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Glucose-independent insulin and glucagon secreted from ventral pancreas sharing a similar pattern in healthy adult rat

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Abstract

Elevated blood glucose concentration due to food intake will trigger insulin secretion from the dorsal pancreas has been extensively studied. This increased intracellular insulin level can stimulate glucagon release from intra-islets. However, the interaction between glucagon and insulin under a fasting state is unknown. To explore the relationship, we partially removed the ventral and dorsal pancreas on wild-type adult rats. The glucose tolerance test was conducted to measure influence of the surgery on the integrity function of the pancreas. The fasting insulin/glucagon level before and after surgery were measured by the ELISA kit. The statistical analyses indicated that the ventral removal of the pancreas had higher fasting glucose than that of dorsal removal. And only the ventral removal group showed significantly increased basal insulin and basal glucagon levels. Our findings showed differential role of the ventral pancreas in response to a glucose-free stimulus and also provided the possible *in vitro* target for developing the anti-hyperglycemic drugs.

Introduction

Insulin secretion from a single pancreatic β-cell is a bi-phase process in response to the glucose stimulus.[1] An early study on rat pancreas found only dorsal pancreas responses to glucose stimulus,[2] and dorsal and ventral parts of the pancreas have different responses to the pancreatic polypeptide or glucagon stimulus.[3] Clinical trials for glucose tolerance tests found that patients with loss of the second phase of insulin secretion are more likely to develop into type 2 diabetes (T2Ds).[4] The regulation of hypertrophy by adipose tissue metabolism seems to be a plausible way to explain the relationship between insulin secretion and insulin resistance in type 2 diabetics patients. However, over-weighted people with T2Ds restored their insulin sensitivity after taking the bypass surgery in a very short time.[5] These emergent metabolic surgery data are less in favor of supporting the leptin hypothesis. Alternately, insulin resistance formation could be viewed as an accumulated process that requires long-term interactions between pancreatic islets and peripheral tissues.[6] To quantitatively measure the insulin resistance in the diagnosis of T2Ds, the hyperglycemic clamp technique has been used intensively in clinical or animal studies.[7-10] Without food intake, the circulating insulin level is low in the blood and it is not produced by islets from the dorsal part of the pancreas.

The measurement of insulin/glucagon secretion from rats requires more attention. The amount of food remaining in the rat gastrointestinal (GI) tract is crucial to the measurement of basal insulin. Overnight fasting on rats could not guarantee a completely clean and empty GI tract, further introduced the bias to subsequent measurement of fasting insulin/glucagon secretion. Besides, rodents cannot vomit and overnight fasting might cause extra stress and an eating disorder that involves appetite perversion demonstrated by eating non-food substances in the rat cage.[11]

In this study, we examined the basal insulin and glucagon secretion pattern on wild-type adult rats in response to surgical intervention. We used the glucose tolerance test (GTT) to validate the normal function of the

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pancreas in response to glucose stimulus for rats after surgery. Our data suggested that the increased fasting insulin and glucagon level from the ventral removal group rendered a synchronized pattern that exists in food-free conditions.

Materials and Methods

Animal preparation and care. All animal experiments followed the guidelines established by the Kunming Medical University Institutional Animal Care and Use Committee (#SYXK K2015-0002, May, 24, 2017). The proper approvals were obtained before the study. Thirteen wild-type adult health male rats aged four weeks and weighing 102 - 153 g formed three groups in our study. Five, five, and three were the number of rats allocated in each group. All rats were fed on a standard diet with distilled water before and after the surgery. Only water was provided to rats within the three days after the surgery. All the rats were kept at a constant temperature of 24.00 ˚C and a light cycle of 12 hr on and 12 hr off. After pancreas surgery, once the rat regained full ambulation and alertness was returned to animal housing. The healthy status of each rat was monitored by checking the infection, pain and changes in weight daily. The infection could be observed if the discharge from incision sites, general lethargy, and/or pain were observed. The pain was indicated by hunched posture, ruffled back fur and absence of ambulation and/or eating behavior. A loss in weight were monitored immediately following surgery up to three days post-surgery.

