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Fasting Induces a Large, Leptin-Dependent Increase in the Intrinsic Action Potential Frequency of Orexigenic Arcuate Nucleus Neuropeptide Y/Agouti-Related Protein Neurons

Kanji A. Takahashi and Roger D. Cone

Vollum Institute and The Center for the Study of Weight Regulation and Associated Disorders (K.A.T., R.D.C.), Oregon Health & Science University, Portland, Oregon 97239; and Department of Neuroscience (K.A.T.), Albert Einstein College of Medicine, Bronx, New York 10461

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The neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus are thought to promote feeding. Here, we demonstrate that feeding state *in vivo*, through a leptin-dependent process, induces large and persistent changes in the electrophysiological activity of these neurons as measured extracellularly *in vitro*. Consistent with an orexigenic role, fasting induced a 4-fold increase in the basal action potential frequency of NPY/AgRP neurons. Leptin, when injected into fasted wild-type mice, induced a dose- and time-dependent decrease in spike frequency, which approached fed levels 2–3 h post treatment. In leptin-deficient (*lep^{ob}/lep^{ob}*) and leptin receptor-deficient (*$lepr^{db}/lepr^{db}$*) mice, NPY/AgRP spike frequency was not significantly increased by fasting, and even in mutant mice fed *ad libitum*, spike frequency was at least as high as in fasted wild-type mice. All recordings included $GABA_A$ and ionotropic glutamate receptor antagonists, suggesting that expression of this modulation is potentially intrinsic and not synaptically dependent. Recorded neurons were unambiguously identified using NPY-Sapphire transgenic mice. This is a remarkably straightforward example of a very robust *in vitro* electrophysiogical effect produced by a simple behavioral manipulation, food restriction. (*Endocrinology* 146: 1043–1047, 2005)

REGULATION OF FOOD intake by the neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus is an area of active investigation (1–3) (reviewed in Refs. 4 and 5). When delivered centrally, NPY is noted for the voraciousness and AgRP for the extreme duration of the induced feeding (6). Chronic infusion of NPY and overexpression of AgRP both lead to obesity. Most NPY-expressing somata in the hypothalamus and all AgRP somata in the brain are located in the arcuate nucleus, where they are coexpressed (7, 8). AgRP acts as an endogenous antagonist of the anorectic effect of α-MSH at melanocortin receptors (9–11). Fasting, mainly through a leptin-dependent process, induces large increases in arcuate nucleus NPY and AgRP expression, and this has been assumed to reflect modulation of the action potential activity of NPY/AgRP neurons (7, 8, 12–14). However, neuropeptide expression as a proxy measure of action potential activity is suggestive but not definitive. Because neuropeptide-containing neurons generally also release a fast neurotransmitter (15), changes in action potential activity could be important independent of changes in neuropeptide expression. In addition, as long as spike activity is not so low as to prevent peptide release, it is possible for changes in neuropeptide expression to alter peptide release without altering spike frequency.

We report direct measurement of arcuate NPY/AgRP neuronal activity in acute slices and its modulation by fasting and leptin signaling *in vivo*. Loose patch extracellular recording was used to measure the basal activity of visually identified neurons without artifactually influencing that activity, and recording solutions contained kynurenic acid and picrotoxin to isolate the cells from synaptic inputs via $GABA_A$ and ionotropic glutamate receptors. A strict cell selection protocol was followed to obtain a large (20–35 cells), unbiased sample of NPY neurons widely distributed in the arcuate nucleus from every mouse in each treatment group.

Materials and Methods

Mice

All mice used in recordings were hemizygous for the NPY-Sapphire transgene in which expression of a Tau-Sapphire (green fluorescent protein variant) fusion protein is directed by NPY genomic elements and selectively expressed in NPY neurons (1). NPY-Tau-Sapphire (NPY-SAP) mice were a kind gift of Dr. Hongyan Liu and Dr. Jeffrey Friedman of Rockefeller University (New York, NY) and were received on a mostly C57Bl/6J background with a smaller CBA contribution (Pinto, S., personal communication). B6.V-Lep^{ob}/J (lep^{ob}/+), B6.Cg-m +/+ $Lepr^{db}$/J ($lepr^{db}$/+), and C57Bl/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Subsequent breeding produced the following mice used for recording: 1) +/+; NPY-SAP Tg/+ (wild type). 2) lep^{ob}/lep^{ob}; NPY-SAP Tg/+ (lep^{ob}/lep^{ob}). 3) $lepr^{db}/lepr^{db}$; NPY-SAP Tg/+ ($lepr^{db}/lepr^{db}$). PCR-based tests of ear-snip DNA were used to genotype mice for the lep^{ob} (16) and $lepr^{db}$ (17) mutations and for the NPY-Sapphire transgene (forward: 5′-GGGCAGAAGGTCCAGTC-3′; reverse: 5′-GGTCTT-

