Supraoptic oxytocin and vasopressin neurons function as glucose and metabolic sensors

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Submitted 21 November 2013; accepted in final form 27 January 2014

Song Z, Levin BE, Stevens W, Sladek CD. Supraoptic oxytocin and vasopressin neurons function as glucose and metabolic sensors. Am J Physiol Regul Integr Comp Physiol 306: R447–R456, 2014. First published January 29, 2014; doi:10.1152/ajpregu.00520.2013.—Neurons in the supraoptic nucleus (SON) produce oxytocin and vasopressin and express insulin receptors (InsR) and glucokinase. Since oxytocin is an anorexigenic agent and glucokinase and InsR are hallmarks of cells that function as glucose and/or metabolic sensors, we evaluated the effect of glucose, insulin, and their downstream effector ATP-sensitive potassium (KATP) channels on calcium signaling in SON neurons and on oxytocin and vasopressin release from explants of the rat hypothalamo-neurohypophyseal system. We also evaluated the effect of blocking glucokinase and phosphatidylinositol 3 kinase (PI3K; mediates insulin-induced insertion of glucose transporter, GLUT4) on responses to glucose and insulin. Glucose and insulin increased intracellular calcium ([Ca$^{2+}$])$_i$. The responses were glucokinase and PI3K dependent, respectively. Insulin and glucose alone increased vasopressin release (P < 0.002). Oxytocin release was increased by glucose in the presence of insulin. The oxytocin (OT) and vasopressin (VP) responses to insulin+glucose were blocked by the glucokinase inhibitor alloxan (4 mM; P ≤ 0.002) and the PI3K inhibitor wortmannin (50 nM; OT: P = 0.03; VP: P ≤ 0.002). Inactivating KATP channels with 200 nM glibenclamide increased oxytocin and vasopressin release (OT: P < 0.003; VP: P < 0.05). These results suggest that insulin activation of PI3K increases glucokinase-mediated ATP production inducing closure of KATP channels, opening of voltage-sensitive calcium channels, and stimulation of oxytocin and vasopressin release. The findings are consistent with SON oxytocin and vasopressin neurons functioning as glucose and “metabolic” sensors to participate in appetite regulation.

Obesity has reached epidemic proportions in the United States and currently gastric bypass is the only effective long-term treatment. In these studies, we investigated a largely overlooked hypothalamic mechanism for appetite regulation that might prove useful for weight intervention. Oxytocin (OT) is an anorexigenic agent that inhibits food intake and reduces body weight following central or peripheral administration (1, 2, 6, 13, 36, 43, 49, 50, 85), and deficits in OT or mutations in the OT receptor (OTR) are associated with obesity in humans (11, 22, 81) and mice (8, 46, 70, 71). Studies on the role of OT in appetite regulation have largely focused on the paraventricular nucleus (PVN) and its hindbrain projections (7, 31, 43, 52, 53, 55, 62). PVN contains both parvocellular and magnocellular OT neurons. Parvocellular OT neurons project to pregastronergic neurons of the autonomic nervous system in the brain stem and spinal cord regulating autonomic functions including gastric motility (16). In contrast, the magnocellular neurons (MCNs) project to the neural lobe of the pituitary and release OT into the peripheral circulation in response to anorexigenic and other stimuli (38, 69). However, the OT MCNs in the supraoptic nucleus (SON) and PVN are also a major source of OT in the brain. They have been shown to project to areas of the brain involved in motivated behaviors [e.g., amygdala and nucleus accumbens (29, 58)]. Since OT neurons have not been described in these regions except in the naked mole rat (57), it is likely that the MCNs provide the ligand for OT receptors in these regions, and could participate in appetite regulation. This possibility has not received attention by investigators studying hypothalamic mechanisms of appetite regulation.

