Laser induced injury caused hyperglycemia-like effect in Drosophila larva: a possible insect model for posttraumatic diabetes

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It has been recognized for decades that severe stress, such as trauma, systemic infection and surgery, triggers the hypermetabolic response or stress hyperglycemia [21, 25, 26, 29]. In clinical practice, special attention is required for diabetes patients in case of infection or injury, commonly known as “sick day management” [4]. Furthermore, admission glucose of trauma patient can be a prognostic indicator for mortality rate [6, 31]. Although mild-stress hyperglycemia is considered a critical survival response and protective against injection by providing a source of fuel for the immune system [14], in many cases, chronic endurance responses lead to persistent hyperglycemia and insulin resistance [28].

Several animal studies as well as studies on patients revealed that trauma stimulates the secretion of inflammatory cytokines, like TNF, IL-1 and IL-6, as well as glucagon, cortisol and catecholamine [5, 13, 15, 16], which may directly mediate the hyperglycemia. Furthermore, phosphorylation of Ser307 in IRS-1, a common indicator of insulin resistance, is significantly elevated in the burn-injured mice [32]. However, the precise mechanisms for insulin resistance produced by burns or other stressor remain largely unknown.

In recent years, the number of investigations into metabolism using Drosophila has sharply increased [11, 20]. Drosophila genome encodes insulin/glucagon homologues as well as leptin equivalent gene, and most of the signaling components are conserved [12, 21, 23]. Here, we set out to establish a novel model of “diabetes of injury” in Drosophila, which will allow us to take advantage of fly genetics to gain insight into its mechanisms. We evaluated metabolisms by measuring trehalose, instead of glucose, because it is a disaccharide composed of two glucose molecules and is the principal blood sugar in many insects. All fly stocks were reared at 25°C on a standard yeast, corn meal and agar medium, under 12 hr:12 hr light:dark conditions unless otherwise stated. The following fly stocks were used: 5053A-Gal4 (DGRC number 107443) [22], NP6310 (DGRC number 113906) [9] and UAS-2XEGFP AH2 (FBst0006874). The laser lesioning of muscle fibers was done in the second instar larvae.

Drosophila larvae body wall muscles consisted of about 580 muscle fibers, each of which can be distinguished from its neighbors by its size, shape and attachment sites [1]. In order to target specific fibers, 5053A-Gad4 strain was chosen to mark, among 30 muscle fibers in each of hemisegments of A2–A7, one particular fibers called ventrolateral body wall muscle 12 (VLM12) (Fig. 1A) [18, 22]. Ablation of muscle fibers was performed as follows: The larvae were anesthetized with ether and immersed in silicon oil (Shin-Etsu Chemical Co., Tokyo, Japan, FL-100) beneath a coverslip. Muscle fibers were identified using GFP fluorescent signal and Nomarski optics. The muscle fiber was lesioned by directing a series of 3.5 nsec laser pulses with a peak power of 170 μJ at 440 nm, generated by a MicroPoint dye laser system (Photonic Instruments Co., Kawasaki, Japan). After muscles were lesioned, the larvae were transferred to grape juice plate supplemented with a thin layer of yeast paste and incubated for 24 hr at 24°C before proceeding to the metabolic measurements. For sham control, the larvae were anesthetized, immersed in silicon oil and allowed to stay under a coverslip for 8 min, which is an average period of time required for lesioning. Trehalose and protein levels were assayed as follows: Whole-larvae trehalose was measured by Trehalose Assay Kit (Megazyme, Wicklow, Ireland, K-TREH). For whole-fly preparation, 5 larvae were collected and briefly rinsed in Ringer’s solution.

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larvae were homogenized by vigorous shaking in the presence of Zirconia beads (NIKKATO Sakai Japan, φ 0.8 YTZ Ball). The resultant homogenate was heated at 70°C for 5 min. and centrifuged at 12,000 rpm for 5 min. The resultant supernatant was used for subsequent measurements. Protein quantity was determined by Quant-iT™ Protein Assay Kits (Invitrogen, Carlsbad, CA, U.S.A.). Because the quantification of trehalose or protein determined here is that of lysate and reflects neither total nor hemolymph trehalose/protein. Therefore, we evaluated the metabolic abnormality by fold change. Similarly, locomotive activity was evaluated as follows: Twenty-four hr after the laser treatment, the larvae were placed on the grape juice plate. The locomotion activity was video recorded for 5 min. The trajectory was measured on tracing film to quantify the distance that each larva traveled. All values are expressed as means ± SE. The statistical