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Abbreviations: AgRP, Agouti-related protein; GABA, γ-aminobutyric acid; NPY, neuropeptide Y; NPY-SAP, NPY-Tau-Sapphire fluorescent protein; PST, pacific standard time; R_{seal}, seal resistance.

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GCAGGGTGTAGTCC-3′; Liu, H., personal communication). Of the uninjected wild-type mice used in the experiments of Fig. 1, five of six mice in both fed and fasted groups were the progeny of the same matings used to produce lep^{ob}/lep^{ob}, NPY-SAP Tg/+ and $lepr^{db}/lepr^{db}$, NPY-SAP Tg/+ mice for recordings. The injected wild-type mice of Fig. 2 were the progeny of wild-type parents, except for five mice distributed among five injection groups that had at least one lep^{ob}/+ parent.

Mice were housed in a rodent facility on a 12-h light, 12-h dark cycle, with lights on at 0715 PST (Pacific standard time), and had *ad libitum* access to water and food (Lab Diet 5001, Rodent Diet; PMI Nutrition International, LLC, Brentwood, MO; fat ≥ 4.5%). All mice were weaned at least 6 d before recording. At least 2 d before recording, mice were acclimated by housing singly in a new cage with a new nestlet and food available only in a ceramic bowl. Twenty-four hours before recording, mice were weighed and placed in a new cage with a new nestlet and a ceramic bowl that either contained five pellets of chow or was left empty.

In the experiments of Fig. 1 (uninjected), 30- to 40-d-old mice were weighed (0752–0803 PST) and decapitated (0801–0810 PST) 1 h, 45 min after lights on, and experiments using mice of the different genotypes were interleaved. In the experiments of Fig. 2 (ip injected), 30- to 48-d-old mice were weighed (0916–0929 PST) and decapitated (0930–0935 PST) 3 h, 15 min after lights on. Recombinant murine leptin (Lot AFP352C, purity 95–99% by SDS-PAGE) was obtained from A. F. Parlow (National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) and stored at −80 C until stock solutions were made. Leptin was dissolved in sterile-filtered PBS (Invitrogen Corp., Carlsbad, CA; adjusted to pH 7.9 with NaOH) at 1 mg/1.5 ml and stored in single-use aliquots at −80 C until injection. For the 5-mg/kg dose, undiluted leptin stock was injected. For lower doses of leptin or controls, injection volume per body weight was kept constant with PBS (pH 7.9). Dose was calculated from body weight 24 h before decapitation, when fasting was started. In both the fed and fasted vehicle-injected groups, three mice were injected at 1 h before decapitation, and another three mice were injected at 3 h before decapitation. Mean spike frequencies were similar at 1 and 3 h (fed 1 h: 0.48 ± 0.02 Hz, fed 3 h: 0.38 ± 0.10 Hz, $P = 0.39$; fasted 1 h: 1.71 ± 0.17 Hz, fasted 3 h: 1.65 ± 0.23 Hz, $P = 0.84$; unpaired, two-tailed, heteroscedastic t tests), and so these groups were combined. Recordings using mice subjected to the various feeding and leptin-injection regimens were interleaved.

In the fasted, 5 mg/kg leptin, 3 h category of Fig. 2, an outlier of 2.19 Hz (indicated by an *open circle*) was more than 6 SDs greater than the mean of the remaining six mice (SD calculated without outlier). Because it was probably the result of experimental error (*i.e.* misinjection of leptin into the intestinal lumen), it was omitted from analysis. Animal protocols were approved by the Oregon Health & Science University Animal Care and Use Committee.

