Insulin signals through the dorsal vagal complex to regulate energy balance

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ABSTRACT

Insulin signaling in the hypothalamus regulates food intake and hepatic glucose production in rodents. Although it is known that insulin also activates insulin receptor in the dorsal vagal complex (DVC) to lower glucose production through an Erk1/2-dependent and PI3K-independent pathway, it is unknown whether DVC insulin action regulates food intake. We here report that a single acute infusion of insulin into the DVC decreased food intake in healthy male rats. Chemical and molecular inhibition of Erk1/2 signaling in the DVC negated the acute anorectic effect of insulin in healthy rats, while DVC insulin acute infusion failed to lower food intake in high-fat fed rats. Finally, molecular disruption of Erk1/2 signaling in the DVC of healthy rats per se increased food intake and induced obesity over a period of two weeks, while a daily repeated acute DVC insulin infusion for 12 days conversely decreased food intake and body weight in healthy rats. In summary, insulin activates Erk1/2 signaling in the DVC to regulate energy balance. (Words: 162)

INTRODUCTION
Hyperphagia and increased body weight are the hallmark features of obesity (1). Obesity has doubled since 1980 and approximately 1.4 billion adults were overweight and 500 million were obese in 2008. Obesity leads to diabetes as an elevated BMI is associated with an increased risk for diabetes (2) while abdominal obesity is a strong predictor of diabetes (3). Laboratories have focused on dissecting the regulatory mechanisms of feeding and weight gain that aim to unveil novel anti-obesity targets. In this regard, the central nervous system (CNS) has received much attention.

CNS integrates signals generated by the pancreas-, white adipose tissue- and GI-derived hormones to regulate energy homeostasis (1;4-6). Alteration of the CNS sensitivity to these hormones leads to uncontrolled feeding behavior, reduced energy expenditure and elevated body weight and adiposity (1;4-6). Central insulin administration reduces food intake and body weight in baboons (7), rats (8), mice (9) and humans (10;11). Intracerebroventricular infusion of insulin into the 3rd ventricle (ICV-3) of mice and rats decreases food intake in males (8;9), while intranasal insulin administration in humans also reduces body fat and food intake in males but not females (10;11). ICV-3 injection of siRNA against the insulin receptors reduces hypothalamic insulin receptor expression and induces hyperphagia in male rats (12), while neuronal insulin receptor knockout female (but not male) mice are hyperphagic (13). These data collectively highlight the hypothalamus as an insulin-responsive region that regulates appetite, but does not limit insulin action to the hypothalamus. This is because intranasal insulin injection in humans does not limit insulin delivery solely to the hypothalamus (10;11) nor is insulin receptor expression only reduced in the hypothalamus of neuronal insulin receptor knockout mice (13).

In fact, insulin receptor knock down in the catecholaminergic neurons of the ventral tegmental area increases body weight and fat mass in mice (14), while direct injection of insulin into the central bed nucleus of the amygdala decreases food intake in healthy but not high-fat fed rodents (15). Insulin responsiveness in the dorsal vagal complex (DVC) and the subsequent control on glucose homeostasis has also been examined (16). First, insulin receptors and the downstream PI3K and Erk1/2 signaling effectors are all expressed in the DVC. Direct insulin injection into the DVC preferentially activates Erk1/2 instead of PI3K-AKT in a dose-dependent manner. Importantly, insulin triggers a PI3K-independent and Erk1/2 dependent signaling cascade in the DVC to lower glucose production in healthy rodents independent of weight changes, while inability of DVC insulin to control glucose develops after three days of high-fed feeding (16).