 Autoradiographic binding of insulin and prominent insulin receptor (InsR) immunohistochemistry was described in SON in the late 1980s (19, 76). Since SON contains only OT and vasopressin (VP) MCNs projecting to neural lobe, and since insulin serves as an appetite-regulating signal in other parts of the hypothalamus, expression of InsR in MCNs raises the possibility that, consistent with an anorexigenic role for OT, MCNs may have the ability to monitor nutrient stores and in turn regulate peptide release to appropriately adjust food intake to maintain body nutrient stores. We assessed the ability of SON MCNs to function as glucose and metabolic sensors as diagrammed in Fig. 1. First, we used quantitative real-time PCR (qRT-PCR) to confirm expression of InsR in SON and to evaluate expression of glucokinase (GK), a hallmark of glucose sensors, to determine whether MCNs expressed molecules characteristic of metabolic sensors. We then evaluated the effect of glucose and insulin on intracellular calcium signaling in SON MCNs and OT and VP release from explants of the rat hypothalamo-neurohypophyseal system (HNS). HNS explants include the SON with their projections to the neural lobe. Since SON contains only MCNs, HNS explants are an excellent preparation for studying responses of MCNs independent of parvocellular OT neurons. Finally, we examined the roles of ATP-sensitive potassium (KATP) channels, GK, and phosphoinositol 3-kinase (PI3K), the kinase that mediates insulin-induced insertion of

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and were approved by the Institutional Animal Care and Use Committee of the University of Colorado Denver. Male Sprague-Dawley rats [CRL: CD(SD)Br; Charles Rivers Laboratories, Wilmington, MA], 150–175 g, were used in all experiments. All protocols were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Colorado Denver.

Detection of mRNA for GK and InsR by qRT-PCR

SON was microdissected using the optic chiasm as a landmark. A rectangular block of tissue immediately rostral to optic chiasm approximately 2 mm wide × 1 mm deep × 3 mm long was removed from each side of the brain using irridectomy scissors (64). Micro-dissected samples of SON were collected into RNA-Later (QIAGEN) and shipped to B. Levin’s laboratory for processing and analysis as described previously (10, 32). Primer sets for cyclophilin, the housekeeping reference gene, GK, and insulin receptor mRNA were designed by reference to published sequences, and their specificity was verified using GenBank and by comparing the sequenced PCR product for cyclophilin, GK, and InsR to these references. For each mRNA species, a pair of conventional primers was used in combination with a sequence-specific 6-carboxyfluorescein (FAM)-labeled probe to allow real-time PCR quantitation using an Applied Biosystems 7700 Sequence Detector set for 40 PCR cycles. The primers for cyclophilin [constitutive gene (39)] were GenBank (NM 017101): forward beginning at 253 bp, CCAAATCGTATTACCACAAACCAAG; reverse beginning at 330 bp, AAGGTGCTGGAATTCTGATGATGC; FAM-labeled probe TCTACGGAGAGGAAATT. Reference standards were created for cyclophilin and InsR from pooled aliquots of arcuate nucleus samples, and these were used to generate standard curves from which quantitative data were read. Data were then expressed as the ratio of GK and InsR to cyclophilin mRNA.

Hypothalamo-Neurohypophyseal Explant Preparation

HNS explants were used for calcium imaging and hormone release studies. Explants were prepared as described previously from male rats (67). HNS explants include the SON neurons, their axons, and axon terminals in the neural lobe as well as organum vasculosum of the lamina terminalis and suprachiasmatic and arcuate nuclei. They do not include the PVN.

Calcium Imaging

HNS explants were loaded with the calcium-sensitive dye Fura-2 AM as described previously (67). They were placed in a recording chamber with the ventral surface up allowing easy visualization of SON neurons using the optic chiasm as an anatomical landmark (67). SON neurons were identified by the size of the cell body (>25 μm in diameter) and their location adjacent to the optic chiasm. Explants were perfused at a rate of 3 ml/min with gassed (95% O2–5% CO2) specially formulated F12 nutrient mixture modified to contain 0.5 mM glucose, 13 mM KCl, and 1.7 mM CaCl2. Fura-2-loaded MNCs were alternately excited with 340 nm and 380 nm UV light from a Xenon Source (Sutter Instruments, Novato, CA). The 380-nm exposure time was between 200 and 500 ms and was tripled for the 340-nm exposure. Emitted light was passed through a ×60 fluorimeter immersion lens attached to an Olympus upright microscope and collected at 510 nm by an intensified charge-coupled device camera (Hamamatsu, Japan). Paired 340- and 380-nm images were acquired every 3 s using Slide-Book software (Intelligent Imaging, Denver, CO) for a period of 100 frames. The 340-to-380 ratio (R) was used as an index of the change in intracellular Ca2+ concentration ([Ca2+]i). Rmax was previously determined in ionomycin-treated explants and far exceeded the highest R obtained with agents studied in these experiment (67). R data are presented as percentage of the basal 340:380 R for each cell determined from the average R of 10 frames preceding drug exposure. Explants were allowed to equilibrate for 1 h.