Electrophysiology

Recording methods were modified from those used earlier (18). Halothane-anesthetized mice were decapitated at a fixed time after lights on (1 h, 45 min—Fig. 1; 3 h, 15 min—Fig. 2). Coronal slices of hypothalamus (200 μm thick) were prepared on a vibrating slicer (VT1000S; Leica Microsystems, Wetzlar, Germany) using standard methods. After slice preparation was complete, at least 1 h was allowed for recovery before use. A slice was transferred to the recording chamber and allowed to equilibrate for 10 min. For the subsequent 40 min, moving deliberately across the arcuate nucleus, as many fluorescent neurons as possible of healthy appearance but of every brightness were subjected to loose-patch (19) extracellular recording of action potential activity. This process was repeated until 5 h, 0 min after decapitation (or earlier if all slices were used). Care was taken to achieve a seal resistance high enough to prevent action potentials from going undetected, and current clamp (I = 0) mode was used to avoid the possibility that current passed through the pipette might influence the recorded cell. Fluorescent cells on the surface of the slice with no overlying tissue were avoided because they were sometimes swollen and were more likely to have been damaged during slicing. The slice holding chamber (submersion type) and recording chamber were maintained at 30 C. Spike frequency for each cell was calculated from the first 120 sec of recording. These were combined to calculate mean spike frequency for each mouse. These means of each mouse, grouped by treatment category, were analyzed by one-way ANOVA and the Newman-Keuls posttest and presented as grand mean ± SEM. Significance level was set at 0.05 for all tests.

Through the recording period, spike frequency did not vary with either time after decapitation, time in recording chamber, or rostral-caudal position. For each mouse, each of these parameters was plotted against spike frequency, and regression lines were drawn to look for potential correlations. The slopes of these relationships, combined for each treatment group of mice, were never significantly different from zero (one sample t test *vs.* a theoretical value of zero, $P > 0.05$).

Saline used for slice preparation and maintenance contained 126.2 mM NaCl, 3.1 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 10 mM glucose, and 16.24 mM sucrose (320 mosm/kg, pH 7.39, when gassed with 95% O_2-5% CO_2). Saline for recording contained 133.2 mM NaCl, 3.1 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 10 mM glucose, and 1.05 mM NaOH (ACS reagents; Mallinckrodt Chemical, Inc., Paris, KY; and Fisher Scientific, Fair Lawn, NJ). Recording saline also contained picrotoxin (0.1 mM; Sigma Chemical Co., St. Louis, MO) and kynurenic acid (Fig. 1, 1.0 mM, Sigma; Fig. 2, 0.9 mM, Tocris Cookson Inc., Ellisville, MO). Patch pipettes were filled with recording saline. The recording chamber was perfused at approximately 2.3 ml/min and was approximately 0.7 ml in volume. To ensure consistency, all equipment in contact with solutions, including the pipette holder and recording chamber, were cleaned daily.

Sapphire-fluorescent neurons were unambiguously identified and patched using epifluorescence and IR-DIC optics (Axioskop 2 FS microscope; Carl Zeiss, GmbH, Jena, Germany). Equipment was optimized for detection of even dim Sapphire fluorescence (Sapphire GFP filter cube; Chroma Technology Corp., Rockingham, VT; and C7500–50 high sensitivity CCD camera and C2400 camera controller; Hamamatsu Photonics, Hamamatsu, Japan), and exposure to near UV excitation light was limited to reduce bleaching. Under these conditions, cells close enough to the surface to be targeted for recording were unambiguously identifiable as fluorescent or not fluorescent in fed mice (*i.e.* fluorescence intensity was not a limiting factor in identification of NPY/AgRP neurons). Perhaps for this reason, although fasting greatly increased the fluorescence intensity of arcuate nucleus neurons (as expected for a transgene controlled by NPY genomic elements), it seemed to not cause a large increase, if any, in the number of patchable fluorescent neurons; however, formal cell counts were not done. Because fluorescent cells were recorded without regard to intensity, we consider it unlikely that fasting-induced increases in fluorescence caused a substantial bias in the selection of cells for recording. After injecting fasted mice with leptin 1–3 h before decapitation, fluorescence intensity remained very high (perhaps due to slow degradation of the Tau-Sapphire fusion protein), eliminating selection bias as an explanation for the large effect on spike frequency.

