The food intake-suppressive effects of glucagon-like peptide-1 receptor signaling in the ventral tegmental area are mediated by AMPA/kainate receptors

Elizabeth G. Mietlicki-Baase,1 Pavel I. Ortinski, Laura E. Rupprecht, Diana R. Olivos, Amber L. Alhadeff, R. Christopher Pierce, and Matthew R. Hayes

1Translational Neuroscience Program and 2Center for Neurobiology and Behavior, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Submitted 26 July 2013; accepted in final form 2 October 2013

Mietlicki-Baase EG, Ortinski PI, Rupprecht LE, Olivos DR, Alhadeff AL, Pierce RC, Hayes MR. The food intake-suppressive effects of glucagon-like peptide-1 receptor signaling in the ventral tegmental area are mediated by AMPA/kainate receptors. Am J Physiol Endocrinol Metab 305: E1367–E1374, 2013. First published October 8, 2013; doi:10.1152/ajpendo.00413.2013.—Glucagon-like peptide-1 receptor (GLP-1R) activation in the ventral tegmental area (VTA) is physiologically relevant for the control of palatable food intake. Here, we tested whether the food intake-suppressive effects of VTA GLP-1R activation are mediated by glutamatergic signaling within the VTA. Intra-VTA injections of the GLP-1R agonist exendin-4 (Ex-4) reduced palatable high-fat food intake in rats primarily by reducing meal size; these effects were mediated in part via glutamatergic AMPA/kainate but not NMDA receptor signaling. Additional behavioral data indicated that GLP-1R expressed specifically within the VTA can partially mediate the intake- and body weight-suppressive effects of systemically administered Ex-4, offering the intriguing possibility that this receptor population may be clinically relevant for food intake control. Intra-VTA Ex-4 rapidly increased tyrosine hydroxylase levels within the VTA, suggesting that GLP-1R activation modulates VTA dopaminergic signaling. Further evidence for this hypothesis was provided by electrophysiological data showing that Ex-4 increased the frequency of AMPA-mediated currents and reduced the paired/pulse ratio in VTA dopamine neurons. Together, these data provide novel mechanisms by which GLP-1R agonists in the mesolimbic reward system control for palatable food intake.

2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid; obesity; glucagon-like peptide-1; glutamate; dopamine; reward; diabetes
compared with aCSF/saline. All data are shown as means ± SE. *P < 0.05.