No study to date has addressed the role of DVC insulin action in the regulation of energy balance (i.e., food intake and body weight gain). In contrast, DVC administration of leptin (17), GLP-1 (18) and leucine (19) lower food intake while administration of insulin into the 4th ventricle decreases
expression of Hap1-Ahi1 (20). The fact that Hap1-Ahi1 is normally up-regulated to stimulate feeding
(21) highlights a potential role of DVC insulin action in energy balance regulation. We here provide
the first evidence, to our knowledge, that acute insulin infusion into the DVC activates Erk1/2 to lower
food intake in healthy but not high-fat fed rats. Direct molecular disruption of DVC Erk1/2 signaling in
normal rats induces hyperphagia and obesity over a period of two weeks, while daily acute repeated
DVC insulin infusion for 12 days lowers food intake and body weight in normal rats. These data
collectively demonstrate that insulin activates Erk1/2 in the DVC to regulate energy balance.

RESEARCH DESIGN AND METHODS
All study protocols were reviewed and approved by the Institutional Animal Care and Use Committee
of the University Health Network.
**Animal Preparation.** Eight-week-old male Sprague-Dawley rats weighing between 270 and 290 g (Charles River Laboratories, Montreal, QC) were housed in individual cages and maintained on a light-dark cycle with access to standard chow and water *ad libitum*. Rats were anesthetized by intraperitoneal (i.p.) injection of ketamine (60 mg/kg) and xylazine (8 mg/kg). A 26-gauge bilateral guide cannula made of stainless steel (Plastics Ones Inc., Roanoke, VA) was stereotactically implanted (David Kopt Instruments, Tujunga, CA) into the DVC targeting the nucleus of solitary tract (0.0 mm on occipital crest, 0.4 mm lateral to midline, 7.9 mm below skull surface) as described (16). The guide cannula was secured in place with mounting screws, cyanoacrylate gel, and dental cement. To create a closed system, the guide cannula was kept free of obstruction by inserting a dummy cannula followed by a dust cap (Plastics One Inc.).

**Virus preparation and injection.** Adenovirus expressing the dominant negative form of MEK1 (MEK1-DN) with a mutated magnesium binding site (D208 mutated to A) to kill the catalytic activity was prepared (16). The purified adenoviruses with pfu of $1.8 \times 10^8$ (MEK1-DN) or $1 \times 10^8$ (GFP) were injected 3 µl per site into the DVC of rats (16).

**Short term feeding study.** Animals whose food intake and body weight returned to baseline after 5 days of DVC surgery underwent feeding studies. Food intake and body weight were measured 48 h (Day 5) and 24 h (Day 6) prior to the feeding experiments (Day 7). On day 7, the rats were fasted at 10 am (6 hrs prior to night cycle) while food intake and body weight were measured. Body weight was monitored at 3 pm and MAPK inhibitor PD98059 (900µM), PI3K inhibitor LY-294002 (5, 10, 50, 100, 250 µM) or wortmannin (20 µM), or saline was infused through the DVC catheter at 0.04 ul/min for 5 minutes (0.2 µl total). At 4 pm (one hour later; start of the dark cycle), insulin (2, 20, 200 or 2000 µU/µL) or saline was infused at 0.04 µL/min for 5 minutes (0.2 µl total) into the DVC and food was returned. Food intake was subsequently measured every 30 minutes for 4 hrs. Food intake and body weight were then monitored at 14, 24, and 48 h after DVC infusion.

A separate group of rats was fed a lard oil-enriched high-fat diet (TestDiet #571R, Purina Mills, Richmond, IN) for 3 days prior to the day of the experiment, with food intake and body weight monitored daily. The composition of the HFD differs from regular chow with respect to total calorie content (5.14 compared to 3.83 kcal g$^{-1}$, respectively), fat content (33% compared to 17%), protein (22% compared to 31%) and carbohydrate content (45% compared to 52%). Food intake and body weight were monitored 72, 48, and 24 h prior to the experiment.

When the feeding study was performed in the presence of viral injection and insulin acute infusion, the rats were injected with the virus (MEK1-DN or GFP) immediately after the brain surgery. Following a 3-day recovery, rats were subsequently pair-fed for an additional 4 days before the feeding
and insulin acute infusion study was performed.

