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TrpC5 mediates acute leptin and serotonin effects via *Pomc* neurons

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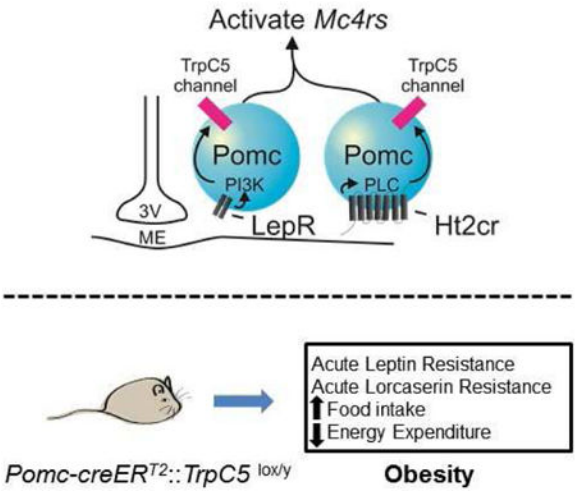
Abstract

The molecular mechanisms underlying acute leptin and serotonin 2C receptor induced hypophagia remain unclear. Here we show that neuronal and pro-opiomelanocortin (*Pomc*)-specific loss of transient receptor potential cation 5 (*TrpC*5) subunits is sufficient to decrease energy expenditure and increase food intake resulting in elevated body weight. Deficiency of *Trpc*5 subunits in *Pomc* neurons was also sufficient to block the anorexigenic effects of leptin and serotonin 2C receptor (*Ht2Cr*) agonists. The loss of acute anorexigenic effects of these receptors was concomitant with a blunted electrophysiological response to both leptin and *Ht2Cr* agonists in arcuate *Pomc* neurons. We also demonstrate that the *Ht2Cr* agonist lorcaserin-induced improvements in glucose and insulin tolerance were blocked by *TrpC5* deficiency in *Pomc* neurons. Together, our results link *TrpC*5 subunits in the brain with leptin- and serotonin 2C receptor-dependent changes in neuronal activity as well as energy balance, feeding behavior, and glucose metabolism.

Graphical abstract

TrpC5 subunits are essential for the negative energy balance associated with Pomc neuronal activation. TrpC5 subunits not only link the acute activities of leptin and serotonin receptors in Pomc neurons, but also modify direct effects on basal metabolism.TrpC5 subunits may provide an endogenous target to manipulate the activity of key neurons involved in the regulation of energy balance and glucose metabolism.



Keywords

melanocortin; obesity; diabetes; transient receptor potential cation channels; thermogenesis; glycemia; patch-clamp; electrophysiology; leptin; serotonin; lorcaserin

Introduction

Leptin receptors (*LepR*s) in the brain produce robust anti-obesity and anti-diabetic effects (Bjorbaek and Kahn, 2004; Coll et al., 2007; Elmquist et al., 1999; Flier, 2006; Friedman, 2004; Spiegelman and Flier, 2001). Similarly, the neurotransmitter serotonin (5-

hydroxytryptamine, 5-HT) contributes to the regulation of feeding behavior, energy expenditure and glucose homeostasis by acting via serotonin 2C receptors (*Ht2Crs*) within the CNS (Giorgetti and Tecott, 2004; Heisler et al., 2003). The hypothalamic arcuate pro-opiomelanocortin (*Pomc*) neurons are vital in mediating both leptin's and serotonin's beneficial effects on metabolism (Balthasar et al., 2004; Berglund et al., 2012; Sohn et al., 2011). Very recently, lorcaserin, an *Ht2Cr* specific agonist became the first FDA-approved weight-loss drug in the last 15 years independent of adverse cardiopulmonary effects. In order to better understand the effectiveness and safety of newly approved anti-obesity drugs it is imperative to delineate cellular mechanisms underlying *Ht2Cr* and *LepR* activity in the brain.

There is an emerging role for *TrpC* channels in the regulation of energy homeostasis by leptin (Qiu et al., 2010). Similarly, activation of *Pomc* neurons by *Ht2Cr*s is mediated by a phospholipase C (*PLC*)-dependent activation of putative *TrpC* channels (Sohn et al., 2011). Single cell RT-PCR of arcuate *Pomc* neurons revealed that *TrpC* 1,4, and 5 subunits predominate in murine *Pomc* neurons (Qiu et al., 2010). However, *TrpC*1 alone may not be sufficient to form a functional ion channel (Strubing et al., 2001), suggesting that *TrpC4 or TrpC5* may be a common target in *Pomc* neurons of these two potent anorexigenic signals, leptin and serotonin. In the current study, we studied the requirement of *TrpC*5 in the acute effects of leptin and serotonin to depolarize hypothalamic *Pomc* neurons. We also examined the requirement of *TrpC*5 subunits in the acute effects of leptin and serotonin on energy expenditure as well as glucose homeostasis.

Results

Neuronal *TrpC*5 subunits are required for proper energy balance

Disturbance of acute leptin or *Ht2Cr* signaling pathways in *Pomc* neurons is correlated with blunted acute anorexia by leptin and serotonin 2C receptor-agonists as well as deficits in energy and glucose homeostasis. (Hill et al., 2008; Xu et al., 2008; Xu et al., 2010b). We hypothesized that leptin and *Ht2Cr*s may acutely activate *TrpC*5 subunits within *Pomc* neurons thus being required *in vivo* for the feeding effects of these receptors as well as regulating energy balance and glucose homeostasis. To determine the metabolic effects of *TrpC*5 subunits within the CNS during postnatal development, we generated a conditional mouse model ($TrpC5^{lox/Y}$) in which we could selectively reduce the expression of *TrpC*5 subunits in key brain sites. This was accomplished by utilizing *TrpC5*-targeted ESCs from the European Union Conditional Mouse Mutagenesis (EUCOMM) program in order to generate conditional $TrpC5^{lox/y}$ mice via the insertion of *loxP* sites surrounding exon 5 of the *TrpC5* gene (Figure 1A). Subsequent breeding to ZP3-cre (Figure 1B), *CamkIIα-cre or Pomc*-creER^{T2} mice generated mice deficient for *TrpC5* in a cell/tissue-type dependent manner.