**Behavioral Experiments**

Nonobese rats (381.7 ± 7.3 g, 11–14 wk of age on the 1st day of experimental testing) were maintained on HFD (60% kcal from fat; Research Diets, New Brunswick, NJ) during and for 2 wk prior to testing. HFD was selected because intra-VTA GLP-1R activation (in which NaCl was replaced with an equiosmolar concentration of sucrose). aCSF contained 130 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 10 mM glucose, 1 mM MgCl2, and 2 mM CaCl2 (pH 7.2–7.4 when saturated with 95% O2 and 5% CO2). Slices were incubated in aCSF at 32–34°C for 45 min and kept at 22–25°C thereafter until transfer to the recording chamber. All solutions had an osmolarity between 305 and 315 mOsm. Slices were viewed under an upright microscope (Eclipse FN1; Nikon Instruments, Melville, NY) with infrared differential interference contrast optics and a ×40 water immersion objective. For recordings, the chamber was perfused continuously at a rate of 1–2 ml/min with oxygenated aCSF heated to 32 ± 1°C using an automated temperature controller (Warner Instruments, Hamden, CT). Picrotoxin (100 μM) was also added to all solutions to block the GABA_A receptors. Recording pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) to a resistance of 4–7 MΩ when filled with the intracellular solution. The intracellular solution contained 145 mM potassium glutamate, 2 mM MgCl2, 2.5 mM KCl, 2.5 mM NaCl, 0.1 mM BAPTA, 10 mM HEPES, 2 mM Mg-ATP, 0.5 mM GTP-Tris, and 1 mM MQ-314 (pH 7.2–7.3 with KOH, osmolarity 280–290 mOsm). VTA dopamine neurons were identified morphologically by large soma sizes as well as the use of a twofold electrophysiological criterion (10, 15). Following the injection of a depolarizing current, the neuron had to exhibit a slow depolarizing shoulder preceding an action potential as well as exhibit an Ip current of >50 pA after a hyperpolarizing step to −120 mV or action potential half-width of >1.3 ms. Ten of 17 recorded neurons passed these criteria and had a mean Ip of 145 ± 30 pA and mean AP half-width of 1.64 ± 0.24 ms. Seven neurons were excluded from analyses. Spontaneous excitatory postsynaptic current (sEPSC) recordings were conducted in whole cell voltage clamp mode (Vh = −70 mV) using a Multi-Clamp700B amplifier (Molecular Devices, Sunnyvale, CA). Ex-4 (1 μM) was applied via the Y-tube perfusion system modified for optimal solution exchange in brain slices. sEPSCs were analyzed after a minimum of 4 min of Ex-4 exposure. Currents were low-pass filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440A acquisition board (Molecular Devices) and pClamp10 software (Molecular Devices). Access resistance (10–30 MΩ) was monitored during recordings by injection of 10 mV hyperpolarizing pulses; data were discarded if access resistance changed by >25% over the course of data collection. For paired/pulse ratio (PPR) experiments, evoked responses with an interstimulus interval of 100
ms were triggered by 100-μs constant-current pulses generated by an A310 Accupulsar (World Precision Instruments) and delivered at 0.2 Hz via a bipolar tungsten stimulation electrode positioned within 100 μm of the recorded cell.

Data Analysis and Statistics

Data were analyzed using Statistica (version 7; StatSoft), StatView for Windows (version 5.0.1; SAS), or Microsoft Excel. The α-level for all tests was set at \( P \leq 0.05 \).

Behavioral studies. Data from each time bin for food intake, meal size, and meal number were analyzed using separate mixed-design ANOVA tests to account for the within-subjects design of the experiments while testing for between-subjects effects of drug treatment(s). Statistically significant main effects and interactions were probed using Student-Newman-Keuls post hoc analyses.

Immunoblot studies. Normalized data were analyzed by one-way ANOVA, with drug condition as a between-subjects factor.

Electrophysiological studies. All analyses of electrophysiological recordings were completed using Clampfit 10 (Molecular Devices). The time constant of decay was based on a monoexponential fit to the decay phase of an average sEPSC trace computed from a minimum of 50 individual sEPSCs. Mean sEPSC frequencies were analyzed from 20-s trace segments. PPRs were calculated by averaging five to 10 responses and dividing the peak amplitude of the second evoked EPSC by the peak amplitude of the first evoked EPSC. Neurons from a total of five animals were analyzed. Statistical comparisons were done using two-tailed paired Student t-tests.

RESULTS

VTA GLP-1Rs Are Activated by a Peripherally Administered GLP-1R Agonist to Control Food Intake

Given that the VTA is a physiologically relevant site of action for palatable food intake (3), this raises the possibility that VTA GLP-1R may also directly mediate the intake-suppressive effects of peripherally administered GLP-1R agonists. To test this possibility, HFD-maintained rats \((n = 15)\) received an intra-VTA infusion of the GLP-1R antagonist Ex-9 (10 μg) or vehicle (200 nl of aCSF), followed by an ip injection of Ex-4 (3 μg/kg) or vehicle (1 ml/kg 0.9% NaCl), and 24-h food intake was measured. This dose of Ex-9 was selected because it has no effect on 24-h food intake or body weight gain when delivered to the VTA (3). Here, intra-VTA GLP-1R blockade with Ex-9 attenuated the anorectic effect of ip Ex-4 \((F_{1,14} = 4.69, P = 0.049; \text{aCSF/Ex-4 vs. Ex-9/Ex-4, } P < 0.01; \text{Fig. 1A})\). Additionally, VTA Ex-9 attenuated the reduction in 24-h body weight gain produced by ip Ex-4 \((F_{1,14} = 5.58, P = 0.03; \text{aCSF/Ex-4 vs. Ex-9/Ex-4, } P < 0.01; \text{Fig. 1B})\). Together, these data suggest that VTA GLP-1Rs partially mediate the intake- and body weight-suppressive effects of systemic Ex-4.

