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Original Article

Melanocortinergic circuits from medial vestibular nuclei to the kidney defined by transneuronal transport of pseudorabies virus

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Abstract: This study was designed to assess whether MC4R signaling existed in vestibular nuclei modulated the activity of kidney by a virally mediated transsynaptic tracing study. Pseudorabies virus (PRV)-614 was injected into the kidney in adult male MC4R-green fluorescent protein (GFP) transgenic mice (n = 5). After a survival time of 5 days, the mice were assigned to humanely sacrifice, and the brainstem were removed and sectioned, and processed for PRV-614 visualization. The neurochemical phenotype of MC4R-GFP-positive neurons was identified using fluorescence immunocytochemical labeling. PRV-614/MC4R-GFP dual labeled neurons were detected in medial vestibular nuclei. Our findings support the hypothesis that there exist melanocortinergic circuits from medial vestibular nuclei to the kidney.

Keywords: Vestibular nuclei, kidney, Melanocortin-4 receptor, pseudorabies virus, transsynaptic tracing

Introduction

Several studies showed that vestibular stimulation elicited distinct changes in blood flow to the forelimb and hindlimb [1]. Previous studies have suggested that the medial vestibular nuclei (MVe) play an important role in the sympathetic control of arterial blood pressure [2-4]. Hao et al revealed direct neuroanatomical evidence to identify catecholaminergic projections from the MVe to the kidney, suggesting that medial vestibulo-renal pathway may be catecholaminergic [5]. However, it is unclear whether there exist melanocortinergic circuits from medial vestibular nuclei to the kidney.

A considerable amount of literature has demonstrated that the melanocortin receptor-4 (MC4R) plays an important role in modulating energy metabolism and blood pressure [6-12]. Recent evidence indicated that the activation of MC4R in brain nuclei played an important role in regulating renal function [13-16]. In present study, we designed to assess whether MC4R signaling existed in the MVe modulated the activity of kidney by a virally mediated transsynaptic tracing study [5, 17-22]. We seek to map the polysynaptic pathways between kidney and MC4R-expressing regions in the MVe, using pseudorabies virus (PRV)-614 in MC4R-green fluorescent protein (GFP) transgenic mice.

Materials and methods

Animal care and use

Male transgenic MC4R-GFP mice weighing between 25 g and 30 g (n = 5), which were first obtained from Dr. Joel Elmquist (UT Southwestern Medical Center, USA), were used for these experiments. Mice were housed under controlled conditions (12 h alternating light-dark cycle, food and water *ad libitum*). Experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of Union Hospital, Tongji

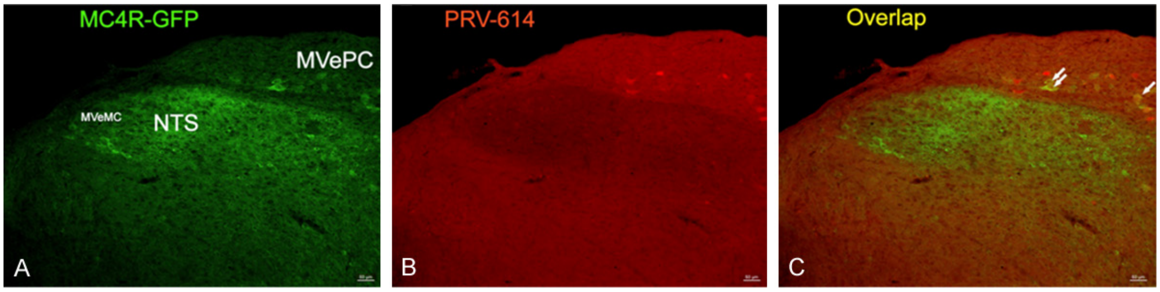

Figure 1. Fluorphor expression in caudal brainstem level at 5 d post-injection of the left kidney. A. MC4R-GFP-immunofluorescent cells are present in the MVePC and MVeMC regions. B. PRV-614 infected neurons were mainly labeled in the MVePC region; C. PRV-614/MC4R-GFP double-labeled neurons located predominantly in MVePC and not in MVeMC. Labeled neurons in MVePC were mapped to transverse sections from the mouse brain atlas of Franklin KB and Paxinos G [31]. NTS, solitary nucleus; MVeMC, the magnocellular medial vestibular nuclei; MVePC, the parvicellular medial vestibular nuclei. Scale bar: 50 μm.

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Microinjection of virus into the kidney

PRV-614 was microinjected into the kidney on male transgenic MC4R-GFP mice using a previously described approach [23, 24]. After mice were anesthetized with isoflurane (1.5%-2%), the skin overlying the kidney was incised by an abdomen midline laparotomy to expose the upper pole of the kidney. 10 mice received a series of injections with PRV-614 into the upper pole of the visualized left kidney (2×10^8 pfu/ml in a total of 1 μl per injection at five injection sites per kidney) using a 10-gauge needle connected to a Hamilton syringe (10 μl) under microscopic guidance. After the final injection, the kidney surface was rinsed twice with sterile saline-soaked swabs, blotted dry, and then returned to the abdominal cavity. The abdominal muscle incision was closed with silk sutures, and the skin incision was closed with stainless steel wound clips.