Fig. 1. Laser ablation of body wall results in increased trehalose levels. 10 muscle ablated (MA): Ten muscle fibers were lesioned using GFP signal as a guide to target ventrolateral body wall muscle 12 (VLM12). VLM12 is GFP labeled in 5053A-Gal4>UAS-2XEGFP (A, allows). Ablation was confirmed by the lack of GFP signal (arrows) (B). 30MA: Thirty muscle fibers, mostly in 1 or 2 abdominal segments, were ablated in EP6310>UAS-2XEGFP, which expresses GFP in all body muscles. Area where muscles were ablated is shown in box. (C). After the ablation, no apparent damages were found in the surrounding tissues: trachea (arrowheads), fat body (*), gut (#) (D). Bars: 200 µm.

Fig. 2. Twenty-four hr after the ablation, the trehalose levels were significantly elevated. 10MA did cause any significant difference, but 30MA treatment resulted in increased overall trehalose level (A). *: P<0.01. In contrast, the protein levels were unchanged for either treatment. (B). The treatment did not affect locomotion activity. P>0.68 for 10MA muscle lesioned; P>0.17 for 30MA lesioned (C). n=4, 10MA control; n=5, 10MA; n=5, 30MA control; n=5, 30MA.
significant differences was evaluated using student’s test. Ablation of ten VLM12 fibers per larva mostly from A2 to A6 segments on both sides did not exert any prominent effect on metabolic and physical activities 24 hr after the operation (Fig. 2). This may be because ten fibers merely correspond to less than 2% of total muscle amount. Therefore, next, we attempted to induce severe damage by targeting more muscle fibers in one or two segments. To achieve this, we screened several NP lines listed as ubiquitous body wall muscle Gal4 strains and identified NP6310 to be one of the most suitable strain for labeling muscles [10]. Lesioning of thirty muscle fibers in a few neighboring segments per larva (Fig. 1C), equivalent to ca. 5% of total muscle mass, resulted in increased whole-body trehalose levels (Fig. 2A). Although the physical activity was unchanged (Fig. 2C), the survival rate of laser-treated larva was 68% (n=31) in comparison to 82% (n=51) for sham control, suggesting the increased sugar level is due to stress from physiological damage rather than dormant muscle activity. It is important to point out that we measured the trehalose levels of whole-body lysate instead of hemolymph trehalose because of technical difficulties to extract sufficient amount of hemolymph from the second instar larvae. Because the total volume of hemolymph in an adult fly is extremely small (ca. 0.1 µl) [7], the contribution of any hemolymph trehalose to the total trehalose content can be regarded as negligible. Assuming that the total volume of hemolymph in larva is similar to that of an adult, the diabetic phenotype observed here may merely reflect the increased storage level of trehalose instead of “plasma” glucose. However, Broughton et al reported the correlation between hemolymph and whole-body trehalose levels [2]. Therefore, similar to a mammalian system, the increased trehalose may indicate one of the stress response and potentially stress diabetes-like phenotype. Recently, isolation of hemolymph from a single fly was reported, which we hope will sophisticate the method and circumvent the issue here [8].

In the Drosophila genome, mammalian inflammatory cytokines, such as TNF, IL-1 and IL-6, are not encoded. However, the dopamine signaling is well conserved, and there are 70–90 dopaminergic neurons in fly larvae [29]. The aberrant dopamine signaling affects basal activity and locomotion similar to human Parkinson’s disease. Because there is no difference was observed for locomotion activity in the muscle-ablated larvae, it is likely that dopamine signaling is not affected. Another possible hormonal mediator is ecdysone, which is a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone. Ecdysone levels sharply increase upon molting and metamorphosis [24]. In addition, ecdysone also plays an important role in egg formation, learning and sleep regulation [10, 17]. Furthermore, insulin and ecdysone are the critical extrinsic regulators of growth for the imaginal disks of insects [27]. Indeed, it is reported that ecdysone negatively regulates growth rates by impeding insulin signaling [4]. Therefore, laser-induced stress may have up-regulated ecdysone levels, resulting in reduced insulin activity.

In summary, to our knowledge, we showed for the first time that trauma or cellular damage leads to increased trehalose levels in Drosophila or insect larva. This could potentially provide a valuable opportunity to investigate the molecular and genetic bases of posttraumatic effect on the metabolism.

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