Adropin acts in the rat paraventricular nucleus to influence neuronal excitability

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Adropin has also been shown to regulate endothelial function via upregulation of endothelial nitric oxide synthase (eNOS) in human umbilical vein endothelial cells, suggesting a role in the maintenance of cardiovascular health (31). Since its discovery, adropin has been associated with multiple disease states and pathophysiological conditions, including diabetes mellitus (1, 4, 46), coronary atherosclerosis (49), myocardial infarction (2, 54) and in obese adolescents with nonalcoholic fatty liver disease (39). Although the hormone has abundant expression in the brain (1, 27), little is known about the central nervous system (CNS) actions of adropin. Recently, Stein et al. (41) showed that intracerebroventricular injection of adropin into the lateral ventricle causes the inhibition of water intake in rats, one of the only known physiological roles for central adropin to date. The exact site at which adropin acts to suppress water intake remains to be determined.

In the same study that revealed the central action of adropin to inhibit water intake, the authors identified a potential receptor through which adropin may signal, the orphan G protein-coupled receptor (GPCR), GPR19 (41). Small interfering RNA (siRNA) knockdown of GPR19 mRNA levels in the medial basal hypothalamus attenuated the adropin-mediated inhibition of water intake in overnight water-deprived rats (41). These results suggest first, that adropin signals through GPR19 and second, it is this receptor in the medial basal hypothalamus that is responsible for the central action of adropin to inhibit water intake. The medial basal hypothalamus contains the paraventricular nucleus (PVN), a critical autonomic control center with well-documented roles in the regulation of fluid balance, energy homeostasis (43), and cardiovascular regulation (8). Additionally, microarray analysis has revealed both Enho and Gpr19 transcripts in the PVN of the rat (18), further implicating this nucleus as a potential site of action for adropin. As such, we sought to determine whether PVN neurons are a target for centrally acting adropin using whole cell patch-clamp electrophysiology to evaluate the effects of adropin on the excitability of PVN neurons.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River, Quebec, Canada) aged postnatal days 21–28 (~50–100 g) were used for all experiments in this study. Animals were housed in a room maintained at 22°C under a 12:12-h light-dark cycle and were provided food and water ad libitum. All animal protocols conformed to the standards of the Canadian Council on Animal Care and were approved by the Queen’s University Animal Care Committee.

Slice preparation. Hypothalamic slices were prepared daily from unanesthetized rats. Rats were decapitated, and their brains were quickly removed and immersed in ice-cold (0–4°C) slicing solution.
consisting of the following (in mM): 87 NaCl, 2.5 KCl, 25 NaHCO₃, 0.5 CaCl₂, 7 MgCl₂, 1.25 NaH₂PO₄, 25 glucose, and 75 sucrose, bubbled with 95% O₂-5% CO₂. The brain was blocked, and 300-µm coronal slices containing the PVN were cut using a vibratome (VT1000S; Leica, Nussloch, Germany). Slices were then incubated at 32°C for a minimum of 1 h in artificial cerebrospinal fluid (aCSF) consisting of the following (in mM): 124 NaCl, 2.5 KCl, 20 NaHCO₃, 2 CaCl₂, 1.3 MgSO₄, 1.24 KH₂PO₄ and 10 glucose, bubbled with 95% O₂-5% CO₂.