Intra-VTA GLP-1R Activation Reduces Palatable High-Fat Diet Intake By Suppressing Meal Size, Effects That Are Mediated By VTA AMPA/Kainate Receptors

The VTA receives afferent glutamatergic projections from other nuclei involved in the control of food intake and other motivated behaviors (16, 29). Importantly, glutamatergic signaling through AMPA/kainate but not NMDA receptors in the MRS reduces feeding (27), leading to the hypothesis that VTA GLP-1R activation reduces food intake via modulation of glutamatergic signaling. Consistent with our hypothesis and our previous results (3), intra-VTA Ex-4 (0.05 μg/100 nl) reduced HFD intake beginning at 3 h postinjection and persisting throughout the remainder of the 24-h measurement period \((n = 22; \text{all ANOVAs } F_{1,21} \geq 6.17, P \leq 0.02; \text{acSF/acSF vs. acSF/Ex-4, all } P < 0.02; \text{Fig. 2A})\). Statistically significant interactions between CNQX and Ex-4 were observed for cumulative food intake beginning at 4 h and lasting until 24 h \((\text{all ANOVAs } F_{1,21} \geq 4.89, P \leq 0.04)\), demonstrating that intra-VTA pretreatment with the AMPA/kainete receptor antagonist CNQX (0.3 μg/100 nl) attenuated the intake-suppressive effect of intra-VTA Ex-4 \((P < 0.04)\). These data suggest that VTA GLP-1R activation reduces food intake in part through glutamatergic AMPA/kainete receptor signaling. Interestingly, intra-VTA administration of CNQX alone increased food intake at early times \((\text{vehicle/vehicle vs. CNQX/vehicle, different at 30 min-1.5 h; all main effects of CNQX } F_{1,21} = 5.20, P \leq 0.04))\), but this hyperphagic effect subsided by the time that a statistically significant interaction between CNQX and Ex-4 was observed at 4 h, suggesting that the AMPA/kainete-mediated attenuation of Ex-4-induced suppression of food intake is not simply the result of competing bidirectional effects. Meal pattern analyses revealed that intra-VTA Ex-4 reduces intake of a palatable high-fat food by decreasing meal size \((\text{from 3 to 24 h, all ANOVAs } F_{1,21} \geq 6.11, P \leq 0.03; \text{acSF/acSF vs. acSF/Ex-4, all } P < 0.03; \text{Fig. 2B})\), with minimal effects on meal frequency \((\text{main effect of Ex-4 at 30 min, 12 h, and 24 h; all ANOVAs } F_{1,21} = 4.46, P \leq 0.05; \text{post hoc comparison of acSF/acSF vs. acSF/Ex-4, } P < 0.03 \text{ at 12 and 24 h only; Fig. 2C})\). Together with previous reports (3, 13), these findings suggest that VTA GLP-1R signaling potentially controls for food intake by modulating the rewarding value of the ongoing meal while having fewer effects on between-meal satiety processes. Pretreatment with intra-VTA CNQX attenuated the meal size-suppressive effect of VTA Ex-4 from 3 to 6 h \((\text{planned post hoc comparisons of acSF/Ex-4 vs. CNQX/Ex-4, all } P < 0.05; \text{Fig. 2B})\), suggesting that this meal size-suppressive effect of VTA GLP-1R activation is also mediated partly by AMPA/kainete receptors.