Pipettes (~1.4–1.8 MΩ) were used to form seals of ≤11 MΩ, and no cell was considered silent unless seal resistance (R_{seal}) was greater than 4 MΩ. R_{seal} was kept low to avoid damaging the patched membrane. Over time, R_{seal} tends to increase as the patched membrane is drawn further into the pipette. When this increase was large, additional strain was prevented by applying a small amount of positive pressure from a short column of water attached via tubing to the pipette holder. Unlike whole-cell patch clamp and sharp microelectrode recording, loose-patch recording does not disturb cytosolic contents and intracellular processes; and neuronal activity is not affected by seal strength or current passed through the pipette. Data were acquired at 10 kHz using an Axopatch 1D amplifier (100× gain, −3 dB filter freq: 5 kHz) and Clampex 8.2 software (Axon Instruments, Union City, CA). Data were analyzed using Mini Analysis Program 5.6.28 (Synaptosoft, Decatur, GA), GraphPad Prism 3.0 (Graphpad Software, Inc., San Diego, CA), and Excel 2000 (Microsoft Corp., Bellevue, WA).

Results

In fed wild-type mice, the basal spike frequency of arcuate nucleus NPY neurons was very low (0.38 ± 0.06 Hz/mouse; Fig. 1, A and B). Fifty-six percent of all neurons from fed wild-type mice had spike frequencies less than 0.1 Hz (Fig. 1C). After a 24-h fast, in which body weight decreased 21.9 ± 1.1% (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.

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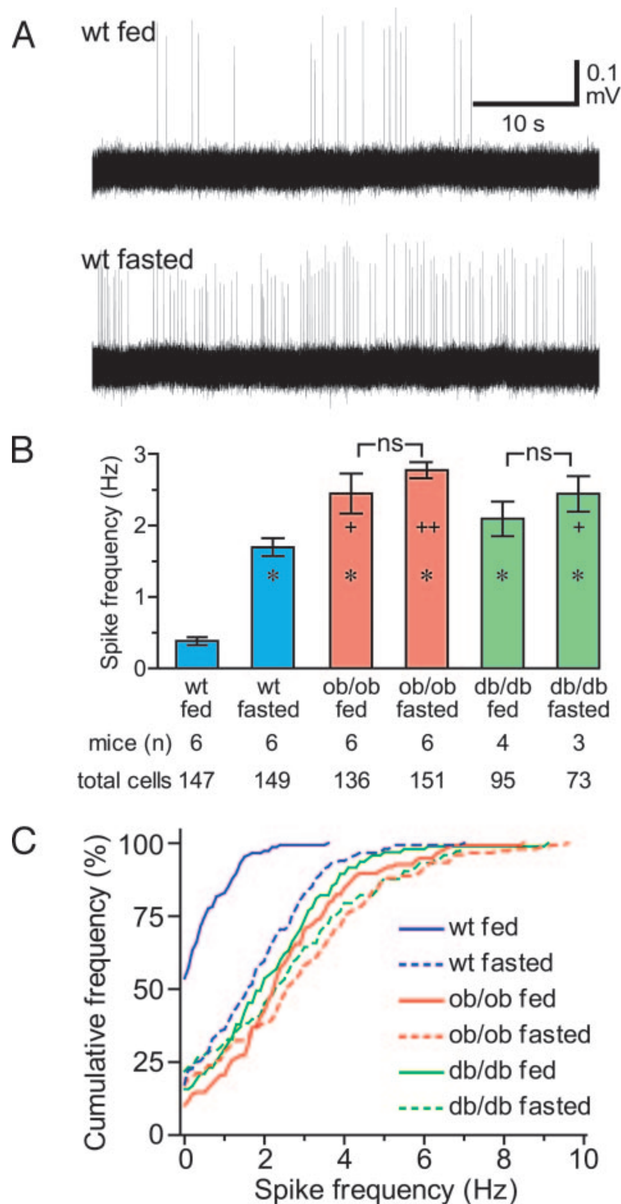

FIG. 1. Fasting for 24 h greatly increases the basal spike frequency of arcuate nucleus NPY neurons in wild-type mice. In lep^{ob}/lep^{ob} (ob/ob) and $lepr^{db}/lepr^{db}$ (db/db) mice, spike frequency was high even in fed animals and was not significantly increased by fasting. A, Typical extracellular recordings of NPY/AgRP neuron spiking from fed and fasted mice. *Scale bars* apply to both traces. wt, Wild-type. B, Grand mean ± SEM of mean frequencies per mouse. Significantly different from fed wild-type mice (*, $P < 0.001$). Significantly different from fasted wild-type mice (+, $P < 0.05$; ++, $P < 0.01$). ns, Not significant. C, Relative distribution of all cells in A by spike frequency, plotted as a cumulative frequency histogram (0.1 Hz bins). In fed wild-type mice, the spike frequency of most NPY neurons was silent or very low. In fasted wild-type mice and in lep^{ob}/lep^{ob} and $lepr^{db}/lepr^{db}$ mice, the entire distribution shifted to higher frequencies.

