Adiponectin Modulates Excitability of Rat Paraventricular Nucleus Neurons by Differential Modulation of Potassium Currents

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The adipocyte-derived hormone adiponectin acts at two seven-transmembrane domain receptors, adiponectin receptor 1 and adiponectin receptor 2, present in the paraventricular nucleus of the hypothalamus to regulate neuronal excitability and endocrine function. Adiponectin depolarizes rat parvocellular preautonomic neurons that secrete either thyrotropin releasing hormone or oxytocin and parvocellular neuroendocrine corticotropin releasing hormone neurons, leading to an increase in plasma adrenocorticotropin hormone concentrations while also hyperpolarizing a subgroup of neurons. In the present study, we investigate the ionic mechanisms responsible for these changes in excitability in parvocellular paraventricular nucleus neurons. Patch clamp recordings of currents elicited from slow voltage ramps and voltage steps indicate that adiponectin inhibits noninactivating delayed rectifier potassium current (I_K) in a majority of neurons. This inhibition produced a broadening of the action potential in cells that depolarized in the presence of adiponectin. The depolarizing effects of adiponectin were abolished in cells pretreated with tetraethyl ammonium (0/15 cells depolarize). Slow voltage ramps performed during adiponectin-induced hyperpolarization indicate the activation of voltage-independent potassium current. These hyperpolarizing responses were abolished in the presence of glibenclamide [an ATP-sensitive potassium (K_{ATP}) channel blocker] (0/12 cells hyperpolarize). The results presented in this study suggest that adiponectin controls neuronal excitability through the modulation of different potassium conductances, effects which contribute to changes in excitability and action potential profiles responsible for peptidergic release into the circulation. (***Endocrinology*** **151: 3154–3162, 2010)**

Central regulation of energy homeostasis and feeding behavior relies on the critical integration of peripheral information with neuronal networks in the hypothalamus. This information describing nutritional intake, storage, and use is transmitted from peripheral organs by signaling molecules that function in the central nervous system (CNS) to coordinate the body's response to macronutrient intake (1). Adiponectin is a 244-amino acid peptide hormone, which is synthesized and released from adipocytes at levels varying inversely with body adiposity (2–4). Adiponectin was originally identified as an insulin-sensitizing hormone and was shown to play important roles in controlling glucose homeostasis and fatty-acid catabolism through its action in metabolic tissues, such as skeletal muscle and the liver (5). Adiponectin deficient animals show insulin resistance, glucose intolerance, and hyperlipidemia, whereas reintroducing the peptide ameliorates these dysfunctions (5–7). When introduced into the brains of rodents, adiponectin has been shown to display a wide variety of effects. In rats, the arcuate nucleus has been shown to mediate adiponectin's induction of feeding through the activation of AMP kinase (AMPK) and acetyl-CoA carboxylase phosphorylation (8), whereas in mice, injections of adiponectin into the lateral ventricle are associated with a reduction in body weight without a preceding decrease in food intake (9). This effect of adiponectin on body weight may be the result of the activation of paraventricular nucleus (PVN) neurons as a ro-

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Abbreviations: aCSF, Artificial cerebral spinal fluid; AdipoR, adiponectin receptor; AMPK, AMP kinase; AP, action potential; BK_{Ca}, large-conductance calcium activated potassium channel; CNS, central nervous system; I_K, delayed rectifier K^+ current; MNC, magnocellular; NE, neuroendocrine; PA, preautonomic; PVN, paraventricular nucleus; TEA, tetraethyl ammonium.

bust induction of c-*fos* in this nucleus accompanied these injections (9).

Two receptors, adiponectin receptor (AdipoR)1 and AdipoR2, through which adiponectin confers its effects have been cloned (10) and localized to distinct nuclei in the CNS, including the area postrema (11), the nucleus of the solitary tract (12), the arcuate nucleus (8), and the PVN (13). In addition to its effects on energy homeostasis, our laboratory has recently demonstrated an increase in peripheral circulation of ACTH concentrations after intracerebroventricular injections of adiponectin into rats and an associated increase in the excitability of corticotropin releasing hormone expressing neurons in the PVN, which in turn are responsible for controlling ACTH release from anterior pituitary cells (14).

The PVN is a bilateral nucleus surrounding the third ventricle and is a critical integrator of information regarding central neuroendocrine (NE) and autonomic function (15). The PVN contains three distinct groups of neurons: type I magnocellular (MNC), type II parvocellular preautonomic (PA), and type III parvocellular NE neurons. In addition to adiponectin's depolarizing actions on corticotropin releasing hormone expressing neurons, the peptide also has been shown to depolarize PA thyrotropin releasing hormone neurons, PA oxytocin neurons (14), and a subset of MNC vasopressin neurons while hyperpolarizing MNC oxytocin neurons (13) and a subgroup of unidentified parvocellular neurons (14). Although these previous studies have shown clear effects of adiponectin on the membrane potential of specific groups of PVN neurons, the underlying mechanisms responsible for these changes in excitability are unknown. This study was therefore designed to investigate the ionic mechanisms through which adiponectin elicits these effects on the neuronal excitability of parvocellular PVN neurons through the modulation of specific ion channels. Using patch clamp techniques, we provide the first evidence that direct modulation of distinct ionic currents in PVN neurons mediates adiponectin's effects on autonomic function.

