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Insulin and leptin excite anorexigenic pro-opiomelanocortin neurones via activation of TRPC5 channels

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Abstract

Pro-opiomelanocortin (POMC) neurones within the hypothalamic arcuate nucleus are vital anorexigenic neurones. Both the insulin receptor and leptin receptor are coupled to activation of phosphatidylinositol-3 kinase (PI3K) to regulate multiple functions that increase POMC neuronal excitability. Using whole-cell recording in several mammalian species, we have found that both insulin and leptin depolarised POMC neurones via activation of transient receptor potential (TRPC)5 channels. TRPC5 channels have been rigorously characterised as the downstream effector based on their biophysical properties, pharmacological profile, and localisation by immunocytochemistry and single-cell reverse transcriptase-polymerase chain reaction. By contrast, insulin and leptin hyperpolarise and inhibit neuropeptide Y/agouti-related peptide neurones via activation of K_{ATP} channels. As proof of principle, insulin given i.c.v. robustly inhibits food intake and increases O_2 utilisation, CO_2 production and metabolic heat production. Therefore, these findings indicate that the depolarisation/excitation of POMC neurones by insulin and leptin is preserved across mammalian species and the activation of TRPC5 channels is likely a major mechanism by which insulin and leptin regulate energy homeostasis in mammals.

Keywords

insulin; leptin; POMC neurone; TRPC5 channel

1 | THE ENIGMA OF INSULIN SIGNALLING IN POMC NEURONES

The hypothalamic arcuate nucleus is one of the primary central nervous system (CNS) regions involved in the regulation of food intake.^{1–3} There are two critical neuropeptide cell groups within the arcuate nucleus controlling feeding behaviour: the orexigenic

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CONFLICT OF INTERESTS

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neuropeptide Y/agouti-related peptide (NPY/AgRP) neurones and the anorexigenic pro-opiomelanocortin (POMC) neurones.^{1–3} Optogenetic or pharmacogenetic stimulation of NPY/AgRP neurones robustly stimulates food intake,^{4,5} whereas optogenetic stimulation of POMC neurones inhibits feeding and weight gain.⁴

POMC and NPY/AgRP neurones within the arcuate nucleus are juxtaposed to and send dendritic projections into the median eminence, which is a circumventricular organ and outside of the blood-brain barrier.⁶ Therefore, these neurones are ideally situated for detecting circulating levels of leptin, insulin and glucose.^{7–9} Cowley et al.⁷ first showed that leptin depolarises POMC neurones via activation of a nonselective cation current, which was consistent with previous studies reporting that leptin increased c-Fos expression in POMC neurones.¹⁰ We later identified that the nonselective cation channel responsible for the depolarising actions of leptin in POMC neurones was a canonical transient receptor potential (TRPC) channel.¹¹

Insulin receptors, like leptin receptors, are expressed in POMC neurones and i.c.v. administration of insulin increases the expression of *Pomc* mRNA.¹² Insulin receptors are also expressed in other unidentified, presumably NPY/AgRP neurones¹² because i.c.v. insulin prevents the increase in arcuate NPY expression induced by fasting.¹³ Insulin binding to its receptor generates phosphorylation of insulin receptor substrate (IRS) proteins, which leads to activation of phosphatidylinositol-3 kinase (PI3K) that has multiple downstream actions in POMC neurones.^{14,15} Similarly, the leptin receptor in POMC neurones is coupled to the Janus kinase-IRS-PI3K signalling pathway, which induces POMC neuronal activation.¹⁶

Although insulin and leptin have similar actions in controlling food intake and energy balance^{12,17} and appear to share a common signalling pathway in POMC neurones,^{14,15} they were thought to have opposing actions on POMC cell excitability.^{16,18,19} To address these issues, we undertook a series of cellular electrophysiological and pharmacological studies aiming to clarify the actions of insulin in POMC neurones from several mammalian species. Our results argue for a common cellular signalling pathway for insulin and leptin in POMC neurones that leads to a robust excitation of these anorexigenic neurones and helps regulate energy homeostasis.