endojournals.org), mean spike frequency per mouse increased over 4-fold to 1.70 ± 0.13 Hz (Fig. 1B). There was a correspondingly large, rightward shift in the frequency distribution of cells from fasted mice (Fig. 1C). Because previous studies had suggested a role for leptin in mediating fasting-induced increases in neuropeptide expression (see *Introduction*, reviewed in Refs. 5 and 14), recordings using leptin-deficient (lep^{ob}/lep^{ob}) and leptin receptor-deficient ($lepr^{db}/lepr^{db}$) mice were interspersed with those using wild-type mice. In fed lep^{ob}/lep^{ob} and $lepr^{db}/lepr^{db}$ mice, NPY spike frequency was at least as high as in fasted wild-type mice. Importantly, in neither mutant strain did fasting significantly increase NPY spike frequency (Fig. 1, B and C). This strongly suggests that in order for fasting to have a large effect on spike frequency, the leptin signaling system must be intact. Alternatively, it is formally possible that developmental defects (20) in lep^{ob}/lep^{ob} and $lepr^{db}/lepr^{db}$ mice account for these results.

These results suggest that fasting increases the spike frequency of NPY neurons by reducing leptin levels. If so, then exogenous leptin should be able to reverse this effect. Recombinant mouse leptin at 1–5 mg/kg or vehicle (PBS, pH 7.9) was injected ip 1–3 h before decapitation of fed or 24-h fasted mice. Leptin induced a dose- and time-dependent decrease in the spike frequency of NPY neurons in fasted mice (Fig. 2 and Supplemental Table 2, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Leptin (5 mg/kg) induced reductions in spike frequency equivalent to 45% (1 h post injection), 71% (2 h post injection), and 90% (3 h post injection) reversals of the fasting-induced increase in controls, revealing a slow time course for this type of inhibition.

Discussion

We have demonstrated that fasting induces a large increase in the basal spike frequency of NPY neurons measured in an *in vitro* slice preparation. Mechanistically, the results from lep^{ob}/lep^{ob} and $lepr^{db}/lepr^{db}$ mice suggest that, in order for

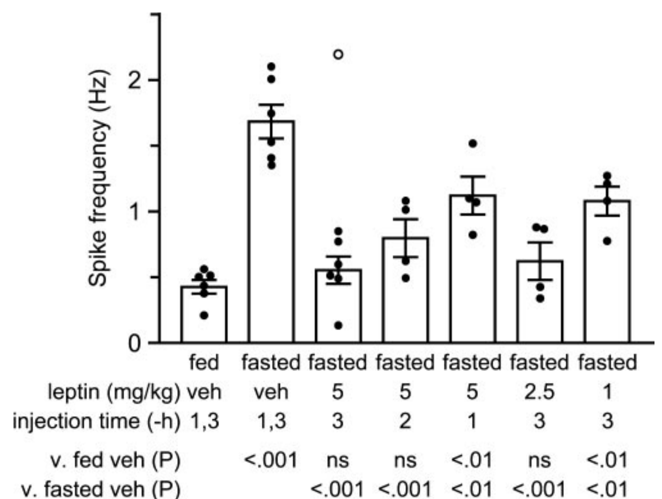

FIG. 2. Leptin injection induces a dose- and time-dependent decrease in the basal spike frequency of arcuate nucleus NPY neurons from 24-h fasted mice. Data displayed as mean spike frequencies of individual mice (*filled circles*, 31.1 ± 0.3 cells/mouse) and grand mean ± SEM. Mice were injected ip with leptin or vehicle 1–3 h before decapitation. In each of the vehicle-injected categories, equal numbers of mice were injected at 1 or 3 h; results were similar and thus were combined (see *Materials and Methods*). Statistical significance *vs.* vehicle (veh)-injected, fed and fasted controls is displayed. One outlier (*open circle*), probably the result of injection error, was not included in the analysis (see *Materials and Methods*). ns, Not significant.