Data analysis. Means ± SE of the percentage values from individual neurons were calculated and plotted. Parametric one-way ANOVA (F value) followed by Student-Newman-Keuls individual mean analysis or Kruskal-Wallis one-way ANOVA on ranks (H
value) followed with Dunn’s individual mean analysis were used to determine significant group differences and paired t-tests were used to compare peak responses to the basal $[\text{Ca}^{2+}]_i$.

**Hormone Release From HNS Explants**

Explants were positioned individually in perifusion chambers having a 500-μl volume and perfused at 2 ml/h as described previously (27) with specially formulated F12 nutrient mixture containing a final glucose concentration of 1 mM. After a 4- to 5-h equilibration period to allow hormone release to stabilize at basal level, explants were either maintained under control conditions or exposed to an increase in glucose (5 mM) or insulin (3 ng/ml) for 1 h followed by the addition of glucose to achieve 5 mM glucose. Since the perifusion medium contained 20% fetal bovine serum (FBS), the amount of glucose and insulin added was adjusted to account for the glucose and insulin concentration in each lot of FBS. Where appropriate, drugs were added to inhibit GK or PI3K, and glibenclamide was used to evaluate the effect of inactivating KATP channels on hormone release. Effluent was collected individually at 20-min intervals using a refrigerated fraction collector maintained at 4°C. VP and OT concentration in the perifusate was determined by radioimmunoassay as described previously (82). Changes in VP and OT release from HNS explants reflects altered release from nerve terminals in the neural lobe, because although VP and OT are released from dendrites in SON and VP from suprachiasmatic nucleus (15), the amount from neural lobe far exceeds these other sources (17).

**Data analysis.** Basal VP/OT release was determined during the hour immediately preceding exposure to insulin, glucose, or drugs. Hormone release in response to experimental manipulations is expressed as a percentage of this initial basal release for each explant. Basal release for the explants included in these studies was $159 \pm 27$ pg/ml for VP and $190 \pm 10$ pg/ml for OT (means ± SE). ANOVA with repeated measures followed by post hoc simple main effects analysis was performed to evaluate changes in hormone release and to compare responses between groups.

**RESULTS**

**Detection of mRNA for GK and InsR by qRT-PCR**

With the use of primers described previously (10, 32), the transcripts for both InsR and GK were found to be abundant in SON. The average number of cycles to detection compared with cyclophilin ($2^{-\Delta Ct}$), the reference housekeeping gene, was 4.9 for InsR and 6.1 for GK, indicating that InsR mRNA is in the order of 3.4% and GK 1.5% as abundant as cyclophilin (assuming equal efficiencies in the PCR reactions). This is consistent with the prominent expression of InsR observed with immunohistochemistry (76). The expression of GK is significant, because it raises the possibility that these neurons may function as glucose sensors (e.g., cells that alter their firing pattern proportionally to the extracellular glucose concentration). Since the insulin-producing β-cells in the pancreas as well as glucose-sensing neurons express GK, GK is considered to be the “gate keeper” for glucosensing (34).

**Fig. 2. Effect of increasing glucose on intracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) in SON neurons.**

A: time course of change in 340-to-380 (340:380) ratio indicative of change in $[\text{Ca}^{2+}]_i$, induced by increasing the glucose concentration from 0.5 to 5 mM. Values shown are means ± SE of 18 SON neurons imaged simultaneously in a single hypothalamo-neurohypophyseal system (HNS) explant. B: peak increase in the 340:380 ratio in response to the addition of glucose in 34 neurons imaged in 2 HNS explants. Values are means ± SE, *$P < 0.001$ by signed rank test between basal and peak response. C: time course of individual neuronal responses to increasing glucose (from 0.5 to 5 mM) in the presence of tetrodotoxin (TTX, 3 μM). In this preparation, the responses were not tightly temporally synchronized as in A. This could reflect neurons at different depths within the preparation. D: peak increase in the 340:380 ratio in response to the addition of glucose in 40 neurons imaged in 2 HNS explants. Values are means ± SE, $t_r = 17,500$, $P < 0.001$, basal to peak response.
dant presence of GK in SON supports the hypothesis that these neurons can monitor extracellular glucose. Since the ventromedial nucleus (VMN) glucose-sensing neurons also express GK and InsR (26), the prominent expression of GK and InsR in SON supports the hypothesis that MNCs may function as glucose sensors similar to VMN neurons. Thus these observations provided the crucial evidence to pursue the subsequent studies on the effects of glucose and insulin on SON neurons and the role of GK and PI3K.

