Diabetic Complications Consortium

Application Title: Brainstem Mechanism of the Vagal Loss in Type 2 Diabetes

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1. Project Accomplishments:

Due to administrative issues the project was started in March 2016, and we have requested and been approved for a no-cost extension. From March to September 2016 we have built animal models and collected tissues for further analysis of protein and gene expression. Some new findings have been used as preliminary data for a NIH RO1 application entitled "Vagal dysregulation of hepatic intermediary metabolism in T2D: role of brainstem TRH", which was submitted in Oct. 2016.

2. Specific Aims:

Specific Aim 1. To identify the morphological and cellular metabolic abnormalities of brainstem DVC in HFD long term fed, obese Wistar rats and T2D GK rats.

Results:

(1) We produced Wistar rat model of high fat diet-fed for 5 months (HFD, containing 45 kcal% high fat diet and 17 kcal% sucrose, Research Diets, Inc, D12451). The control Wistar rats and genetically developed type 2 diabetic (T2D) Goto--Kakizaki (GK) rats were fed with normal diet (ND) at the same duration. The body weight, food intake, blood glucose levels, lean mass and fat mass were assessed weekly. The Table shows the differences between ND-fed and HFD-fed Wistar groups in the fifth week. At the end of the fifth months, tissues including the dorsal vagal complex, rostral ventrolateral medulla, and the raphe nuclei in the brainstem, the hypothalamus, the liver, the adrenal glands and the serum of ND-fed Wistar, HFD-fed Wister and ND-fed GK rats were collected and stored in -80ºC. Currently brainstem protein and total RNA extraction are performed for Western blot and real-time PCR analyses.

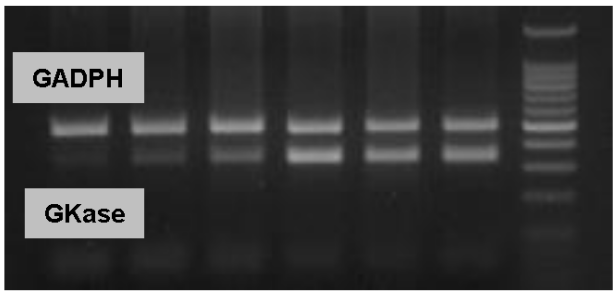
Diet	n	Body weight (g)	Daily food intake (g)	Glucose (mg/dL)	Lean mass (g)	Fat mass (g)
ND	11	354.8±9.7	25.0±1.3	99.8±1.5	302.1±7.4	30.5±2.1
HFD	10	397.6±7.9*	16.7±0.1*	123.4±4.3*	317.7±4.9	56.8±5.4*

*P<0.05 vs ND

(2) The liver RNAs was extracted and gene expression of specific enzymes involved in metabolism shows significant differences between ND-fed Wistar rats and GK rats.

Fold changes of liver enzyme gene expression by RT-PCR in GK rats compared to Wistar rats

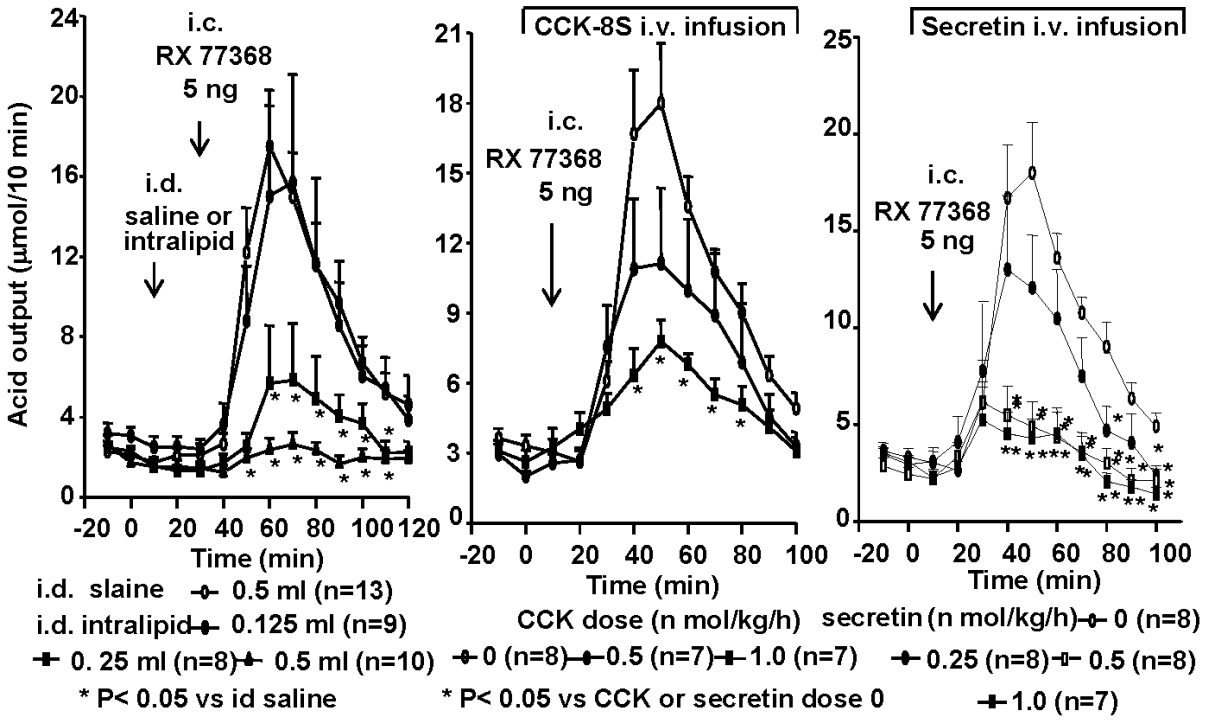
Enzyme	Glucokinase (GKase)	Glucose-6-phosphatase (G6P)	Phosphoenolpyruvate Carboxykinase (PEPCK)
Function involved	Glycolysis	Glycogenolysis	Gluconeogenesis
Fold change GK/Wistar	+ 1.9	+ 1.2	+ 7.1



Increased hepatic glucokinase gene expression in GK rats vs Wistar rats by PCR.

Specific Aim 2. To demonstrate an impaired TRH function in DVC neurons of HFD-fed Wistar and T2D GK rats.

Results: Intracisternally injected (ic) TRH analog acts in the dorsal vagal complex (DVC) to active vagal efferent function and thereby stimulates gastric acid secretion. We performed animal experiment to test the effects of intraduodenal infusion of lipid, or intravenous infusion of cholecystokinin (CCK) or secretin on the gastric acid stimulatory action of ic TRH analog. The results show that increase blood lipids, CCK or secretin dose-dependently abolished ic TRH analog-induced gastric acid secretion, indicating that circulating lipids and gut hormones released by meal inhibits TRH action in the DVC. Further study will be performed in HFD-fed animals.



Dose-dependently inhibited ic TRH analog RX 77368-inducd gastric acid secretion by intraduodenal infusion of intralipid or intravenous infusion of CCK-8 or secretin in rats.

3. Publications:

1. Ko M, Marvizon JC, Chen W, Pisegna J, Germano P, Yang H: Potentiated hyperglycemic response to intrathecal injection of TRH analog, substance P and neuropeptide Y in type 2 diabetic Goto-Kakizaki rats. (in preparation)
2. Yang H, Chen A, Gernamo P, Ho S, Pisegna J: Altered gene expression in brainstem dorsal vagal complex in high fat died-fed rats and type 2 diabetic Goto-Kakizki rats. (in preparation)