Materials and Methods

Ethical information

Procedures involving the use of animals in this study were approved by the Queen's University Animal Care Committee and conformed to the standards of the Canadian Counsel on Animal Care.

Preparation of hypothalamic slices

Hypothalamic coronal slices (300 μm) containing PVN were taken on the day of recording from male postembryonic d 19–26 Sprague Dawley rats (Charles River, Quebec, Canada), maintained on a 12-h light, 12-h dark cycle, and provided water and food *ad libitum*. Rats were immobilized, decapitated, and their brains quickly removed and placed in 4 C slicing solution consisting of (in mM) 87 NaCl, 2.5 KCl, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 25 glucose, and 75 sucrose, bubbled with 95/5% O_2/CO_2 for 3–5 min. Brains were then trimmed to size, mounted on a stage immersed in slicing solution, and coronal slices containing the PVN were cut using a vibratome (Leica, Nussloch, Germany). Slices were incubated in a 95/5% O_2/CO_2 holding chamber containing artificial cerebral spinal fluid (aCSF) consisting of (in mM) 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose for at a minimum of 1 h before recording.

Electrophysiological recordings

PVN neurons were submerged in aCSF, anchored to the bottom of the recording chamber, and visualized using a ×40 water-immersion objective mounted to an upright Nikon E600FN microscope outfitted with infrared differential interference contrast optics (Nikon, Tokyo, Japan). Electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL), pulled using a micropipette puller (P97 Sutter instruments Co., Novato, CA) to resistances of 2–3 MΩ. Electrodes were filled with internal recording solution consisting of (in mM) 130 K^+-gluconate, 10 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, 5.5 EGTA, 10 HEPES, 2 NaATP with pH adjusted to 7.2 using KOH. Whole-cell recording configurations were made by applying slight negative pressure on to the end of the patch pipette after the formation of a gigaohm seal, breaking through the cell membrane and obtaining continuity with the internal contents of the cell. Current clamp data were obtained with a Multiclamp 700B patch clamp amplifier (Molecular Devices, Palo Alto, CA) recorded in real-time using the acquisition software Spike 2 (version 6) and Signal (version 4) (Cambridge Electronic Devices, Cambridge, UK). Data were acquired at 5 kHz and filtered at 10 kHz using a Micro1401 mkII interface (Cambridge Electronic Devices). Data were stored for off-line analysis. Capacitance transients and series resistance error was minimized at the start of all recordings. Parvocellular PVN neurons (PA or NE) were identified and separated from MNC neurons using a current pulse protocol designed to identify the presence of a low-threshold spike (PA) or nonaccommodating spike firing pattern (NE), electrophysiological fingerprints which clearly distinguish them from MNC neurons (distinct transient potassium conductance) as described by Luther *et al.* (16). These parvocellular neurons were not further subdivided in the present analysis, because our initial voltage ramp recordings showed similar responses to adiponectin in both groups of parvocellular neurons. After establishing a stable control membrane potential for at least 100 sec, an experiment-specific peptide or drug, dissolved in aCSF, was bath applied at a rate of 2 ml/min through gravity-fed perfusion. In current clamp recordings, responsiveness of neurons to treatment was assessed by averaging all points in time blocks of 100 sec for up to 1000 sec after initial application of treatment. The maximum mean voltage change was compared with baseline control mean voltage and was counted as responsive if the value was greater than two times the SD value of the control membrane potential. After washout of the treatment, cells were recorded until membrane potential returned to baseline values. A calculated junction potential of −14.4 mV was added to the membrane potential of recorded neurons. Cells that did not show at least a partial (50%) recovery toward baseline control membrane potential after drug treatments were omitted from the analysis.

Analysis of action potentials (APs) was performed by signal averaging all APs over a given time frame and meaning the data