VTA NMDA Receptors are not Required to Mediate the Food Intake-Suppressive Effects of Intra-VTA GLP-1R Activation

In contrast to the findings for CNQX, intra-VTA pretreatment with the NMDA receptor antagonist MK-801 (0.05 μg/100 nl) did not attenuate the food intake-suppressive effects of intra-VTA Ex-4 \((n = 8); \text{significant main effect of Ex-4 from 3 to 24 h, all ANOVAs } F_{1,7} \geq 8.40, P < 0.03; \text{no statistically significant interaction between MK-801 and Ex-4 at any time (all ANOVAs } F_{1,7} \geq 3.38, P > 0.05; \text{Fig. 3A})\). Although VTA Ex-4 again reduced meal size in this experiment \((\text{significant main effect of Ex-4 from 4 to 24 h, all ANOVAs } F_{1,7} \geq 6.66, P < 0.05; \text{Fig. 3B})\), MK-801 pretreatment did not attenuate this effect. Importantly, the Ex-4-induced meal pattern effects observed in this experiment were comparable with those observed in the CNQX experiment, with Ex-4-induced suppression of food intake being driven predominantly by meal size reductions, with no significant effects on meal frequency. Worth noting is that the dose of MK-801 used here had a statistically significant main effect at early times for cumulative food intake \((30–90 \text{ min, all ANOVAs } F_{1,7} \geq 5.75, P < 0.05))\) and meal
size (60 min, $F_{1,7} = 12.04, P = 0.01$), suggesting that the dose was appropriately selected from previous literature examining NMDA-mediated effects on food intake (17). However, in the current experiments, blockade of NMDA receptors by MK-801 did not reverse or attenuate the suppression of food intake by VTA Ex-4 administration. Collectively, these results indicate that the food intake- and meal size-suppressive effects of GLP-1R activation in the VTA are mediated by glutamatergic signaling through AMPA/kainate but not NMDA receptors.

**Intra-VTA GLP-1R Activation Increases VTA Tyrosine Hydroxylase Levels**

Given the present findings and the role of MRS dopamine signaling in the control of food intake (7, 28, 35), we hypothesized that GLP-1R activation in the VTA modulates dopaminergic signaling. Because TH is the rate-limiting step in dopamine synthesis, we tested whether VTA GLP-1R activation would alter TH levels within the VTA. Compared with vehicle treatment, intra-VTA administration of Ex-4 (0.05 μg/100 nl) increased VTA TH levels at 15 min postinjection ($n = 11, F_{1,9} = 5.12, P = 0.050$; Fig. 4).

**GLP-1R Expressed on Presynaptic Glutamatergic Terminals in the VTA Modulate the Activity of VTA Dopaminergic Neurons**

The finding that VTA GLP-1R activation increased TH levels in the VTA, together with our behavioral studies indicating a glutamatergic AMPA/kainate-mediated attenuation of VTA Ex-4-induced suppression of food intake, led us to hypothesize that activation of GLP-1R modulates the activity of VTA dopaminergic neurons via AMPA/kainate receptors. Thus, using patch clamp electrophysiology, we recorded pharmacologically isolated AMPA/kainate receptor-mediated sEPSCs in VTA dopamine neurons before and during bath application of Ex-4 (1 μM). Ex-4 increased sEPSC frequency significantly in VTA dopamine neurons [Fig. 5, A, B, and D; $n = 10; t(9) = 3.08, P = 0.01$], suggesting a presynaptic effect of GLP-1R activation. sEPSC decay, time, peak amplitude, and charge transfer were unchanged by Ex-4 (Fig. 5, C and D), arguing against postsynaptic effects of GLP-1R activation. To confirm the presynaptic locus of GLP-1R effects on VTA dopamine neurons, we analyzed the PPR before and during Ex-4 bath application. Corroborating the sEPSC frequency findings, Ex-4 reduced the PPR [$n = 6; t(5) = 3.77, P = 0.01$; Fig. 5, E and F], consistent with a presynaptic mechanism. To examine a possible effect of GLP-1R activation on VTA dopamine neuronal firing (1), we analyzed the relationship between action potential frequency and injected current (f-I). Ex-4 induced a slight but nonsignificant reduction in the mean f-I relationship ($n = 4, P > 0.05$; Fig. 6). Together, these results show that activation of presynaptic GLP-1R, expressed putatively on glutamatergic terminals, increases synaptic excitation of VTA dopamine neurons via AMPA/kainate receptors.