When fed a chow diet, neuron- [*CamkIIα* –cre; in which Cre recombinase is expressed widely in the forebrain and hindbrain (Casanova et al., 2001)] or Pomc- [*Pomc*-creER^{T2}-cre -allows temporal control of *cre* recombinase activity in *Pomc* neurons (Berglund et al., 2013)] specific deficiency of *TrpC*5 subunits resulted in an age-dependent increase in body weight (Figures 1C and 1D). The increased body weight was more pronounced in mice

deficient for *TrpC*5 subunits in *Pomc* neurons alone (Figure 1D). Age and weight matched *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ males were hypometabolic, as demonstrated by significant decreases in energy expenditure (Figures 1E-1H) independent of altered activity levels (Figure 1I). Components of total energy expenditure include energy required for physical activities and basal metabolism. In particular, *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice exhibited decreased heat production (Figure 1H) suggestive of decreased metabolic rate. *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice also showed increased ad libitum food intake (Figure 1J).

Neuron- and *Pomc*-specific *TrpC5* deficiency blunts the acute anorexia by *LepR* and *Ht2Cr* activation

Wildtype, *TrpC5*KO (Riccio et al., 2009) and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice were fasted overnight and intraperitoneally (i.p.) injected with either saline or leptin (5 mg/kg). Consistent with previous reports (Hill et al., 2008), i.p. injections of leptin reduced food intake in wildtype mice (at 1h) compared to the saline-injected group (Figures 2A and 2B). However, i.p. injections of leptin failed to decrease food intake in *TrpC5*KO and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice (at 1h) versus the saline-injected group (Figures 2A and 2B).

We performed a similar series of experiments with both the non-selective *Ht2Cr* agonist (***meta*-Chlorophenylpiperazine** – mCPP) (3 mg/kg) and lorcaserin (1, 3, and 6 mg/kg). As reported previously, the *Ht2Cr* agonists significantly reduced food intake in wildtype mice versus saline-injected groups (at 1h, 4h, and/or 6h Figures 2C-2F). However, i.p. injections of mCPP or lorcaserin failed to suppress food intake (at 1h, 4h, and/or 6h) in *TrpC5*KO, *CamkIIα*::*TrpC5*$^{lox/Y}$ and/or *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice compared to saline injections. Of note 3mg/kg of lorcaserin appears to be a threshold dose for the acute biological activity of food intake. Taken together, *TrpC*5 subunits are required in *Pomc* neurons for the acute anorexia induced by leptin receptor and *Ht2Cr* activation.

*TrpC*5 subunits are required for the acute activation of *Pomc* neurons by leptin

Whole-cell patch-clamp recordings were made in 58 arcuate *Pomc* neurons from *Pomc*-hrGFP mice (Parton et al., 2007; Ramadori et al., 2010; Sohn et al., 2011). 26 *Pomc* neurons were from wildtype and, 32 were in *Pomc* neurons deficient for *TrpC5* subunits (Riccio et al., 2009). *Pomc* neurons from wildtype mice (n= 26) had a resting membrane potential of −41.5 ± 1.3 mV, a mean input resistance of 1260 ± 80 MΩ, and overshooting action potentials. When compared to values obtained from wildtype *Pomc* neurons, *Pomc* neurons deficient for *TrpC 5* exhibited a hyperpolarized mean resting membrane potential (−46.3± 0.9 mV; 1085 ± 53 MΩ; n=32; $p<0.05$; supplemental figure 1A). Analogous results were obtained in *Pomc* neurons from *Pomc*-creER^{T2} vs *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice (RMP = -44.3 ± 1.0mV from wildtype Pomc neurons, n=24; and RMP = -48.0 ± 1.4mV in *Pomc* neurons from *Pomc*-creER^{T2}::*Trpc5*$^{lox/Y}$ mice n = 22; $p<0.05$; supplemental figure 1A).

Similar to previous reports (Hill et al., 2008; Sohn et al., 2011), *Pomc* neurons that express *LepRs* were targeted from *Pomc*-hrGFP::*LepR*-cre-tdtomato (PLT) mice (Sohn et al., 2011; Sun et al., 2016; Williams et al., 2014) to test the acute cellular effects of leptin (Figures 3A to 3E). Leptin (100 nM) depolarized 70.6 % (12 out of 17) of wildtype *Pomc* neurons that

express *LepRs* by 6.7 ± 0.5 mV (n=12, Figures 3F, 3J and supplemental table 1). We found 1 cell (5.9 %) that was hyperpolarized by -10 mV, while the remaining 4 cells (23.5 %) were not responsive to leptin (0.3 ± 0.3 mV, n=4). Analysis of current-voltage relationships revealed that leptin decreased input resistance by 21.4 ± 3.0 % (n=12, from 1.2 ± 0.1 GΩ in control ACSF to 0.9 ± 0.1 GΩ in leptin) with a reversal potential of -26.0 ± 2.2 mV (n=12) (Figures 3G and 3H). These results confirmed that leptin activates a non-selective cation conductance to depolarize *Pomc* neurons.

In contrast, leptin failed to depolarize any of 15 *Pomc* neurons which express *LepRs* from *TrpC*5 knockout (PLT5KO) mice (Figures 3I, 3J and supplemental table 2): 14 cells (93.3 %) remained unresponsive to leptin (-0.1 ± 0.5 mV), while 1 cell (6.7 %) was hyperpolarized by -9 mV. Analogous results were obtained in recordings from *Pomc* neurons selectively deficient for *TrpC5* subunits (from *Pomc*-creER^{T2}::*TrpC5* mice; supplemental figure 1B). These data suggest that *TrpC*5 subunits underlie the leptin-induced activation of a non-selective cation conductance resulting in the depolarization of *Pomc* neurons. Notably, the hyperpolarizing effects of leptin remained unchanged in *Pomc* neurons lacking *TrpC*5 subunits and these effects were accompanied by decreased input resistance with a reversal potential close to E_K. Similar results were obtained in ventral premammillary nucleus (PMv) neurons which express leptin receptors (supplemental material and supplemental figure 2). Thus, the null *TrpC5* gene does not affect the functional expression of leptin receptors or other channels in our model.

After recordings, the sections were fixed in formalin to determine the location of recorded cells within the arcuate nucleus, as described previously (Sohn et al., 2011). Since acute leptin responses show a distinct distribution pattern (Williams et al., 2010), we targeted cells of similar region within the arcuate nucleus in both wildtype and *TrpC*5 knockout mice (Figure 3K).