2 | INSULIN AND LEPTIN EXCITE ARCUATE POMC NEURONES VIA EVOLUTIONARILY CONSERVED ACTIVATION OF TRPC5 CHANNELS

We have targeted POMC neurones in the hypothalamic arcuate nucleus of guinea pigs using visualised patch recording, which were identified as *Pomc* neurones based on a single-cell reverse transcriptase-polymerase chain reaction (RT-PCR), and POMC-EGFP neurones from *Pomc*^{EGFP} mice.²⁰ The biophysical properties of the POMC neurones from both guinea pig and mouse are quite analogous.^{8,21,22} Similar to leptin, insulin depolarises and increases the action potential firing of the guinea pig and mouse POMC neurones (Figure 1). By contrast, insulin hyperpolarises and inhibits the firing of NPY/AgRP neurones (see below). In the presence of tetrodotoxin to block synaptic input and under whole-cell voltage clamp conditions, both insulin and leptin induce an inward current in POMC neurones (Figure 1).

The double-rectifying current-voltage (I/V) relationship with a reversal potential of -10 mV is the tell-tale sign of TRPC5 channel activation.²³ Other pure insulin reagents, such as purified bovine and recombinant human insulin, also depolarise mouse POMC neurones so the response is not unique to purified guinea pig insulin.²⁰ We then investigated whether pure insulin also depolarises and excites POMC neurones in the rhesus macaque. For these experiments, we were able to utilise slices from two control adult male monkeys that were part of a Oregon National Primate Research Center Alcohol Center Grant study. We targeted arcuate neurones utilising visualised patch recording and recorded from eight neurones. Five of the cells were depolarised by activation of a TRPC-like current (Figure 2A, B) and two of these neurones were identified *post-hoc* by scRT-PCR as *Pomc* neurones (Figure 2D). One of the rhesus macaque neurones exhibited biophysical characteristics typical of guinea pig/mouse NPY/AgRP neurones (high input resistance, low capacitance)²⁴ and indeed was hyperpolarised by insulin (Figure 2C). Single-cell RT-PCR confirmed that this neurone was not *Pomc* positive. Therefore, insulin depolarises mouse, guinea pig and monkey POMC neurones via activation of a cationic conductance, with the response appearing to be conserved across mammalian species.

3 | COMMON SIGNALLING PATHWAY FOR LEPTIN AND INSULIN IN POMC NEURONES

Given that insulin depolarises POMC neurones, we have utilised several pharmacological blockers to further characterise this mixed cation conductance. 2-Aminoethyl diphenylborinate, which is a potent blocker of TRPC3, 4, 5 and 6 channels,²⁵ almost completely blocks (by 83%) the insulin-induced currents in POMC neurones. In heterologous expression systems, La^{3+} ($100 \mu\text{mol L}^{-1}$) potentiates TRPC4 and TRPC5²⁶ but inhibits TRPC1, 3, 6 and 7 channels.²⁵ Indeed, La^{3+} greatly potentiates the insulin-induced current by five-fold (Figure 3A). Furthermore, rosiglitazone, an enhancer of TRPC5 channel activity in adipocytes,²⁷ potentiates the TRPC channel current by two-fold, and the I/V exhibits a more pronounced double-rectifying plot that reverses at -10 mV,²⁰ which is consistent with the enhancement of a TRPC-mediated cationic conductance.