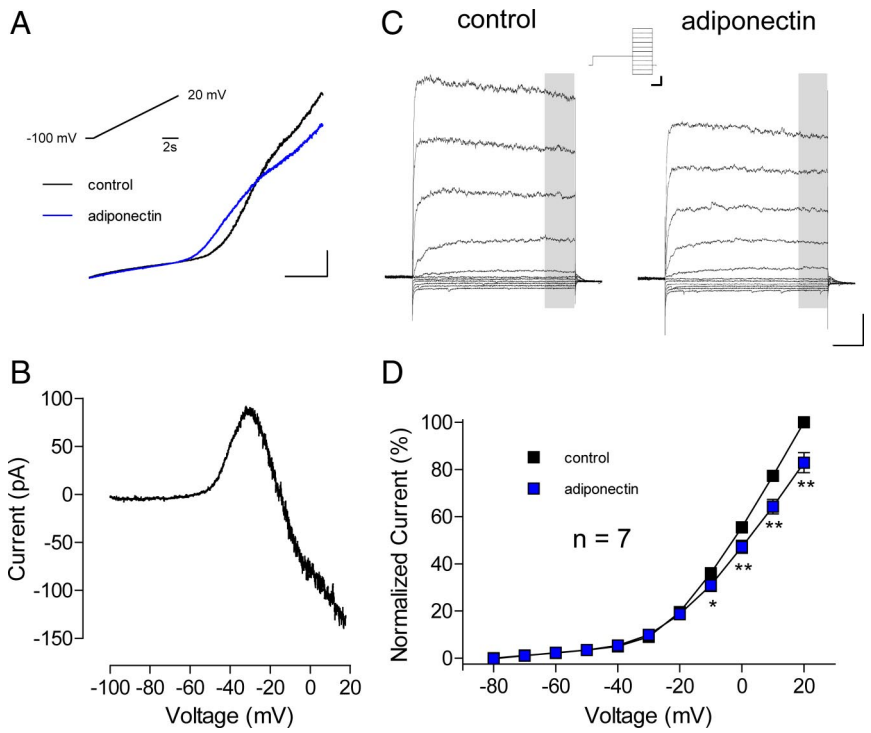

FIG. 1. Adiponectin inhibits a voltage-gated potassium conductance. A, Representative traces of currents elicited from slow voltage ramps (12 mV/sec) starting from a holding potential of −100–20 mV (*inset*). *Black current traces* represent current before the bath application of 10 nM adiponectin, whereas *blue traces* represent currents after. Currents shown are an average of three ramps (*Scale bars*: 20 mV, 100 pA). B, Difference current obtained from subtracting currents obtained during control (*black*) from adiponectin-induced currents (*blue*). C, Voltage clamp currents obtained from performing 500-msec voltage steps (−80–20 mV) after a 1-sec conditioning pulse to −40 mV from a holding potential of −60 mV (*step protocol inset*; *Scale bars*: 250 msec, 20 mV) in the absence (*left*) and presence (*right*) of 10 nM adiponectin. *Gray boxes* represent the data points used to measure average steady-state current (*Scale bars*: 150 pA, 100 msec). D, I–V, Plot of normalized steady-state current, averaged across all cells tested at each voltage step in control conditions (*filled black boxes*) and after treatment with bath application of 10 nM adiponectin (*filled blue boxes*). Significant differences in steady-state current are indicated by *asterisks*. *, $P < 0.05$; **, $P < 0.005$, paired *t* test.

points in time to provide an average profile. Signal averages of APs before and after adiponectin were normalized to the peak of the rising phase and the trough of the repolarization phase. Comparison in the AP repolarization times before and after adiponectin were made by averaging data points taken every 0.6 msec from the peaks of the normalized APs.

Signal version 4 was used for stimulation and data acquisition in all voltage clamp experiments. Series resistance was routinely compensated up to 50% in these neurons. Peak steady-state outward potassium current of the delayed rectifier subtype was measured by averaging all current data points elicited during the final 100 msec of the voltage step (*gray bar* in Fig. 1C). Slow voltage ramps (12 mV/sec) were run in most cells from −105–20 mV. Difference currents were obtained from subtracting control currents from currents elicited after treatment with adiponectin. When used for regression analysis, difference current data points were obtained from averaging 100 data points surrounding the voltage of interest.

Chemicals and peptides

With the exception of those listed below, all chemicals used to make solutions in these experiments were purchased from Sigma Pharmaceuticals (Oakville, Ontario, Canada). Tetrodotoxin was purchased from Alomone Labs (Jerusalem, Israel). Lyophilized recombinant human globular adiponectin was purchased from Phoenix Pharmaceuticals (Burlingame, CA), reconstituted in filtered molecular grade water, and made in 20 μM aliquots for daily use. Glibenclamide was purchased from Tocris Biosciences (Cedarlane Laboratories, Burlington, Ontario, Canada).

Statistical analysis

Data obtained in these experiments were statistically analyzed using GraphPad Prism version 5.1 (GraphPad, San Diego, CA).

Results

Slow voltage ramps (−100–20 mV, 12 mV/sec) were performed on identified parvocellular PVN neurons in the absence and presence of adiponectin to assess the peptide's effect on whole-cell current properties. Initial recordings were obtained from 15 parvocellular PVN neurons and grouped according to their expression of a similar "difference current" (adiponectin, control current) in response to adiponectin. The predominant response observed (7/15 neurons) showed a difference current, indicating a large decrease in outward current seen at depolarized levels of the slow voltage ramp (Fig. 1, A and B).

Adiponectin inhibits delayed rectifier K^+ current (I_K) in parvocellular PVN neurons

Voltage-gated K^+ conductances have been shown to be important for regulation of neuronal excitability, and modulation of these currents can have significant effects on the membrane potential of neurons (17). Therefore, based on the adiponectin-induced decrease in outward current observed in the depolarized range of our voltage ramps, we proceeded to investigate the specific ionic conductance that was responsible for this effect. Whole-cell voltage clamp recordings were made from a total of 13 parvocellular neurons within the PVN. A pulse protocol was used to isolate the noninactivating delayed rectifier current (I_K) from fast-inactivating outward potassium current by subjecting cells to a 1-sec prepulse to −40 mV followed by 500-msec voltage steps in increments of 10 mV from −80 mV to 20 mV (Fig. 1C, *inset*). This protocol avoids the activation of fast-inactivating outward potassium current and therefore allows direct measurement of