The acute mCPP-induced activation of *Pomc* neurons requires *TrpC*5 subunits

To test the acute cellular effects of *Ht2Cr*s, we targeted *Pomc* neurons that do not express *LepR*s from PLT mice (Figures 4A to 4E), as previously described (Sohn et al., 2011). We confirmed that mCPP (4 μM) depolarized 55 % (5 out of 9) of wildtype *Pomc* neurons that do not express *LepR*s by 6 ± 0.6 mV (n=5, Figures 4F, 4J, and supplemental table 1). The remaining 4 cells (45 %) were not responsive to mCPP (0.4 ± 0.2 mV, n=4). Application of current steps revealed that mCPP decreases input resistance by 14.8 ± 4.8 % (n=5, from 1.5 ± 0.1 GΩ in control ACSF to 1.3 ± 0.1 GΩ in mCPP) with a reversal potential of -14 ± 4.8 mV (n=5) (Figures 4G and 4H). In contrast, mCPP failed to depolarize all *Pomc* neurons tested from *Trpc*5 knockout (PLT5KO) mice (0.1 ± 0.2 mV, n=19, Figures 4I, 4J, and supplemental table 2). Similar to the acute leptin effects in the current study, we targeted cells in similar region and responses were mapped within the arcuate nucleus (Figure 4K).

The lorcaserin-induced depolarization of *Pomc* neurons is dependent upon *TrpC*5

We previously confirmed that the acute effects of mCPP on *Pomc* neurons were mediated by *Ht2Cr*s (Berglund et al., 2013; Xu et al., 2010a). Thus, the observed cellular effects of mCPP in the present study should depend upon *Ht2Cr* activation, and the lack of mCPP effects on

Pomc neurons from the *TrpC*5 knockout mice indicate that *Trpc*5 is required for the acute activation of *Pomc* neurons by *Ht2Cr*s.

However, to further examine this activity, we used lorcaserin (a specific *Ht2Cr* agonist recently approved for chronic weight management). Similar to results obtained with mCPP, lorcaserin (4 μM) depolarized 50 % (6 out of 12) of wildtype *Pomc* neurons that do not express *LepRs* by 7.0 ± 0.7 mV (n=6, Figures 5A, 5E, and supplemental table 1). The remaining 6 cells (50 %) were not responsive to lorcaserin (0.3 ± 0.2 mV, n=6). Application of current steps confirmed that lorcaserin decreased the input resistance by 25.6 ± 6.5 % (n=6, from 1.6 ± 0.1 GΩ in control ACSF to 1.2 ± 0.2 GΩ in lorcaserin) with a reversal potential of -13.1 ± 3.2 mV (n=6) (Figures 5B and 5C). Similar results were observed in *Pomc* neurons which express *Ht2Cr*s (from *Pomc*∷ *Ht2Cr*-cre∷tdtomato [P2CT] mice; supplemental material, supplemental figures 3, 4, and supplemental table 3).

As expected, lorcaserin failed to depolarize any of 12 *Pomc* neurons from *TrpC*5 knockout (PLT5KO) mice (Figures 5D, 5E, and supplemental table 2). 11 cells (91.7 %) remained unresponsive to lorcaserin (0.4 ± 0.2 mV), while 1 cell (8.3 %) was hyperpolarized by -8 mV. A summary of the location of targeted cells was mapped throughout the rostro-caudal extent of the arcuate nucleus (Figure 5F). Similar results were obtained in recordings from *Pomc* neurons selectively deficient for *TrpC5* subunits (from *Pomc*-creER^{T2}∷*TrpC5* mice; supplemental figure 1C and supplemental table 4). These data confirm that *TrpC*5 subunits underlie the *Ht2Cr*-mediated activation of non-selective cation conductance and the depolarization of *Pomc* neuron membrane potential.

Discussion

Neuronal or *Pomc*-specific *TrpC*5 deficiency resulted in a positive energy balance which contributed to excess weight gain. In addition to these physiological aberrations, global deficiency of *TrpC*5 as well as neuron- or *Pomc*-specific deficiency of *TrpC*5 alone abrogated the acute anorexigenic effects of both leptin and serotonin 2C receptor agonists. *TrpC*5 subunits were also required for the acute effects of lorcaserin to improve both glucose and insulin tolerance (Supplemental Figures 5 and 6). On a cellular level, deficiency of *TrpC*5 subunits abrogates the acute effects of both leptin and serotonin 2C receptors to activate arcuate *Pomc* neurons. Together, these data demonstrate that *TrpC*5 subunits in arcuate *Pomc* neurons are important regulators of the pharmacological effects of both leptin and serotonin as well as link critical molecular mechanisms with recently developed anti-obesity pharmacotherapeutics, such as lorcaserin.

Multiple signaling cascades are activated in response to leptin receptor activation. In particular, phosphoinositol 3 kinase (*PI3K*) has been suggested to be required for the acute effects of leptin to stimulate *Pomc* neuronal activity via a putative activation of *TrpC* channels (Al-Qassab et al., 2009; Hill et al., 2008; Qiu et al., 2010). Pharmacological inhibition of *PI3K* or targeted disruption of *PI3K* regulatory subunits in *Pomc* neurons alone blunted the suppression of feeding elicited by central leptin administration (Hill et al., 2008; Niswender et al., 2001). Moreover, mice deficient for *PI3K* catalytic activity exhibited central leptin resistance, increased adiposity, diet-induced obesity, and impaired glucose

regulation (Al-Qassab et al., 2009; Hill et al., 2009). Importantly, these data are analogous to the observations detailed in the current study, supporting a role for the *PI3K-TrpC*5 pathway in the acute and chronic regulation of energy balance and glucose homeostasis.

