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TRPCing around the Hypothalamus

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Abstract

All of the canonical transient receptor potential channels (TRPC) with the exception of TRPC 2 are expressed in hypothalamic neurons and are involved in multiple homeostatic functions. Although the metabotropic glutamate receptors have been shown to be coupled to TRPC channel activation in cortical and sub-cortical brain regions, in the hypothalamus multiple amine and peptidergic G protein-coupled receptors (GPCRs) and growth factor/cytokine receptors are linked to activation of TRPC channels that are vital for reproduction, temperature regulation, arousal and energy homeostasis. In addition to the neurotransmitters, circulating hormones like insulin and leptin through their cognate receptors activate TRPC channels in POMC neurons. Many of the post-synaptic effects of the neurotransmitters and hormones are regulated in different physiological states by expression of TRPC channels in the post-synaptic neurons. Therefore, TRPC channels are key targets not only for neurotransmitters but circulating hormones in their vital role to control multiple hypothalamic functions, which is the focus of this review.

Keywords

TRPC channels; GnRH; kisspeptin; neurokinin B; POMC; orexin; 17β-estradiol; leptin; insulin

1. Introduction

The mammalian canonical transient receptor potential (TRPC) channel family consists of seven members, TRPC1–7 that appear to function as receptor-operated channels, analogous to the TRP channels involved in *Drosophilia* phototransduction (Clapham, 2003). With the exception of TRPC 2, these channels are widely distributed in the mammalian brain (Venkatachalam and Montell, 2007). The TRP channels are made of subunits with six membrane-spanning domains that co-assemble as tetrameric complexes similar to what has

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been described for K^+ channels (Clapham et al., 2005; Clapham et al., 2001). TRPC channels co-assemble as heteromeric channels consisting of the TRPC 1, 4 and 5 sub-family (Plant and Schaefer, 2003; Strubing et al., 2001) as well as TRPC 3, 6 and 7 sub-family (Berg et al., 2007; Trebak et al., 2003). Interestingly, TRPC 4 and 5 share ~73% homology, and TRPC 3, 6 and 7 share ~75% homology (Clapham, 2003). The functional distinction between these channel subtypes in CNS neurons has been problematic because of a lack of selective pharmacological reagents (Clapham et al., 2005). However, a unique feature of the heteromeric complexes of TRPC 1 + 4 or TRPC 1 + 5 subunits expressed in HEK cells is current-voltage relationship with a characteristic negative slope conductance and pronounced outward rectification (Clapham, 2003; Strubing et al., 2001). This biophysical characteristic has been exploited to identify TRPC 1 + 4 channel activation by kisspeptin in GnRH neurons (Zhang et al., 2008), and TRPC 1 + 5 channel activation by leptin and insulin in arcuate POMC and kisspeptin neurons (Qiu et al., 2011; Qiu et al., 2010; Qiu et al., 2014). However, it is also important to use molecular techniques like single-cell RT-PCR to help verify expression of TRPC channel transcripts in individual hypothalamic neurons to complement the pharmacological and biophysical characterization (Table 1).

The mammalian TRPC channels can be activated by G protein-coupled receptors and receptor tyrosine kinases (see Ambudkar and Ong, 2007; Clapham, 2003 for review). TRPC channels are one of the major targets for group I metabotropic glutamate receptor (mGluR1) signaling in CNS neurons (Bengtson et al., 2004; Berg et al., 2007; Faber et al., 2006; Tozzi et al., 2003). For example, in substantia nigra dopamine neurons TRPC 1 and 5 channels are highly expressed, and the mGluR1 agonist dihydroxyphenylglycine-induced current yields a double-rectifying (“S” shape) current-voltage plot (Tozzi et al., 2003). In addition, the peptide cholecystokinin via its receptor (CCK2) activates TRPC 1, 4 and 5 channels in amygdala neurons with the characteristic double-rectifying I/V (Meis et al., 2007). Both the mGluR1 and CCK2 receptors are Gq-coupled to PLC activation which leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP_3). A unique feature of TRPC 4 and 5 channels is that they are sensitive (potentiated) by the lanthanide lanthanum (La^{3+}), which blocks TRPC 3, 6 and 7 channels (Clapham et al., 2005). This unique pharmacology has been used to characterize TPRC 5 signaling in POMC neurons (Qiu et al., 2010; Qiu et al., 2014). Classically, the TRPC 3, 6 and 7 sub-family is DAG sensitive (Ambudkar and Ong, 2007; Clapham, 2003; Clapham et al., 2005). Interestingly, TRPC channels are minimally Ca^{2+} selective, but can associate with Orai calcium channels to form calcium release-activated calcium channels (see below) (Birnbaumer, 2009).

2. Calcium homeostasis and TRPC channel activity

TRPC channels form either receptor-operated cation channels (activated by membrane delimited receptors) or store-operated calcium channels (activated by depletion of calcium stores), which is dependent on their association with the endoplasmic reticulum protein stromal-interaction molecule 1 (STIM1) and Orai calcium channels (Birnbaumer, 2009; Salido et al., 2011). STIM1 is localized to the endoplasmic reticulum (ER) membrane of cells and its N-terminal domain contains an EF-hand that protrudes into the lumen of the endoplasmic reticulum to sense changes in ER Ca^{2+} concentrations (Salido et al., 2011).

Upon depletion of endoplasmic reticulum Ca^{2+}, STIM1 undergoes a conformational change, oligomerizes and then interacts with plasma membrane Orai and TRPC channels to become plasma membrane calcium release-activated calcium (I_{crac}) channels (Huang et al., 2006; Salido et al., 2011; Yuan et al., 2007). In cerebellar Purkinje neurons, activation of mGluR1 receptors evokes IP_3 receptor-mediated Ca^{2+} release from endoplasmic reticulum stores, activation of STIM1, recruitment of plasma membrane TRPC 1 and Orai channels, and the refilling of endoplasmic reticulum Ca^{2+} stores (Hartmann et al., 2014). This is critical for Purkinje neuron excitability since specific deletion of *Stim1* in Purkinje neurons causes significant motor impairments in mice (Hartmann et al., 2014). The STIM1 Ca^{2+} sensing is also involved in the control of L-type Ca^{2+} channel activity in hippocampal pyramidal neurons (Dittmer et al., 2017). Depolarization by glutamate activates L-type calcium channels and release of Ca^{2+} from the endoplasmic reticulum stores that activates STIM1, which drives aggregation of the calcium channels to inhibit L-type channel activity (Dittmer et al., 2017).

