A Correlative Relationship between Neuropathic Pain and Insulin Resistance in Zucker Fatty Rats: Role of Downregulation of Insulin Receptors

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Introduction

Type-2-diabetes (T2D) is a complex metabolic disorder frequently accompanied with painful diabetic neuropathy, among many other complications. Approximately one in three people with diabetes is affected by diabetic neuropathy. However, the exact relationship between neuropathic pain and T2D remains unclear.

The genetically leptin-receptor deficient Zucker diabetic fatty (ZDF) rats develop obesity, insulin resistance, and T2D naturally. In this report, we used ZDF rats as a diabetic model and Zucker lean (ZL) rats as control to study a correlative relationship between the progression of T2D and changes in nociceptive threshold, and examined a possible involvement of insulin receptors expression in the central nervous system in this process.

Methods

ZDF (fa/fa,n=35) and ZL (+/fa,n=21) rats entered experimental procedures at 6 weeks of age. Chronic constriction injury (CCI) was produced by placing loose ligations at left sciatic nerve. Tactile sensitivity threshold was examined by applying von Frey filaments and thermal hypersensitivity to radiant heat using a 390 Analgesia Meter.

Random glucose concentrations were tested using Ascensia Breeze Blood Glucose Monitoring System from tail tip blood samples between 9-10AM. Hemoglobin A1c (HbA1c) was tested by ELISA. Fresh tissues of spinal cord dorsal horn from the lumber enlargement and skeletal muscles were collected 2 weeks following operation for Western blotting and perfused samples were used for immunohistochemical staining to detect the qualitative expression of insulin receptor.

Raw data from blood glucose tests, $HbA1_C$, and nociceptive sensitivity tests were analyzed in SigmaPlot, by using one way repeated measures analysis of variance (ANOVA) across testing time points to detect overall differences among treatment groups. Data from ELISA and Western blots were analyzed by using *Student t*-test to detect differences between treatment groups. The data are presented as mean±SE. Differences were considered to be statistically significant at level of α=0.05. To determine the association of nociceptive threshold to glucose concentration in ZDF rats, data were analyzed using SigmaPlot by comparing raw data at different time points (0, 2, and 6 weeks) in separate CCI and naïve groups.

Results

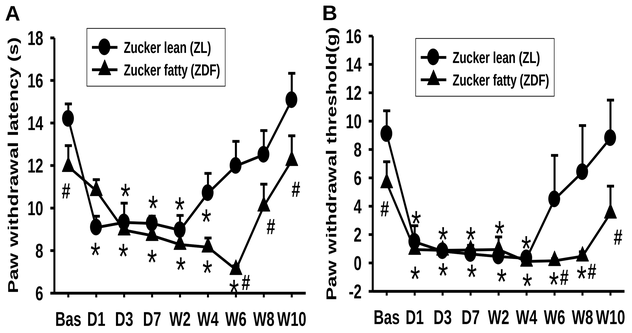

Fig.1. Differential nociceptive thresholds and nociceptive hypersensitivity recovery periods between ZDF and ZL rats. Naïve ZDF rats show a lower baseline nociceptive threshold to thermal (A) and mechanical stimuli (B). There is also a prolonged recovery period for thermal (A) and mechanical hypersensitivity (B) after CCI operation in ZDF rats. *, *P*<0.05 vs baseline (Bas) of the same group; #, *P*<0.05 vs ZL at the same time point. Bas, baseline data before operation; D, post-operation time in day; W, post-operation time in week.

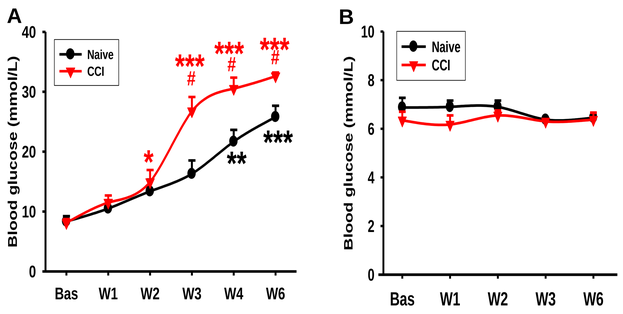

Fig.2. Influence of CCI on blood glucose concentration. There is an accelerated progression of hyperglycemia in ZDF (A) but not ZL rats (B) after CCI operation. *, **, ***, *P*<0.05, 0.01, 0.001 vs baseline (Bas) of the same group, respectively; #, *P*<0.05 vs Naïve group at the same time point. CCI, chronic sciatic nerve constriction injury group of rats. W, Experimental time in week.

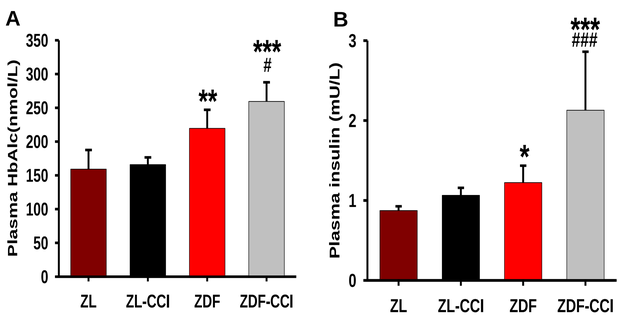

Fig.3. Differential influence of CCI on the concentration of HbA1c and insulin in ZDF and ZL rats. CCI induced higher HbAlc (A) and hyperinsulinemia (B) in ZDF but not ZL rats 6 weeks after CCI operation. ZL, Zucker lean rats; ZDF, Zucker fatty rats. CCI, chronic sciatic nerve constriction injured ZL (ZL/CCI) or ZDF rats (ZDF/CCI). *, **, ***, *P*<0.05, 0.01, 0.001 vs naïve ZL, respectively; #, ###, P<0.05, 0.001 vs naïve ZDF, respectively.