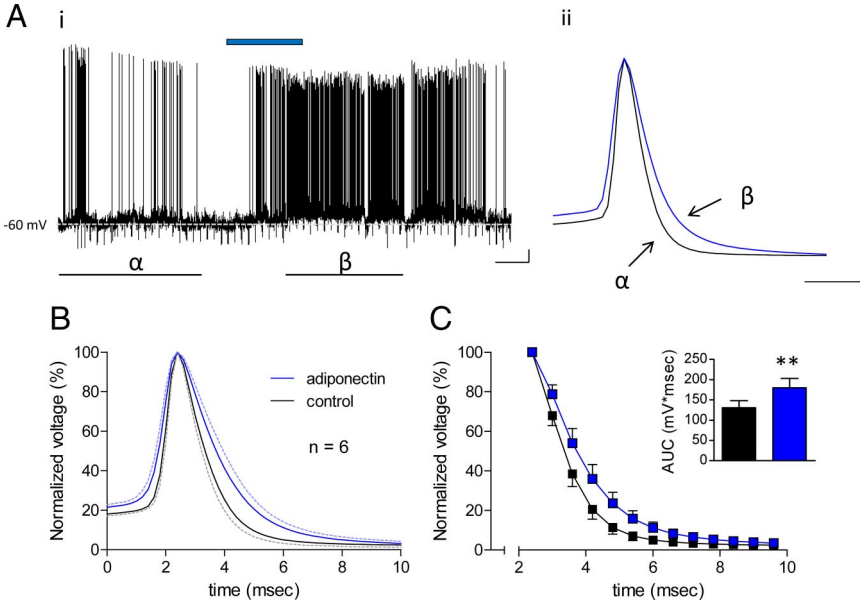

FIG. 2. AP broadening accompanies the depolarizing effects of adiponectin. A, i, Current clamp recording representing a increase in excitability and depolarizing response to bath application of 10 nM adiponectin (*blue bar*) along with recovery to baseline after washout (*scale bars*, 50 sec, 10 mV). *Gray hatched bar* indicates control membrane potential before adiponectin treatment. α And β represent regions of current clamp recording used for AP shape analysis. ii, Overlay of normalized APs for the current clamp trace on the *left*, recorded during the control phase [average of all spikes in α (*black line*)] and after treatment with 10 nM adiponectin [average of all spike in β (*blue line*)] (*Scale bars*, 2 msec). Note: AP averages were normalized to the peak of the AP and the trough of the repolarization phase. AP profiles after adiponectin treatment reflect initiation from depolarized potentials compared with control. B, Plot of normalized APs averaged over six neurons comparing AP profiles before (control, *black*) and after depolarization to bath application of 10 nM adiponectin (adiponectin, *blue*). *Hatched bars* represent SE measurement of the neurons tested in each respective color. C, Plot showing normalized repolarization rate of APs in control phase (*black*) *vs.* adiponectin treatment (*blue*). *Inset*, Bar graph showing the calculated area under the curve (AUC) for control (*black*) compared with adiponectin repolarization (*blue*). **, $P < 0.005$.

I_K (18). These recordings were made in the presence of 1 μM tetrodotoxin to inhibit the activation of sodium currents. After the establishment of control I_K amplitudes, the effect of bath administration of 10 nM adiponectin on sustained outward I_K was assessed. Of the 13 recorded neurons, the predominant effect of adiponectin was to cause a decrease in the steady-state I_K (7/13 cells, Fig. 1C). Examination of the normalized I–V relationship in the absence and presence of adiponectin suggested that the delayed rectifier I_K is statistically different from control beginning at the −10-mV voltage step, and the effects of adiponectin become progressively larger up to the 20-mV peak voltage step, which shows a decrease of 18 ± 4% (n = 7; *, $P < 0.05$, paired *t* test) (Fig. 1D). In the remaining parvocellular neurons tested, steady-state I_K was increased (3/13) or unaffected (3/13).

Adiponectin modulates AP shape in cells that depolarize

We next examined whether these actions of adiponectin on I_K are associated with a change in the shape of the AP, as would be predicted, in parvocellular neurons that depolarized in response to the peptide. Analysis of AP shape was carried out using neurons from a previously developed database of adiponectin responsive cells (14). Neurons for analysis were selected based on the criteria of having spontaneous APs during baseline control membrane potential recordings and showing a depolarization concurrent with an increase in AP frequency in response to 10 nM adiponectin (Fig. 2A, i). Six neurons fulfilled these criteria and were examined for changes in AP shape in response to adiponectin-induced depolarization. Current clamp recordings of APs were signal averaged and normalized to peak amplitude providing an average AP shape before and after adiponectin treatment (Fig. 2A, ii). Adiponectin induced significant spike broadening (Fig. 2B), increasing the time course of recovery from the peak of the AP through the repolarization phase (area under the curve: adiponectin, 179.8 ± 23.6 mV*msec *vs.* control, 130.5 ± 17.7 mV*msec; n = 6; **, $P < 0.01$ paired *t* test) (Fig. 2C, *inset*). A significant difference in normalized voltage of the repolarization phase was first seen at 0.6 msec after the peak of the AP (control, 67.9 ± 5.0%; n = 6 *vs.* adiponectin, 78.7 ± 4.7%; n = 6; $P < 0.005$, paired *t* test) and continued to be significantly different for 4.2 msec (Fig. 2C). These results suggest that during depolarization, adiponectin acts to broaden AP width through the inhibition of I_K.