STIM1 is also dynamically regulated by other endogenous activity. Phosphorylation of STIM1 is required for oligomerization, and 17β-estradiol (E_2) inhibits the phosphorylation of STIM1 and consequently its interaction with plasma membrane Orai and TRPC channels and hence store-operated Ca^{2+} entry (Salido et al., 2011; Yuan et al., 2007). Pathologically, *Stim1* mRNA is up-regulated in human glomerular mesangial cells in a diabetic (hyperglycemia) model (Chaudhari et al., 2014) and in smooth muscle cells of hypertensive mice that exhibit vascular dysfunction and high (systolic) blood pressure (Kassan et al., 2016). Therefore, STIM1 regulates plasma membrane calcium (and TRPC) channels in both physiological and pathological conditions.

Plasma membrane voltage-gated calcium channels can also be part of TRPC channel signaling complexes in neurons under normal physiological conditions (Hartmann et al., 2014; Sun et al., 2017). In substantia nigra dopamine neurons the activities of TRPC 1 and L-type calcium (Cav1.3) channels are coupled (Sun et al., 2017), and TRPC 3 and P/Q-type calcium (Cav2.1) channels are linked in cerebellar Purkinje neurons (Hartmann et al., 2014). The T-type calcium channel Cav3.1 underlies burst firing in hypothalamic kisspeptin neurons (Zhang et al., 2013b) and facilitates TRPC 4 channel activation in GnRH neurons (Zhang et al., 2013a; Zhang et al., 2008). In POMC neurons it appears that the T-type calcium channel is coupled to TRPC 5 channel activation by insulin (Qiu et al., 2018a). Therefore, voltage-gated calcium channels can be part of the TRPC channel signaling complex.

3. TRPC channels in reproductive circuits

3.1 Gonadotropin Releasing Hormone (GnRH) neurons

The somas of GnRH neurons are localized to the medial septum, rostral preoptic area, anterior hypothalamus and medial basal hypothalamus with the exception that in mice and rats there are a few scattered GnRH neurons in the medial basal hypothalamus (Silverman et al., 1987; Silverman et al., 1979). The majority of GnRH neurons project to the median eminence where they release GnRH peptide into the portal blood in a pulsatile manner to control gonadotropin secretion from the pituitary gland and ultimately fertility (Clarke and

Cummins, 1985; Levine et al., 1985; Levine and Ramirez, 1980). Because GnRH cell bodies are widely scattered throughout the hypothalamic-septum continuum, the control of the synchronous firing of these neurons, the so-called "pulse generator," had been debated over two decades (see (Moenter et al., 2003) for review). Then in 2003 the puberty peptide kisspeptin was discovered and its ability to stimulate GnRH release via signaling through GPR54, *a.k.a.* Kiss1R (De Roux et al., 2003; Seminara et al., 2003). Indeed, kisspeptin is the most potent and efficacious neuropeptide/neurotransmitter to excite GnRH neurons (Han et al., 2005; Pielecka-Fortuna et al., 2008; Zhang et al., 2008), and Kiss1 neurons may be the presynaptic pacemaker neurons that drive GnRH neurons (Figure 1) (Liu et al., 2011; Qiu et al., 2016; Wang et al., 2016; Zhang et al., 2015). Although single action potential-generated calcium influx is sufficient to spark the release of classical neurotransmitters, burst firing or tetanic stimulation is required for the release of neuropeptides. High frequency electrical stimulation is required to evoke peptide release as originally demonstrated in the frog ganglion by Jan *et al.* (Jan et al., 1979) (see Arrigoni and Saper, 2014 for review). In this respect, high frequency electrical stimulation of anteroventral periventricular/periventricular preoptic nucleus Kiss1 ($Kiss1^{AVPV/PeN}$) neurons increases GnRH cell firing, which is absent in Kiss1R knockout mice and blocked by Kiss1R partial agonist peptide 234 (Liu et al., 2011). High frequency photostimulation (20 Hz) of $Kiss1^{AVPV/PeN}$ neurons releases kisspeptin, which excites GnRH neurons directly (Qiu et al., 2016). This high-frequency photostimulation of $Kiss1^{AVPV/PeN}$ neurons evokes a slow EPSP in GnRH neurons that is characterized by the tell-tale double rectifying I/V plot and antagonism by the Kiss1R partial agonist peptide 234 (Qiu et al., 2016; Roseweir et al., 2009; Zhang et al., 2008).

Pharmacologically, Kisspeptin-54 and the smaller peptide fragments (*e.g.*, kisspeptin 14, 13 and 10) bind with low nanomolar affinities to rat and human Kiss1R (GPR54) expressed in Chinese hamster ovary cells and are Gq-coupled to stimulate PIP_2 hydrolysis, Ca^{2+} mobilization, arachidonic acid release, and phosphorylation of mitogen-activated protein kinase (Kotani et al., 2001). In native GnRH neurons, kisspeptin causes excitation primarily through activation of TRPC channels and to a lesser extent inhibition of inwardly rectifying K^+ channels (Pielecka-Fortuna et al., 2008; Zhang et al., 2008). The activation of TRPC channels in GnRH neurons by kisspeptin is not affected by buffering intracellular calcium levels by the calcium chelators EGTA (ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid) or BAPTA (1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid) or by depleting intracellular calcium stores (Zhang et al., 2013a). Therefore, store release of calcium does not appear to play a critical role in the kisspeptin-mediated activation of TRPC channels. However, the kisspeptin-activated TRPC current is attenuated by the general calcium channel blocker Cd^{2+} and by the low voltage-activated calcium channel blocker Ni^{2+}, but not by the high voltage-activated calcium channel blocker amlodipine. This would indicate that low voltage-activated (T-type) calcium channels are a trigger for activating TRPC channels in GnRH neurons and are part of the TRPC signaling complex as described above. However, reducing extracellular calcium to nominally calcium free has no effect on the kisspeptin-activated TRPC current, an indication that very little calcium is needed to spark the opening of TRPC channels in GnRH neurons. This is consistent with the small, but persistent, T-type calcium channel current around the resting membrane potential of GnRH neurons (Zhang et al., 2009). Therefore, kisspeptin excites GnRH neurons primarily through

the opening of a TRPC channel that is independent of intracellular calcium store release, but appears to be dependent on calcium influx through T-type calcium channels. This ensures fast and sustained depolarization of GnRH neurons.