Results

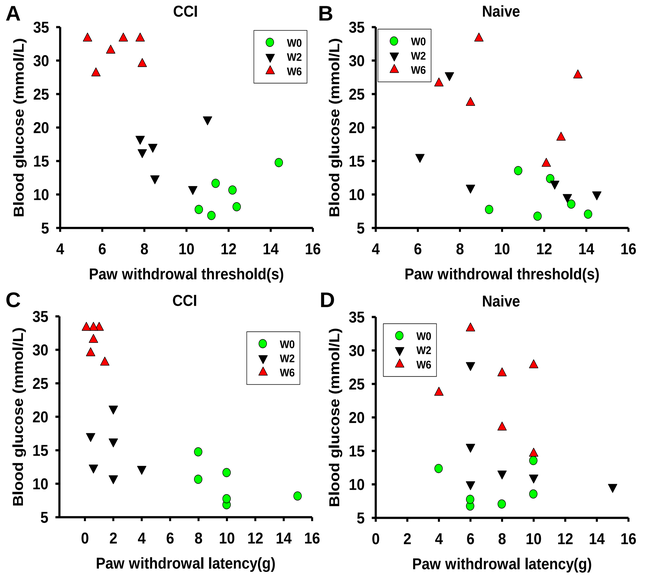

Fig.4. Association between nociceptive threshold and glucose concentration. Both thermal hyperalgesia threshold (A) and mechanical allodynia latency (C) are negatively associated to blood glucose concentration. There is a strong correlation in CCI operated (A, C) and a moderate correlation in naïve ZDF rats (B,D). This suggests that CCI may deteriorate the glucose metabolism dysfunction (hyperglycemia) in ZDF rats. CCI, chronic sciatic nerve constriction injured ZDF rats. W0, W2, W6, Week 0, 2, and 6 respectively.

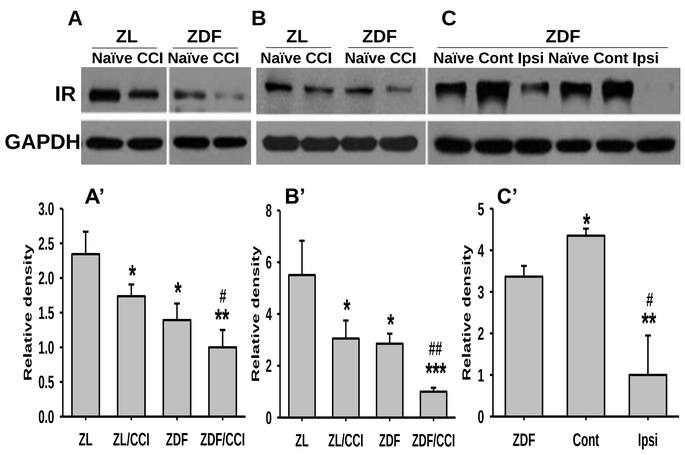

Fig.5. Expression of IR in spinal cord dorsal horn (A) and skeletal muscles of naïve and CCI rats (B,C). Western blots showing a lower expression level of insulin receptor (IR) in spinal cord (A) and skeletal muscles of naïve ZDF rats as compared with that in naïve ZL rats (B). Two weeks after CCI operation the expression of IR was further downregulated in both ZL and ZDF rats, with a stronger impact in ZDF rats (B). ZL, Zucker lean rats; ZDF, Zucker diabetic fatty rats. CCI, chronic constriction injured ZL (ZL/CCI) or ZDF rats (ZDF/CCI). *, **, ***, *P*<0.05, 0.01, 0.001, respectively, vs naïve ZL rats. #, ##, *P*<0.05, 0.01, respectively, vs naïve ZDF rats. When compared within ZDF rats alone, the expression of IR in the skeletal muscle directly innervated by CCI operated nerve was significantly down-regulated while it was somewhat up-regulated in the contralateral skeletal muscle (C). ZDF, naïve ZDF rats. *, **, *P*<0.05, 0.01, respectively, *vs* naïve ZDF rats. #, *P*<0.05, *vs* contralateral.

Immunohistochemistry

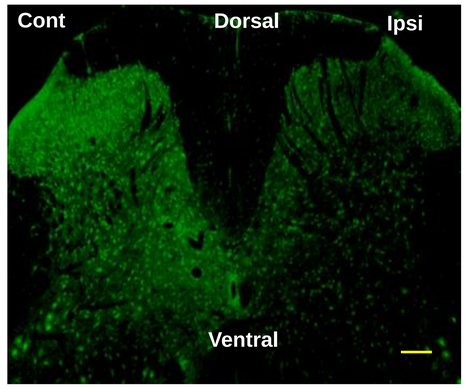

Fig.6. Expression of IR-positive cells in the spinal cord of a ZL rat two weeks after CCI. There is a down-regulated expression of IR-immunopositive cells (green) in the spinal cord ipsilateral to CCI operation. Cont, Control lateral to CCI operation; Ipsi, Ipsilateral to CCI orepation. Dorsal, Dorsal part of spinal cord; Ventral, Ventral part of spinal cord. Bar, 250μm.

Conclusions

1. The relationship between insulin resistance and chronic pain in ZDF rats is bidirectional and an impaired IR signaling system may be implicated in this reciprocal relationship.

2. Nerve injuries in genetically susceptible individuals may accelerate the development of insulin resistance as in type 2 diabetes. A downregulated expression of insulin receptor in the skeletal muscle innervated by the injured nerve is one of the underlining mechanisms.

Acknowledgement

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