Over the past decade, a major effort has been made to identify the critical signalling molecules in POMC and NPY/AgRP neurones, and one common convergent point for both insulin receptor and leptin receptor signalling is PI3K, and specifically p110 β .^{14,16,28} The leptin-mediated excitation (inward current) of POMC neurones is abrogated by inhibition of PI3K activity,^{11,16,28} and we have shown that blockade of PI3K prevents the insulin-mediated depolarisation.²⁰ Activation of PI3K generates phosphatidylinositol-3,4,5-triphosphate, which appears to contribute to the translocation and activation of phospholipase C (PLC) γ at the plasma membrane.^{29–31} Through its phospholipase catalytic domains, PLC γ hydrolyses phosphatidylinositol 4,5-bisphosphate, which affects multiple channels, including TRPC channels.^{32,33} In addition, in hippocampal neuronal cultures, nerve growth factor and brain-derived neurotrophic factor activate PI3K and increase the insertion of TRPC5 channels into the plasma membrane from intracellular vesicular pools,³⁴ all of which also may be involved in actions of insulin and leptin in POMC neurones. Because PI3K is essential for mediating the effects of leptin^{14,16} and is also critical for the

membrane insertion of TRPC channels,³⁴ we also examined the role of PI3K in the insulin-induced inward current. The selective PI3K inhibitor wortmannin (100 nmol L⁻¹) almost completely blocks (by 94%) both the insulin- and leptin-induced inward currents (Figure 3A, B).

TRPC1, 4 and 5 mRNAs are the predominant subfamily members expressed in POMC neurones.^{11,20} We have used immunocytochemical staining to identify TRPC5 channel protein in POMC neurones, and the TRPC5 channel subtype is co-localised with β -endorphin in the majority of POMC neurones in the arcuate nucleus.²⁰ Also, our quantitative PCR analysis comparing expression of TRPC5 mRNA in POMC versus NPY/AgRP neurones reveals that TRPC5 mRNA is highly expressed in POMC neurones but is expressed at many-fold lower levels in NPY/AgRP neurones. Taken together, it appears that the insulin- and leptin-induced excitation of POMC neurones occurs via a receptor-mediated activation of PI3K and the opening of TRPC5 channels.

4 | INTRACEREBROVENTRICULAR INSULIN DECREASES FOOD INTAKE AND INCREASES METABOLISM

As proof of principle, we have also evaluated the efficacy of centrally administered insulin to modulate energy intake and energy expenditure in male and late follicular female guinea pigs fed a standard, grain-based diet. Insulin delivered directly into the third cerebroventricle (i.c.v.) decreases food intake (Figure 4). These centrally mediated, insulin-induced changes in food intake are correlated with alterations in energy expenditure (ie, increased O₂ consumption, CO₂ production and metabolic heat production). As further evidence of the activational effects of insulin in the hypothalamic arcuate nucleus, we have measured increased expression of the immediate early gene *c-fos* in the insulin-treated guinea pigs and specifically in POMC neurones.²⁰ Therefore, the insulin-induced increase in POMC cell excitability boosts transcriptional activity, presumably in POMC neurones to decrease energy intake and increase energy expenditure. Importantly, because i.c.v. insulin is acting centrally within the hypothalamus to alter feeding behaviour, there is no effect on serum glucose levels or cholesterol levels, although serum triglycerides are significantly reduced by i.c.v. insulin, which is consistent with findings indicating that insulin infused into the hypothalamus suppresses lipolysis in rats.³⁵

The increase in POMC cell excitability induced by insulin translates into heightened transcriptional activity and presumably downstream postsynaptic actions of POMC peptides to regulate food intake and energy expenditure. Our findings in guinea pigs indicating that i.c.v. insulin decreases food intake over 8 hours are comparable with previous findings in mice^{12,17} and rats.³⁶ Moreover, the catabolic effects of insulin in mice are blocked by melanocortin receptor 3, 4 antagonists.¹² Collectively, these findings are consistent with the excitation/activation of POMC neurones by insulin to reduce appetite in a number of mammalian species.

