.
neurons (n = 6) (Fig. 5D–F).

### 3.4. Micro-superfusion over the RVLM area, hyperpolarizing effects of Klotho and depolarizing effects of FGF23

A total of 13 IML neurons were examined. The MP and FAP in these neurons under aCSF were $-43.5 \pm 5.2$ mV (range $-35.3$ mV to $-53.6$ mV) and $0.5 \pm 0.6$ Hz (range $0.0$ Hz to $1.2$ Hz) respectively. To determine whether the klotho-induced changes in RVLM neuron activities were transmitted to the IML neurons, the RVLM areas were micro-superfused with a Klotho solution (200 pmol/L), and changes in the MP and FAP of the IML neurons were observed. Accordingly, micro-superfusion over the RVLM area with a klotho solution caused hyperpolarization of the IML neurons at the Th2 level (n = 7) (Fig. 6A–C). The latency, which is the period from the end of micro-superfusion over the RVLM areas to the beginning of the changes in IML neuron MP, was...
Na+/K+-ATPase-immunoreactive neurons which was ascertained in six rats. In the RVLM area was subsequently determined by histologically analyzing immunoreactivity for Na+/K+-ATPase in six rats. In the RVLM area shown in Fig. 7A, we counted the number of Klotho and FGF23 immunoreactive; however, complete overlap was not observed (Fig. S1D). Accordingly, most of the Klotho-immunoreactive neurons in the RVLM area also exhibited Na+/K+-ATPase immunoreactivity (Fig. 7F-H).

3.5. Immunoreactivity, expression of Klotho and FGF23 on RVLM neurons

To histologically determine the presence of Klotho, Na+/K+-ATPase, FGF23, and FGFR1, immunofluorescence staining was performed at the end of the recordings, with our results subsequently confirming their presence in RVLM neurons. The presence of Klotho and FGF23 was also examined in separate experiments (Figs. 7F, J, N, S1A, D). In the low magnification image, we show the RVLM area (Fig. 7A). Lucifer Yellow staining was performed after completing whole-cell recordings of the bulbospinal RVLM neurons. Moreover, four of the five Lucifer Yellow-stained neurons, which showed hyperpolarization during Klotho superfusion, exhibited immunoreactivity for TH (Fig. 7B–E), suggesting that C1-catecholaminergic RVLM neurons have sensitivity for Klotho.

After confirming the presence of Klotho, the presence of Na+/K+-ATPase in the RVLM area was subsequently determined by histologically analyzing immunoreactivity for Na+/K+-ATPase in six rats. In the RVLM area shown in Fig. 7A, we counted the number of Klotho and Na+/K+-ATPase-immunoreactive neurons which was ascertained in six 20-μm-thick transverse sections (Fig. 7I). In the RVLM, most of the Klotho-immunoreactive neurons also exhibited Na+/K+-ATPase-immunoreactivity; however, complete overlap was not observed (Fig. S1A–C). Accordingly, most of the Klotho-immunoreactive neurons in the RVLM area also exhibited Na+/K+-ATPase immunoreactivity (Fig. 7F-H).

Five RVLM neurons showing depolarization during FGF23 superfusion were examined for FGFR1 and Klotho immunoreactivity. Accordingly, our results showed that all five neurons were located in the RVLM and exhibited FGFR1 and Klotho immunoreactivity (Fig. 7J–M), suggesting Klotho to be a component of FGFR1 in the RVLM neurons.

After confirming the presence of FGFR1, the presence of FGF23 in the RVLM area was determined by histologically analyzing immunoreactivity for FGF23 in five rats. We counted the number of FGF23 and FGFR1-immunoreactive RVLM cells in the RVLM area in five transverse sections (Fig. 7Q). In the RVLM, most of the FGF23-immunoreactive neurons also exhibited FGFR1-immunoreactivity; however, complete overlap was not observed (Fig. S1D–F). Accordingly, most of the FGFR1-immunoreactive neurons in the RVLM area also exhibited FGF23 immunoreactivity (Fig. 7N–P).

4. Discussion

The RVLM contains neurons that play a key role in controlling sympathetic vasomotor tone and BP. Increased activity of the RVLM presympathetic neurons is transmitted to the IML in the spinal cord, where peripheral sympathetic nerves activated, thus causing BP elevation (Pilowsky and Goodchild, 2002) and decreased activity of these neurons leads to BP reduction (Zanzinger et al., 1995). In this study, we examined how Klotho or FGF23 changes the activities of RVLM neurons.

The results presented herein confirmed the presence of Klotho in RVLM neurons. Indeed, studies have determined the presence of the Klotho gene in the brain (Koh et al., 2001), indicating that Klotho is produced therein. Moreover, the presence of Klotho is observed in the choroid plexus, cerebellum, and hippocampus (Vo et al., 2018).
However, to our knowledge, no reports have shown the presence of klotho in the RVLM. Given our results showing that Klotho hyperpolarizes bulbospinal RVLM neurons, we believe that Klotho in the RVLM could act as an anti-hypertensive protein by mediating a reduction in sympathetic nerve activity. A previous study indicated that the level of Klotho in the brain decreases with age (Duce et al., 2008). Thus, the decreased effect of Klotho on the RVLM neurons may be associated with hypertension among elderly individuals.