Electrophysiology. Brain slices were transferred to a recording chamber continuously perfused with carbogenated (95% O₂-5% CO₂) aCSF heated to 32°C at a rate of 1.5–2 mL/min. PVN neurons were visualized using a ×40 water-immersion objective mounted on an upright microscope fitted with infrared differential interference contrast optics (Scientifica, East Sussex, UK). Patch electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL) and pulled on a micropipette puller (P-97; Sutter Instrument, Novato, CA) to resistances between 2.5 and 5 MΩ when filled with the intracellular solution consisting of the following (in mM): 125 potassium glutonate, 10 KCl, 2 MgCl₂, 0.1 CaCl₂, 5.5 EGTA, 10 HEPES, 2 NaATP, and adjusted to pH 7.2 with KOH. For voltage-clamp recordings of inhibitory post synaptic currents (IPSCs), 140M KCl was substituted for potassium glutonate. After establishment of a high-resistance seal (minimum 1 GΩ), a brief pulse of suction was applied to rupture the membrane and obtain whole cell access. Whole cell recordings were obtained using a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA), filtered at 2.4 kHz, and acquired at a sampling rate of 10 kHz using a Micro1401 mk II interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK) for offline analysis. PVN neurons were characterized on the basis of their electrophysiological fingerprint in response to a standard current pulse protocol (45). Neurons that expressed a large A-current were classified as magnocellular (MNC) (45); those that expressed low-threshold calcium spikes were classified as preautonomic (PA) (42), and those that did not express either of these characteristics were classified as neuroendocrine (NE) (32). To determine the effect of adropin on the excitability of PVN neurons, adropin was bath-applied to cells for 120 s. A neuron was classified as being responsive to adropin in the voltage-clamp and current-clamp configurations if the change in membrane potential in the absence of adropin was at least twice the amplitude of the standard deviation of the baseline membrane potential obtained during the 100-s period immediately before peptide application. A calculated liquid junction potential of 15 mV (for the potassium gluconate-based intracellular solution) has been subtracted from all reported membrane potentials. The same criteria as described above were used for analyzing whole cell currents in the voltage-clamp configuration.

Postsynaptic currents [excitatory postsynaptic currents (EPSCs) and IPSCs] were recorded in the voltage-clamp configuration at a holding potential of −60 mV. Clamping the membrane at −60 mV eliminated CT-mediates currents (when using the potassium gluconate-based intracellular solution) to ensure that IPSCs were not present during EPSC recordings. Miniature postsynaptic currents (mEPSCs and mIPSCs) were recorded in the presence of 1 μM TTX. IPSCs were isolated by application of the broad-spectrum glutamate receptor antagonist kynurenic acid (KA; 1 mM). Administration of KA and KA + biccuculline methiodide (BMI, 10 μM) at the end of EPSC and IPSC recordings, respectively, successfully abolished all postsynaptic IPSC events (data not shown). EPSCs and IPSCs were analyzed using Mini Analysis Program 6.07 (Synaptosoft, Decatur, GA). Events were quantified on the basis of shape (fast rising phase followed by slow exponential decay) and amplitude (threshold defined as four times the baseline electrical noise of each recording). Each detected event was inspected visually to exclude obvious false IPSCs.

Chemicals and drugs. All drugs were freshly prepared in aCSF on the day of experimentation and applied to PVN slices via bath perfusion. KA, BMI, and all salts used to prepare the slicing solution, aCSF, and intracellular recording solution were obtained from Sigma Pharmaceuticals (Oakville, Ontario, Canada). Adropin ⁳¹⁴–⁷⁶ was obtained from Phoenix Pharmaceuticals (Belmont, CA) and TTX citrate from Alomone Laboratories (Jerusalem, Israel).

Statistical analysis. All data are expressed as means ± SE. Group comparisons were made using paired and unpaired Student’s t-tests or a one-way ANOVA with a post hoc Tukey’s test for multiple comparisons. The proportion of responsive neurons was analyzed using a χ²-test. Postsynaptic events were grouped into 100-s bins and compared preadropin and postadropin application. Cumulative probability plots for event amplitude and interevent interval were generated from these data and compared using the Kolmogorov–Smirnov (KS) test for analysis of individual neurons, while group significance was determined using the Wilcoxon matched-pairs signed-rank test. All statistical analyses were performed using GraphPad Prism 6.07 (La Jolla, CA), and in all statistical tests, the level of significance was set at P < 0.05.