It should be noted that, a previous report demonstrated that global deficiency of *TrpC*5 subunits (*Trpc5*KO) fails to alter body weight (Riccio et al., 2009). However, numerous reports have demonstrated that global deficiency of various genes may result in compensatory adaptation (Luquet et al., 2007; Zeltser et al., 2012). In the current study, global *TrpC5* deficiency resulted in improved glucose tolerance whereas neuronal- or *Pomc*-specific loss of *TrpC5* failed to alter basal glucose tolerance (Supplemental Figures 5 and 6). Despite the improved glucose tolerance, global *TrpC5* deficiency did not improve insulin tolerance, suggesting that *Trpc5* subunits might ameliorate only specific components of the glucose-stimulated insulin response. Together, these data may suggest that inhibition of *TrpC5* subunits in the periphery (possibly including *TrpC5* subunits in adipose tissue) may improve glucose homeostasis; however loss of *TrpC5* subunits in *Pomc* neurons might impair energy balance and blunt targeted improvements in glucose metabolism. While these data support possible mechanisms for improved energy balance and glucose metabolism via *TrpC5* subunits, they also highlight the utility of cell type targeted strategies to better identify roles for cellular signaling in energy balance and glucose metabolism.

Another salient finding is that *TrpC*5 subunits in *Pomc* neurons alter basal metabolism and feeding behavior. Several reports using chemo- and opto- genetic strategies have highlighted the role of acutely modulating melanocortin cellular activity and resulting changes in feeding behavior and body weight (Aponte et al., 2011; Zhan et al., 2013). However, identification of humoral signals linked to endogenous signaling cascades and channels which may acutely alter metabolism has remained undefined. Moreover, leptin's and serotonin's acute beneficial effects on metabolism are due at least in part to the acute activity of melanocortin neurons. In the current study we propose that activation of *TrpC5* in arcuate *Pomc* neurons, as occurs in response to *LepRs* or *Ht2Crs*, represents a physiological cellular correlate to the light/chemical-induced activation of channelrhodopsins/DREADDs in *Pomc* neurons previously demonstrated to stimulate a negative energy balance (Aponte et al., 2011; Zhan et al., 2013). Moreover, in addition to the pharmacological activity of leptin and *Ht2Cr* agonists; *Trpc*5 subunits contribute to a basal activity of melanocortin neurons which alters metabolic rate and feeding behavior.

Ht2Crs are known to regulate energy homeostasis and body weight. This regulatory impact is perhaps most notable due to the anti-obesity drug d-fenfluramine (d-Fen) which was identified to act via *Ht2Cr* (Vickers et al., 1999; Xu et al., 2008). However, the cellular mechanism required for *Ht2Cr*-expressing neurons to mediate effects on energy homeostasis remains unclear. This is topical due to the recent approval by the FDA of the anti-obesity drug, lorcaserin, which targets *Ht2Cr* for chronic weight management (Harlan et al., 2011; Martin et al., 2011). Additionally, other *Ht2Cr* agonists are also being tested in humans to treat obesity further reinforcing a need for mechanistic insights on how these compounds exert their effects (Rodgers et al., 2012). Importantly, lorcaserin has also been associated with improvements of blood glucose levels in patients with type 2 diabetes (O'Neil et al., 2012). Notably, in the current study lorcaserin-induced acute improvements in glucose and

insulin tolerance were observed and required *TrpC*5 subunits in *Pomc* neurons. This observation suggests potential weight-independent effects of lorcaserin and *TrpC* channels on blood glucose levels, which should be studied further.

It should be noted that in addition to classic neuropeptides (α-MSH, β-endorphin, NPY, AgRP, etc.), there is an emerging role for the fast acting neurotransmitters (glutamate and GABA) in regulating energy balance and glucose metabolism(Krashes et al., 2014; Liu et al., 2012; Pinto et al., 2004; Tong et al., 2008; Yang et al., 2011). Arcuate *Pomc* neurons co-express glutamate (Collin et al., 2003; Kiss et al., 2005); independent of NPY/AgRP and to a variable extent GABA (Hentges et al., 2004; Hentges et al., 2009; Horvath et al., 1997; Ovesjo et al., 2001; Wittmann et al., 2013; Yee et al., 2009). It's currently unclear how *TrpC5* dependent activation of arcuate *Pomc* neurons might differentiate between the release of either neurotransmitters or neuropeptides. Similarly, recent work suggest a sensory component of Pomc or NPY/AgRP neuronal activity which is likely independent of endogenous peptidergic signals (Betley et al., 2015; Chen et al., 2015). Although currently unclear, involvement of pharmacological levels of leptin and serotonin 2C receptor agonists might act on these sensory cues influencing feeding behavior. Thus the role of either neurotransmitters or neuropeptides in the *Pomc* specific *TrpC5* dependent regulation of metabolism warrants further investigation.

In summary, activation of arcuate *Pomc* neurons results in a negative energy balance (Aponte et al., 2011; Zhan et al., 2013). Numerous peptides and neurotransmitters have been demonstrated to acutely stimulate the activity of arcuate *Pomc* neurons, including leptin and serotonin. Although several signaling mechanisms have been identified, the channel required for these activities has remained largely undefined. In the current study we demonstrate that *TrpC*5 subunits are essential for the negative energy balance associated with *Pomc* neuronal activation. *TrpC*5 subunits not only link the acute activities of leptin and serotonin receptors in Pomc neurons, but also modify direct effects on basal metabolism. Similar to opto- and chemo-genetic strategies, *TrpC*5 subunits may provide an endogenous target to manipulate the activity of key neurons involved in the regulation of energy balance and glucose metabolism.

Methods

Animals

Male (4- 16-week-old) pathogen-free *CamkIIα*-cre, *Pomc*-creER^{T2}, *Pomc*-hrGFP::*LepR*-cre::tdtomato (PLT), *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato (P2CT), *TrpC5KO,* and *TrpC5*$^{fl/y}$ (described below) mice (Berglund et al., 2013; Parton et al., 2007; Ramadori et al., 2010; Riccio et al., 2009; Sohn et al., 2011; Sun et al., 2016) were used for all experiments. All mice were housed under standard laboratory conditions (12 hr on/off; lights on at 7:00 a.m.) and temperature-controlled environment with food and water available ad libitum. All experiments were performed in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals, and approved by the University of Texas Institutional Animal Care and Use Committee.

Generation of a cre-conditional TrpC5 allele (*TrpC5*$^{fl/y}$)

The *TrpC5* targeted clone obtained from EUCOMM was injected into blastocysts to obtain highly chimeric mice. These mice were then bred to obtain germline transmission of the targeted *TrpC5* allele. The *TrpC5*$^{lox/Y}$ mice were generated following flp-mediated removal of the neo cassette leaving two *lox*P sequences flanking exons 5 of the *TrpC5* gene. Deletion of exon 5 creates a frameshift mutation.