5 | PHYSIOLOGICAL SIGNIFICANCE OF A COMMON SIGNALLING MECHANISM FOR INSULIN AND LEPTIN

The pleiotropic effects of leptin and insulin in POMC neurones are most critical for both the short-term (excitability) and long-term (transcription) modulation of POMC neuronal activity and the control of food intake and energy expenditure. Our electrophysiological findings are congruent with the effects of optogenetic and pharmacogenetic stimulation of NPY/AgRP neurones to rapidly increase food intake^{4,5} and prolonged stimulation of POMC neurones to attenuate food intake and weight gain.⁴ POMC and NPY/AgRP neurones in the arcuate nucleus have been identified as major CNS targets of both insulin and leptin.^{15,37,38} Insulin and leptin depolarise the same population of POMC neurones in guinea pigs and mice,²⁰ and the common CNS-mediated (anorexigenic) actions of these two hormones with respect to regulating energy homeostasis argue for the convergence of these two metabolic hormone signalling pathways onto one effector system (ie, TRPC5 channel activation). Indeed, leptin acting through a hypothalamic-dependent mechanism is capable of maintaining euglycaemia in the absence of insulin,³⁹ and conditional deletion of *Trpc5* in POMC neurones results in a decrease in energy expenditure, as well as an increase in food intake and weight gain in male mice.⁴⁰

Therefore, we conclude that insulin and leptin bind to their cognate receptors to converge on the IRS-PI3K-PLC γ signalling pathway, resulting in TRPC5 channel activation (Figure 5); phosphorylation of IRS proteins activates PI3K that subsequently activates PLC γ 1 to augment TRPC channel activity and POMC neuronal excitability. Because it is evident that insulin and leptin are essential for regulation of food intake and energy expenditure, this signalling pathway, and in particular the sine qua non TRPC5 channels, represent potential targets for the critical uncoupling events leading to insulin and leptin resistance in obesity and type II diabetes.

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References

1. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron*. 1999; 22:221–232. [PubMed: 10069329]
2. Gao Q, Horvath TL. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci*. 2007; 30:367–398. [PubMed: 17506645]
3. Hill JW, Elmquist JK, Elias CF. Hypothalamic pathways linking energy balance and reproduction. *Am J Physiol Endocrinol Metab*. 2008; 294:E827–E832. [PubMed: 18285524]
4. Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nat Neurosci*. 2011; 14:351–355. [PubMed: 21209617]