Single-cell RT-PCR analysis shows that GnRH neurons express primarily TRPC 1,4,5 channels (Table 1) (Zhang et al., 2008). TRPC 1 channels form heteromeric complexes with TRPC 4 and/or TRPC 5 channels (Plant and Schaefer, 2003; Strubing et al., 2001). Quantitative PCR analysis shows that *Trpc4* mRNA is expressed at levels 4-fold higher than *Trpc1* and *Trpc5* in GnRH neurons, and *Trpc4* mRNA expression is significantly increased in E2-treated, ovariectomized mice (Bosch et al., 2013). Phosphatidylinositol 4,5-bisphosphate is an important regulator of TRPC channels, and hydrolysis of PIP_2 is required for kisspeptin-induced TRPC channel activation in GnRH neurons (Zhang et al., 2013a). In addition to PIP_2 depletion, kisspeptin activation of TRPC channels is also dependent on the non-receptor tyrosine (cSrc) kinase activation (Fig. 1), since both global tyrosine kinase inhibitors such as genistein and the specific cSrc kinase inhibitor PP2 (4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-*d*]pyrimidine) attenuate (inhibit) kisspeptin currents in GnRH neurons (Zhang et al., 2013a). cSrc kinase directly regulates TPRC 4 channel activity through tyrosine phosphorylation, which causes rapid insertion of TRPC 4 channels into the plasma membrane (Odell et al., 2012). Therefore, cSrc kinase appears to be a key signaling molecule in the kisspeptin-mediated activation of TRPC 4 channels in GnRH neurons, and Kiss1R activation of TRPC 4 channels appears to be a major cellular mechanism by which kisspeptin causes a robust, sustained depolarization of GnRH neurons to generate a surge for maintaining reproductive function (d'Anglemont de Tassigny and Colledge, 2010). TRPC 5 channels are also expressed in the pituitary gonadotropes and mediate, at least in part, the depolarizing effects of GnRH (Götz et al., 2017).

3.2 Arcuate Kiss1 ($Kiss1^{ARH}$) neurons

As stated earlier since the discovery of the pulsatile release of GnRH, the source of the hypothalamic "pulse generator" activity had been an open question. More recently, $Kiss1^{ARH}$ neurons (*a.k.a* KNDy neurons since they co-express neurokinin B and dynorphin) have been proposed to be the "pulse-generator" neurons that stimulate pulsatile secretion of GnRH (Figure 1) (Lehman et al., 2010; Navarro et al., 2009), and unilateral optogenetic stimulation of $Kiss1^{ARH}$ neurons is efficacious to generate pulsatile release of LH (driven by pulsatile GnRH) in the mouse (Han et al., 2015). Therefore, to study the synaptic connections between Kiss1 neurons, we have utilized a $Kiss1^{Cre}$ mouse expressing channel rhodopsin unilaterally in Kiss1 neurons (Kiss1-Cre mice were injected with an AAV-DIO-ChR2:mCherry virus) to study the circuitry (Qiu et al., 2016). These injections labeled ChR2-mCherry-positive (Kiss1) cells and fibers ipsilaterally, but only ChR2-mCherry-positive fibers in the contralateral ARH. In addition to the ChR2:mCherry labeling ipsilaterally, control AAV-DIO-YFP was injected into the contralateral ARH in order to identify Kiss1 neurons for whole cell recordings. Low frequency (1 Hz) photostimulation of the ChR2 fibers evoked a glutamatergic fast EPSC in the contralateral ARH, but high frequency stimulation (20 Hz) generated a peptidergic slow EPSP, which is mediated by neurokinin B based on the blockade by tachykinin receptor 3 (TacR3) antagonists. The slow EPSP was blocked by sodium channel blocker tetrodotoxin (TTX) but rescued with the

addition of the K^+ channels blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA), demonstrating direct communication between $Kiss1^{ARH}$ neurons on both sides (Cousin and Robinson, 2000; Petreanu et al., 2009). The TacR3 is coupled to the activation of TRPC channels (Figure 2). Moreover, dual patch recording revealed that $Kiss1^{ARH}$ neurons are excited simultaneously with photostimulation of the contralateral $Kiss1^{ARH}$ neuronal input even with the median eminence transected (Qiu et al., 2016). Thus, high frequency autoexcitation of $Kiss1^{ARH}$ neurons ipsilaterally is able to recruit $Kiss1^{ARH}$ neurons bilaterally, which is dependent on TRPC channel activation (Figure 2), to induce synchronization of this critical neural network that underlies the pulse generator activity in mammalian females (Clarkson et al., 2017; Qiu et al., 2016).