**Conditioned taste aversion (CTA) test.** Rats underwent DVC surgery and after 3 days of recovery (Day 3), rats underwent the conditioned taste aversion test as described (22). Rats were habituated to 1 h daily access to water on Day 3. During this hour, two bottles (each containing unflavored water) were placed in each cage. After 7 days, all rats received two bottles containing 0.1% saccharin solution instead of water. Immediately following this 1 h exposure, rats received an injection of saline or insulin (2 mU/µL; 400 µU per site) into the DVC. Independent rats were i.p. injected either with LiCl (22mg/kg) as positive or saline as negative control. On the following day, rats received 1 h access to two bottles with unflavored water. In the subsequent and final 2 days, a two-bottle choice test was given in which all rats were allowed 1 h access to water and the 0.1% saccharin solution. The position of the two bottles in each cage was switched on the second day of the test.

**Long term feeding study.** Rats were injected with virus (MEK1-DN or GFP) into the DVC 3 days after DVC surgery. Food intake, body weight and water consumption were monitored over an 18-day period.

**Feeding study with repeated daily insulin infusion into the DVC.** Rats were subjected to DVC surgery and had a 1 week recovery before starting daily repeated DVC infusion. Insulin (2 mU/µl) or saline was infused at 4 pm for 12 days. Body weight and food and water intake were measured daily at the same time of the DVC infusion. Fat pads were removed and measured on day 12.

**Statistical analysis.** Analysis was done with GraphPad Prism by two-way ANOVA to compare across the groups followed by a Bonferroni post hoc test to compare between the groups or one-way ANOVA to compare across the groups followed by Tukey post hoc test to compare between groups. Statistical analysis was accepted as significant with a p value of < 0.05. Data are presented as means +, - or ± SEM.

**RESULTS**

**Single acute DVC insulin infusion lowers food intake.** We monitored food intake and body weight of healthy rats that received a single acute insulin or saline infusion into the DVC (Fig. 1A). Food restricted (6 h) rats were treated with different concentrations of insulin ranging from 2 mU/µl (400 µU per site) to 2 µU/µl (0.4 µU per site) or saline and food was given back immediately after the DVC
administration. All rats which received DVC insulin ate less compared to the saline-infused rats, with a significant difference detected as early as 90 min after infusion (Fig. 1B). This difference was lost within 14-24 h following DVC insulin infusion (Supplementary Table 1). A trend towards a drop in body weight at 14 h post DVC single acute insulin infusion was detected (Supplementary Table 1).

Two groups of rats that received DVC insulin or saline underwent CTA testing (Fig. 1C) and the rats did not show a preference for saccharin (Fig. 1D). In contrast, rats that received i.p. LiCl injection drank a significant less percentage of saccharin as compared to those that received i.p. saline (Fig. 1D). Thus, the acute anorectic effect of DVC insulin is not caused by malaise.

**DVC Erk1/2 activation.** We decided to carry out all subsequent studies with insulin infused at 2 mU/µl (400 µU per site) into the DVC to mimic the insulin dose used in the hypothalamic feeding studies. Insulin administered at 2mU/µl into the ICV-3rd (e.g. targeting the hypothalamus) was demonstrated to equally effective to lower feeding in rats (8). Of note, we have previously validated that DVC insulin infused at 2 µU per site activates Erk1/2 but not PI3K-AKT and such Erk1/2 activation lowers hepatic glucose production (16). However, when the dose of insulin infused into the DVC increases to near 200-400 µU per site, both Erk1/2 and AKT in the DVC are activated (16). These findings open up the possibility that both of these signaling pathways could be involved in the ability of DVC insulin (2 mU/µl) to regulate feeding.

Co-infusion of MAPK inhibitor PD98059 with insulin fully abolished the ability of DVC insulin to lower food intake (Fig. 2A), while DVC PD98059 infusion *per se* did not alter food intake as compared to saline-treated rats (Fig. 2A). The current dose of PD98059 administered into the DVC would negate the ability of insulin to activate Erk1/2 as a blockade on insulin-Erk1/2 activation was previously described with a lower dose of PD98059 (16).