5. Krashes MJ, Koda S, Ye C, et al. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest*. 2011; 121:1424–1428. [PubMed: 21364278]
6. Horvath TL. The hardship of obesity: a soft-wired hypothalamus. *Nat Neurosci*. 2005; 8:561–565. [PubMed: 15856063]
7. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in arcuate nucleus. *Nature*. 2001; 411:480–484. [PubMed: 11373681]
8. Ibrahim N, Bosch MA, Smart JL, et al. Hypothalamic proopiomelanocortin neurons are glucose-responsive and express K-ATP channels. *Endocrinology*. 2003; 144:1331–1340. [PubMed: 12639916]
9. Faouzi M, Leshan R, Björnhom M, Hennessey T, Jones J, Münzberg H. Differential accessibility of circulating leptin to individual hypothalamic sites. *Endocrinology*. 2007; 148:5414–5423. [PubMed: 17690165]
10. Elias CF, Aschkenasi C, Lee C, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron*. 1999; 23:775–786. [PubMed: 10482243]
11. Qiu J, Fang Y, Rønnekleiv OK, Kelly MJ. Leptin excites proopiomelanocortin neurons via activation of TRPC channels. *J Neurosci*. 2010; 30:1560–1565. [PubMed: 20107083]
12. Benoit SC, Air EL, Coolen LM, et al. The catabolic action of insulin in the brain is mediated by melanocortins. *J Neurosci*. 2002; 22:9048–9052. [PubMed: 12388611]
13. Schwartz MW, Marks JL, Sipols AJ, et al. Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology*. 1991; 128:2645–2647. [PubMed: 2019270]
14. Xu AW, Kaelin CB, Takeda K, Akira S, Schwartz MW, Barsh GS. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest*. 2005; 115:951–958. [PubMed: 15761497]
15. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature*. 2006; 443:289–295. [PubMed: 16988703]
16. Hill JW, Williams KW, Ye C, et al. Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. *J Clin Invest*. 2008; 118:1796–1805. [PubMed: 18382766]
17. Brown LM, Clegg DJ, Benoit SC, Woods SC. Intraventricular insulin and leptin reduce food intake and body weight in C57BL/6J mice. *Physiol Behav*. 2006; 89:687–689. [PubMed: 16979194]
18. Plum L, Ma X, Hampel B, et al. Enhanced PIP₃ signaling in POMC neurons causes K_{atp} channel activation and leads to diet-sensitive obesity. *J Clin Invest*. 2006; 116:1886–1901. [PubMed: 16794735]
19. Williams KW, Margatho LO, Lee CE, et al. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J Neurosci*. 2010; 30:2472–2479. [PubMed: 20164331]
20. Qiu J, Zhang C, Borgquist A, et al. Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels. *Cell Metab*. 2014; 19:682–693. [PubMed: 24703699]
21. Qiu J, Bosch MA, Tobias SC, et al. Rapid signaling of estrogen in hypothalamic neurons involves a novel G protein-coupled estrogen receptor that activates protein kinase C. *J Neurosci*. 2003; 23:9529–9540. [PubMed: 14573532]
22. Qiu J, Fang Y, Bosch MA, Rønnekleiv OK, Kelly MJ. Guinea pig kisspeptin neurons are depolarized by leptin via activation of TRPC channels. *Endocrinology*. 2011; 152:1503–1514. [PubMed: 21285322]
23. Clapham DE. TRP channels as cellular sensors. *Nature*. 2003; 426:517–524. [PubMed: 14654832]
24. Smith AW, Bosch MA, Wagner EJ, Rønnekleiv OK, Kelly MJ. The membrane estrogen receptor ligand STX rapidly enhances GABAergic signaling in NPY/AgRP neurons: role in mediating the anorexigenic effects of 17 β -estradiol. *Am J Physiol Endocrinol Metab*. 2013; 305:E632–E640. [PubMed: 23820624]
25. Clapham DE, Julius D, Montell C, Schultz G. International union of pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. *Pharmacol Rev*. 2005; 57:427–450. [PubMed: 16382100]