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feeding state to greatly impact spike frequency, it is necessary for leptin signaling to be intact. Our injection experiments demonstrate that increasing leptin *in vivo* (although probably to supraphysiological levels) is sufficient to reduce spike frequency in fasted mice. Taken together, these data suggest that deviations from energy homeostasis modulate the spike activity of arcuate NPY/AgRP neurons through changes in leptin. According to this model, homeostatic levels of leptin tonically suppress the activity of NPY/AgRP neurons, perhaps directly, given that arcuate nucleus NPY neurons have been reported to express leptin receptor (21). Fasting, by reducing circulating leptin, alleviates this inhibition and causes spike frequency to increase. Genetic ablation of leptin or leptin receptor eliminates this inhibition entirely, driving spike frequency even higher and making feeding state irrelevant. This model predicts that clamping leptin at normal fed levels would be sufficient to prevent a fasting-induced increase in spike frequency. Our data do not address whether leptin acts alone in inducing these changes in spike frequency; leptin may act indirectly through, or in concert with, additional hormones, transmitters, or other inputs. Insulin, for example, when intracerebroventricularly administered, can prevent the normal fasting-induced increase in NPY expression (22). In addition, it is possible that synaptic input *in vivo* is involved in inducing these changes in firing or that non-$GABA_A$, nonionotropic glutamate receptor-driven synaptic activity might be involved in expression of these changes *in vitro*.

After ip injection in mice, leptin levels have been reported to peak after a delay of 30–60 min in serum (23, 24) and 30 min in brain (23). In contrast, inhibition of spiking developed rather slowly with a 3-h time course. Also, the effects of fasting and leptin injection were very persistent, lasting unchanged for several hours *in vitro*, even while bathed in saline containing a superphysiological concentration (10 mM) of glucose (25). These properties of slow onset and persistence are consistent with an effect on transcription or translation. In this regard, it has been reported that leptin regulation of feeding and body weight are dependent on leptin receptor-long form activation of the STAT3 transcription factor (26), and leptin-induced transcriptional effects have been reported in NPY neurons (27, 28).

We have described a homeostatic process in which orexigenic NPY/AgRP neurons sense negative energy balance via reduced leptin signaling and respond by increasing spike frequency. This form of spike frequency regulation is persistent, surviving several hours after initiation *in vivo*, and is observable even in the presence of $GABA_A$ and ionotropic glutamate receptor antagonists. *In vivo*, arcuate NPY neurons are doubtless also subject to numerous other forms of regulation that might not be detected by this assay, either because they are more transient in effect or because they require $GABA_A$ or ionotropic glutamate receptor activity (2–4, 29, 30). Recently, Pinto *et al.* (1) described the effects of the ob mutation on $GABA_A$ and ionotropic glutamate receptor-mediated synaptic inputs to arcuate nucleus NPY neurons. They reported that spontaneous, CNQX/AP5-sensitive excitatory postsynaptic currents in whole-cell recordings of arcuate NPY neurons were 1.5 times higher in frequency in lep^{ob}/lep^{ob} mice, suggesting that leptin can influence NPY neurons via their synaptic inputs. However, in our recordings, where the inputs recorded by Pinto *et al.* (1) were blocked by antagonists, intrinsic spike frequency was 6.4 times higher in fed lep^{ob}/lep^{ob} mice relative to fed wild-type mice, suggesting that leptin has a much greater, nonsynaptic effect directly on the NPY/AgRP neurons.

These methods and results will be useful in the further study of feeding and energy homeostasis. As shown here for leptin, the long-term effects of potential regulators can be reliably tested by *in vivo* injection and *in vitro* extracellular recording of spike frequency. One advantage of spike frequency recording is its sensitivity. The fasting-induced increase in spike frequency reported here, although quite large, can be caused by a change in membrane potential too small to be detected above experimental variability using a more invasive procedure such as whole-cell patch clamp. Recording spike activity extracellularly is an excellent way to quickly screen potential modulators of neuronal activity without the artifacts associated with other recording methods, but when applied to NPY/AgRP neurons, it must be remembered that in fed animals, the spike activity of most neurons will be extremely low or silent (Fig. 1B). Thus, an anorectic compound that reversed the fasting-induced increase in NPY-AgRP activity would be of great interest, but such a substance might have no measurable effect in slices from fed mice.

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Address all correspondence and requests for reprints to: Kanji A. Takahashi, Albert Einstein College of Medicine, Rose F. Kennedy Center, Room 703, 1410 Pelham Parkway South, Bronx, New York 10461. E-mail: katakaha@aecom.yu.edu.