Tamoxifen treatment to induce adult-onset ablation of *TrpC5* in *Pomc* neurons

Tamoxifen (0.15mg/g; Sigma-Aldrich) dissolved in corn oil (Sigma-Aldrich) was administered i.p. for 5 consecutive days to 6-weekold male *TrpC5*$^{fl/y}$ mice (controls) and *TrpC5*$^{fl/y}$ × *Pomc*-cre:*ERT2* littermate mice. Corn oil was used as a vehicle control.

Electrophysiology

Whole-cell patch-clamp recordings from *Pomc*-hrGFP neurons with or without leptin receptors were maintained in hypothalamic slice preparations and data analysis were performed as previously described (Hill et al., 2008). Briefly, 4- to 16-week-old male mice were anesthetized and transcardially perfused with a modified ice-cold artificial CSF (ACSF) (described below), in which an equiosmolar amount of sucrose was substituted for NaCl. The mice were then decapitated, and the entire brain was removed, and immediately submerged in ice-cold, carbogen-saturated (95% O2 and 5% CO2) ACSF (126 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl2, 2.5 mM CaCl2, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 5 mM glucose). Coronal sections (250 μm) were cut with a Leica VT1000S Vibratome and then incubated in oxygenated ACSF at room temperature for at least 1 hr before recording. Slices were transferred to the recording chamber and allowed to equilibrate for 10–20 min before recording. The slices were bathed in oxygenated ACSF (32°C–34°C) at a flow rate of ~2 ml/min.

The pipette solution for whole-cell recording was modified to include an intracellular dye (Alexa Fluor 594 or Alexa Fluor 350) for whole-cell recording: 120 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 5 mM EGTA, 1 mM CaCl2, 1 mM MgCl2, and 2 mM MgATP, 0.03 mM Alexa Fluor 594 or Alexa Fluor 350 hydrazide dye (pH 7.3). Epifluorescence was briefly used to target fluorescent cells, at which time the light source was switched to infrared differential interference contrast imaging to obtain the whole-cell recording (Zeiss Axioskop FS2 Plus or Nikon FN1 equipped with a fixed stage and a QuantEM:512SC electron-multiplying charge-coupled device camera). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices), low-pass filtered at 2–5 kHz, and analyzed offline on a PC with pCLAMP programs (Molecular Devices). Recording electrodes had resistances of 2.5–5 MΩ when filled with the K-gluconate internal solution. Input resistance was assessed by measuring voltage deflection at the end of the response to a hyperpolarizing rectangular current pulse steps (500 ms of −10 to −50 pA).

Leptin (100 nM; provided by A.F. Parlow, through the National Hormone and Peptide Program), mCPP (4 μM, Sigma Aldrich), and Lorcaserin (4 μM, provided by Kathryn Cunningham), were added to the ACSF for specific experiments. Solutions containing leptin, mCPP, or lorcaserin were typically perfused for 2–4 min. A drug effect was required to be

associated temporally with peptide application, and the response had to be stable within a few minutes. A neuron was considered depolarized or hyperpolarized if a change in membrane potential was at least 2 mV in amplitude.

Analyses of leptin-, mCPP- and lorcaserin-induced hypophagia

Leptin (5 mg/kg; provided by A.F. Parlow, through the National Hormone and Peptide Program)), mCPP (3 mg/kg; Sigma-Aldrich), lorcaserin (1, 3, and 6 mg/kg; proved by Kathryn Cunningham), and vehicle (sterile saline) were administered i.p. in a counterbalanced manner to chow-fed 18-hour overnight-fasted mice as previously described (Berglund et al., 2013; Williams et al., 2014; Xu et al., 2010b). Food intake was measured hourly for 6 hours and then a single measurement at 24 hours.

Analyses of lorcaserin-induced alterations in glucose tolerance test (GTT) and insulin tolerance test (ITT)

For GTTs mice were intraperitoneally injected with 1.5 g/kg body weight of dextrose after an overnight fast. Mice were injected with lorcaserin (1.5 mg/kg) or vehicle 45 min prior to glucose injection. We drew blood samples at 0, 15, 30, 60, 90, and 120 min time points and measured glucose levels with a Bayer contour glucometer.

For ITTs, mice were fasted for 3 hours with water ad libitum. Mice were injected with lorcaserin (1.5 mg/kg) or vehicle 45 min prior to insulin injection. After measurement of basal levels of glucose, insulin (1.2 U/kg, Eli Lilly and Company) was administrated i.p. Blood glucose levels were monitored at given time points after insulin injection.

Statistics

Statistical analysis was carried out using GraphPad 5 (GraphPad) software. All data were evaluated using a 2-tailed Student's *t* test or ANOVA where appropriate with a *P* value of less than 0.05 being considered significant. In all instances, data are presented as mean ± SEM. Degrees of freedom (DF) for *t* statistics are marked as $t_{(DF)}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Leptin- and serotonin-induced activation of arcuate Pomc neurons requires TrpC5 subunits
2. Acute effects of leptin and serotonin on food intake require TrpC5 subunits
3. TrpC5 subunits are a physiological mechanism in the CNS to regulate metabolism