We have developed an adenovirus expressing the dominant-negative form of MEK1 (MEK1-DN) (16). MEK1 is MAPK kinase that phosphorylates and activates ERK1/2. Direct injection of MEK1-DN into the DVC negates the ability of insulin to activate Erk1/2 in the DVC (16). We here injected the MEK1-DN into the DVC and evaluated the effect of DVC insulin. DVC MEK1-DN reversed the ability of DVC insulin to lower food intake as compared to the GFP injected rats (Fig 2B).

Next, DVC insulin was co-infused with PI3K inhibitors LY294002 or wortmannin. Neither DVC LY294002 nor wortmannin infusion negated the ability of insulin to lower food intake (Fig. 2B). However, DVC LY294002 or wortmannin alone also lowered food intake to a similar extent as insulin (Fig. 2B). We then performed a dose-response study for DVC LY294002 infusion and discovered that administering LY294002 as low as 5 µM into the DVC still lowered food intake to a similar extent as insulin (Fig. 2C). A previous study has reported that hypothalamic administration of LY294002 at 10
μM is sufficient to negate insulin’s control on glucose production (23).

Thus, insulin signals through an Erk1/2-dependent and PI3K-AKT independent pathway to acutely lower food intake. However, the role of DVC PI3K-AKT signaling in appetite regulation remains ambiguous.

**High-fat feeding.** Rats were fed with a lard oil-enriched high-fat diet (HFD) for 3 days prior to undergoing the feeding study (Fig. 3A). Rats fed with this HFD for 3 days develop hyperphagia and hypothalamic insulin resistance, and exhibit increased body weight gain after one week (24;25). Thus, this 3-day HFD model was chosen to evaluate whether DVC is a site of insulin resistance that contributes to the early onset of diet-induced obesity. The caloric intake of HFD fed rats was significantly greater than that of regular chow rats (171.8±4.3 vs. 119.5±3.5 kcal/day; p<0.001). Contrary to the acute DVC insulin response in rats fed with a regular chow diet (Fig. 1B), DVC insulin infusion failed to lower food intake in HFD rats (Fig. 3B). Together with the fact that 3-day HFD impairs the ability of DVC insulin to activate Erk1/2 (16), these findings indicate that HFD impairs DVC insulin signaling to lower appetite.

To better address whether bidirectional changes of DVC insulin-Erk1/2 signaling alter food intake and body weight, we disrupted DVC Erk1/2 signaling for 18 days or performed daily repeated acute DVC insulin infusion for 12 days in healthy rats.

**Molecular disruption of Erk1/2 signaling in the DVC.** We injected MEK1-DN or GFP viral control directly into the DVC of healthy rats and monitored daily food intake and body weight (Fig. 3C). Upon immunohistiochemical analysis of GFP injected rats, we first confirmed that the virus was localized to the DVC (mainly within the nucleus of the solitary tract) (Supplementary Fig.1). Strikingly, DVC MEK1-DN (vs. GFP) increased cumulative food intake starting at day 6 after viral injection (Fig. 3D) and such effect on food intake was sustained until day 18 (Fig. 3E). The increase in food intake of DVC MEK1-DN rats was associated with body weight gain that became significant 9 days after viral injection (Fig. 3F). The body weight of DVC MEK-DN rats remained elevated up until 18 days (Fig. 3F). No difference in the amount of water intake was detected between the groups (data not shown).