The tachykinins comprise a series of structurally related peptides that are derived from alternative processing of three *Tac* genes and are expressed throughout the nervous and immune systems (Steinhoff et al., 2014). Tachykinins interact with three neurokinin G protein-coupled receptors, *Tacr1, Tacr2* and *Tacr3*. *Tacr1* and *Tacr3*, but not *Tacr2*, mRNA are expressed in $Kiss1^{ARH}$ neurons based on single cell RT-PCR analysis (Navarro et al., 2015), and the TacR3 agonist senktide depolarizes $Kiss1^{ARH}$ neurons, which is blocked by TacR3 antagonists (Navarro et al., 2011; Qiu et al., 2016). As proof of principle, we recently investigated the effects of senktide to activate TRPC 5 channels directly. These channels are expressed in $Kiss1^{ARH}$ neurons, and bath application of the TacR3 agonist generated an inward current, which was blocked by the TRPC channel blocker 2-aminoethoxydiphenyl borate (2-APB) (Figure 2). The I/V plot for the senktide-induced cation current showed the typical characteristics of TRPC 5 channels with a reversal of -10 mV (Figure 2). $Kiss1^{ARH}$ neurons co-express NKB and dynorphin, and high-frequency firing of $Kiss1^{ARH}$ neurons co-releases NKB and dynorphin (Qiu et al., 2016). NKB binds to TacR3 in neighboring $Kiss1^{ARH}$ neurons to open TRPC 5 channels to cause a robust depolarization (slow EPSP); co-released dynorphin feedbacks to bind to presynaptic κ-opioid receptors to limit the release of NKB to discrete bursts of activity and presumably postsynaptic TacR3 desensitization (Qiu et al., 2016). The combination of the two peptide neurotransmitters coordinates the synchronous firing of $Kiss1^{ARH}$ neurons that drives the pulsatile release of GnRH into the median eminence (Figure 1) (Campos and Herbison, 2014; Clarkson et al., 2017; Qiu et al., 2016).

4. TRPC channels in other homeostatic circuits

4.1 Orexin neurons and arousal

Orexin neurons are maintained in a depolarized state, independent of synaptic input, by a cation current generated by the expression of TRPC channels (Table 1) (Cvetkovic-Lopes et al., 2010). These spontaneously firing orexin neurons are thought to maintain arousal states (Adamantidis et al., 2007; Saper et al., 2001) and are inhibited during sleep states by GABAergic synaptic input (Eggermann et al., 2003). The orexin neurons project to the tuberomammillary histamine neurons, and high frequency photostimulation of ChR2-expressing orexin neurons evokes a slow EPSC in these histamine neurons that is abrogated by a selective orexin receptor 2 antagonist (Schone et al., 2014). Although the underlying cellular (channels) mechanism mediating the slow EPSC has not been elucidated, it is

possibly mediated by TRPC channels. The orexin neurons also excite local lateral hypothalamic GABA neurons that inhibit the REM sleep-promoting melanin-concentrating hormone (MCH) neurons. High frequency optogenetic stimulation of orexin neurons inhibits MCH cell firing, which is presumed to be via these inhibitory GABA interneurons (Apergis-Schoute et al., 2015). In addition, Thyrotropin-releasing Hormone (TRH), which increases arousal states and locomotor activity, also excites the local GABA neurons, and based on the pharmacological profiling with a number of channels blockers is thought to be mediated by TRPC channels (Zhang and Van den Pol, 2012).

Therefore, TRPC channels play a prominent role in the orexin-MCH neuronal circuitry in controlling arousal/sleep states (Table 1).

4.2 Preoptic neurons and temperature regulation

The preoptic area of the hypothalamus is involved in many physiological and behavioral homeostatic processes (McKinley et al., 2015). One main function of the preoptic area is to govern core body temperature in response to changes in ambient temperature, which is achieved in part by controlling brown adipose tissue thermogenesis. Thermoregulatory neurons of median preoptic nucleus (MnPO) are a target at which histamine modulates body temperature, and MnPO GABAergic neurons tonically inhibit warm-sensitive neurons in the medial preoptic area (Morrison et al., 2014). Histamine fibers from the tuberomammillary nucleus densely innervate the MnPO, and activation of H3 histamine receptors reduces the firing frequency of MnPO GABAergic neurons by augmenting an A-type current conducted by Kv4.2 channels, which decreases inhibitory input to the warm-sensitive neurons and ultimately sympathetic drive for brown adipose tissue (BAT) thermogenesis (Lundius et al., 2010; Sethi et al., 2011). In addition, histamine activates H1 and H2 receptors in MnPO glutamatergic neurons, which increases their firing frequency and excitatory input to the warm-sensitive neurons and BAT thermogenesis (Lundius et al., 2010; Tabarean et al., 2012). The activation of H1 histamine receptors on MnPO glutamatergic neurons excites these neurons through TRPC 1,5 channels (Tabarean, 2012). Interestingly, $Kiss1^{ARH}$ neurons project to the medial preoptic area (Krajewski et al., 2010; Yeo and Herbison, 2011), and local infusion of a selective neurokinin B agonist activates preoptic neurons and reduces core body temperature (Dacks et al., 2011). The TacR3 is highly expressed in the medial preoptic area (Dacks et al., 2011) and presumably is coupled to TRPC channel activation (Figure 2). Neurokinin B expression is up-regulated in post-menopausal states and is implicated in the etiology of menopausal hot flashes (Rance et al., 2013) such that TacR3 antagonists are in phase 2 clinical trials for treatment of postmenopausal hot flushes (Prague et al., 2017). Therefore, TRPC channels play a critical role in the control of temperature homeostasis.

4.3 POMC neurons and energy homeostasis

In the early part of this century it was discovered that leptin excites/depolarizes POMC neurons by opening non-selective cation channels, which is a vital action of the metabolic hormone to suppress food intake (Cowley et al., 2001). Ten years later it was discovered that TRPC 5 channels constitute the non-selective cation channels mediating the response, which became the focus of numerous metabolic studies (Gao et al., 2017; Qiu et al., 2011; Qiu et