Glucose tolerance test. All the rats were fasted overnight (16 hr) before conducting GTT. The injected glucose concentration was calculated as 2.00 g of glucose per kg of body weight. The total intraperitoneal (IP) injection volume was computed as follows:

$$Volume\ (\mu L) = 10.00 \times body\ weight\ (g)$$

The blood glucose levels were measured at 15, 30, 60, and 120 min after the IP injection. More details of conducting GTT and IP injection could be found in references.[12,13]

Pancreatectomy. To first identify the ventral and dorsal regions of pancreas, we removed the entire pancreas from one rat and used it as a reference. We followed the pancreas isolation protocol as described by others.[14] The pre-mixed ketamine (90.00 mg kg^{-1}; Sigma-Aldrich, St. Louis, USA) and xylazine (10.00 mg kg^{-1}; Sigma-Aldrich) at ratio three-over-one were selected to anesthetize the rat with the volume unit of 0.10 mL per 100 g.[15] To improve the post-surgery survival rate, the heating pad was used to keep the rat body temperature during the surgery. To suture the surgical wound, the non-absorbable braided silk 6/0 (Suturing Doctor, Beijing, China) was used. To prevent the post-surgery infection, ampicillin (Sigma-Aldrich) was injected into the rat abdomen before suturing the muscle.[16] The minimum weight of 0.02 and 0.08 g of the pancreas was removed from the ventral and dorsal parts, respectively. The overall surgery time was around 20 to 40 min per rat.

Blood withdrawal and protein extraction. To detect the insulin at a very lower level, an extra amount of blood sample was needed to be collected. We performed the retro-orbital bleeding twice, before and after surgery. The interval between the two collections was set as seven days.[17] A small amount of pre-mixed anesthetizing drugs were administered to rats via IP injection before blood collection. The total blood sample collected was less than 0.50 mL per withdrawal retro-orbitally.[18] On the other hand, tail withdrawal was used for glucose measurements to detect the blood sugar level in the GTT.

Fasting insulin and glucagon measurement. Insulin secretion from the impaired subject is low.[19] To measure this number from fasting surgery-free rat, we selected the rat ultrasensitive insulin ELISA kit (sensitivity up to 0.107 ng mL^{-1}; ALPCO, Salem, USA). We selected the Levenberg-Marquardt method to fit the five-point data and find the standard coefficient: A = 0.059, B = 1.75, C = 835, D = 1,7e + 05, and G = 0.994, respectively. In case the calibration curve failed to capture the data points at the low concentration, we used a numerical interpolation method to find coefficient A. The data analysis was coded in Matlab (MathWorks, Natick, USA). After the required 24 hr incubation time, the fasting glucagon ELISA test was the same as the fasting insulin ELISA test.

Statistical analysis. Numerical data are presented as the mean ± standard errors of the mean (SEM). All the analyses were conducted on user-defined Matlab code and the figures were generated by Python (version 3.7; Python Software Foundation, New York, USA). The *p*-value was set at 0.05 or less to be considered significant.

Results

The rat pancreas was a thin layer that covered the biliary, duodenal and gastrosplenic potion. To differentiate the ventral and dorsal pancreas, we first created the reference structure by injecting the trypan blue from the bifurcation portion of the common bile duct (CBD; Fig. 1A) to match the morphology description in reference.[20] The bifurcation of the ventral and dorsal pancreatic duct from CBD had variations[21] Unless other specified, we differentiated the ventral and dorsal pancreas as shown in Figure. 1B. The area ratio between the ventral and dorsal pancreas was one-third. To match this ratio, we removed on average 0.02 g pancreas from the medial side of CBD per rat and 0.08 g pancreas from the lateral side of the spleen per rat.