Depolarizing effects of adiponectin are blocked in tetraethyl ammonium (TEA)

Based on the observed inhibition of the outward rectifying I_K by adiponectin, we next tested the hypothesis that depolarizing effects of adiponectin result from inhibition of I_K, by assessing membrane potential responses of parvocellular neurons to bath administration of 10 nM adiponectin in the presence of the potassium channel blocker TEA (Fig. 3A, i–iii). Current clamp recordings were obtained from 15 parvocellular neurons. Administration of TEA alone induced a depolarizing shift (≥2 mV) in the membrane potential of 14/15 neurons (example Fig. 3A, i) (mean depolarization, 6.4 ± 4.1 mV; n = 14) (Fig. 3B, *inset*), suggesting that baseline membrane potential in par-

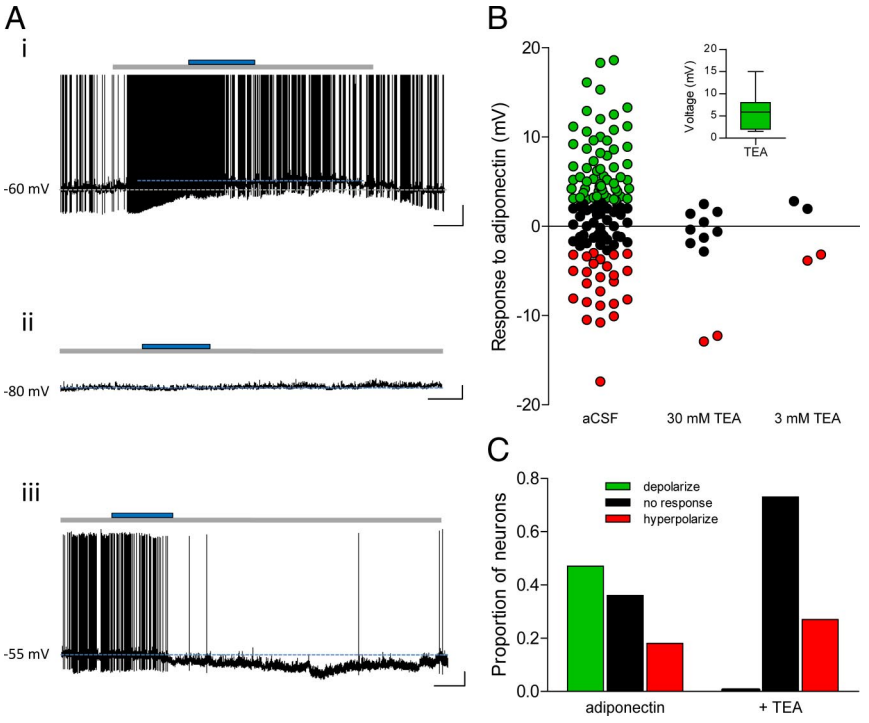

FIG. 3. Adiponectin-induced depolarization is abolished in TEA. A, Current clamp recordings showing the effect of bath application of 10 nM adiponectin (*blue bar*) in the presence of 30 mM TEA (*gray bar*). *Blue dashed lines* in i–iii represent the baseline membrane potential used to compare against the effects of adiponectin. Current clamp recordings in i and ii show no response to adiponectin treatment, whereas iii shows a hyperpolarizing response to the peptide (*Scale bars*: 10 mV, 100 sec). Note: In i, the *gray dashed liner* represents baseline membrane potential before the bath application of 30 mM TEA followed by a depolarization to the new baseline potential (*blue line*). B, Scatter-dot plot of the response amplitude to adiponectin observed in the presence of either 30 or 3 mM TEA compared with aCSF. *Green filled circles* indicate a depolarizing response, *black filled circles* are nonresponders, whereas *red filled circles* are cells that hyperpolarized. *Inset*, Box and Whisker plot of the effect of TEA alone on the membrane potential of parvocellular neurons. C, *Bar graph* showing the proportion of neurons responding to adiponectin in the presence and absence of bath administration of 10 nM adiponectin. *Red bars* indicate a hyperpolarizing response, *black bars* indicate no response, whereas *green bars* indicate a depolarizing response.

vocellular neurons is in part mediated by a TEA-sensitive current. Whereas in the presence of TEA, a stable membrane potential was obtained for a minimum of 50 sec (using small negative holding current in 5 cells), and the effects of bath perfusion of 10 nM adiponectin on membrane potential was assessed (Fig. 3A, i–iii). No depolarizing effects of adiponectin were observed (0 of 15 cells tested; Fig. 3, B and C) in the presence of either 30 or 3 mM TEA, although hyperpolarizing responses to the peptide were still observed (4/15 cells). We performed additional experiments, in which we tested the effect of 10 nM adiponectin in the presence of 100 nM iberiotoxin used to block large-conductance calcium activated potassium channels (BK_{Ca}). Both depolarizing (9.1 ± 1.7 mV, 2/6) and hyperpolarizing (−3.5 ± 0.3 mV, 2/6) responses were maintained in the presence of the potassium channel blocker, suggesting that BK_{Ca} is not involved in mediating the effects of adiponectin. These results support the conclusion that the depolarizing effects of adiponectin are mediated through the inhibition of a TEA-sensitive current, whereas the hyperpolarizing effects are not.

