Supplemental Material

***TrpC5* is required for the distributed activation of neurons by leptin**

In addition to activity within melanocortin neurons, leptin activates neurons within the ventral premammillary nucleus (PMv); an effect attributed to leptin regulating the reproductive axis in response to metabolic and odorant stimuli (Leshan et al., 2009; Williams et al., 2011). PMv neurons that express *LepRs* were targeted from PLT mice (Sohn et al., 2011; Williams et al., 2014) to test the acute cellular effects of leptin (Supplemental Figures 2A to 2D). Similar to previous reports(Leshan et al., 2009; Williams et al., 2011), leptin (100 nM) depolarized 55.5 % (5 out of 9) of wildtype PMv neurons that express *LepRs* by 6.4 ± 0.7 mV (n=5, Supplemental Figure 2). We found 1 cell (5.9 %) that was hyperpolarized by -8 mV, while the remaining 3 cells (33.3 %) were not responsive to leptin (0.3 ± 0.3 mV, n=3). Analysis of current-voltage relationships revealed that leptin decreased input resistance by 23.3 ± 7.4 % (n=5, from 0.93 ± 0.1 GΩ in control ACSF to 0.73 ± 0.1 GΩ in leptin) with a reversal potential of -17.5 ± 5.2 mV (n=5). These results confirmed that leptin activates a non-selective cation conductance to depolarize PMv neurons what express *LepRs*.

In contrast, leptin failed to depolarize any of 10 PMv neurons that express *LepRs* from *TrpC*5 knockout (PLT5KO) mice (supplemental Figures 2F to 2H): 7 cells (70 %) remained unresponsive to leptin (-0.1 ± 0.1 mV), while 3 cells (30 %) were hyperpolarized by -7.3 ± 1.2mV. These data suggest that *TrpC*5 subunits underlie the leptin-induced activation of a non-selective cation conductance resulting in the depolarization of PmV neurons that express *LepRs*.

Acute leptin and insulin responses in *Pomc* cells are in distinct populations from that of *Ht2Cr*-expressing Pomc neurons

To further delineate the acute *Ht2Cr*-induced activity of *Pomc* cells, we specifically labeled *Ht2Cr*-expressing *Pomc* neurons using a transgenic approach. We generated *Pomc*-hrGFP:: *Ht2Cr*-cre::tdtomato (P2CT) reporter mice (see Experimental Procedures). These P2CT mice enabled identification of neurons expressing *Pomc*-hrGFP (green), *Ht2Cr*-cre::tdtomato (red), and *Pomc*-hrGFP:: *Ht2Cr*-cre::tdtomato (green/red) in the arcuate nucleus (Supplemental Figures 3A to E). *Pomc* neurons from P2CT mice were then examined for the acute effects of lorcaserin, leptin, and insulin as measured by whole-cell patch clamp electrophysiology.

As expected, lorcaserin failed to alter the membrane potential of *Pomc*-hrGFP (green) neurons that did not express the *Ht2Cr* (−0.4 ± 0.4mV; n=7). In current-clamp configuration, 11 of 21 (52.3 %) *Pomc*-hrGFP:: *Ht2Cr*-cre::tdtomato (green/red) neurons from P2CT mice were depolarized in response to lorcaserin (4μM; 8.24 ± 0.5mV, n=11, Supplemental Figure 3F). Consistent with previous reports and results in the present study, the lorcaserin-induced depolarization was accompanied by a 20.7 ± 6.6% decrease in input resistance (1467± 247MΩ in control ACSF to 1058± 161 MΩ in lorcaserin, n = 9; Supplemental Figure 3G). Moreover, extrapolation of the linear slope conductance revealed a reversal potential of −19.2 ± 1.7 mV (Supplemental Figure 3H). The membrane potential of the remaining *Pomc*-hrGFP:: *Ht2Cr*-cre::tdtomato (green/red) neurons remained unchanged (0.3 ± 0.4 mV, n=10) in response to lorcaserin.