al., 2010; Qiu et al., 2014; Sohn et al., 2011). POMC and the orexigenic NPY/AgRP neurons are major CNS targets of insulin and leptin actions (Belgardt and Bruning, 2010; Morton et al., 2006; Qiu et al., 2014; Schwartz et al., 2000). Insulin depolarizes mouse, guinea pig and monkey POMC neurons in both males and females via activation of TRPC 5 channels (Figure 3), and hyperpolarizes NPY/AgRP neurons via activation of K_{ATP} channels (Qiu et al., 2018b; Qiu et al., 2014), activities that are complementary for mediating the anorexigenic effects of insulin. In addition, the serotonin $5HT_{2C}$ receptor is Gq-coupled to activate TRPC 5 channels in POMC neurons (Sohn et al., 2011), and deletion of TRPC 5 channels specifically in POMC neurons results in a decrease in energy expenditure and increase in food intake and weight gain in male mice (Gao et al., 2017). The increase in POMC cell excitability induced by insulin translates into heightened transcriptional activity —*i.e.*, an increase in c-Fos expression in the arcuate nucleus and specifically in POMC neurons following *icv* insulin (Qiu et al., 2014). Insulin delivered directly into the third ventricle uniformly decreases food intake in guinea pigs (Qiu et al., 2014), mice (Benoit et al., 2002; Brown et al., 2006) and rats (Clegg et al., 2011). The insulin-induced decrease in food intake is correlated with alterations in energy expenditure as manifested by increases in O_2 consumption, CO_2 production and metabolic heat production (Qiu et al., 2014). The catabolic effects of insulin are blocked by melanocortin receptor 3, 4 antagonists, which is evidence that actions of insulin are mediated by POMC neurons (Benoit et al., 2002). Although optogenetic and pharmacogenetic stimulation of NPY/AgRP neurons rapidly increases food consumption (Aponte et al., 2011; Krashes et al., 2011), prolonged stimulation of POMC neurons attenuates food intake (Aponte et al., 2011). The effects of leptin and insulin in POMC neurons are vital for both the short term (excitability) and long term (transcriptional) modulation of POMC neuronal activity and the control of food intake and ultimately energy homeostasis.

In POMC neurons the insulin receptor (InsR) couples to phosphoinositide 3-kinase (PI3K) p110β activation (Al-Qassab et al., 2009; Xu et al., 2005), and the InsR-mediated excitation of POMC neurons is abrogated by inhibition of PI3K activity (Al-Qassab et al., 2009; Hill et al., 2008; Qiu et al., 2010; Qiu et al., 2014). Activation of PI3K generates PIP_3, which stimulates phospholipase C (PLC) and protein kinase B (Akt) (Bae et al., 1998; Falasca et al., 1998; Qiu et al., 2014; Rameh et al., 1998). PLC also hydrolyzes PIP_2, which modulates TRPC 4, 5 channel activity (Qiu et al., 2014; Rodríguez-Menchaca et al., 2012; Zhang et al., 2013a). In addition, it is thought that PI3K rapidly increases the insertion of TRPC 5 channels into the plasma membrane from intracellular vesicular pools to further boost depolarization and Ca^{2+} entry into neurons (Figure 3) (Bezzerides et al., 2004). Collectively, all of these PI3K-mediated effects are critically involved in the activation of TRPC 5 channels by insulin actions in POMC neurons (Qiu et al., 2018b).

Insulin resistance (IR) is at the core of the metabolic syndrome and causes abnormal insulin signaling in cells throughout the body. Neurons, similar to fat and muscle cells, can develop hyperinsulinemia-induced IR, which results in severe injury to the nervous system as seen in diabetic neuropathies (Kim and Feldman, 2012). Moreover, males exhibit a higher incidence of metabolic syndrome than women in early adult life, but this sex difference diminishes sharply in hypo-estrogenic states (Gustafsson et al., 2011; Janssen et al., 2008). Exposing rodents to a high fat diet over 6-10 weeks invariably causes diet-induced obesity (DIO) and

dramatic alterations in multiple physiological systems. The DIO model has been exploited for electrophysiological studies of NPY/AgRP and POMC neurons (Baver et al., 2014; Konner and Bruning, 2012; Parton et al., 2007; Plum et al., 2006; Tsaousidou et al., 2014). Diet-induced obese *Pomc*^{EGFP} mice have been used for comprehensive cellular and molecular studies in order to dissect deficits in the insulin signaling pathway (Qiu et al., 2018a). Although there are no differences in the resting membrane potential or input resistance between the control and DIO male groups, there is a significant attenuation in the insulin response (TRPC channel-mediated current) in POMC neurons from DIO males, which is an indication that these anorexigenic neurons have become insulin resistant (Qiu et al., 2018a).

Interestingly, TRPC 5 channels are fully expressed and operational in DIO males since the thiazolidinedione rosiglitazone (Bon and Beech, 2013) is able to fully activate the channels in POMC neurons (Qiu et al., 2018a). The inward current generated by rosiglitazone mimics insulin's effects in control animals with the same reversal potential, indicative of activation of a non-selective cationic (*i.e.*, TRPC) channel. Therefore, it is clear that TRPC 5 channels are not downregulated in POMC neurons from DIO males, but rather there is an uncoupling of insulin receptors from channel activation. Indeed, there is no difference in *Trpc5* mRNA expression in POMC neurons from DIO versus control males based on qPCR of POMC neurons (Qiu et al., 2018a).

However, there is a pronounced sex difference in the response to insulin with diet-induced obesity. Similar to the males, there are no differences in the resting membrane potential or input resistance of POMC neurons between control and DIO female mice; but in contrast to males, the steady-state response (inward current) to insulin is not attenuated with DIO (Qiu et al., 2018a). The reversal potential for the insulin-induced inward current is the same for both control fed and DIO females and as expected is antagonized by the TRPC channel blocker 2-APB (Clapham et al., 2005). Moreover, insulin robustly depolarizes and increases the firing activity of POMC neurons from DIO females similar to the controls. Hence, there is no attenuation of the insulin response (*i.e.*, TRPC 5 channel activation) in POMC neurons from DIO, proestrous females, which indicates that there is a clear sex difference in the development of insulin resistance by POMC neurons in obesity.

In order to explore the role of the gonadal steroids in preserving the insulin response in DIO females, we ovariectomized female *Pomc*^{EGFP} mice after they had been on a high fat diet for 10 weeks, and one week following ovariectomy, we gave an estradiol benzoate treatment (*s.c.* injection regimen that yields proestrous serum levels of E_2) (Bosch et al., 2013) or oil vehicle. In contrast to ovariectomized females fed a control diet, POMC neurons from ovariectomized, DIO females are completely resistant to insulin such that there is no inward current (Qiu et al., 2018a). However, the TRPC 5 channel opener rosiglitazone is able to generate a robust inward current indicating that TRPC 5 channels are expressed and functional in POMC neurons from DIO, ovariectomized females. In contrast, POMC neurons from E_2-treated, ovariectomized, DIO females maintain their sensitivity to insulin—*i.e.*, insulin induces a robust inward current and depolarizes POMC neurons. Therefore, in the absence of E_2 there appears to be an uncoupling of InsR from activating TRPC 5 channels in obese females, but the insulin response is rescued with E_2 replacement.