Adiponectin hyperpolarizes PVN neurons through modulation of a voltage independent K^+ conductance

We have shown previously that adiponectin hyperpolarizes and inhibits AP firing in a subpopulation of parvocellular PVN neurons (14). To investigate the ionic mechanisms responsible for the inhibitory effects of adiponectin, we used a current clamp and voltage clamp technique, which allows us to examine both the underlying currents and membrane potential response to bath application of 10 nM adiponectin. The recording protocol consists of first voltage clamping neurons and performing slow voltage ramps from −105 to −70 mV (12 mV/sec) to obtain a profile of the baseline currents. Next, the recording is switched to current clamp to record membrane potential and the activity of the cell. Bath application of 10 nM adiponectin is administered in the current clamp configuration, and the response is observed. Neurons are once again voltage clamped, and the same voltage ramps are performed to measure resultant currents in the presence of adiponectin (Fig. 4A, i and ii). This technique was successfully performed on four neurons that showed clear hyperpolarizations in response to adiponectin. Linear regression analysis of the grouped difference currents (adiponectin, control) for these cells confirms a current reversal of −108.4 mV for the adiponectin-induced current (Fig. 4B), a value near the equilibrium potential for potassium (−105 mV) calculated using the Nernst equation, where $[K]_o = 2.5$ mM and $[K]_i = 140$ mM. These data suggest that adiponectin hyperpolarizes cells through the activation of voltage-independent potassium current.

Adiponectin-induced hyperpolarization is blocked in glibenclamide

Based on these data showing the adiponectin-induced difference current reversed at the equilibrium potential for potassium, we hypothesized that hyperpolarizing effects of adiponectin resulted from the modulation of the ATP-sensitive potassium conductance K_{ATP}. We therefore un-

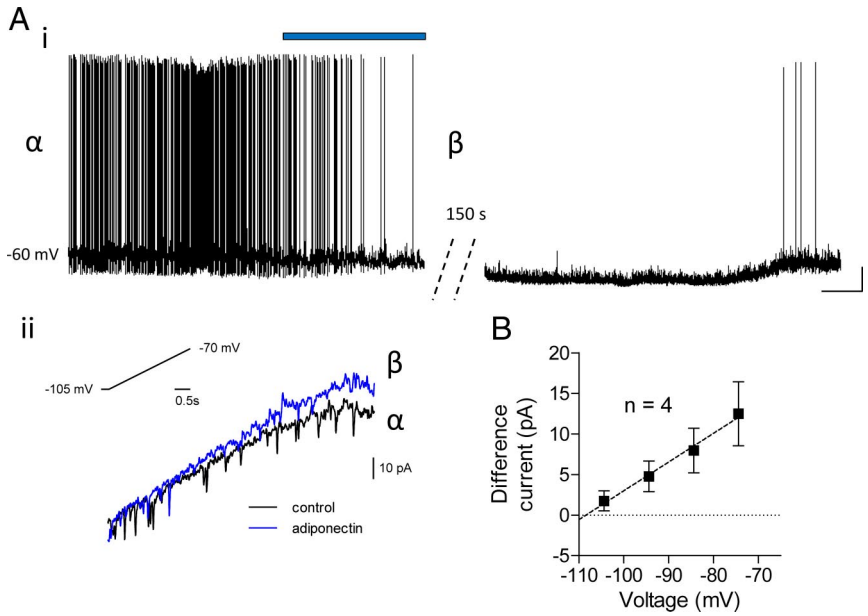

FIG. 4. Adiponectin activates a potassium conductance in hyperpolarized neurons. A, Representative current clamp/voltage clamp recording from the same neuron. i, Current-clamp recording of a parvocellular neuron hyperpolarizing in the presence of bath administration of 10 nM adiponectin (*blue bar*). α, Control voltage-clamp recording as shown in ii; β, period of time performing the same voltage clamp protocols in the presence of 10 nM adiponectin. Time spent in voltage-clamp configuration during β is indicated by the *hatched bars* (*scale bars*, 10 mV, 25 sec). ii, Representative currents obtained during control (α, *black*) and after adiponectin-induced hyperpolarization (β, *blue*) from the current-clamp recording in i. Currents are an average of three (five-point smoothed) ramps performed during the times indicated. *Inset*, Voltage clamp ramp protocol used to elicit the currents shown. B, *Graph* showing the mean average difference current obtained from subtracting control currents from adiponectin-induced currents. *Plotted points* are an average of 100 difference current data points around the voltage of interest averaged from all cells tested (mean ± SEM). Linear regression (*black dashed line*) intersects when Y = 0 near −108 mV.