**Effect of 5 mM Glucose on [Ca2+]i.**

Increasing the glucose concentration to 5 mM in F12 containing 3 mM K+ increased [Ca2+]i in some, but not all, SON neurons (data not shown). With the use of responsiveness to VP or OT as criteria for OT or VP phenotypic identification of the neurons (12), 44% of OT neurons and 74% of VP neurons showed responses. However, when the K+ concentration in the perifusate was increased to 13 mM to move neurons closer to their depolarization threshold (80), increasing the glucose concentration from 0.5 to 5 mM reliably increased [Ca2+]i in all of the MNCs recorded (Fig. 2, A and B). The response was sustained in the presence of tetrodotoxin (TTX, Fig. 2, C and D) indicating that the glucose response is not synaptically mediated. Since all the MNCs responded to an increase in glucose with an increase in [Ca2+]i, they would be classified as “glucose-excited.” It should be noted, however, that these experiments were not optimally designed to reveal “glucose-inhibited” neurons.

**Effect of 5 mM Glucose and Insulin on OT and VP Release.**

As seen in Fig. 3A, VP release from HNS explants was stimulated by increasing glucose alone (glucose group from hour 4.6 to end; \( F_{\text{treatment}} = 25.74, P < 0.0005; F_{\text{time}} = 4.91, P < 0.0001 \)), insulin alone (first hour in the Ins+glucose group; \( F_{\text{treatment}} = 14.47, P = 0.0035; F_{\text{time}} = 2.67, P = 0.032; F_{\text{interaction}} = 2.81, P = 0.026 \), and the response to insulin was sustained when glucose was increased in the presence of insulin (Ins+glucose group from hour 4.6 to end; \( F_{\text{treatment}} = 11.26, P = 0.001; F_{\text{time}} = 5.13, P < 0.0001 \)). OT release was significantly increased during combined exposure to insulin and 5 mM glucose (\( F_{\text{time}} = 5.17, P < 0.0001; F_{\text{interaction}} = 3.57, P < 0.0001 \)) Thus glucose and insulin stimulated VP release from the posterior pituitary, and OT release was increased by increasing the glucose concentration in the presence of insulin.

**Role of K_A TP Channels in OT and VP Neurons.**

To assess the effect of inactivating K_A TP channels on OT and VP release, HNS explants were incubated in glibenclamide (200 nM), a blocker of K_A TP channels, for 3 h. Glibenclamide was solubilized in ethanol and a comparable amount of ethanol was added to the control explants. As shown in Fig. 4, the addition of glibenclamide resulted in a gradual and sustained increase in OT and VP release during the duration of exposure to glibenclamide that reached statistical significance after 2 h (OT: \( F_{\text{treatment}} = 17.24, P = 0.002; F_{\text{time}} = 6.32, P < 0.0001; F_{\text{interaction}} = 3.87, P < 0.0001 \); VP: \( F_{\text{treatment}} = 3.86, P = 0.07; F_{\text{time}} = 10.98, P < 0.0001; F_{\text{interaction}} = 2.64, P = 0.005 \)). Thus the presumptive depolarization resulting from inactivation of K_A TP channels is sufficient to increase OT and VP release.

To assess the role of K_A TP channels in the glucose-induced increase in [Ca2+]i in SON neurons, the effect of glucose was evaluated in the absence and presence of diazoxide (0.4 mM). As shown in Fig. 5, increasing the glucose concentration from 0.5 to 5 mM resulted in a robust and sustained elevation in [Ca2+]i in all of the OT and VP neurons. Increasing the glucose concentration to 5 mM in F12 containing 3 mM K+ in the presence of insulin (Ins+glucose group from hour 4.6 to end; \( F_{\text{treatment}} = 11.26, P = 0.001; F_{\text{time}} = 5.13, P < 0.0001 \)). OT release was significantly increased during combined exposure to insulin and 5 mM glucose (\( F_{\text{time}} = 5.17, P < 0.0001; F_{\text{interaction}} = 3.57, P < 0.0001 \)) Thus glucose and insulin stimulated VP release from the posterior pituitary, and OT release was increased by increasing the glucose concentration in the presence of insulin.
induced increase in \([\text{Ca}^{2+}]_i\) is dependent on closure of KATP channels. This suggests that the glucose-induced increase in calcium is most likely due to depolarization-induced opening of voltage-dependent calcium channels, but our studies do not test that directly.