RESULTS

Adropin influences the excitability of PVN neurons. Whole cell current-clamp recordings were obtained from 84 PVN neurons. These cells exhibited a mean resting membrane potential of −61.8 ± 0.8 mV, a mean input resistance of 931 ± 47 MΩ, and action potential amplitudes greater than 60 mV. Bath application of 10 nM adropin elicited responses in 68% of cells (n = 57), of which 58% depolarized (5.2 ± 0.3 mV; n = 49; Figs. 1A and 2B) and 10% hyperpolarized (−3.4 ± 0.5 mV; n = 8; Figs. 1B and 2B). The remaining 32% (n = 27) of tested PVN neurons did not respond to adropin application. Depolarizations were usually accompanied by an increase in firing frequency of action potentials, while hyperpolarizations were usually accompanied by a decrease in action potential firing frequency. There was no correlation between the baseline membrane potential of neurons and the polarity of the response to adropin (depolarizing cells, −62.9 ± 1.1 mV vs. hyperpolarizing cells, −56.8 ± 2.9 mV, paired Student’s t-test, P = 0.16). The effects of adropin concentrations between 1 pM and 100 nM on the membrane potential of PVN neurons were assessed and found to be concentration-dependent (100 pM, 3.9 ± 0.2 mV; n = 13; 10 nM, 5.2 ± 0.3 mV, n = 49; 100 nM, 5.9 ± 1.5 mV, n = 6), as illustrated by the concentration-response curve presented in Fig. 3 (EC₅₀ = 86.5 pM). The magnitudes of hyperpolarizing responses were included in the concentration analysis as absolute values. Although no neurons tested responded to 1 pM adropin (1.1 ± 0.2 mV; n = 6), these cells were included in the analysis to complete the concentration-response relationship. On the basis of this concentration-response curve, we chose 10 nM as an appropriate concentration of adropin for subsequent experiments.

We also assessed the effects of adropin on whole cell currents in the voltage-clamp configuration in an additional 29 PVN neurons. Application of 10 nM adropin resulted in changes to whole cell currents in 59% of cells (n = 17), of which 52% exhibited an inward current (−7.5 ± 1.0 pA; n = 15; Figs. 1C and 2C), and 7% exhibited an outward current (6.5 ± 1.7 pA; n = 2; Figs. 1D and 2D) at a holding potential of −60 mV. Overall, a similar proportion of PVN neurons responded to adropin in the voltage-clamp and current-clamp configurations (χ² = 1.97, P = 0.37).
Adropin has similar effects on magnocellular, preautonomic, and neuroendocrine neurons in the PVN. As there are three distinct neuronal subpopulations within the PVN, we determined whether adropin differentially influences PVN neurons from each of these populations. We categorized neurons on the basis of their electrophysiological fingerprint in response to a current pulse protocol (45) (see MATERIALS AND METHODS). Bath application of 10 nM adropin to MNC neurons (n = 29) elicited responses in 72% of cells (n = 21), with 65% depolarizing (5.3 ± 0.5 mV; n = 19), and 7% hyperpolarizing (−2.3 ± 0.1 mV; n = 2). Adropin (10 nM) treatment to PA neurons (n = 30) caused 67% to depolarize (4.7 ± 0.3 mV; n = 20) and 10% to hyperpolarize (−4.3 ± 1.0 mV; n = 3), whereas the remaining 23% (n = 7) showed no response. Finally, 10 nM adropin caused responses in 52% of NE neurons (n = 13/25), with 40% depolarizing (5.9 ± 0.9 mV; n = 10) and 12% hyperpolarizing (−3.2 ± 0.6 mV; n = 3). Overall, a similar proportion of MNC, PA, and NE neurons either depolarize or hyperpolarize in response to adropin (χ² = 5.3, P = 0.26; Fig. 2A). Furthermore, the magnitude of response to adropin within each of these three populations did not differ (one-way ANOVA, P = 0.29 and P = 0.34 for depolarizations and hyperpolarizations, respectively). As it appears that adropin does not differentially influence PVN neurons from each subpopulation, all cells were grouped together for subsequent analyses.

Adropin elicits direct effects on PVN neurons. To determine whether the observed effects of adropin on the membrane...
TTX (χ² = 0.31, P = 0.86; Fig. 4C). Furthermore, the magnitude of response of both depolarizations and hyperpolarizations did not differ in aCSF containing TTX compared with normal aCSF (unpaired Student’s t-test, P = 0.89; Fig. 4D). These observations suggest adropin elicits direct, postsynaptic effects on PVN neurons.