Interestingly, 8 of 16 *Pomc*-hrGFP neurons not expressing *Ht2Cr*s (green cells) were depolarized by 6.3 ± 1.1 mV in response to leptin (Supplemental Figures 4A to C). The leptin-induced depolarization was accompanied by a 24.3 ± 3.9 % decrease in input resistance (from 1448 ± 147 MΩ resistance in control ACSF to 1095 ± 124 MΩ in leptin) with a reversal potential of −18.9 ± 1.3 mV. Similarly, 8 of 16 *Pomc*-hrGFP neurons not expressing *Ht2Cr*s (green cells)

were hyperpolarized by -7.4 ± 0.9 mV in response to insulin (Supplemental Figure 4D to F). The insulin-induced hyperpolarization was accompanied by a 25.2 ± 2.9 % decrease in input resistance (from 1323± 73 MΩ resistance in control ACSF to 1012 ± 45MΩ in insulin) with a reversal potential of −91.2 ± 2.1 mV. However, none of the *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato (green/red) neurons from P2CT mice responded to leptin (0.2 ± 0.3 mV, n=6; $t_{(12)} = 4.661$, $p < 0.05$) or insulin (0.3 ± 0.3 mV, n=6; $t_{(12)} = 7.124$, $p < 0.05$). Together, these data support the hypothesis that the acute effects of leptin, insulin, and serotonin are largely segregated in distinct arcuate *Pomc* neurons and that leptin and serotonin require *TrpC5* subunits for the activation of arcuate melanocortin neurons (Supplemental Figures 3I and 4G).

Neuron-specific *TrpC5* deficiency abrogates *Ht2Cr*-dependent improvements in glucose and insulin tolerance

Melanocortin signaling plays a critical role in the regulation of blood glucose levels; an effect that at least in part is dependent upon *Ht2Cr*s on Pomc neurons (Berglund et al., 2013; Nonogaki et al., 1998; Xu et al., 2010a). Moreover, lorcaserin has been suggested to improve blood glucose levels; however the cellular mechanism has largely been undefined. In order to examine the requirement of *TrpC*5 signaling in the *Ht2Cr*-dependent improvements of glucose and insulin tolerance, we examined lorcaserin-induced changes in blood glucose levels from *CamkIIα::Trpc5*$^{lox/Y}$, *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$, and *TrpC*5 knockout (*TrpC5*KO) mice.

Fed and fasted glucose levels were unchanged in *CamkIIα::TrpC5*$^{lox/Y}$, *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$, and *TrpC*5 knockout mice (Supplemental Figure 5). Similarly, *CamkIIα::TrpC5*$^{lox/Y}$ and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ displayed normal glucose tolerance; including glucose excursion and in the glucose incremental area under the curve (iAUC). However, *TrpC*5 knockout mice exhibited an improved glucose tolerance in response to a

glucose bolus suggesting a possible contribution of *TrpC5* subunits in the periphery to regulate glucose homeostasis (Supplemental Figure 5A). An insulin tolerance test (ITT) revealed no significant differences among the three groups (Supplemental Figure 6). To evaluate whether a single dose of lorcaserin can acutely alter glucose sensitivity in mice, we performed a glucose tolerance test (GTT) in lean mice that received lorcaserin (1.5mg/kg), a dose which fails to alter acute food intake (Figure 2). Lorcaserin (or vehicle) injected (I.P.) 45 min before the start of the GTT resulted in improved glucose tolerance of wildtype mice compared to littermate controls (Supplemental Figures 5B, 5F, and 5J). Importantly, lorcaserin failed to improve glucose tolerance in *TrpC*5 knockout, *CamkIIα::TrpC5*$^{lox/Y}$, and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice (Supplemental Figures 5C, 5G, and 5K). Similarly, the lorcaserin-induced improvement of glucose levels in response to an ITT were abrogated in *TrpC*5 knockout, *CamkIIα::TrpC5*$^{lox/Y}$, and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice (Supplemental Figure 6). These data support a *TrpC*5-dependent acute regulation of glucose metabolism by lorcaserin.

Supplemental Figures

Supplemental Figure 1 (related to Figures 3, 4, and 5). Electrophysiological characteristics of *Pomc* neurons deficient for *TrpC5* subunits. (A) Developmental and adult deletion of *TrpC5* subunits in *Pomc* neurons results in a hyperpolarized basal membrane potential. (B) and (C) *Pomc* neurons deficient for *TrpC5* subunits fail to depolarize to leptin or lorcaserin. (D) Summary of responses observed in response to leptin or lorcaserin.

Supplemental Figure 2 (related to Figure 3). *Trpc*5 subunits are required for the acute leptin-induced depolarization of leptin receptor expressing neurons of the PMV. (A) Brightfield illumination of *LepR-cre*::*tdtomato* neuron in the PMV from *PLT* mice. (B) The same neuron under Alexafluor 594 (*tdtomato*) illumination. (C) Complete dialysis of Alexa Fluor 350 from the intracellular pipette. (D) Merge image illustrates colocalization of tdtomato and Alexa Fluor 350 indicative of a *LepR* neuron of the PMV. (E) Electrophysiological study demonstrates a *LepR-cre*::*tdtomato* (red) neuron in the PMV from *PLT* mice that depolarized in response to leptin (100nM). (F) demonstrates a current clamp recording of a *LepR-cre*::*tdtomato*::*Trcp5*$^{lox/Y}$ (red) neuron in which leptin fails to induce a depolarization. (G) Histogram summarizing the acute effect of leptin on the membrane potential of *LepR* neurons in the PMV which express or do not express Trpc5 subunits (n= 9-10 per group). (H) Table of the electrophysiological responses to leptin from *LepR* neurons of the PMV which express or do not express Trpc5 subunits.