**Role of GK in OT and VP Neurons**

To determine whether the glucose-induced increase in \([\text{Ca}^{2+}]_i\) and the stimulation of OT and VP release by glucose and insulin are GK dependent, we used alloxan (4 mM) to block GK. As shown in Fig. 6, the glucose-induced increase in \([\text{Ca}^{2+}]_i\) was essentially abolished in the presence of alloxan, and alloxan prevented the glucose plus insulin-induced increase in OT (F\(_{\text{treatment}} = 18.04, P = 0.0017; F_{\text{time}} = 2.63, P = 0.0051; F_{\text{interaction}} = 2.667, P = 0.0045) and VP release (F\(_{\text{treatment}} = 17.75, P = 0.0001; F_{\text{time}} = 2.97, P = 0.0013; F_{\text{interaction}} = 4.331, P < 0.0001). Thus GK mediates the glucose-induced increase in \([\text{Ca}^{2+}]_i\); and the stimulation of OT and VP release induced by the combined increase in glucose and insulin.

**Role of PI3K in OT and VP Neurons**

Since insulin activation of PI3K is required for insulin-induced translocation of the GLUT4 glucose transporter to the plasma membrane (60), we used wortmannin (50 nM), a PI3K inhibitor, to determine whether the insulin-induced increase in \([\text{Ca}^{2+}]_i\) and the stimulation of OT and VP release by insulin and glucose are PI3K dependent. As shown in Fig. 7, in the presence of 5 mM glucose, the insulin-induced increase in \([\text{Ca}^{2+}]_i\) was significantly reduced by 10 or 50 nM wortmannin, and no increase in OT and VP release was detected in the presence of 50 nM wortmannin in response to simultaneously increasing the glucose and insulin concentrations of the perfusate to 5 mM and 3 ng/ml, respectively (OT: F\(_{\text{treatment}} = 6.289, P = 0.031; F_{\text{time}} = 2.887, P = 0.0023; F_{\text{interaction}} = 2.808, F = 0.0029; VP: F\(_{\text{treatment}} = 9.75, P = 0.0019; F_{\text{time}} = 5.416, P < 0.0001; F_{\text{interaction}} = 3.141, P < 0.0001). Thus the responses to insulin reflect PI3K-mediated intracellular events that could include insulin-induced insertion of GLUT4 transporters into the membrane. However, PI3K-dependent insulin actions that are independent of GLUT4 translocation exist and are not excluded in these experiments (61).

**DISCUSSION**

The ability of glucose and insulin to alter VP and OT release from the neurohypophysis is consistent with the possibility that the MNC neurons can function as metabolic sensors. Although
dendritic release of VP and OT also may be affected, the intact HNS explant does not allow us to explore that possibility. An important aspect of these findings is that the reported effects of glucose and insulin reflect responses to physiologically relevant concentrations of glucose and insulin. Glucose concentrations measured in the hypothalamus range from 0.5 mM in fasting rats to 5 mM in severely hyperglycemic rats with 2.5 mM representing postingestive normoglycemia (63). Thus the changes in glucose utilized in these experiments represented the full physiological range of hypothalamic glucose. Insulin is transported into the brain by a saturable transport mechanism that is physiologically and regionally regulated (3). Hypothalamic insulin levels are among the highest in the brain at 0.4 ng/g (4). Thus it is reasonable, given the diffusion barriers inherent in in vitro preparations, that the glucose and insulin concentrations employed in these experiments resulted in local fluctuations that SON neurons might encounter in vivo.

Using qRT-PCR, we found that InsR and GK are prominently expressed in rat SON. This confirmed the earlier immunohistochemical report showing dense InsR expression and the in situ hybridization study showing GK expression in SON neurons (45, 76). Since some of the glucose-sensing neurons in VMN also express GK and InsR, expression of these molecules in SON neurons is consistent with the hypothesis that MNCs function as glucose sensors. In VMN neurons, glucose induces depolarization via GK-mediated inactivation of K_{ATP}. Specifically, GK-mediated glycolysis is likely to result in ATP production, closure of K_{ATP} channels, and depolarization (34).