Importantly, E_2 treatment significantly down-regulated the expression of *Stim1*, which as discussed above (Section 2), shifts TRPC 5 channels from being store-operated to receptor (InsR)-operated channels (Birnbaumer, 2009; Salido et al., 2011).

Deletion of both insulin and leptin receptors in POMC neurons causes overt systemic insulin resistance in both male and female mice (Hill et al., 2010). If left untreated, insulin-deficiency leads to hyperglycemia, polyuria, ketoacidosis and death as seen in type 1 diabetes. However, insulin-deficient rodents are viable with leptin monotherapy, and this life-saving therapy is effective in mice expressing leptin receptors only in hypothalamic POMC and GABAergic neurons (Fujikawa et al., 2013). Coppari and colleagues (Fujikawa et al., 2013) postulated that leptin and insulin must engage the same hypothalamic circuitry to maintain glucose homeostasis and hepatic function, which at the cellular level means that insulin and leptin engage a common effector such as TRPC 5 channels to excite POMC neurons to maintain homeostatic function (Qiu et al., 2014). In addition, TRPC 3 channels are involved in mediating the glucose-mediated excitation of ventromedial hypothalamic neurons (Table 1) (Chretien et al., 2017), which provide an additional excitatory drive to POMC neurons (Conde et al., 2017; Tong et al., 2007). Therefore, TRPC channels play a pivotal role in maintaining energy homeostasis.

5. Conclusions

Although TRPC channels are probably one of the major targets for group I metabotropic glutamate receptor (mGluR1) signaling in CNS neurons (Bengtson et al., 2004; Berg et al., 2007; Faber et al., 2006; Tozzi et al., 2003), they are key players in multiple homeostatic functions in the hypothalamus. As such they are activated by G protein-coupled receptors similar to other CNS structures (see Ambudkar and Ong, 2007; Clapham, 2003 for review) and in addition by tyrosine kinase receptors, which can be expressed in the same neuron. In $Kiss1^{ARH}$ neurons, for example, leptin and insulin through their cognate receptors signal via PI3K to activate TRPC 5 channels in different metabolic states (Qiu et al., 2011; Qiu et al., 2014), whereas the Gq-coupled TacR3 activates TRPC 5 channels in reproductive states (Figure 2) (Qiu et al., 2016). Likewise in POMC neurons, the serotonin $5HT_{2C}$ receptor is Gαq-coupled to activate TRPC 5 channels in POMC neurons (Sohn et al., 2011), and deletion of TRPC 5 channels specifically in POMC neurons results in a decrease in energy expenditure and increase in food intake and weight gain in male mice, a clear metabolic phenotype (Gao et al., 2017). Furthermore, the importance of preserving this critical metabolic signaling pathway is exemplified by the fact that E_2 is able to protect females from the development of insulin resistance through preserving TRPC 5 channel coupling to the insulin receptor (Figure 3). Therefore, there are multiple systems that impinge on TRPC channel signaling in hypothalamic peptidergic neurons. A future challenge will be to develop specific tools (*e.g.*, selective pharmacological agents, genetic engineering) for probing TRPC channel function in different physiologic and pathophysiologic states.

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Highlights

- TRPC channels are highly expressed in hypothalamic neurons
- TRPC channels maintain the excitability of hypothalamic neurons
- Multiple G protein-coupled receptors are coupled to TRPC channels
- Receptor tyrosine kinases activate TRPC channels
- Receptor coupling to TRPC channels depends on physiological state