Adropin has no effect on the amplitude or frequency of postsynaptic events in PVN neurons. Our experiments with TTX suggested adropin acts directly on PVN neurons. However, to rule out the possibility that adropin was influencing glutamatergic and/or GABAergic neurotransmission at the level of the synapse, we conducted voltage-clamp experiments to examine the effects of adropin on postsynaptic currents (EPSCs and IPSCs) in PVN neurons. A total of 15 cells were tested for analysis of EPSCs. Adropin (10 nM) was without effect on the amplitude of EPSCs in 11 cells (0.2 ± 0.1 pA; KS test, P > 0.05). In the remaining four cells, adropin (10 nM) had slight, but significant, effects on EPSC amplitude, with three cells increasing amplitude (1.6 ± 0.1 pA; KS test, P < 0.05) and one cell decreasing amplitude (−1.3 ± 0.3 pA; KS test, P < 0.0001). However, when comparing all cells together, these effects on EPSC amplitude did not reach statistical significance as a group (n = 15; Wilcoxon signed-rank test, P = 0.07; Fig. 5, A and B). Adropin (10 nM) also had significant effects on EPSC frequency in four cells tested, of which, two increased frequency (5.4 ± 1.6 Hz; KS test, P < 0.05) and two decreased frequency (−1.2 ± 0.5 Hz; KS test, P < 0.05). Again, however, these effects were not statistically significant as a group (n = 15; Wilcoxon signed-rank test, P = 0.54; Fig. 5, A and C). An additional 9 PVN neurons were tested following pretreatment of 1 μM TTX to examine the effects of adropin on mEPSCs. Adropin (10 nM) application had no effect on the amplitude (0.2 ± 0.1 pA; n = 9; KS test, P > 0.05; Fig. 5, D and E) or frequency (0.6 ± 0.1 Hz; n = 9; KS test, P > 0.05; Fig. 5, D and F) of mEPSCs in any of the cells tested (n = 9).

To examine whether adropin influences GABAergic neurotransmission, we next investigated the effect of adropin on the amplitude and frequency of IPSCs. Out of 11 cells tested, 1 cell increased IPSC amplitude (10.1 ± 0.1 pA; KS test, P = 0.0001) and 1 cell decreased IPSC amplitude (−3.8 ± 0.1 pA; KS test, P < 0.0001) in response to 10 nM adropin application. However, when comparing all cells together, these effects on IPSC amplitude did not reach statistical significance as a group (n = 11; Wilcoxon signed-rank test, P = 0.52; Fig. 6, A and B). Additionally, the frequency of IPSCs was unaffected by 10 nM adropin application in all cells tested (0.4 ± 0.1 Hz; n = 11; KS test, P > 0.05; Fig. 6, A and C). Finally, adropin was without effect on the amplitude or frequency of mIPSCs in 6 of 12 PVN neurons (n = 6; KS test, P > 0.05). In five of the six affected cells, three increased mIPSC amplitude (5.2 ± 1.4 pA; KS test, P < 0.05), and two decreased mIPSC amplitude (−10.51 ± 4.8 pA; KS test, P < 0.05). However, when comparing all cells together, these effects on mIPSC amplitude did not reach statistical significance as a group (n = 12; Wilcoxon signed-rank test, P = 0.91; Fig. 6, D and E). One of the cells that decreased amplitude also exhibited a decrease in mIPSC frequency (−2.8 ± 0.4 Hz; KS test, P = 0.008), while a separate cell increased mIPSC frequency only (2.3 ± 0.4 Hz; KS test, P = 0.008), with no change in mIPSC amplitude. Again, these effects on mIPSC frequency failed to reach
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...altering glutamatergic and/or GABAergic synaptic transmission, as opposed to clamp experiments further support the conclusion that adropin is acting on the cell membrane of PVN neurons, as opposed to altering glutamatergic and/or GABAergic synaptic transmission.

DISCUSSION

In the present study, we describe novel electrophysiological effects of adropin in the CNS. Our data demonstrate that adropin directly influences the excitability of neurons in the PVN. It appears that adropin is primarily an excitatory peptide in the PVN, as the majority of responsive neurons were depolarized in response to adropin application. However, a subset of neurons did exhibit hyperpolarizations. These heterogeneous effects were observed in similar proportions in all three neuronal subpopulations (MNC, PA, and NE) of the PVN. The effects of adropin were shown to be direct, postsynaptic, as they were maintained in the presence of TTX and did not influence the frequency or amplitude of postsynaptic currents. Together, these results suggest the PVN as a site of action for central adropin.