26. Strübing C, Krapivinsky G, Krapivinsky L, Clapham DE. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron*. 2001; 29:645–655. [PubMed: 11301024]
27. Sukumar P, Sedo A, Li J, et al. Constitutively active TRPC channels of adipocytes confer a mechanism for sensing dietary fatty acids and regulating adiponectin. *Circ Res*. 2012; 111:191–200. [PubMed: 22668831]
28. Al-Qassab H, Smith MA, Irvine EE, et al. Dominant role of the p110 β isoform of PI3K over p110 α in energy homeostasis regulation by POMC and AgRP neurons. *Cell Metab*. 2009; 10:343–354. [PubMed: 19883613]
29. Rameh LE, Rhee SG, Spokes K, Kazlauskas A, Cantley LC, Cantley LG. Phosphoinositide 3-kinase regulates phospholipase C γ -mediated calcium signaling. *J Biol Chem*. 1998; 273:23750–23757. [PubMed: 9726983]
30. Falasca M, Logan SK, Lehto VP, Baccante G, Lemmon MA, Schlessinger J. Activation of phospholipase C γ by PI 3-kinase-induced PH domain-mediated membrane targeting. *EMBO J*. 1998; 17:414–422. [PubMed: 9430633]
31. Bae YS, Cantley LG, Chen C-S, Kim S-R, Kwon K-S, Rhee SG. Activation of phospholipase C- γ by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem*. 1998; 273:4465–4469. [PubMed: 9468499]
32. Rodríguez-Menchaca AA, Adney SK, Zhou L, Logothetis DE. Dual regulation of voltage-sensitive ion channels by PIP $_2$. *Front Pharmacol*. 2012; 3:170. [PubMed: 23055973]
33. Zhang C, Bosch MA, Rønnekleiv OK, Kelly MJ. Kisspeptin activation of TRPC4 channels in female GnRH neurons requires PIP $_2$ depletion and cSrc kinase activation. *Endocrinology*. 2013; 154:2772–2783. [PubMed: 23744639]
34. Bezzerides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE. Rapid vesicular translocation and insertion of TRP channels. *Nat Cell Biol*. 2004; 6:709–720. [PubMed: 15258588]
35. Scherer T, O'Hare J, Diggs-Andrews K, et al. Brain insulin controls adipose tissue lipolysis and lipogenesis. *Cell Metab*. 2011; 13:183–194. [PubMed: 21284985]
36. Clegg DJ, Gotoh K, Kemp C, et al. Consumption of high-fat diet induces central insulin resistance independent of adiposity. *Physiol Behav*. 2011; 103:10–16. [PubMed: 21241723]
37. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000; 404:661–671. [PubMed: 10766253]
38. Belgardt BF, Brüning JC. CNS leptin and insulin action in the control of energy homeostasis. *Ann NY Acad Sci*. 2010; 1212:97–113. [PubMed: 21070248]
39. Fujikawa T, Berglund ED, Patel VR, et al. Leptin engages a hypothalamic neurocircuitry to permit survival in the absence of insulin. *Cell Metab*. 2013; 18:431–444. [PubMed: 24011077]
40. Gao Y, Yao T, Deng Z, et al. TrpC5 mediates acute leptin and serotonin effects via Pomc neurons. *Cell Rep*. 2017; 18:583–592. [PubMed: 28099839]

FIGURE 1.

Leptin and insulin excite guinea pig pro-opiomelanocortin (POMC) neurones. (A) Leptin (100 nmol L^{-1}) depolarised POMC neurones and increased their firing frequency. (B) Under voltage clamp conditions with an internal solution containing $130 \text{ mmol L}^{-1} \text{ Cs}^+$ -gluconate and the exchange of Cs^+ for the external Na^+ and K^+ , the leptin-induced current reversed at approximately 0 mV and exhibited the double rectification characteristic of transient receptor potential (TRPC)1/TRPC5 heteromeric channels.²⁶ (C) Similarly, guinea pig insulin (20 nmol L^{-1}) depolarised POMC neurones and increased their firing frequency. (D) Under voltage-clamp conditions with an internal solution containing $130 \text{ mmol L}^{-1} \text{ Cs}^+$ -gluconate and in the presence of Ca^{2+} and K^+ blockers in the extracellular bath, the insulin-induced current reversed at approximately 0 mV and exhibited less pronounced double rectification without the external Cs^+ block. I/Vs were generated by digital subtraction. (B) Modified from with permission from Qiu et al.¹¹ (C, D) Modified with permission from Qiu et al.²⁰

FIGURE 2.

Insulin excites nonhuman primate pro-opiomelanocortin (POMC) neurones. Whole-cell current and voltage clamp recordings were done using a normal internal solution in arcuate neurones in slices (300 μ m) from two adult rhesus macaque males. Eight neurones were tested with purified guinea pig insulin (20 nmol L⁻¹). Five of the eight neurones were depolarised and one neurone was hyperpolarised by insulin. The eight neurones were harvested, and post hoc single cell reverse transcriptase-polymerase chain reaction (scRT-PCR) analysis was performed: two of the depolarised neurones were identified as *Pomc* neurones and the hyperpolarised neurone was a non-*Pomc* neurone. (A) In current clamp, insulin depolarised a POMC neurone and induced action potential firing. (B) Under voltage clamp conditions, the I/V curve revealed that insulin generated an inward current that reversed at approximately -20 mV (*Pomc* neurone from A). (C) Insulin (20 nmol L⁻¹) hyperpolarised and inhibited the firing of a non-*Pomc* (presumably neuropeptide Y/agouti-related protein) neurone. (D) scRT-PCR identification of recorded arcuate neurones.