dertook additional current clamp experiments to determine whether pretreatment with glibenclamide (a K_{ATP} channel inhibitor) abolished hyperpolarizing effects of adiponectin on PVN parvocellular neurons. Recordings were obtained from 12 parvocellular neurons and examined first for their response to bath application of 500 nM glibenclamide, which depolarized 6/12 neurons (control, -51.5 ± 1.4 mV *vs.* glibenclamide, -46.02 ± 0.4 mV; n = 6; paired *t* test, $P < 0.005$), suggesting that in a population of PVN neurons, this conductance contributes to resting membrane potential. After pretreatment with glibenclamide, a stable baseline was obtained for a minimum of 100 sec before bath administration of 10 nM adiponectin (Fig. 5A, i and ii). No hyperpolarizing responses to adiponectin were observed in this group of 12 neurons, with depolarizing responses observed in 7/12 cells (7.4 ± 1.8 mV, n = 7) and five cells being unaffected (-0.2 ± 0.9 mV, n = 5) (Fig. 5, B and C). The fact that no hyperpolarizing responses to adiponectin were observed in the presence of glibenclamide (0/12 neurons) supports the conclusion that a glibenclamide-sensitive current (likely K_{ATP}) is responsible for adiponectin-induced hyperpolarization in parvocellular neurons on the PVN.

Discussion

In this study, we present evidence showing that depolarizing and hyperpolarizing effects of adiponectin on the excitability of parvocellular PVN neurons are mediated through the differential modulation of separate potassium currents. Specifically, adiponectin depolarizes and increases excitability in a subpopulation of PVN neurons through the inhibition of a TEA-sensitive potassium current, effects that are accompanied by changes in the shape of the AP. In contrast, the hyperpolarizing effects of adiponectin on PVN neurons appear to be mediated through the activation of a glibenclamide-sensitive leak potassium conductance. These experiments describe new and potentially critical roles that these ion channels play in mediating the effects of adiponectin on parvocellular neurons in the PVN and identify these channels as final pathways through which adiponectin may influence energy homeostasis and feeding behavior, through actions in the CNS.

Potassium currents make important contributions to the regulation of neuronal excitability, firing patterns of individual neurons and AP shape (17). The inhibition of sustained outward potassium conductance observed through voltage clamp recordings, both slow voltage ramps and voltage step protocols, suggested that adiponectin's effects on these channels would also be reflected by changes in AP shape when examined in current clamp. Analysis of current clamp data obtained from our previous study examining the effects of adiponectin on the excitability of PVN neurons (14) suggests that AP shape is significantly different in cells that depolarize in the presence of adiponectin. Normalized APs showed a significantly prolonged duration when compared with control confirming the inhibitory effects on potassium current manifested as changes in AP profile. Similar effects have previously been reported to occur after blockade of I_K with TEA in physiological systems (19), as well as in computer simulations (20). Changes in the kinetics of potassium currents responsible for the repolarization of the AP can have profound consequences at the synaptic terminal, inducing more inward calcium current and increasing the efficacy of synaptic transmission in central synapses (21). Spike broadening