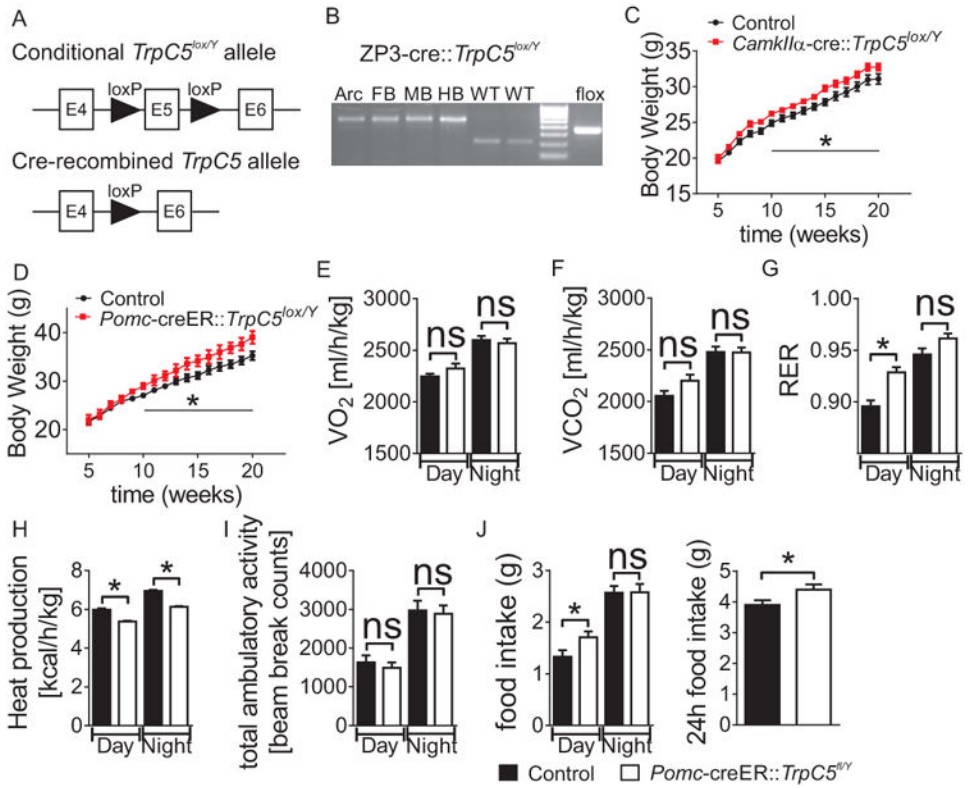

Figure 1.

Body weight and metabolic assessment of male WT and *Pomc*-creER^{T2}::*TrpC5*$^{lox/Y}$ mice on chow diet. (A) Schematic of genomic DNA region around exon 5 of *TrpC5* in mice carrying a targeted *TrpC5* allele (*TrpC5*$^{lox/Y}$). After Cre-mediated excision of exon 5, a PCR amplification product identifying the deleted *TrpC5* allele (*TrpC5*$^{lox/Y}$) becomes detectable. (B) PCR amplification products from genomic DNA using primers detect wild type, floxed, and deleted *TrpC5*. Tissues were dissected from a ZP3-cre::*TrpC5*$^{lox/Y}$ mouse. From left to right; lane 1 Arcuate nucleus (positive for recombination); lane 2 forebrain (positive for recombination); lane 3 midbrain (positive for recombination); lane 4 hindbrain (positive for recombination); lane 5 and 6 wildtype hypothalamus (negative for recombination); lane 6 DNA ladder; and lane 7 hypothalamus from floxed mouse negative for ZP3-cre. Body weight curve of (C) male *CamkIIα*-cre::*TrpC5*$^{lox/Y}$ and (D) male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice (*p<0.05). (E-I) Depicts (E) unchanged oxygen consumption - VO_2, (F) unchanged carbon dioxide production - VCO_2, (G) increased respiratory exchange ratio - RER (H) decreased heat production, and (I) unchanged ambulatory activity in male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice. (J) Male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice exhibited increased food intake in the light cycle which resulted in hyperphagia over 24h. For (A)–(I), n = 8-15 per group; *p < 0.05. Error bars indicate SEM. Note: mice used in (E-I) were age-matched male littermates (8 weeks of age), and had comparable body weight and lean mass.

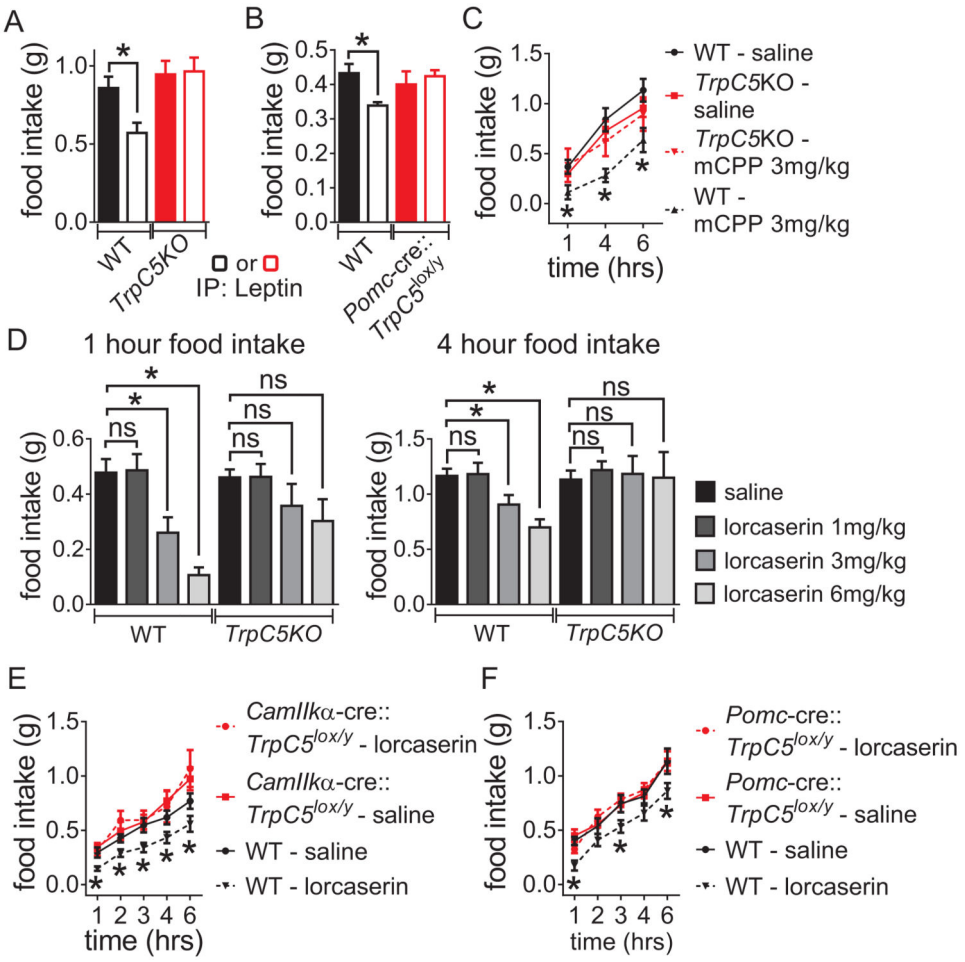

Figure 2.