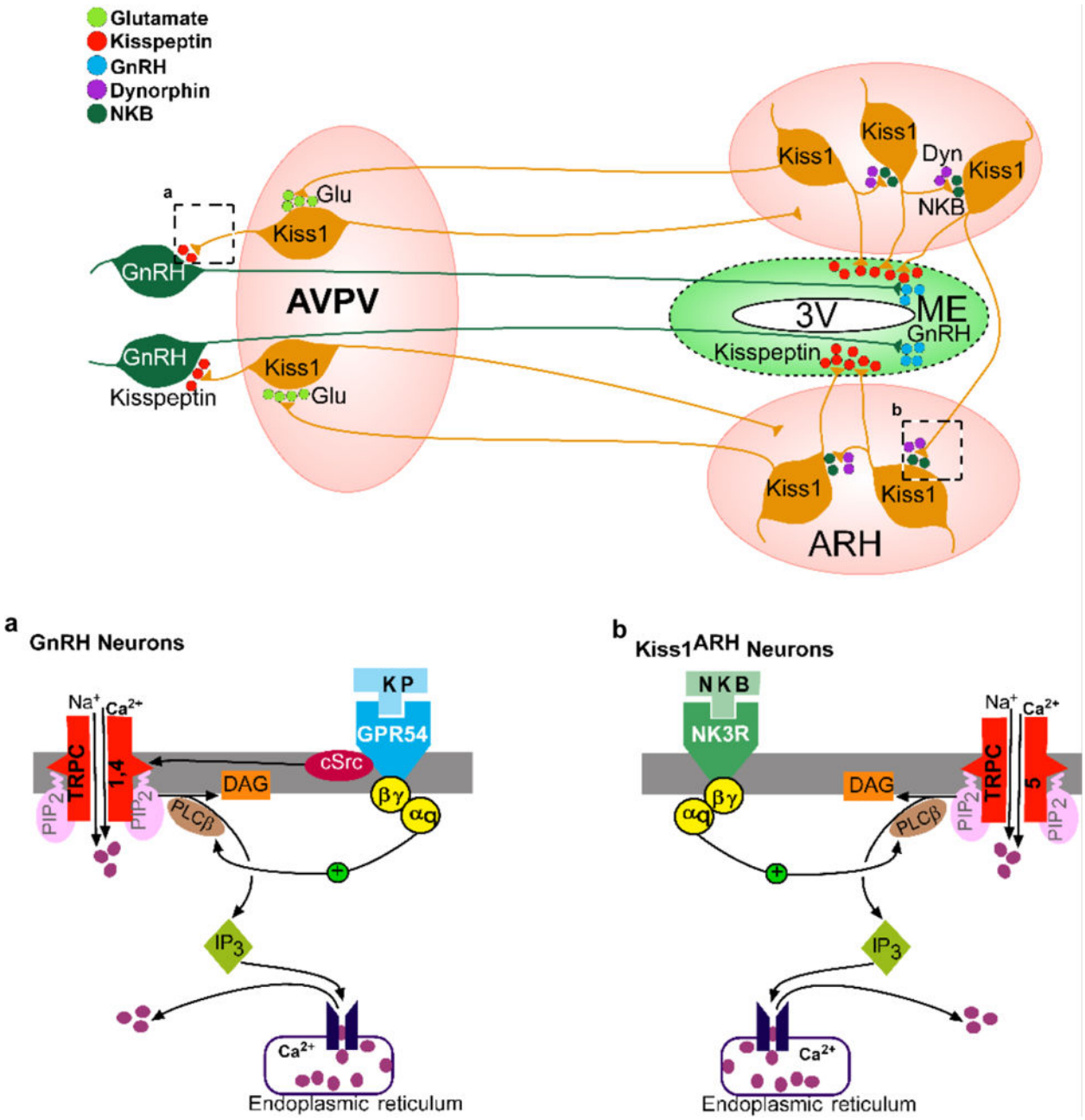

Figure 1. A model by which activation of Kiss1 neurons governs GnRH neuronal excitability through TRPC channels

Activation of Kiss1 neurons in the ARH releases neurokinin B (NKB) that depolarizes and recruits other Kiss1^{ARH} neurons. Dynorphin is co-released and acts presynaptically to modulate (inhibit) the release of NKB. Together the two peptides govern the synchronous activity of Kiss1^{ARH} neurons and promote kisspeptin release that stimulates GnRH release in the median eminence (ME). Kiss1^{ARH} neurons also communicate with the Kiss1$^{AVPV/PeN}$ neurons via the fast neurotransmitter glutamate, which stimulates burst-firing of Kiss1$^{AVPV/PeN}$ neurons. Activation of these rostral Kiss1 neurons releases kisspeptin to robustly excite GnRH neurons via activation of the GPR54 (Kiss1R) signaling cascade,

thereby stimulating the release of GnRH at the time of the preovulatory surge. Kisspeptin, GPR54, NKB, TacR3 and GnRH are all required for normal fertility. [Modified from Fig. 12, Qiu *et al.*, 2016. *Elife* doi: 10.7554/eLife.16246.] (**a**) Kisspeptin excites GnRH neurons through TRPC 4 channels. Kisspeptin binds to the Gq-coupled GPR54 receptor to activate phospholipase Cβ (PLCβ), which catabolizes PIP2, potentiates TRPC channel activity and inhibits the inwardly rectifying K^+ channel activity. cSRC kinase, which is activated by kisspeptin/GRP54 signaling, potentiates the activity of TRPC 4 channels; and (**b**) Neurokinin B excites $Kiss1^{ARH}$ neurons through TRPC 5 channel activation.