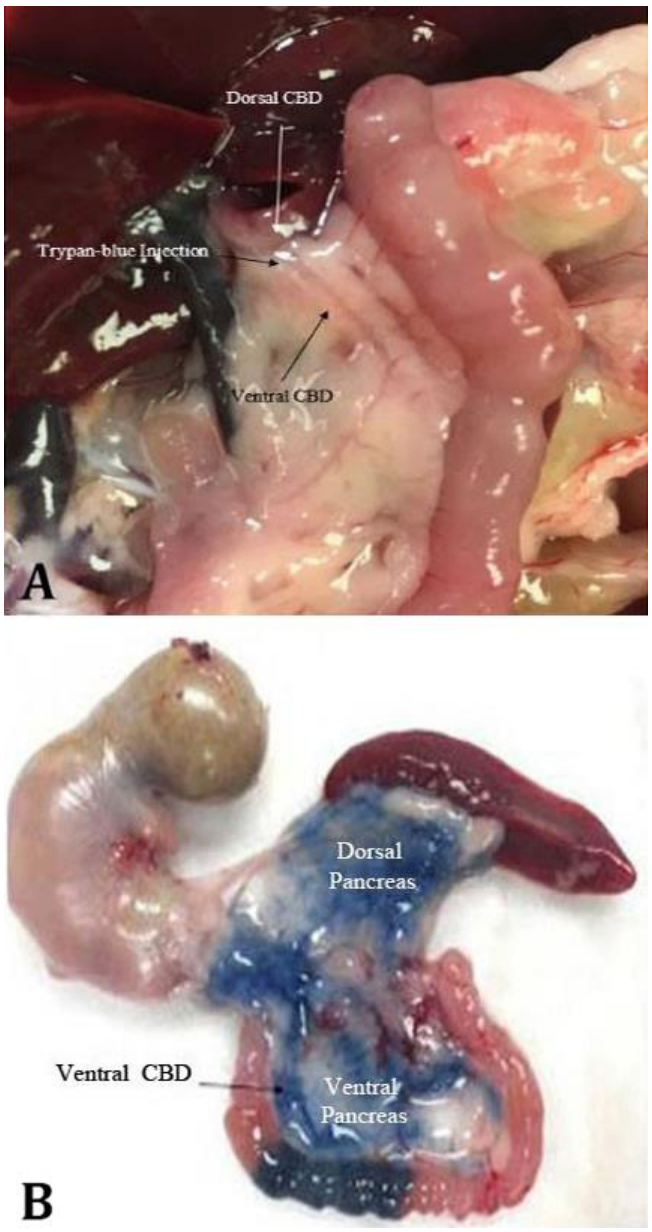

Fig. 1. Anatomic position of pancreas removal surgery on wild-type rats. **A)** Anatomic bifurcation of CBD used for differentiating ventral and dorsal pancreas. **B)** Stained and isolated rat pancreas.

The GTT plot in Figure 2 shows that the ventral and dorsal pancreas removal groups had a similar blood glucose fluctuation curve in terms of intraperitoneal glucose injection within 2 hr. Rats undergoing ventral removal surgery had increased fasting glucose levels from 83.60 mg dL^{-1} to 107.90 mg dL^{-1} (p = 0.00), was returned to the low glucose level at the end of GTT. For dorsal removal rats, the procedure did not affect their fasting glucose level statistically, (p = 0.93). However, the dorsal removal group failed to restore their glucose level initially after two hours of IP injection.

Without food intake, the insulin level secreted by rodents was below 10.00 ng mL^{-1}.[22,23] For the ventral removal group, we obtained the average insulin level as 0.11 ng mL^{-1} (before surgery) and 0.26 ng mL^{-1} (after surgery). For the dorsal removal group, the average insulin level was 0.12 ng mL^{-1} (before surgery and 0.54 ng mL^{-1} (after surgery). For the sham group, the average insulin level was 0.36 ng mL^{-1} (before surgery) and 0.73 ng mL^{-1} (after surgery). Only the ventral removal group had a p = 0.03. The graphical comparison between ventral and dorsal removal group are given in Figure 3. The error bar represents the stand error of the mean. For the healthy adult rat, the fasting glucagon secretion was around 100 pg mL^{-1} within 24 hr.[24] Our ELISA tests showed (Fig. 4) the average glucagon secretion was increased from 200.74 pg mL^{-1} to 326.74 pg mL^{-1} for ventral removal rats (p = 0.01). However, for dorsal removal rats, the fasting glucagon level was increased to 317.60 pg mL^{-1} (p = 0.05).

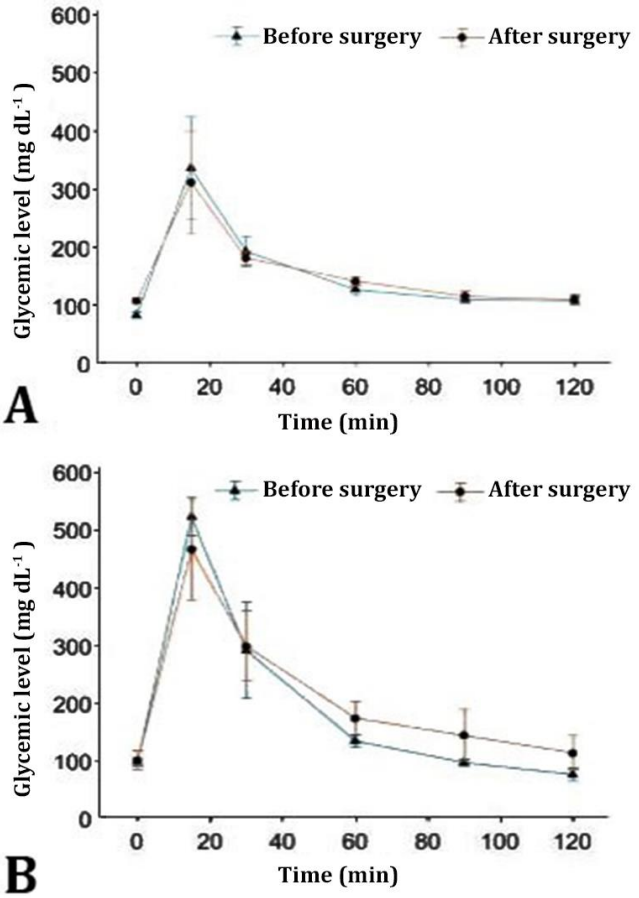

Fig. 2. The GTT test on rats before and after surgery shows the intact pancreatic function in response to a glucose stimulus. **A)** Ventral removal rats had higher initial glucose levels (fasting), however, the overall GTT response curve was not changed too much. **B)** Dorsal removal rats had a higher glucose level after 2 hours of injection; however, the overall GTT response curve was not changed too much.