Leptin-, mCPP-, and lorcaserin-induced hypophagia is blunted in mice deficient for *TrpC5* subunits. (A) and (B) Food consumption was measured 1 hour after leptin administration (5mg/kg, i.p.) and compared with food consumption in each animal following saline administration. (C) and (D) Cumulative food intake measured at 1, 4 and/or 6 hours after administration of *Ht2Cr* agonists mCPP (3mg/kg) or lorcaserin (1, 3, or 6mg/kg). (E) and (F) Food intake measured in response to lorcaserin (3mg/kg, i.p.). For (A)–(F), n = 9-14 per group; *p < 0.05.

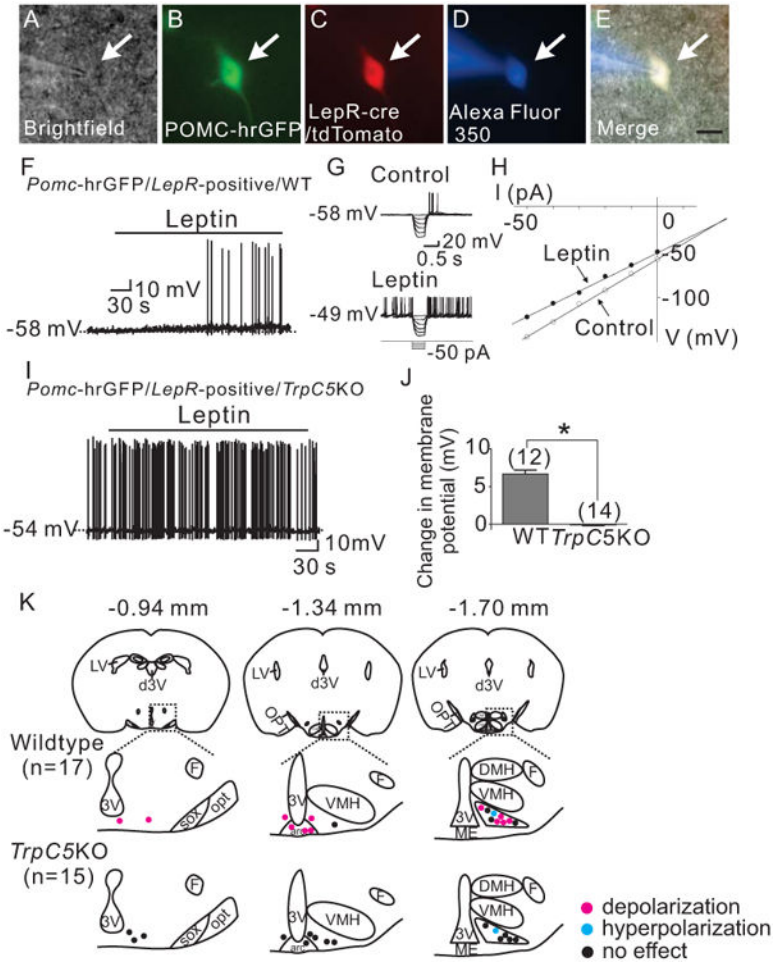

Figure 3.

*Trpc*5 subunits are required for the acute leptin-induced depolarization of arcuate *Pomc* neurons. (A) Brightfield illumination of *Pomc*-hrGFP::*Lepr*-cre::*tdtomato* neuron from *PLT* mice. (B) and (C) The same neuron under FITC (hrGFP) and Alexafluor 594 (*tdtomato*) illumination. (D) Complete dialysis of Alexa Fluor 350 from the intracellular pipette. (E) Merge image illustrates colocalization of hr-GFP, *tdtomato*, and Alexa Fluor 350 indicative of a *Pomc* neuron which expresses *Lepr*s. (F) Electrophysiological study demonstrates a *Pomc*-hrGFP::*Lepr*-cre::*tdtomato* (green/red) neuron that is depolarized in response to leptin (100nM). (G) Traces showing decreased voltage deflection and increased action potential frequency after leptin application. (H) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to leptin application. Shown are responses before (control) and during leptin application. (I) demonstrates a current clamp recording of a *Pomc*-hrGFP::*Lepr*-cre::*tdtomato*::*TrpC5KO* (green/red) neuron in which leptin fails to induce a depolarization. (J) Histogram summarizing the acute effect of leptin on the membrane potential of *Pomc* neurons which express leptin receptors as well as express or do not express Trpc5 subunits (n= 12-14 per group). (K) Rostro-caudal and medio-lateral distribution of electrophysiological responses to leptin from *Pomc* neurons which express leptin receptors as well as express or do not express Trpc5 subunits.