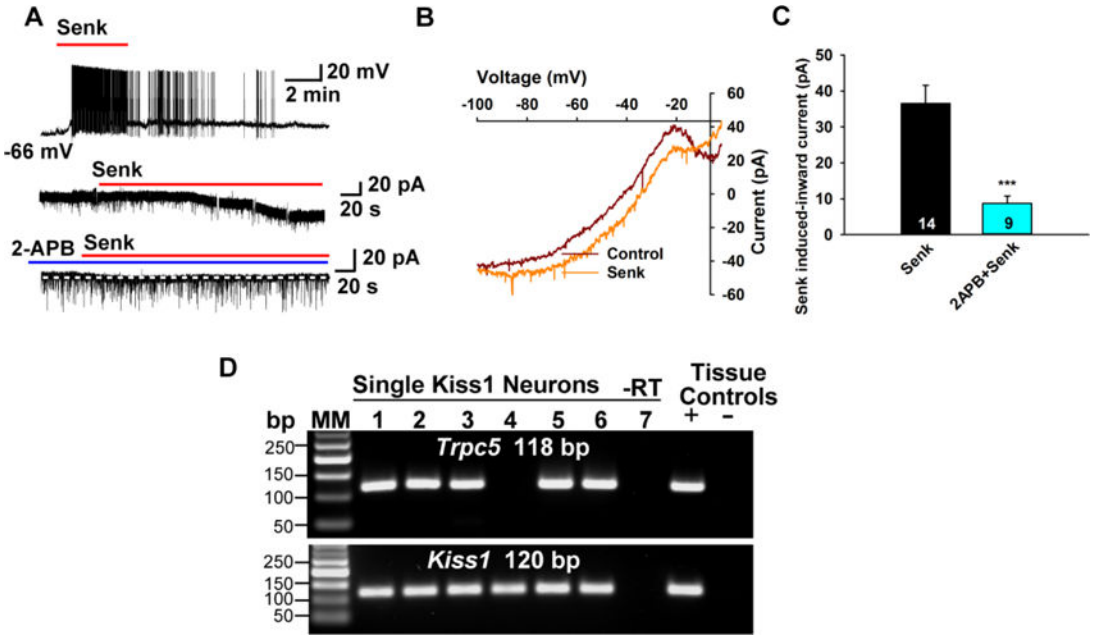

Figure 2. TacR3 agonist excites Kiss1^{ARH} neurons through TRPC5 channels

(**A**) TacR3 agonist senktide (500 nM) depolarizes Kiss1^{ARH} neurons in female mice and induces firing (upper trace). In voltage clamp senktide induces an inward current (middle trace). V_{hold} = −60 mV. The inward current is blocked by TRPC channel blocker 2-aminoethyl diphenylborinate (2-APB; 100 μM) (lower trace). (**B**) The I-V relationship for the senktide (Senk)-induced inward current reverses at −10 mV and exhibits double rectification. (**C**) Summary of the effects of TRPC channel blocker 2-APB on the senktide-induced inward currents. The TRPC channel blocker is applied 15 min before the application of senktide (500 nM). V_{hold} = − 60 mV. ***$p < 0.005$, different from the senktide control group. Data points represent the mean ± SEM. Unpaired two-tailed *t* test, $t_{(21)} = 4.234$, $p = 0.0004$. Cell numbers tested are indicated. (**D**) Representative gel images illustrating the mRNA expression of *Trpc5* channel subunit in Kiss1^{ARH} neurons harvested from female mice. The expected size of PCR products for *Kiss1* and *Trpc5* are indicated. MM is the molecular marker; −RT indicates a harvested Kiss1 neuron without RT; + indicates positive tissue control (with RT); – indicates negative tissue control (without RT) using cDNA from mouse medial basal hypothalamic tissue. RT, reverse transcriptase.

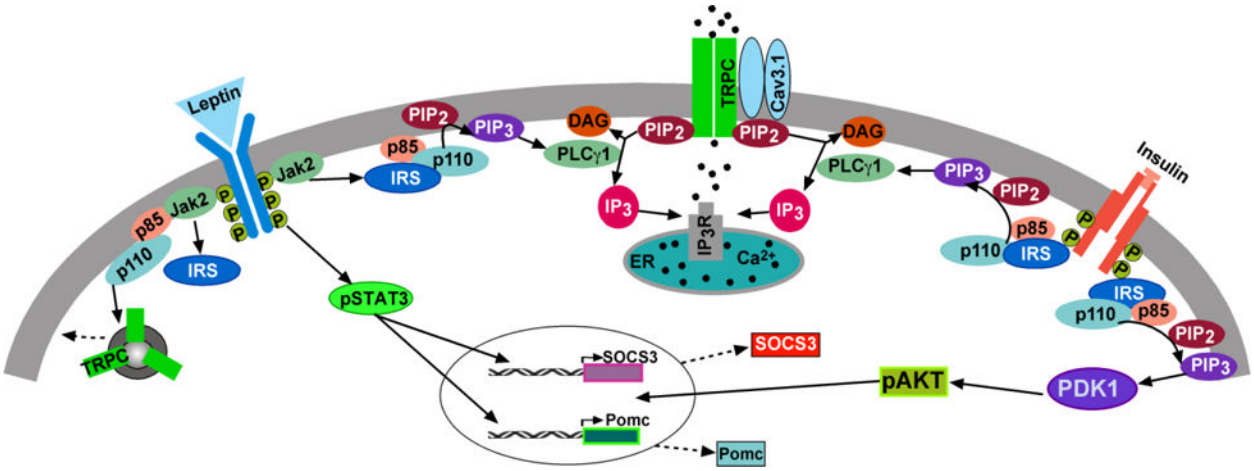

Figure 3. A cellular model of insulin (and leptin) signaling via TRPC 5 channel activation in POMC neurons

Insulin signals via IRS-PI3K to activate TRPC 5 channels in POMC neurons, which generates a robust inward cationic current to depolarize POMC neurons and increase their excitability. Similarly, leptin binding to its receptor (LRb) triggers the recruitment of the tyrosine kinase Janus kinase (JAK) 2, leading to the activation of the JAK/signal transducer and activator of transcription (STAT) signaling pathway and simultaneously activation of PI3K, which also activates TRPC 5 channels. PI3K (p85/p110) will also accelerate the rapid insertion of TPRC 5 channels into the plasma membrane. PDK1, 3-phosphoinositide–dependent protein kinase-1. [Modified from Fig. 8, Qiu *et al.*, 2018a. *Endocrinology* 159, 647-664.]

Table 1

Summary of TRPC channel subtype expression and their cell-type dependent effects

CELL TYPE	EXPRESSION LEVEL OF TRPC CHANNEL	EFFECT ON CELL	STIMULATOR	PHYSIOLOGICAL FUNCTION	REFERENCE
GnRH neurons (m)	4>1>5	Increases excitability	Kisspeptin	Reproduction	Zhang 2008, 2013; Bosch 2013
POMC neurons (m, g)	5>1>4>7>6 (m)	Increases excitability	Leptin, insulin,	Energy homeostasis; anorexigenic	Qiu 2010 (m); Qiu 2014 (g); Gao 2017 (m)
ARH Kiss1 neurons (m,g)	5 (m)	Increases excitability	Senktide, insulin	Reproduction	Qiu (new data, m); Qiu 2014
Hypocretin/Orexin neurons (r)	4,5>7>1>3	Increases excitability	Unknown, but controls excitability	Arousal	Cvetkovic-Lopes 2010
Vasopressin neurons (r)	4	Increases excitability	Water deprivation	Body fluid homeostasis	Nedungadi 2014
Glutamatergic MnPO neurons (m)	1,5,7; PCR only	Increase excitability	Histamine	Thermoregulation	Tabarean 2012
Glucose-excited neurons in MBH (m)	3	Increases excitability	Glucose	Energy homeostasis	Chretien 2017
PMV neurons (m)	Putative, pharmacology only	Increases excitability	Leptin	Reproduction	Williams 2011
GABAergic neurons in LH (m)	Putative, pharmacology only	Increases excitability results in MCH neuron inhibition	TRH	Anorexic and arousal actions	Zhang 2012

ARH, arcuate nucleus; GnRH, gonadotropin- releasing hormone; LH, lateral hypothalamus ; MBH, mediobasal hypothalamus ; MCH, melanin-concentrating hormone; MnPO, median preoptic nucleus; PCR, polymerase chain reaction ; PMV, ventral premammillary nucleus ; POMC, pro-opiomelanocortin; TRH, thyrotropin-releasing hormone. (m), mouse; (r), rat; (g), guinea pig.