Diabetic Complications Consortium

Application Title: Model of acute painful diabetic neuropathy after rapid glycemic control

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1. Project Accomplishments:

In this project, we have shown that a form of treatment-induced painful neuropathy of diabetes (TIND) or acute painful diabetic neuropathy of rapid glycemic control (APDNRGC) in response to insulin can be modeled in mice. Streptozotocin (STZ)-mice developed a chronic persistent allodynia that lasted at least 72 hours after the last insulin injection for STZ-mice receiving 1U insulin daily. Blood glucose levels were transiently decreased for 1 hours after daily insulin injection but remained in the hyperglycemic range for the rest of 24hours. The protracted insulin-induced allodynia developed after a minimum of 8 days of daily insulin injections. To verify that the allodynia detected after daily injection of insulin was dependent on the activation of the insulin-signaling pathway and subsequent phenotypic changes, administration of an AKT inhibitor was started once allodynia was confirmed. AKT inhibition alleviated insulin-induced tactile allodynia acutely for 3 hours, demonstrating a role of the insulin-signaling pathway in the etiology of TIND. However, the effect was not maintained chronically. This model will allow us to understand mechanisms involved in TIND and develop new therapeutic approaches.

Specific Aims:

Specific Aim: Develop a model of APDNRG in type 1 diabetes, establish the role played by insulin and identify if structural changes contribute to the painful neuropathy.

Study 1: Effect of chronic insulin injection

After 16 weeks of diabetes, STZ-mice were injected daily, 5 days a week, with 1U insulin subcutaneously (sc) and tactile responses were assessed 24 hours after the last injection, twice a week for 50 days, along with blood glucose levels. A significant ($p<0.001$) and persistent pain response or allodynia to von Frey filaments stimulation was detected by day 8 after the initial insulin injection, and up to 50 days (Fig. 1A). Allodynia was detected 72h after the last insulin injection on

day 43 (Fig. 1A), demonstrating a chronicity of the effect rather than an acute effect of insulin injections. Weekly testing of the mice did not induce allodynia as demonstrated by non-allodynic responses of STZ mice that were tested weekly in parallel with the STZ mice receiving insulin (Fig. 1A). Similarly, hyperalgesia to thermal stimulus was detected in STZ mice receiving 1U insulin daily (Fig. 1B) while blood glucose levels remained hyperglycemic 24 hours after insulin injections (Fig 1C). Blood glucose levels decreased by 57±12% 1hour post-daily insulin injection and rose back to levels similar to baseline by 6 hours post injection (Fig 2D), indicating that insulin induces a pain response in diabetic mice independent of blood glucose levels, glycemic excursion or hypoglycemia.