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References

1. **Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL** 2004 Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 304:110–115
2. **Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S** 2004 Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 145:2607–2612
3. **van den Top M, Lee K, Whyment AD, Blanks AM, Spanswick D** 2004 Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. Nat Neurosci 7:493–494
4. **Barsh GS, Schwartz MW** 2002 Genetic approaches to studying energy balance: perception and integration. Nat Rev Genet 3:589–600
5. **Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. Nature 404:661–671
6. **Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LH, Woods SC, Seeley RJ** 2000 Long-term orexigenic effects of AgRP-(83—132) involve mechanisms other than melanocortin receptor blockade. Am J Physiol Regul Integr Comp Physiol 279:R47–R52
7. **Hahn TM, Breininger JF, Baskin DG, Schwartz MW** 1998 Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nat Neurosci 1:271–272
8. **Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T** 1998 The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc Natl Acad Sci USA 95:15043–15048
9. **Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen**

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W, Woychik RP, Wilkison WO, Cone RD 1994 Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371:799–802

10. **Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD** 1997 Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385:165–168
11. **Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS** 1997 Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278:135–138
12. **Sanacora G, Kershaw M, Finkelstein JA, White JD** 1990 Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 127:730–737
13. **Chua Jr SC, Brown AW, Kim J, Hennessey KL, Leibel RL, Hirsch J** 1991 Food deprivation and hypothalamic neuropeptide gene expression: effects of strain background and the diabetes mutation. Brain Res Mol Brain Res 11:291–299
14. **Korner J, Savontaus E, Chua Jr SC, Leibel RL, Wardlaw SL** 2001 Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J Neuroendocrinol 13:959–966
15. **Hokfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M** 2000 Neuropeptides—an overview. Neuropharmacology 39:1337–1356
16. **Namae M, Mori Y, Yasuda K, Kadowaki T, Kanazawa Y, Komeda K** 1998 New method for genotyping the mouse Lep(ob) mutation, using a polymerase chain reaction assay. Lab Anim Sci 48:103–104
17. **Horvat S, Bunger L** 1999 Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay for the mouse leptin receptor (Lepr(db)) mutation. Lab Anim 33:380–384
18. **Takahashi KA, Smart JL, Liu H, Cone RD** 2004 The anorexigenic fatty acid synthase inhibitor, C75, is a nonspecific neuronal activator. Endocrinology 145:184–193
19. **Roberts WM, Almers W** 1992 Patch voltage clamping with low-resistance seals: loose patch clamp. Methods Enzymol 207:155–176
20. **Bouret SG, Draper SJ, Simerly RB** 2004 Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304:108–110
21. **Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P** 1996 Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. J Neuroendocrinol 8: 733–735
22. **Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP** 1992 Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology 130:3608–3616
23. **Van Heek M, Mullins DE, Wirth MA, Graziano MP, Fawzi AB, Compton DS, France CF, Hoos LM, Casale RL, Sybertz EJ, Strader CD, Davis Jr HR** 1996 The relationship of tissue localization, distribution and turnover to feeding after intraperitoneal 125I-leptin administration to ob/ob and db/db mice. Horm Metab Res 28:653–658
24. **Ahren B, Baldwin RM, Havel PJ** 2000 Pharmacokinetics of human leptin in mice and rhesus monkeys. Int J Obes Relat Metab Disord 24:1579–1585
25. **McNay EC, Sherwin RS** 2004 From artificial cerebro-spinal fluid (aCSF) to artificial extracellular fluid (aECF): microdialysis perfusate composition effects on in vivo brain ECF glucose measurements. J Neurosci Methods 132:35–43
26. **Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers MG, Jr.** 2003 STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421:856–859
27. **Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK** 1999 Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23:775–786
28. **Shimizu-Albergine M, Ippolito DL, Beavo JA** 2001 Downregulation of fasting-induced cAMP response element-mediated gene induction by leptin in neuropeptide Y neurons of the arcuate nucleus. J Neurosci 21:1238–1246
29. **Mizuno TM, Makimura H, Silverstein J, Roberts JL, Lopingco T, Mobbs CV** 1999 Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. Endocrinology 140:4551–4557
30. **Makimura H, Mizuno TM, Isoda F, Beasley J, Silverstein JH, Mobbs CV** 2003 Role of glucocorticoids in mediating effects of fasting and diabetes on hypothalamic gene expression. BMC Physiol 3:5

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