It is unknown whether adropin can cross the blood-brain barrier (BBB), although, its size and lipophobic nature suggest that it would not readily diffuse from the circulation into the CNS. Thus, it is more likely that the effects of this peptide in the brain arise from locally produced adropin. Adropin has abundant expression in the CNS, including many areas of the midbrain and hindbrain (1, 27), and microarray data have identified both *Enho* and *Gpr19* transcripts in the PVN (18).

Our concentration-response relationship for the effects of adropin on the excitability of PVN neurons correlates with a measurement of ~2.5 ng/mg of adropin in rat brain tissue (1). Furthermore, microarray analysis identified both *Enho* and *Gpr19* transcripts in the supraoptic nucleus of the rat (18), suggesting a definite effect on MNC neurons. Interestingly, microarray analysis failed to detect expression of the *Enho* transcript in the PVN of dehydrated rats (18), consistent with the hypothesis that the inhibitory effect of adropin on water intake is mediated by the PVN. There is also recent evidence that adropin is expressed in rat brain endothelial cells in an in vitro BBB model (52), providing an additional source of adropin in the brain.

On the basis of the varied responsiveness of PVN neurons to adropin, our data suggest the existence of three distinct populations of neurons with respect to adropin sensitivity, within all three neuronal subpopulations of the PVN. The differential effects of adropin on MNC neurons may reflect the peptide acting on separate MNC neurons that express oxytocin (OT), vasopressin (VP), or OT/VP-coexpressing neurons (23, 50). Both OT and VP have roles in salt and water balance (7, 25) and may be involved in adropin’s inhibitory effect on water intake. However, these hormones have also been implicated in metabolic homeostasis. OT receptor-deficient mice exhibit an obese phenotype with elevated fasting triglycerides, suggesting OT has a role in lipid metabolism (44), whereas knockdown of the VP V1A and V1B receptors alters both glucose and lipid metabolism in mice (19, 20, 33). PA neurons also express OT and VP, as well as corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH), but project to auton...

![Fig. 4. The effects of adropin on PVN neurons are maintained in the presence of TTX. A and B: representative current-clamp recordings from two PVN neurons in slice preparation, pretreated with 1 μM TTX (horizontal light gray bars), showing that bath-application of 10 nM adropin (horizontal dark gray bars) caused a depolarization (A), while a different PVN neuron (B) exhibited a hyperpolarization. Both neurons showed a return to baseline membrane potential (dashed line) following washout of adropin. C: bar graph illustrating the percentage of PVN neurons that depolarize, hyperpolarize, or do not respond to bath application of 10 nM adropin in the absence and presence of 1 μM TTX. The proportion of responsive neurons did not change with recordings in TTX (χ² = 0.31, P = 0.86). D: bar graphs summarizing the mean change in membrane potential ± SE following application of 10 nM adropin during depolarizations (left: adropin, 5.2 ± 0.3 mV, n = 49 vs. adropin + TTX, 4.7 ± 1.0 mV, n = 8) and hyperpolarizations (right: adropin, −3.4 ± 0.5 mV, n = 8 vs. adropin + TTX, −5.8 mV, n = 1) in the absence and presence of 1 μM TTX.](https://example.com/filename.png)
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Adropin is an integral component of the neuroendocrine system and may also be involved in the stress response, as NE neurons in response to adropin application suggest this. The depolarizations observed in NE neurons in response to adropin application suggest this peptide may also be involved in the stress response, as NE neurons are an integral component of the neuroendocrine system.

Our results are consistent with those obtained for nesfatin-1, which has both depolarizing and hyperpolarizing effects on PVN neurons (36), and also inhibits both pharmacologically and physiologically driven thirst when injected intracerebroventricularly (53). This inhibition is likely occurring via the PVN, as an ANG II-induced dipsogenic response can be produced after a reduction in immunoreactive nesfatin-1 in this nucleus (53). Similarly, central administration of hydrogen sulfide, which depolarizes the majority of PVN neurons (26), has been shown to cause an initial decrease in water intake in water-deprived rats, while also increasing plasma levels of OT, VP, and corticosterone (6). With the wide range of physiological functions of specific PVN neurons, many of which overlap, it is difficult to ascertain which specific functions central adropin is influencing. However, our results suggest that the inhibitory effect of central adropin on water intake is likely mediated by neurons in the PVN and that this peptide has roles in additional endocrine and autonomic functions, likely related to its physiological effects in the periphery.