Supplemental Figure 3 (related to Figure 5). Lorcaserin Activates *Pomc*-hrGFP/ *Ht2Cr*-positive Neurons Via *TrpC5* Channel

(A-E) Identification of *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato cells for whole-cell patch-clamp recordings (*arrow* indicates the targeted cell. Scale bar =50 μm). (A) Brightfield illumination of *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato neuron from P2CT mice. (B)& (C) show the same neuron under FITC (hrGFP) and Alexa Fluor 594 (tdtomato) illumination. Complete dialysis of Alexa Fluor 350 from the intracellular pipette is shown in (D) and the merged image of the targeted *Pomc* neuron in (E). (F) Current-clamp recording demonstrates that application of lorcaserin (4μM) induces a depolarization of *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato neuron. Dashed line indicates the resting membrane potential. (G-H) The lorcaserin-decreased input resistance and its I-V relationship in *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato neuron.

(I) Images show the distribution of *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato neurons responded to lorcaserin from control mice.

Supplemental Figure 4 (related to Figures 3, 4, and 5). Distinct Effects of Leptin and Insulin on Separate *Pomc*-hrGFP/*Ht2Cr*-negative Neurons

(A) Electrophysiological recording demonstrates a leptin-induced depolarization of *Pomc*-hrGFP::*Ht2Cr*-negative neuron from P2CT mice. Dashed line indicates the resting membrane potential. (B-C) Decrease of input resistance and I-V relationship obtained from leptin-induced depolarization of *Pomc*-hrGFP::*Ht2Cr*-negative neurons. (D) Representative tracing shows that *Pomc*-hrGFP::*Ht2Cr*-negative neuron is hyperpolarized by insulin. (E-F) Decrease of input resistance and I-V relationship obtained from insulin-induced hyperpolarization of *Pomc*-hrGFP::*Ht2Cr*-negative neurons. (G) Images indicate the location of separate *Pomc*-hrGFP::*Ht2Cr*-negative neurons that responded to leptin and insulin.

Supplemental Figure 5 (related to Figures 2 and 5) Lorcaserin-induced activation of *Pomc* neurons improves systemic glucose sensitivity (A-L) Glucose tolerance test (GTT) performed in the presence of Saline or lorcaserin (1.5mg/kg, administered 45 min before glucose bolus) on (A-D) WT and TrpC5KO mice, (E-H) WT and *CamkIIα* –cre::*TrpC5*$^{lox/Y}$ mice, and (I-L) WT and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$. For (A)–(L), n = 8-16 per group; $^{*}p < 0.05$.

Supplemental Figure 6 (related to Figures 2 and 5) Lorcaserin-induced activation of Pomc neurons improves systemic insulin sensitivity (A-L) Insulin tolerance test (ITT) performed in the presence of Saline or lorcaserin (1.5mg/kg, administered 45min prior to insulin bolus) on (A-D) WT and TrpC5KO mice, (E-H) WT and *CamkIIα* –cre::*TrpC5*$^{lox/Y}$ mice, and (I-L) WT and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$. For (A)–(L), n = 9-15 per group; $^{*}p < 0.05$.

Supplemental Table 1 (related to Figure 3) Baseline resting membrane potential and action potential frequency of *Pomc* neurons from WT and Pomc-hrGFP::LepRcre::tdtomato (PLT) mice.

Supplemental Table 2 (related to Figure 3) Baseline resting membrane potential and action potential frequency of *Pomc* neurons from WT and Pomc-hrGFP::LepRcre::tdtomato::TrpC5KO (PLT5KO) mice.

Supplemental Table 3 (related to Figure 4) Baseline resting membrane potential and action potential frequency of *Pomc* neurons from WT and Pomc-hrGFP::HT2Cr-T2A-icre::tdtomato.

Supplemental Table 4 (related to Figure 5) Baseline resting membrane potential and action potential frequency of *Pomc* neurons from WT, *Pomc*-creER^{T2}::tdtomato, and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$::tdtomato mice.