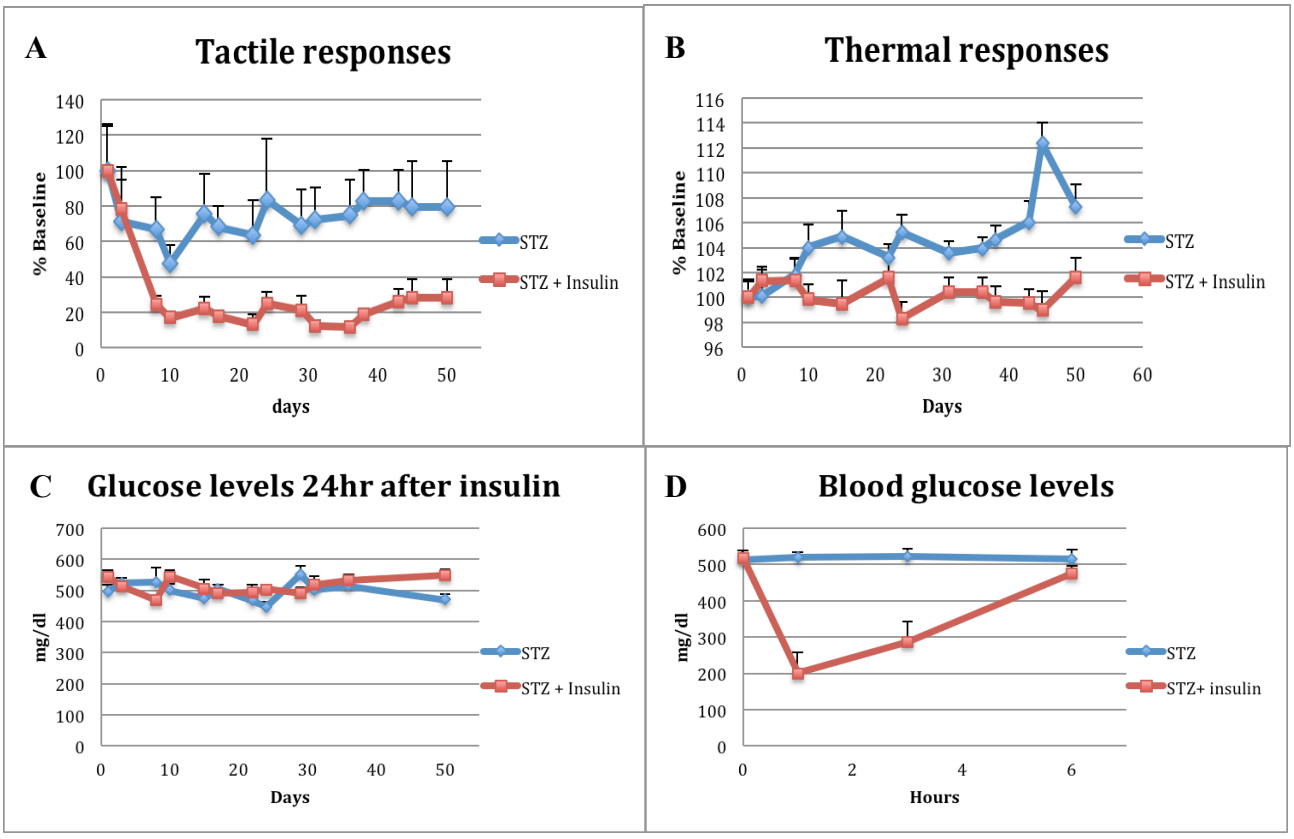

Figure 1: (A) Tactile responses to von Frey filaments 24 hrs after the last injection for STZ mice (STZ) and STZ mice receiving daily 1U insulin injection sc (STZ + insulin). n=10. **(B)** Thermal responses 24 hrs after the last injection for STZ mice (STZ) and STZ mice receiving daily 1U insulin injection sc (STZ + insulin). n=10. **(C)** Blood glucose levels 24hrs after injection of 1U insulin and **(D)** blood glucose levels at 1, 3 and 6 hrs following 1U insulin injection for STZ mice (STZ) and STZ mice receiving daily 1U insulin injection sc (STZ + insulin). n=10.

Study 2: Role of insulin-signaling pathway

The role of insulin, rather than of glucose changes, in TIND, demonstrated in Study 1, was further investigated with an inhibitor of AKT, a downstream kinase of the insulin-signaling pathway.

The first result of this study is the reproducibility of the development of allodynia induced by insulin. In both Study 1 and 2, insulin-induced allodynia developed by day 8 and persisted at least 50-60 days (Fig. 1A and 2A).

Starting on day 17 after the initial insulin injection, once insulin-induced allodynia was confirmed, AKT inhibitor IV at 1 mg/kg was injected daily to STZ mice receiving 1U insulin. Inhibition of AKT alleviated insulin-induced allodynia acutely (Fig 2 B) with a peak efficacy of 3hrs, confirming the role of the insulin-signaling pathway in the etiology of TIND. However, the beneficial effect of AKT inhibition did not last more than 4hours and did not translate to a chronic effect (Fig 2A).

In addition, we have tested the effect of insulin cessation. Withdrawal of insulin after 43 days of daily injection and confirmed allodynia, tactile responses recovered and returned to baseline levels after more than 30 days without insulin injection, suggesting phenotypic changes. These changes will be explored in further studies, which are the basis of a new application to NIH, currently under review.