As both depolarizing and hyperpolarizing responses were still observed in similar proportions in synaptic isolation after pretreatment with TTX, it is likely adropin acts directly on these neurons in the PVN. However, the PVN contains glutamate interneurons within the nucleus itself (3, 9), as well as GABA interneurons located in the halo zone surrounding the PVN (37). These interneurons have roles in mediating the effects of multiple peptides in the PVN. For example, the excitatory effects of orexin (13), ANG II (28), and prokineticin 2 (55), and the inhibitory effects of adrenomedullin (12) on MNC neurons have been shown to be mediated by glutamate and GABA interneurons, respectively. Additionally, GABA interneurons can mediate excitatory effects of peptides through disinhibition, a proposed mechanism by which ANG II influences PA neurons (30). TTX-resistant excitatory/inhibitory effects mediated by glutamate/GABA can occur at the synaptic level on postsynaptic currents (EPSCs) or miniature EPSCs (mEPSCs) in PVN neurons.

Fig. 5. Adropin has no effect on the amplitude or frequency of excitatory postsynaptic currents (EPSCs) or miniature EPSCs (mEPSCs) in PVN neurons. A: representative voltage-clamp traces from an individual PVN neuron (held at –60 mV) before (top trace) and after (bottom trace) application of 10 nM adropin. B and C: cumulative probability plots of the neuron in A showed there was no change in the amplitude (B) or interevent interval (C) of EPSCs over a 100-s period after application of 10 nM adropin (dashed line) compared with 100 s of baseline (continuous line) (KS test, P > 0.05). Insets: summary graphs showing that mean amplitude (B) and frequency (C) of EPSCs were unaltered after adropin application. D: representative voltage-clamp traces from a separate PVN neuron (held at –60 mV), pretreated with 1 μM TTX (horizontal gray bars), before (top trace) and after (bottom trace) application of 10 nM adropin. E and F: cumulative probability plots of the neuron in D showed there was no change in the amplitude (E) or interevent interval (F) of mEPSCs over a 100-s period after application of 10 nM adropin (dashed line) compared with 100 s of baseline (continuous line) (KS test, P > 0.05). Insets: summary graphs showing that mean amplitude (E) and frequency (F) of mEPSCs were unaltered after adropin application.
either the presynaptic or postsynaptic membrane (17, 35, 40). Changes in quantal release (action potential-independent) of these neurotransmitters at the presynaptic terminal affect the frequency of postsynaptic events, whereas changes in postsynaptic receptor expression or kinetics affect the amplitude of postsynaptic events. This modulation of synaptic transmission can also occur indirectly via retrograde messengers, as is the case for glucocorticoid signaling in MNC and parvocellular neurons (10, 11). Activation of a postsynaptic GPCR by glucocorticoid causes endocannabinoid synthesis, which feeds back to glutamate terminals to decrease glutamate release, observed as a decrease in EPSC frequency in the postsynaptic neuron (10, 11). A divergent glucocorticoid-induced signaling pathway also facilitates GABA release via retrograde signaling of nitric oxide at GABA terminals, causing an increase in IPSC frequency in the postsynaptic neuron (11). In our experiments on postsynaptic events, adropin had no effect on the amplitude or frequency of either EPSCs or IPSCs in PVN neurons. Together, our voltage-clamp and TTX experiments suggest the influence of adropin on the membrane potential of PVN neurons is not due to any changes in glutamatergic and/or GABAergic synaptic transmission; rather, it is a direct effect on the PVN neurons themselves.

The cellular mechanisms through which adropin acts to influence the membrane potential of PVN neurons remain to be elucidated. Since the effects of adropin occur soon after perfusion onto the slice, it is likely that such effects are a consequence of GPCR-mediated modulation of ion channel properties, presumably through the candidate adropin receptor, GPR19. In the periphery, adropin activates eNOS in endothelial cells via the ERK1/2 and phosphatidylinositol 3-kinase (PI3K)-Akt pathways (31). While in the brain, overexpression of GPR19 enhances the phosphorylation of ERK and Akt in dissociated hippocampal neurons (22). As such, these pathways may underlie the potential mechanism through which adropin may signal via GPR19 to affect the membrane potential of PVN neurons.