**DISCUSSION**

The present data support the hypothesis that VTA GLP-1R signaling regulates the control of palatable high-fat food intake. Behavioral data presented here indicate that VTA GLP-1R activation reduces intake of palatable high-fat diet primarily by
reducing meal size, with minimal and inconsistent effects on meal frequency. Additionally, the results demonstrate that the anorectic effects of intra-VTA GLP-1R activation are mediated in part by glutamatergic AMPA/kainate but not NMDA receptor signaling. The intake-suppressive effect of Ex-4 was observed from 3 to 24 h postinjection, and this hypophagic response was attenuated by the AMPA/kainate receptor antagonist CNQX at each of these times. The Ex-4-induced hypophagia was not completely blocked by AMPA/kainate receptor antagonism, highlighting the possibility that the feeding effects of intra-VTA GLP-1R activation are also mediated by additional nonglutamatergic mechanisms that persist regardless of the presence of glutamatergic receptor antagonists. Although the independent contributions of AMPA receptors vs. kainate receptors cannot be elucidated by these studies, the finding that AMPA/kainate receptors rather than NMDA receptors mediate the food intake- and meal size-suppressive effects of VTA GLP-1R activation is consistent with previous results demonstrating a role for AMPA/kainate but not NMDA receptors in MRS-mediated feeding (27).

Immunoblot results indicate that intra-VTA Ex-4 increases levels of TH within the VTA. This finding suggests an upregulation of dopamine production in the VTA as a result of VTA GLP-1R activation. Further support for this idea is revealed by electrophysiological data demonstrating that presynaptic GLP-1R activation increases AMPA receptor-mediated glutamatergic transmission onto the VTA dopamine neurons. The fact that Ex-4 increased VTA dopamine neuron activity is interesting given that intra-VTA GLP-1R activation potently suppresses food intake, an effect often associated with reduced dopaminergic signaling in the MRS. There are several hypothetical possibilities for these seemingly discrepant data. One potential explanation is that GLP-1R excitation of VTA dopaminergic neurons may lead to dopamine-induced activation of D2 autoreceptors within the VTA (2), having the subsequent net effect of decreasing dopamine signaling in the MRS. Another possibility is that GLP-1R signaling in the VTA somehow replaces the hedonic within-meal-promoting signals of continued ingestion of a palatable food, thus reducing the likelihood of further feeding. This would be consistent with evidence demonstrating that VTA GLP-1R activation reduces rats’ motivation to obtain a palatable food in an operant paradigm (13) as well as present findings showing suppression in meal size by VTA GLP-1R activation. Finally, it is possible that the GLP-1-producing preproglucagon neurons in the hindbrain coexpress glutamate, and thus cotransmission of GLP-1 and glutamatergic signals may occur in the VTA from the same axon.
Traditionally, fluctuations in MRS dopamine signaling have been examined over a very short time scale (i.e., ms to h) with regard to influences on appetitive behaviors (18, 31, 36), whereas alterations on the scale of several hours to days have not been as carefully characterized. Together with our previous report (3), present data demonstrate that the food intake-suppressive effects of intra-VTA GLP-1R activation occur hours after drug administration for high-fat diet but within 20 min for a sucrose solution (3). Therefore, VTA GLP-1R modulation of food intake may add levels of complexity and depend in part on macronutrient composition and/or the physical properties (solid vs. liquid) of the palatable food being ingested. Another important question raised by the electrophysiological data presented here is the neuroanatomic location of the glutamatergic cell bodies that project to the VTA and putatively express GLP-1R on their presynaptic terminals. Several nuclei implicated in the control of food intake provide glutamatergic input to the VTA (e.g., prefrontal cortex, bed nucleus of the stria terminalis, lateral hypothalamus) [reviewed by Geisler and Wise (16)], and although the present data cannot determine which source(s) is relevant to the effects of GLP-1R in the VTA, further investigation is certainly warranted and will provide more insight into the mechanism of action of GLP-1R signaling in the VTA.