Fig. 6. GK dependence of the effects of glucose on [Ca^{2+}], and Ins + glucose on OT and VP release. A: time course of Ca^{2+} response to glucose (5 mM) in the presence of 13 mM KCl in the absence (glucose) or presence of alloxan (AOX.glucone; 4 mM AOX). The basal medium contains 0.5 mM glucose. Arrows indicate when glucose was administered. B: comparison of the peak responses under these conditions. Glucose (5 mM) induced an increase in [Ca^{2+}]; in the majority of SON neurons in the presence of 13 mM KCl (to increase resting membrane potential). The response was abolished when cells were pretreated with GK inhibitor AOX (*H = 59.260, P ≤ 0.001). Data were combined from neurons imaged in 2 explants. Total number of neurons = 40. C and D: effect of alloxan on OT and VP release, respectively. Addition of alloxan (4 mM) 1 h before increasing the perifusate glucose and insulin concentrations to 5 mM and 3 ng, respectively, did not alter basal release of OT (198 ± 25 pg/ml) or VP (169 ± 46 pg/ml), but it prevented the increase in OT and VP release induced by the addition of glucose and insulin. **P < 0.01, ***P < 0.0001. n = 6 explants per group.
We found that similar mechanisms regulate OT and VP secretion from the neurohypophysis. Specifically, inhibition of K<sub>ATP</sub> channel activity with glibenclamide was sufficient to increase OT and VP secretion, and the ability of glucose to increase [Ca<sup>2+</sup>]<sub>i</sub> is dependent on closure of K<sub>ATP</sub> channels. This is consistent with the reported expression of the K<sub>ATP</sub> channels Kir6.1 and 6.2 in SON (14, 73). Furthermore, the increase in [Ca<sup>2+</sup>]<sub>i</sub> induced by glucose and the increase in OT and VP release induced by combined exposure to increases in glucose and insulin are GK dependent. Finally, since insulin-mediated membrane insertion of GLUT4 is PI3K dependent (9, 47, 48), the ability of wortmannin to significantly reduce the calcium response to insulin and to prevent combined glucose and insulin-stimulated OT and VP release, is consistent with the hypothesis that one role of InsR in SON neurons is to increase glucose transport potentially via inducing translocation of glucose transporters to the membrane (34, 75). However, since we have not demonstrated that insulin increases glucose uptake, it remains possible that the insulin actions, although PI3K dependent, are independent of changes in glucose uptake. Insulin has been shown to activate K<sub>ATP</sub> channels in VMN and arcuate neurons via PI3K (68). However, this results in mem-

Fig. 7. Effect of the phosphatidylinositol 3 kinase (PI3K) inhibitor wortmannin (WMN) on the Ins-induced increase in [Ca<sup>2+</sup>]<sub>i</sub>, and insulin with glucose (Ins+glucose)-induced OT and VP release. A: time course of Ca<sup>2+</sup> response to Ins (3 ng/ml) in the presence of 13 mM KCl in the absence (Ins) or presence of WMN (50 nM, WMN.Ins) The medium contained 5 mM glucose. Arrows indicate when Ins was administered. B: comparison of the peak responses under these conditions. Ins (3 ng/ml) induced an increase in [Ca<sup>2+</sup>]<sub>i</sub>, in the majority of SON neurons in the presence of 5 mM glucose and 13 mM KCl (to increase resting membrane potential) (*t = 820.0, P < 0.001). The peak response was greatly reduced in the experiments when cells were pretreated with WMN (#t = 42.879, P < 0.001; $t = 18.299, P < 0.001). Data were combined from neurons imaged in 2 explants. Total number of neurons = 40 in each experiment. C and D: effect of WMN on OT and VP release, respectively. Addition of WMN alone 1 h before increasing the perfusate glucose and insulin concentrations to 5 mM and 3 ng/ml, respectively, did not alter basal release of OT (175 ± 22 pg/ml) or VP (231 ± 20 pg/ml), but it prevented the increase in OT and VP release induced by the addition of glucose and insulin. ****P < 0.0001. n = 6 explants per group.
brane hyperpolarization, which is not consistent with our findings that insulin increases [Ca\textsuperscript{2+}], and hormone release. Thus, although future studies should evaluate the effect of insulin on glucose uptake, our findings are consistent with the model shown in Fig. 1. Compartmentalization of InsR with other membrane proteins may allow for selective actions in various cell types (60).