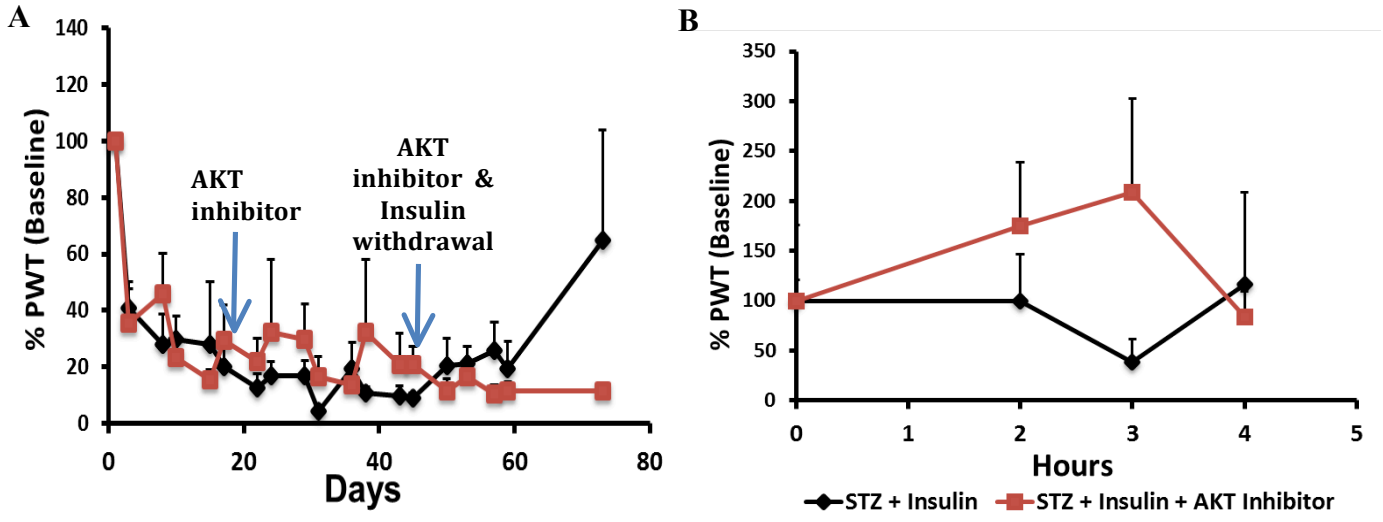

Figure 2: (A) Time course of tactile responses in STZ mice treated daily with 1U insulin or 1U insulin + AKT Inhibitor initiated 17 days after insulin neuritis onset. **(B)** Time course of tactile responses for 4 hours following insulin+ vehicle and insulin+ AKT inhibitor injections. Measurements taken on day 17 of insulin + AKT inhibitor treatment, day 38 of insulin treatment. N=6, mean+SEM

Study 3: Effect of C peptide supplementation

The lack of C peptide when exogenous insulin is injected may contribute to TIND. Injection of C peptide at 1.3 mg/kg may prevent the development of insulin-induced allodynia. This study is currently in progress.

Study 4: Effect of chronic insulin injection on nerve structure and vasculature.

Motor nerve conduction velocity (MNCV) and nerve and skin blood flow were measured on STZ-mice receiving saline or daily injection of insulin (Study 1) for 50 days. Sciatic nerve and foot skin were collected at study termination (day 51) and processed for light microscopy and immunohistochemistry. Daily injection of insulin did not alter MNCV in STZ mice (Fig 3A) as it was shown in insulin-deficient diabetic mice receiving low levels of constant insulin not sufficient to fully correct blood glucose (Yorek et al., 2014) and in insulin-deficient diabetic rats with insulin implants (Calcutt et al., 1996). Blood flow tend to be reduced in sciatic nerve and skin of STZ mice receiving daily injection of insulin (Fig 3B), consistent with the preliminary assessment of blood vessels in sciatic nerve of STZ mice receiving daily insulin (1.8 ±0.2 vs 0.9±0.1 vessels/mm^2, STZ vs STZ+ insulin respectively, n=3-4, in progress). Quantification of small sensory intraepidermal nerve fibers (IENF) and with the neuronal growth-associated marker GAP43 for assessing nerve growth are in progress.

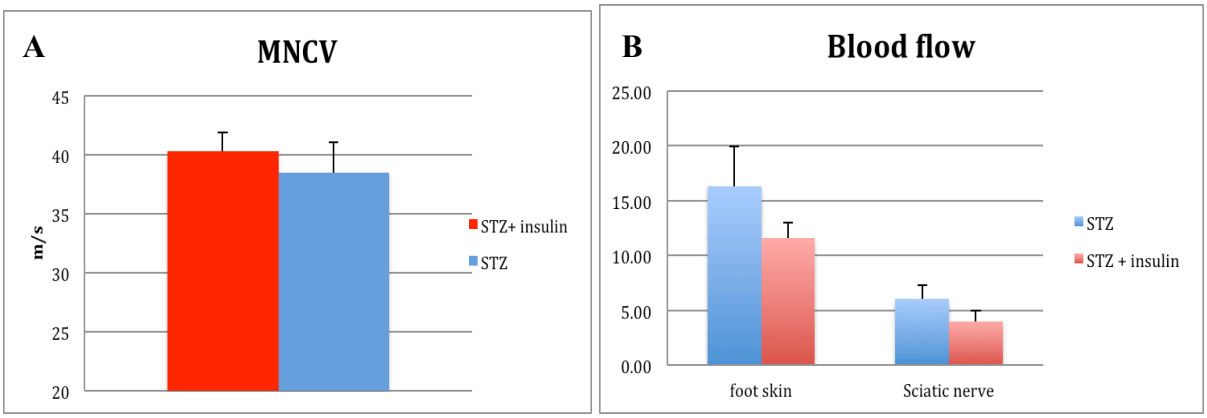

Figure 3: (A) Motor nerve conduction velocity and **(B)** foot and nerve blood flow in STZ mice (STZ) and STZ mice receiving daily 1U insulin injection sc (STZ + insulin). n=10.

2. Publications:

A manuscript is in preparation, waiting for completion of the histological assessments.