Fluorescence immunohistochemistry and tissue analysis

At 5 d after PRV-614 injection into the upper pole of the left kidney, the mice were sacrificed under deep anesthesia with ketamine hydrochloride and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde-borate fixative (pH 9.5). Brainstems were removed and postfixed for 2 h in 4% paraformaldehyde-borate and overnight in a 30% sucrose solution at 4°C. Postfxed brainstems were sliced into 30 μm coronal sections on a freezing-stage sledge microtome. Immunofluorescence studies were carried out to determine expression of MC4R-GFP in vestibular nuclei slices. According to published protocols [25, 26]. The sections were pre-incubated for 1 h in 2% normal donkey serum followed by incubation for 24 h with a chicken polyclonal anti-GFP (1:1000) primary antibodies in 0.01 M PBS containing 0.5% Triton-X 100 at 4°C. Slices were then washed with PBS 3 times for 10 minutes and incubated for 2 h in Alexafluor 488-conjugated anti-chicken IgG (1:1000); then they were washed several times at room temperature. Sections were washed, mounted onto slides and cover slipped with mounting media. To identify immunohistochemical co-localization of MC4R-GFP and kidney-related neurons, an Olympus IX81 photomicroscope was used. Double labeled neurons were merged by using Adobe Photoshop.

Results and discussion

We used a fluorescence immunohistochemistry to characterize the chemical neuroanatomical substrate of MVe innervating the kidney in the mouse. The medial vestibular nuclei divide into magnocellular (MVeMC) and parvicellular subfields (MVePC) [27]. At five days after PRV-614 injection in the kidney, PRV-614 infected neurons were mainly labeled in MVePC; PRV-614/MC4R-GFP double-labeled neurons located predominantly in MVePC and not in MVeMC (**Figure 1**).

The study of Hao et al reported that PRV-614 infected neurons were retrogradely labeled in MVeMC and MVePC; PRV-614/tyrosine hydroxylase (TH) double-labeled neurons located pre-

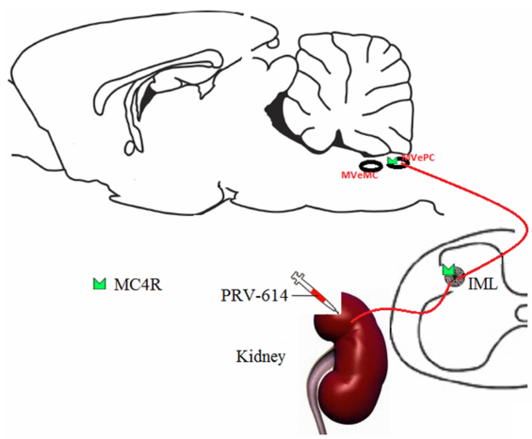

Figure 2. Summary diagram showed that the melanocortinergic pathway from the MVe to the kidney. It is speculated that there exist melanocortinergic circuits from medial vestibular nuclei to the kidney by intermediolateral column (IML) of spinal cord. IML, intermediolateral column; MC4R, the melanocortin-4 receptor; MVeMC, the magnocellular medial vestibular nuclei; MVePC, the parvicellular medial vestibular nuclei; PRV-614, pseudorabies virus-614. Some drawings were taken from Y Hao (Epilepsy Behav 2014).

dominantly in MVeMC and not in MVePC, whereas PRV-614/tryptophan hydroxylase (TPH) neurons were not localized in MVeMC and MVePC [5]. Previous experiments have demonstrated that the vestibular system contributes to regulating sympathetic nervous system activity [28]. Sugiyama et al explored that role of the rostral ventrolateral medulla in the patterning of vestibular system influences on sympathetic nervous system outflow to the upper and lower body, and found that there existed the anatomical patterning of vestibulo-sympathetic reflexes [29]. Concurrently, Kerman et al also showed anatomic patterning in the expression of vestibulosympathetic reflexes [30]. Based these data, we inferred that there existed melanocortinergic circuits from medial vestibular nuclei to the kidney by the sympathetic signals (**Figure 2**).

Taken together, the data derived from these studies extends our understanding of the role of vestibular MC4R in two ways. First, it provides neuroanatomical confirmation of the melanocortinergic circuits from medial vestibular nuclei to the kidney. Second, it provides new insights into whether vestibular sympathoactivation modulates renal function. How the melanocortinergic-sympathetic signals control renal function remains to be determined.

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Disclosure of conflict of interest

None.

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