**Daily repeated acute infusion of insulin into the DVC.** Conversely, rats received daily repeated acute infusion of insulin (2 mU/μl) or saline into the DVC for 12 days (Fig. 4A). DVC insulin substantially reduced cumulative food intake (Fig. 4B) and body weight (fig. 4C), and had a strong tendency to lower total fat mass with the most significant reduction detected in visceral fat (Fig. 4D). Together with the loss-of-function experiments targeting Erk1/2 signaling, these gain-of-function experiments targeting DVC insulin signaling demonstrated that insulin-Erk1/2 signaling in the DVC regulates food intake and body weight.
DISCUSSION

We have first demonstrated that insulin action in the DVC lowers food intake in male rats. To date, the regulation of appetite by brain insulin action appears to be sex-specific as hypothalamic insulin administration in rats and intranasal insulin delivery in humans only lowers food intake in healthy males but not females (8;10;11). Similarly, leptin and GLP-1 receptor signaling in the DVC lowers feeding in male rats (17;18). Although we have yet to explore the sex-specificity of insulin action in the DVC, we do demonstrate that insulin given at 2 µU per site into the DVC is sufficient to not only lower glucose production in male rats (16), but also to lower food intake. This is in contrast to previous
findings in which a much higher insulin dose in the hypothalamus is required to regulate feeding than glucose homeostasis (8;23).

Also in contrast, insulin signals via a PI3K-independent and Erk1/2 signaling-dependent pathway in the DVC to lower glucose production (16). At a insulin dose that activates both Erk1/2 and PI3K-AKT in the DVC (16), we here report that it is still Erk1/2 signaling in the DVC that is necessary for insulin to lower feeding. Importantly, bidirectional changes of insulin-Erk1/2 signaling in the DVC for approximately two weeks are sufficient to alter food intake and body weight, strengthening the role of DVC insulin-Erk1/2 signaling. Together with a recent report that indicates DVC Erk1/2 signaling is necessary for leucine to regulate feeding (19), these findings collectively highlight the integrative and sufficient role of DVC Erk1/2 signaling in the regulation of energy balance.

The neuronal population that mediates DVC insulin to regulate feeding remains unknown. Given that both insulin and leptin signal through the PI3K in the hypothalamus to regulate feeding (1) and that leptin action in the DVC lowers feeding (17), future studies are warranted to begin addressing a potential overlap between insulin and leptin receptor co-expressing neurons in the DVC. Although the bilateral cannula was inserted to the DVC targeting the nucleus of the solitary tract, other regions such as the dorsal motor nucleus and the area postrema within the DVC could be possible targets of insulin action. Also, the role of PI3K signaling in the DVC remains unclear. Not only did chemical inhibition of DVC PI3K fail to negate the anorectic effect of insulin, it mimicked the effect of insulin, to lower food intake. This finding suggests basal PI3K activity in the DVC promotes feeding to maintain energy balance. Although this working hypothesis remains to be tested, it is noteworthy that enhanced PI3K activation in the ventromedial hypothalamic SFd1 neurons likewise promotes hyperphagia and obesity in mice (26). Of note, the role of IGF-1 receptor in the DVC in mediating the anorectic effect of insulin remains to be explored as insulin could interact with IGF-1 receptor (27).

HFD impairs the ability of DVC insulin infusion to lower food intake. This finding is of importance as it unveils an extra-hypothalamic site of insulin resistance to control feeding. Given that HFD disrupts the ability of insulin to activate Erk1/2 in the DVC (16) and that disruption of DVC Erk1/2 in healthy rats induces hyperphagia and obesity, future studies aimed at characterizing the signaling events involved in insulin-mediated Erk1/2 activation could prove useful to restore DVC insulin action to lower body weight gain in obesity. The mechanisms responsible for HFD-induced DVC insulin resistance may mirror the events of hypothalamic insulin resistance, with an induction of inflammation and ER stress being implicated in the development of insulin resistance (6). Lastly, it is possible that DVC insulin resistance increases food intake via alterations in hedonic food reward signaling, as intact brain insulin signaling is demonstrated to decrease food reward in non-obese.
humans and rodents (28;29). Future studies are needed to address these working hypotheses.

In summary, the current set of data unveils the DVC as a novel site of insulin action capable of lowering food intake and body weight and suggests that DVC insulin resistance can contribute to weight gain in obesity.