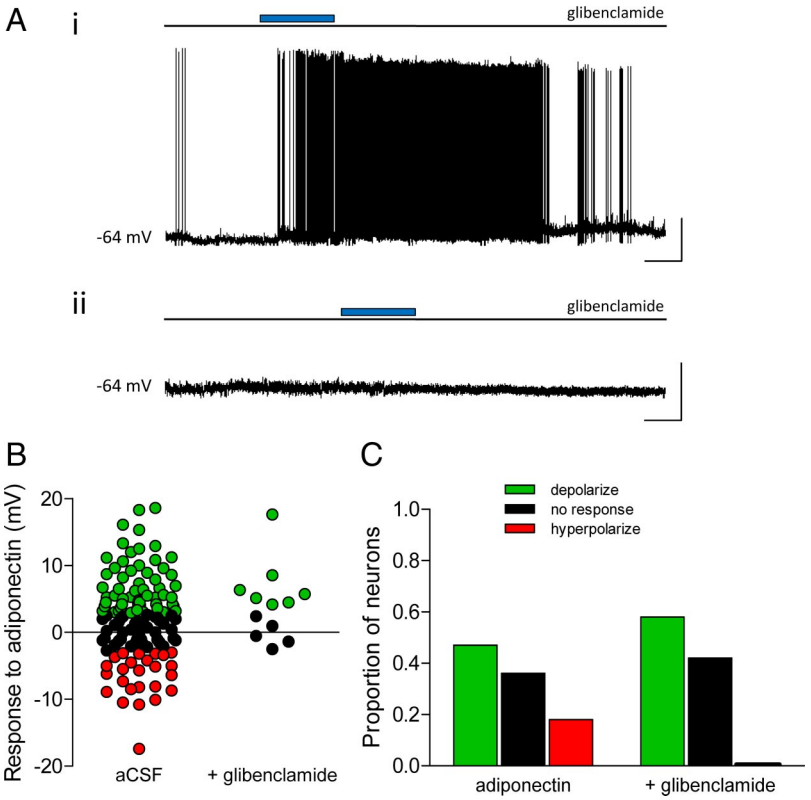

FIG. 5. Glibenclamide inhibits adiponectin-induced hyperpolarization. A, Representative current clamp traces showing a parvocellular neuron that depolarizes (i) and parvocellular neuron that does not respond (ii) to bath application of 10 nM adiponectin (*blue bar*) in the constant presence of 500 nM glibenclamide (*black line*) (*scale bars,* 20 mV, 50 sec). B, Scatter-dot plot showing the response to adiponectin in the presence of 500 nM glibenclamide compared with aCSF. *Green data points* indicate a depolarizing response. *Black points* are those that did not respond, and *red points* indicate a hyperpolarizing response to adiponectin. C, Histogram showing the proportion of neurons that depolarize (*green*), do not respond (*black*), or hyperpolarize (*red*) in response to adiponectin in aCSF (adiponectin) or when the K_{ATP} channel is inhibited by bath application of glibenclamide (+glibenclamide).

in neurohypophysial neurons has also been shown to be intimately associated with frequency-dependent facilitation at presynaptic terminals, increasing release rates of synaptic vesicles containing neurotransmitters (22). The inhibition of I_K by adiponectin could also act as a peptide-specific mechanism to favor the exocytosis of large dense core vesicles containing hypothalamic peptides to be released into the hypophyseal portal circulation. Finally, we were able to demonstrate that TEA, which at concentrations used in these studies blocks, I_K in PVN neurons (23), abolished depolarizing effects of adiponectin in PVN, whereas such effects were still observed in the presence of the BK_{Ca} blocker iberiotoxin, supporting the conclusion that this TEA-sensitive current mediates the depolarizing effects of adiponectin in this population of neurons.

In contrast, our data suggest that adiponectin hyperpolarizes neurons through the activation of a glibenclamide-sensitive potassium conductance. Glibenclamide is a membrane permeable sulfonylurea, which is used clinically to treat type 2 diabetes because of its specific inhibition of K_{ATP} channels, depolarizing pancreatic β-cells, and inducing the release of insulin (24). K_{ATP} channels are heteromultimers of inwardly rectifying potassium channel subunits ($K_{ir}6x$) and sulfonylurea receptors (25) and are expressed in a variety of tissues, including the CNS (24). These channels are inhibited by increasing concentrations of intracellular ATP independent of membrane potential (24) and have been shown to be critical for the sensing of glucose in neurons of the ventromedial hypothalamus (26). Recent microarray analysis indicates that mRNA encoding genes for *Kir6.2*, *sur1*, and *sur2* are present in the PVN (27), suggesting that these cells have the capacity to form functional K_{ATP} channels. That glibenclamide can block the hyperpolarizing effects of adiponectin supports the conclusion that these effects are most likely the result of either AdipoR1 or AdipoR2 receptor-mediated activation of K_{ATP} channels, although potential roles for alternative glibenclamide- sensitive conductances cannot be ruled out. The specific nature of this second messengers involved in such channel modulation is unknown at the current time.

Adiponectin has been shown to stimulate phosphorylation of AMPK in peripheral and CNS tissue through AdipoR1 activation (8, 10, 28). Activated AMPK transitions a cell from energy-consuming anabolic processes to catabolic pathways, which generate ATP (29). Elevation in cellular ATP concentrations would be expected to have an inhibitory effect on K_{ATP} channels and therefore potentially contradict the results obtained in these experiments. However, Yamauchi *et al.* (28) suggest that maximal phosphorylation of AMPK occurs at 15 min after exposure to adiponectin in C2C12 myocytes, whereas our results show the effect on cell membrane excitability usually occurs within 200 sec of bath application of the peptide. This suggests that the modulation of ion channels occurs before the phosphorylation of AMPK.

The physiological relevance of these actions of adiponectin in PVN are not yet fully understood, although the differential effects that we have previously reported on different cell phenotypes (14) suggest an integrative role for this adipokine. Although the concentrations of adiponectin that we have shown to influence PVN neurons (10 nM) are well within the normal physiological range [up

to 100 nM (30)] reported in the circulation, available data suggest that adiponectin does not cross the blood-brain barrier (31). Such observations suggest that this adipokine may also be produced centrally, and thus the adiponectin that normally influences PVN neurons may be locally released from neurons, glial cells, or even vascular endothelial cells. This speculation is supported by recent evidence showing adiponectin mRNA in chicken and mouse brain (32, 33). In conclusion, we have shown that adiponectin controls the excitability of parvocellular PVN neurons through the modulation of potassium currents. Specifically, we have demonstrated that adiponectin-induced depolarization occurs as a consequence of the inhibition of a TEA-sensitive current and that increases in excitability are associated with a broadening of the AP. Conversely, the adiponectin-induced hyperpolarizing responses are due to the activation of a glibenclamide-sensitive current. This study provides the first evidence of specific conductances responsible for conferring adiponectin's effects on CNS neurons controlling energy homeostasis and autonomic function and thus may also identify membrane events that underlie adiponectin actions in other cell types.

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