Our studies did not demonstrate a differential effect of glucose or insulin on OT and VP neurons. How is this compatible with the evidence that OT is the neurohypophyseal hormone associated with satiety in rats (49–51)? It is consistent with our prior findings that most physiological stimuli and neurotransmitters elicit similar responses from MNC OT and VP neurons. In previous experiments with HNS explants, the two hormones show similar responses to osmolarity as well as glutamatergic, adrenergic, purinergic, and peptidergic agents (21, 27, 28, 41, 42, 65, 66). Similarly, in vivo, both hormones are released by hypertonicity and hypovolemia with only sucking, gastric distention, and peripherally administered cholecystokinin (CCK) identified as stimuli that selectively activate OT neurons or specifically stimulate OT secretion in rats (54, 56). These OT-specific stimuli discriminate between OT and VP neurons via effenter pathways that selectively activate the OT MNCs not as a result of expression of a particular receptor or ion channel being limited to the OT neuron (23, 24). The selectivity of the anorexigenic ef fectors for OT versus VP neurons is species dependent: While peripheral administration of CCK induces OT release in rats, in monkeys and humans, peripheral CCK stimulates VP release but not OT release (39, 78). Species variation in OT and VP receptor expression in target brain areas may also contribute to the anorexigenic specificity of OT in rats versus VP in primates.

The hypothesis that, in addition to anorexigenic effects generated by peripheral OT (1, 2, 6, 13, 36, 43, 49, 50, 85), OT-induced anorexia partially reflects MNC-derived activation of OT receptors in the motivated behavior circuit is plausible based on the evidence for differential localization of OT and VP in receptors in forebrain regions that participate in appetite regulation (44, 59) as well as the recent evidence that MNC OT neurons innervate these regions (29, 58). OT receptors are present in multiple regions of the motivated behavior circuitry including the amygdala, nucleus accumbens, prefrontal cortex, and ventral pallidum (caudate/putamen) (59), and all of these areas have been implicated in the motivated and hedonic components of appetite regulation (5, 33). Evidence for innervation of the amygdala and nucleus accumbens by OT neurons that also project to the posterior pituitary (e.g., MNCs) has been obtained in elegant studies utilizing fluorogold and pseudorabies virus to retrogradely label neurons projecting to nucleus accumbens and amygdala (29, 58). Both studies identified neurons in SON (which only contains MNCs) that project both to the limbic region and the posterior pituitary. Thus it is likely that axon collaterals from OT MNCs provide a substrate for OT receptors in these limbic regions. This suggests that glucose and insulin may have similar effects on OT release in the motivated behavior circuitry to that reported here on neurohypophyseal hormone release.

While our studies specifically targeted the metabolic sensor capability of the MNCs by examining the responses to glucose and insulin, these neurons also are activated in response to feeding (25, 35, 40), gastric distention (56), activation of vagal afferents (72), and refeeding after an overnight (83) or 48 h fast (35). They also respond to other molecules involved in appetite regulation including leptin (20), ghrelin (18, 84), cholecystokinin (23, 24), nesfatin (30, 37), and prolactin-releasing peptide (83). This further supports a role for these neurons in appetite regulation.

Perspectives and Physiological Significance

Although involvement of insulin in mediating glucose uptake by the neurohypophyseal system has been recognized since the late 1980s when Gary Robertson and colleagues reported that in untreated, insulin-dependent diabetics glucose became an osmotic stimulus for VP release (79) and Unger et al. (77) reported prominent expression of InsR in the SON, the possibility that the neurohypophyseal system monitors metabolic status and contributes to appetite regulation was not investigated and the anorexigenic actions of OT were attributed primarily to parvocellular OT neurons and hindbrain mechanisms (7). However, the intractability of the recent obesity epidemic has led to the realization that a highly redundant and distributed system is involved in appetite regulation. Thus, while the evidence presented here that neurohypophyseal neurons can monitor nutrient and metabolic status and potentially act on OT receptors in the motivated behavior circuitry may simply represent an additional layer of redundancy, it also might represent a previously under appreciated opportunity to enhance anorexigenic actions, because an integrated peripheral and central release of OT has the potential to influence peripheral metabolism (6) as well as both motivated/hedonic and homeostatic pathways mediating food intake.

GRANTS

This work was supported by grants from the National Institutes of Health to C. D. Sladek (R21-HD072428) and to B. E. Levin (RO1-DK53181).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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OXYCOTIN AND VASOPRESSIN NEURONS: GLUCOSE/METABOLIC SENSORS


APJ-Regul Integr Comp Physiol • doi:10.1152/ajpregu.00520.2013 • www.ajpregu.org

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