Acknowledgments

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No potential conflicts of interest relevant to this article were reported.
B.M.F. conducted and designed the experiments, performed data analyses, and wrote the manuscript. A.B., M.A.A., F.A.D., J.T.Y.Y. assisted with experiments. T.K.T.L. supervised the project, designed the experiments and edited the manuscript.

Figure Legends

Figure 1. Insulin administration into the DVC inhibits food intake. A. Experimental procedure and feeding protocol. B. Rats fasted for 6 hrs were infused with the reported amounts of insulin and saline into the DVC at 0.04 µl/min for 5 minutes (0.2 µl total). Food was returned and intake was measured every half hour for 4 h. Data are presented as mean ± SEM; n = 13 for saline, n = 11 for 2 mU/µl insulin and n = 5 for the other insulin concentration. * p value saline versus insulin 2mU/µl; # p value saline versus insulin 200 µU/µl; $ p value saline versus insulin 20 µU/µl; † p value saline versus insulin 2 µU/µl. 1 symbol p< 0.05, 2 symbols p<0.01, 3 symbols p<0.001. C. Experimental procedure and conditioned taste aversion protocol. D. Data are presented as % of saccharine over the total liquid ingested. Data are presented as mean + SE. n = 4 per group. * p< 0.05.
Figure 2. Inhibition of DVC Erk1/2 signaling negates the ability of insulin to lower food intake. Rats fasted for 6 hrs were infused with A. MAPK inhibitor (PD98059, 900 µM) into the DVC at 0.04 µl/min for 5 minutes (0.2 µl total). An hour later, rats were infused with insulin (2 mU/µl, 400 µU per site) or saline into the DVC at 0.04 µl/min for 5 minutes. Food was returned and intake was measured every half hour for 4 hrs. Data are presented as mean ± SEM; n = 13 for saline, n = 11 for insulin 2 mU/µl, n = 6 for insulin or saline with PD98059, n = 7 for insulin or saline with LY294002, n = 4 for insulin or saline groups with Wortmannin. * p value insulin versus saline; + p value insulin versus PD98059 + saline; # p value insulin versus PD98059 + insulin; $ p value saline versus LY294002 + saline; ^ p value saline versus LY294002 + insulin; ~p value saline versus Wortmannin + saline; & p value saline versus Wortmannin + insulin. B. Rats injected with an adenovirus overexpressing MEK1-DN or GFP into the DVC were fasted for 6 hrs and subsequently infused with insulin (2 mU/µl) or saline into the DVC at 0.04 µl/min for 5 minutes. Food was returned and intake was measured every half hour for 4 hrs. Data are presented as mean ± SEM; n = 6 for MEK1-DN with saline or insulin, n = 5 for GFP with saline or insulin. • p value GFP-insulin versus GFP-saline; ° p value GFP-insulin versus MEK1-DN-insulin; • p value GFP-insulin versus MEK1-DN-saline. C. Same protocol was used as panel A but PI3K inhibitors (LY294002, 500 µM or Wortmannin 20 µM) were infused instead. D. Rats fasted for 6 hrs were infused with various doses of LY294002, saline or insulin. Food was given back and food intake was measured every half hour for 4 h. Data are presented as mean ± SEM. n = 13 for saline; n = 11 for insulin at 2 mU/µl; n = 5 for various doses of LY294002 (5, 10, 50, 100 and 250 µM). * p value saline versus insulin 2 mU/µl; ~ p value saline versus 5 µM LY294002 + saline; + p value saline versus 10 µM LY294002 + saline; $ p value saline versus 50 µM LY294002 + saline; #p value saline versus 100 µM LY294002 + saline; ^ p value saline versus 250 µM LY294002 + saline. 1 symbol p< 0.05, 2 symbols p<0.01, 3 symbols p<0.001.