That the intake-suppressive effects of a peripherally administered GLP-1R agonist are attenuated by blockade of VTA GLP-1R highlights the intriguing possibility that VTA GLP-1Rs are not only physiologically relevant for the control of food intake (3) but may also be a clinically relevant site of action for peripherally administered GLP-1R ligands in humans. Interestingly, we observed that unilateral VTA administration of Ex-9 was sufficient to attenuate the food intake- and body weight-suppressive effects of systemically administered Ex-4. Although not statistically different, the mean 24-h food intake and body weight of the Ex-9/Ex-4 group were lower than that of the vehicle/vehicle group, suggesting that the contralateral VTA and other CNS GLP-1R-expressing nuclei are

Fig. 5. A: representative spontaneous excitatory postsynaptic current (sEPSC) traces from VTA dopamine neurons before and during Ex-4 application. B: Ex-4 increases sEPSC frequency. Effect of Ex-4 on sEPSC averages (C) is expressed as % difference from baseline recorded before Ex-4 application (D). E: Ex-4 reduces paired/pulse ratio (PPR). F: quantified PPR results (gray lines represent individual neuron responses before and during Ex-4 application; black line is the mean). Quantified data are shown as means ± SE. *P < 0.05.

Fig. 6. A: voltage responses of a VTA dopamine neuron to a −140-pA and a +140-pA current step illustrate a slight decrease in action potential (AP) firing during Ex-4 treatment. B: quantified frequency vs. injected current (f-I) data (means ± SE) shows that the effect of Ex-4 on the f-I relationship is not significant (P > 0.05).
also mediating in part the 24-h anorectic effect produced by IP Ex-4 (see Ref. 19 for review). It is worth noting that whereas GLP-IR agonists can induce nausea/malaise when administered systemically (4, 9, 25, 26), GLP-IR activation in the VTA reduces food intake in the absence of nausea/malaise (3, 13). Therefore, the novel findings presented here, together with a growing body of basic science evidence, suggest that VTA GLP-IR may be a potential future clinical target for obesity pharmacotherapies to selectively reduce palatable food intake independent of nausea/malaise (3, 13).

In summary, the data presented here provide complementary behavioral, molecular, and electrophysiological evidence that AMA/pain/kainate receptor signaling in the VTA mediates at least in part the food intake-suppressive effects of VTA GLP-IR activation. Given that NTS GLP-1-producing neurons are activated by gastrointestinal derived, vagally mediated satiation signals (34) and NTS neurons project monosynaptically to the VTA (3), the present data provide a novel mechanism by which increased satiation signaling may decrease the rewarding value of an ongoing meal (5, 6).

ACKNOWLEDGMENTS

Valuable technical assistance was provided by Shaila Berlas, Lauren McGrath, Orianne Montaubin, David Reiner, Line Stensland, Frank Wang, and Derek Zimmer.

GRANTS

This research was supported by DA-031747 (P. I. Ortsinski), DA-22339 and DA-18678 (R. C. Pierce), DK-097954 (E. G. Mietlicki-Baase), and DK-096139 and DK-085435, the University of Pennsylvania Research Foundation, and the Diabetes Research Center (DK-19525) (M. R. Hayes).

DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS


REFERENCES

3. Alhadeff AL, Rupprecht LE, Hayes MR. GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. Endocrinology 153: 647–658, 2012.

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00413.2013 • www.ajpendo.org