Representative gel illustrating the mRNA expression of *Pomc* in one of two arcuate neurones that were depolarised by insulin. The expected size for the *Pomc* PCR product is 197 bp. The cell that was hyperpolarised by insulin did not express *Pomc* but did express *Gapdh*, confirming the presence of mRNA following the harvesting. +, -, positive and negative tissue controls reacted with (+) and without (-) reverse transcriptase. H₂O, a water blank control was also included. MM, molecular markers

FIGURE 3.

Insulin and leptin responses require transient receptor potential (TRPC) channel activation and phosphatidylinositol-3 kinase (PI3K). (A) Summary of the effects of PI3K inhibitor wortmannin (Wort) (100 nmol L^{-1}), 2-aminoethyl diphenylborinate (2-APB) ($100 \mu\text{mol L}^{-1}$) and TRPC4/5 channel enhancers La^{3+} and rosiglitazone (Rg) ($100 \mu\text{mol L}^{-1}$) on the insulin (20 nmol L^{-1})-induced inward current. La^{3+} ($100 \mu\text{mol L}^{-1}$) augmented the current by five-fold and rosiglitazone augmented the current by approximately two-fold. $V_{\text{hold}} = -60 \text{ mV}$. The mean $\pm$ SEM of the inward currents is shown. *** $P < .001$; ** $P < .01$; * $P < .05$ significantly different from the control group (black bar). Cell numbers are indicated. (B) Summary of the effects of the Janus kinase inhibitor AG490 ($10 \mu\text{mol L}^{-1}$), Wort (100 nmol L^{-1}), phospholipase C (PLC) inhibitor U73122 ($20 \mu\text{mol L}^{-1}$) and its inactive analogue U73343 ($20 \mu\text{mol L}^{-1}$) and the PLC γ inhibitor ET-18-OCH3 ($15 \mu\text{mol L}^{-1}$) on the leptin-induced inward current. $V_{\text{hold}} = -60 \text{ mV}$. ** $P < .01$, U73122 vs U73343 group; *** $P < .001$ vs the leptin control group. Cell numbers are indicated. (A) Modified with permission from Qiu et al.²⁰ (B) Modified with permission from Qiu et al.¹¹

FIGURE 4.

Guinea pig insulin (i.c.v.) inhibits food intake (A) and increases energy expenditure (B–D) insulin decreases food intake. (B–D) Insulin increases O₂ consumption (B), as well as CO₂ (C) and metabolic heat production (D). Bars represent mean ± SEM of the incremental hourly food intake, O₂ consumption, CO₂ production and metabolic heat production in animals fed a standard, grain based diet and treated with either insulin (4 mU, i.c.v.) or its 0.9% saline vehicle (2 µL, i.c.v.). The asterisk shows values from insulin-treated animals that are significantly different ($P < .05$, repeated-measures, multifactorial ANOVA least significant difference) than those from vehicle-treated controls (n=5 in each group). Modified with permission from Qiu et al.²⁰

FIGURE 5.

A cellular model of insulin and leptin signalling in pro-opiomelanocortin (POMC) neurones. On the basis of our findings and other published data, we propose that the insulin and leptin receptors couple to the IRS-PI3K-PLC γ signalling pathway resulting in the activation of transient receptor potential (TRPC)5 channels, which generates a robust inward cationic current to depolarise POMC neurones and increase their excitability. PI3K (p85/p110) will also accelerate the insertion of TRPC5 channels into the plasma membrane (see text). Modified with permission from Qiu et al.²⁰ DAG, diacylglycerol; IP₃R, inositol trisphosphate receptor; IRS, insulin receptor substrate; Jak2, Janus kinase; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol-3,4,5-triphosphate; PLC γ 1, phospholipase C γ 1