Figure 3. High-fat fed feeding negates the ability of DVC insulin to lower food intake while molecular disruption of DVC Erk1/2 signaling causes obesity. A. Experimental procedure and high-fat feeding protocol. B. Rats fasted for 6 hrs were infused with insulin (2 mU/µl) or saline into the DVC at 0.04 ul/min for 5 minutes. Food was returned and intake was measured every half hour for 4 hrs. Data are presented as mean ± SE. n = 5 per group. C. Experimental procedure and feeding protocol. Rats were injected with MEK1-DN or GFP virus into the DVC 3 days after brain surgery. D. Cumulative food intake from day 0 to day 9. E. Cumulative food intake from day 9 to day 18. F. Increase in body weight recorded over 18 days. Data are presented as mean + or − SEM; n = 8 for MEK1-DN; n = 9 for GFP. * p<0.05, ** p<0.01, *** p<0.001.
Figure 4. Daily repeated acute infusion of insulin into the DVC lowers food intake, body weight and fat deposition. A. Experimental procedure and feeding protocol. Rats were injected with insulin (2mU/µl) or saline everyday at 4 pm. B. Cumulative food intake recorded over a 12-day period. C. Relative daily increase in body weight over a 12-day period. E. Relative fat mass at end of 12-day period. Data are presented as mean ± or – SEM; n = 6 for insulin treated rats; n = 5 for saline treated rats. * p< 0.05, ** p<0.01, *** p<0.001.

References


Diabetes

Figure 1

191x157mm (300 x 300 DPI)
Figure 2
Figure 3

230x161mm (300 x 300 DPI)
Figure 4
### Supplementary Table 1

Food intake and body weight at 14h, 24h and 48h post DVC injections.

<table>
<thead>
<tr>
<th></th>
<th>Food Intake (g)</th>
<th>Body Weight (% increase)</th>
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<tbody>
<tr>
<td></td>
<td>14h</td>
<td>24h</td>
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<tr>
<td>DVC-saline (n=13)</td>
<td>33.35 ± 0.96</td>
<td>35.79 ± 0.95</td>
</tr>
<tr>
<td>DVC-Insulin 2 mU/µl (n=11)</td>
<td>28.12 ± 1.24</td>
<td>33.3 ± 4.16</td>
</tr>
<tr>
<td>DVC-Insulin 200 µU/µl (n=5)</td>
<td>27.18 ±3.40</td>
<td>29.44 ± 2.85</td>
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<tr>
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<td>29.2 ± 1.76</td>
<td>31.03 ± 2.1</td>
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<tr>
<td>DVC-PD9809+Saline (n=6)</td>
<td>29.13 ± 2.57</td>
<td>31.2 ± 1.95</td>
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<td>31.41 ± 1.21</td>
<td>32.11 ± 1.20</td>
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<tr>
<td>DVC-LY294002+insulin 400 µU/site (n=7)</td>
<td>24.741 ± 1.86</td>
<td>26.62 ± 2.31</td>
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The food intake of the indicated groups of rats was measured 14, 24 and 48 h after the DVC injection. The body weight is expressed as % increase over the body weight recorded at time 0 (i.e., before DVC injection).
Supplementary Figure 1. Localization of GFP adenovirus. Rats implanted with a bilateral DVC catheter were infected with the adenovirus expressing the GFP the day of the surgery. Rats were then subjected to cardiac perfusion with 4% paraformaldehyde. Paraformaldehyde-fixed DVC tissues were frozen in liquid nitrogen and stored at −80 °C until sectioning using a cryostat (Leica CM1950, Leica Microsystems, Nussloch, Germany) at −20 °C. Coronal sections of 10 μm were taken from the frozen tissues. For immunohistochemical staining, slides were washed in Tris-buffered saline (TBS) plus 0.025% Triton X-100 with gentle agitation. The cover slip was then mounted using ProLong Gold Antifade reagent with DAPI. Immunostaining was detected with a fluorescence microscope (QImaging, Olympus IX71). In the figure the GFP, Dapi and merge of the two staining are shown. The upper panels (A, B, C) show a snapshot of the squared red area in the scheme (G). The lower panels (D, E F) show a snapshot of the blue squared area in the scheme (G).