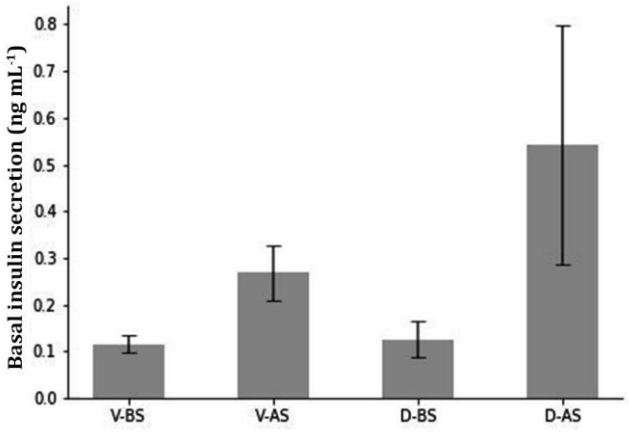

Fig. 3. Fasting insulin level was increased significantly in the ventral removal group (p = 0.03), however, there was no significant difference in fasting insulin secretion in the dorsal removal group (p = 0.21). Notes: V-BS: Ventral removal group before surgery. V-AS: Ventral removal group after surgery. D-BS: Dorsal removal group before surgery. D-AS: Dorsal removal group after surgery.

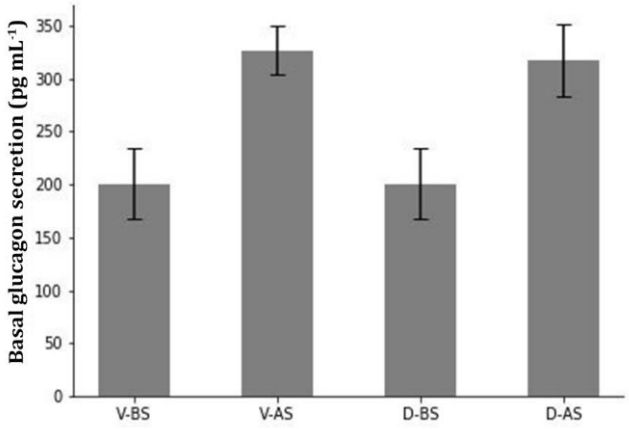

Fig. 4. Fasting glucagon level was increased significantly in the ventral removal group (p = 0.01), however, there was no significant difference in fasting glucagon secretion in the dorsal removal group (p = 0.05). Notes: V-BS: Ventral removal group before surgery. V-AS: Ventral removal group after surgery D-BS: Dorsal removal group before surgery. D-AS: Dorsal removal group after surgery.

Discussion

In the past decades, food intake induced insulin/ glucagon secretion from the dorsal pancreas has been extensively studied. However, the contribution of the ventral pancreas on insulin/glucagon secretion without food stimulus is less reported. The genetically modified rodents do not necessarily follow the same path as humans when developing T2Ds. To explore the role of the ventral pancreas without food intake, we decided to remove the pancreas on normal healthy wild-type rats rather than genetic modification.

For normal healthy adult rats, our GTT data showed surgery did not impair the function of glucose-stimulus insulin secretion for both ventral and dorsal removal groups. Compared to the ventral removal group, after 2 hours of IP injection, the dorsal removal group showed higher restoring glucose levels after surgery. However, the ventral removal group had a higher fasting glucose level after surgery. We hypothesized that high fasting glucose was due to the increased fasting glucagon on ventral removal rats. Our subsequent ELISA tests on basal insulin and basal glucagon showed that only basal insulin and glucagon levels were increased in the ventral removal group.

Such synchronized behavior for basal insulin and glucagon from the pancreas was activated by ventral removal surgery. We suggested that to compensate for the loss of islet from the ventral removal, the remaining islet on the ventral region will release more insulin and glucagon without food intake. This secretion pattern between basal insulin and basal glucagon from the ventral pancreas provided direct evidence to understand how insulin resistance was generated in response to the food-free stimulus.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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