Alternatively, adropin may signal through an additional, still unidentified receptor. There are several peptides, including orexin (38), adiponectin (51), and cholecystokinin (21), in which its CNS effects are mediated through two different receptors. It has already been suggested that GPR19 may form heterodimers with additional GPCRs in plasmalogen-mediated signaling (22). If an additional GPCR exists for adropin, it may form a heterodimer with GPR19 to produce the differential effects observed in PVN neurons, similar to how adiponectin signals in MNC neurons. Neurons that express both adiponectin receptors (AdipoR1 and AdipoR2) hyperpolarize, whereas neurons that express only AdipoR2 depolarize (23).

Fig. 6. Adropin has no effect on the amplitude or frequency of inhibitory postsynaptic currents (IPSCs) or miniature IPSCs (mIPSCs) in PVN neurons. A: representative voltage-clamp traces from an individual PVN neuron (held at −60 mV), pretreated with 1 mM kynurenic acid (KA; horizontal gray bars), before (top trace) and after (bottom trace) application of 10 nM adropin. B and C: cumulative probability plots of the neuron in A showed there was no change in the amplitude (B) or interevent interval (C) of IPSCs over a 100-s period after application of 10 nM adropin (dashed line) compared with 100 s of baseline (continuous line) (KS test, P > 0.05). Insets: summary graphs showing that mean amplitude (B) and frequency (C) of IPSCs were unaltered after adropin application. D: representative voltage-clamp traces from a separate PVN neuron (held at −60 mV), pretreated with 1 mM KA and 1 μM TTX (horizontal gray bars), before (top trace) and after (bottom trace) application of 10 nM adropin. E and F: cumulative probability plots of the neuron in D showed there was no change in the amplitude (E) or interevent interval (F) of mIPSCs over a 100-s period after application of 10 nM adropin (dashed line) compared with 100 s of baseline (continuous line) (KS test, P > 0.05). Insets: summary graphs showing that mean amplitude (E) and frequency (F) of mIPSCs were unaltered after adropin application.
While our experiments support the hypothesis that the PVN is a site of action for central adropin, additional studies are required to fully elucidate the physiological relevance of the actions of adropin on specific PVN neurons. In particular, in vivo experiments focused on hormone secretion in response to adropin administration would further our understanding of the physiological effects of central adropin. Employment of single-cell RT-PCR together with electrophysiological recordings would allow not only for connection of the molecular phenotype of individual PVN neurons to one of the heterogeneous responses to adropin, but also for determination of whether GPR19 is the receptor through which adropin signals in the PVN. Finally, additional voltage-clamp studies determining which ion channels are affected by adropin would aid in elucidating a cellular mechanism for adropin signaling.

**Perspectives and Significance**

In conclusion, our experiments have demonstrated that adropin directly influences the excitability of neurons from each subpopulation of the PVN, suggesting this hypothalamic nucleus is a novel site at which central adropin acts. Our results support the hypothesis that central adropin inhibits water intake through actions in the PVN. Furthermore, as the majority of neurons from each subpopulation of the PVN were affected, adropin is likely to have additional roles in the CNS. While additional studies are required to bridge the gap between our electrophysiological data and the physiological actions of adropin, our findings further solidify adropin as a hormone with actions in the PVN. Furthermore, as the majority of the majority of neurons from each subpopulation of the PVN were affected, adropin is likely to have additional roles in the CNS. While additional studies are required to bridge the gap between our electrophysiological data and the physiological actions of adropin, our findings further solidify adropin as a hormone with actions in the brain.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

S.P.L. and A.V.F. conception and design of research; S.P.L. performed experiments; S.P.L. analyzed data; S.P.L. and A.V.F. interpreted results of experiments; S.P.L. drafted manuscript; S.P.L. prepared figures; S.P.L. and A.V.F. edited and revised manuscript. S.P.L. and A.V.F. approved final version of manuscript and agree to be accountable for all aspects of this work.

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