Patients with CKD exhibit significantly low levels of serum Klotho (Koh et al., 2001; Nitta et al., 2014), which is associated with hypertension in humans (Zhou et al., 2015) and mice (Takenaka et al., 2019). Although studies have yet to confirm whether patients with CKD...
have decreased Klotho production in the brain, a previous study did show an association between CSF and serum levels of Klotho (Vo et al., 2018). Considering the results of these studies, it might therefore be the case that Klotho levels in the CSF of CKD patients are reduced. The mechanisms by which Klotho suppresses RVLM neuron activities remain unknown. However, a previous study indicated that Klotho increases Na+/K+-ATPase activity using Xenopus oocytes (Sopjani et al., 2011). In this study, the hyperpolarizing effect of Klotho was suppressed during ouabain superfusion, while the depolarizing effect of ouabain significantly increased during Klotho superfusion. These results suggest that the hyperpolarizing effect of Klotho on RVLM neurons is mediated by Na+/K+-ATPase activation. Therefore, Na+/K+-ATPase may exist downstream of the Klotho signaling pathway in these neurons.

Our results showed that FGF23 was present in RVLM neurons and that FGFR1 was present in most of the RVLM neurons that possessed FGF23. Given that SU5402 (a FGFR1 antagonist) hyperpolarized RVLM neurons, FGF23 in the RVLM is thought to activate RVLM neurons. To our knowledge, few reports have determined the presence of FGF23 in neurons, however, several studies have suggested increased CSF levels of FGF23 among patients with CKD (Marebwa et al., 2018; Haffner and Leifheit-Nestler, 2017). Accordingly, FGF23 levels are increased in the frontal lobe of CKD patients (Marebwa et al., 2018), where FGF23 is shown to act directly on hippocampal neurons of CKD patients (Haffner and Leifheit-Nestler, 2017). A previous study showed that higher serum levels of FGF23 were independently associated with increased BP (Akhabue et al., 2018). Based on the aforementioned studies, we believe that the increased levels of FGF23 in the CSF of patients with CKD could stimulate RVLM and thus sympathetic nerve activity leading to hypertension in these patients.

Previous reports have shown that patients with CKD exhibit hypertension through increased sympathetic nerve activity (Campese, 1997; Cottone et al., 1995; Koomans et al., 2004). One of our previous studies supporting this mechanism suggested that accumulated uremic toxins, such as uric acid, indoxyl sulfate, and methylglyoxaline, in such patients could cause hypertension by acting on RVLM neurons (Oshima et al., 2015). The present study found that RVLM neuron activity was suppressed by Klotho and stimulated by FGF23. Another study found that patients with CKD had decreased Klotho and increased FGF23 (Nitta et al., 2014). Thus, the results presented herein may also support the aforementioned mechanism wherein increased sympathetic nerve activity causes hypertension in patients with CKD.

In conclusion, our results showed that Klotho was present in bulbospinal RVLM neurons and suppressed the activities of the same neurons via Na+/K+-ATPase, suggesting that klotho in the RVLM may act as an antihypertensive protein. In contrast, FGF23 was also present in the RVLM neurons and increased the activities of these neurons via FGFR1, suggesting that FGF23 in the RVLM may act as a pressor protein.

4.1. Limitations of the present study

The preparation used for these experiments is suitable for newborn rats. However, the activities of RVLM neurons and expression of receptors for the proteins may change with age. Therefore, these results may not reflect the physiology of the adult rats. Further investigation is needed to determine the effects of Klotho and FGF23 in the RVLM the adult rat.

In this study, we used a TTX solution to block synaptic transmission. However, TTX blocks the transmission dependent on APs but does not block spontaneous neurotransmitter release which is independent of presynaptic APs (Kavalali, 2015). In addition to TTX, bicuculline, strychnine and kynurenic acid should be used to block all neurotransmitters from other neurons. Therefore, although the recorded RVLM neurons showed hyperpolarization during superfusion with Klotho dissolved in a TTX solution and depolarization during superfusion with FGF23 dissolved in a TTX solution, these results may not sufficiently demonstrate that the reactions are direct effects of these proteins on the recorded neurons. Further studies are needed.

4.2. Perspectives and significance

The effects of Klotho and FGF23 in the central nervous system are not well known. The present study confirmed the presence of Klotho and FGF23 in RVLM neurons and that Klotho in the RVLM may act as an antihypertensive protein, whereas FGF23 in the RVLM may act as a pressor protein. However, the signal pathways in RVLM neurons stimulated by these proteins are not well known. Furthermore, change of amount of these proteins in RVLM neurons in CKD patients is not proved. Further studies are needed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autneu.2020.102640.

Declaration of competing interest

None.

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