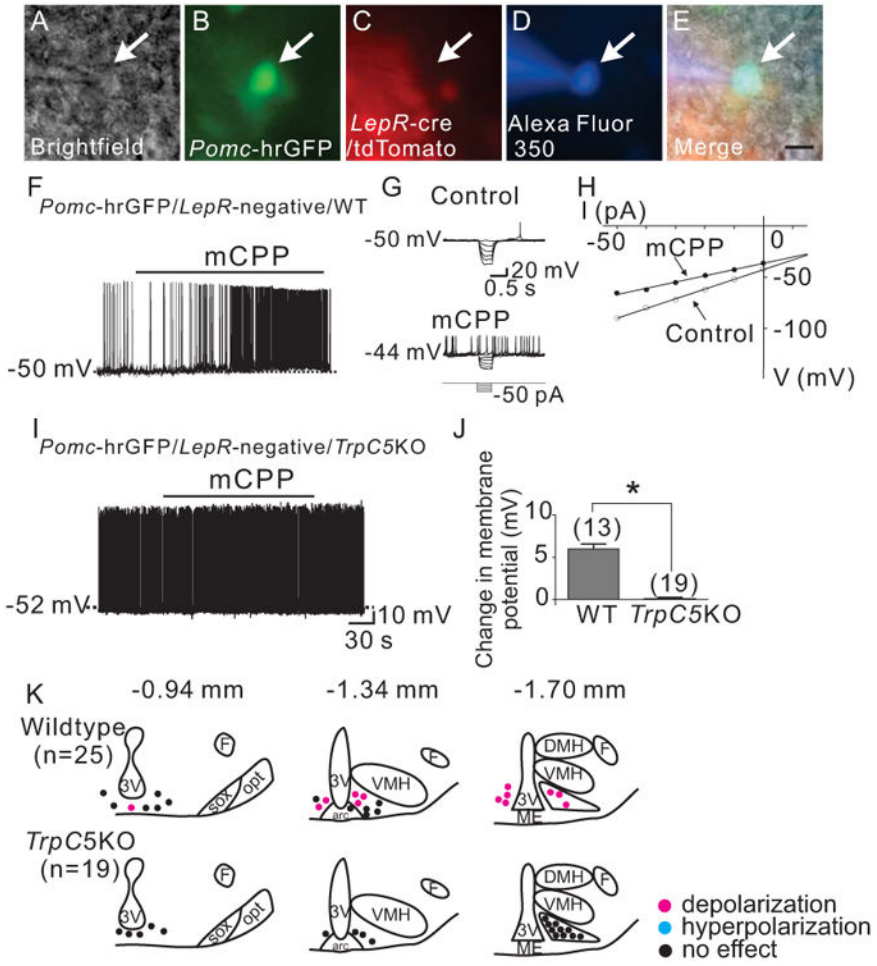

Figure 4.

*Trpc*5 subunits are required for the acute mCPP-induced depolarization of arcuate Pomc neurons. (A) Brightfield illumination of *Pomc*-hrGFP neuron from *PLT* mice. (B) and (C) The same neuron under FITC (hrGFP) and Alexafluor 594 (*tdtomato*) illumination. (D) Complete dialysis of Alexa Fluor 350 from the intracellular pipette. (E) Merge image illustrates colocalization of hr-GFP and Alexa Fluor 350 indicative of a *Pomc* neuron which does not express *Lepr*s. (F) Electrophysiological study demonstrates a *Pomc*-hrGFP (green) neuron from *PLT* mice that depolarized in response to mCPP (4μM). Traces showing decreased voltage deflection and increased action potential frequency after mCPP application. (H) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to mCPP application. Shown are responses before (control) and during mCPP application. (I) demonstrates a current clamp recording of a *Pomc*-hrGFP:: *Trcp5* $^{-/Y}$ (green) neuron in which mCPP fails to induce a depolarization. (J) Histogram summarizing the acute effect of mCPP on the membrane potential of *Pomc* neurons which do not express leptin receptors as well as express or do not express Trpc5 subunits (n= 13-19 per group). (K) Rostro-caudal and medio-lateral distribution of electrophysiological responses to mCPP from *Pomc* neurons which do not express leptin receptors as well as express or do not express Trpc5 subunits.

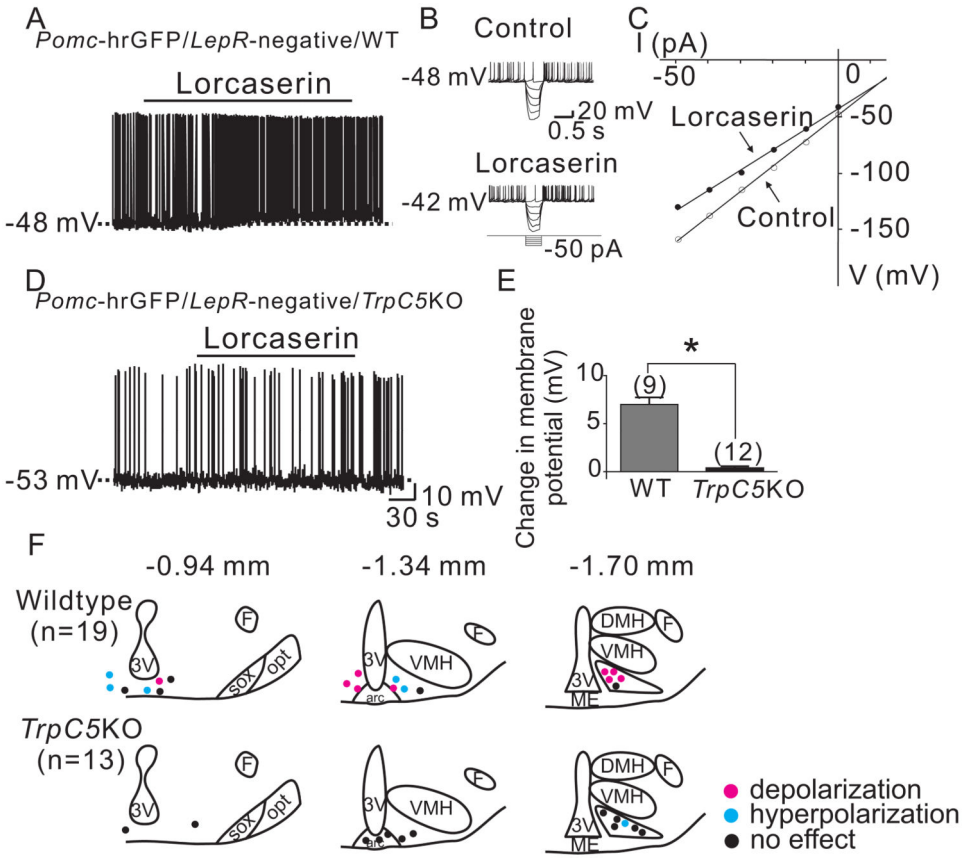

Figure 5.

*Trpc*5 subunits are required for the acute lorcaserin-induced depolarization of arcuate Pomc neurons. (A) Electrophysiological study demonstrates a *Pomc*-hrGFP (green) neuron from *PLT* mice that depolarized in response to lorcaserin (4μM). (B) Traces showing decreased voltage deflection and increased action potential frequency after lorcaserin application. (C) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to lorcaserin application. Shown are responses before (control) and during lorcaserin application. (D) Demonstrates a current clamp recording of a *Pomc*-hrGFP::*Trcp5*$^{lox/Y}$ (green) neuron from *PLT* mice in which lorcaserin fails to induce a depolarization. (E) Histogram summarizing the acute effect of lorcaserin on the membrane potential of *Pomc* neurons which do not express leptin receptors as well as express or do not express *TrpC5* subunits (n= 9-12 per group). (F) Rostro-caudal and medio-lateral distribution of electrophysiological responses to lorcaserin from *Pomc* neurons which do not express leptin receptors as well as express or do not express *TrpC5* subunits.