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NPY presynaptic actions are reduced in the hypothalamic mpPVN of obese (*fa*/*fa*), but not lean, Zucker rats *in vitro*

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1 Neuropeptide Y (NPY) profoundly enhances feeding when injected intracerebroventricularly, or directly into hypothalamic nuclei, such as the paraventricular nucleus (PVN). Paradoxically, NPY has a reduced action on feeding in obese Zucker rats relative to lean Zucker rats, although the obese rats have much higher levels of hypothalamic NPY expression. GABAergic inputs to a subpopulation of medial parvocellular PVN (mpPVN) neurons are sensitive to NPY. Here, we tested the hypothesis that the blunted eating response to NPY observed in obese Zucker rats will be reflected in a reduced NPY action at mpPVN GABAergic synapses.

2 'Blind' whole-cell patch-clamp recordings made from mpPVN neurons in acute brain slices of lean and obese Zucker rats revealed GABAergic inhibitory postsynaptic currents (IPSC) responses which were inhibited by NPY. While the maximum response in the obese Zucker rats was significantly less than in lean Zucker or Sprague–Dawley rats, there was no difference in the EC_{50}.

3 Experiments using blocking concentrations of Y_1- or Y_5-receptor antagonists revealed no differences between lean and obese Zucker rats in the contributions of either of these receptors to the total NPY response in mpPVN.

4 NPY is less effective at the mpPVN GABA synapse in obese than in lean Zucker rats. This is not associated with a change in the proportion of Y_1 or Y_5 receptors mediating the NPY response, and is consistent with the downregulation of NPY receptors or a reduction in receptor–effector coupling, and with the reduced sensitivity of obese rats to NPY.
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Abbreviations: IPSC, inhibitory postsynaptic current; mpPVN, medial parvocellular paraventricular nucleus; pNPY, porcine NPY; PVN, paraventricular nucleus of the hypothalamus; NPY, neuropeptide Y; Y_1, NPY - Y_1 receptor; Y_5, NPY - Y_5 receptor

Introduction

Neuropeptide Y (NPY) is one of the most potent orexigens known, and may be a physiological regulator of food intake (Clark *et al*., 1984; Levine & Morley, 1984; Stanley & Leibowitz, 1985). The paraventricular nucleus (PVN) is one of several NPY-sensitive hypothalamic nuclei involved in the regulation of energy balance and food intake (Stanley & Leibowitz, 1985). Although the mechanisms by which NPY regulates energy balance remain unknown, we have recently demonstrated specific actions of NPY in the PVN, using whole-cell patch-clamp recordings in brain slices. Specifically, we have shown that NPY suppresses GABAergic synaptic input to neurons in the medial parvocellular aspect of the paraventricular nucleus (mpPVN) (Cowley *et al*., 1999; Pronchuk *et al*., 2002); these synapses are potentiated by the anorectic peptide α-MSH (Cowley *et al*., 1999). Animals with deficient leptin signaling, such as the obese (*fa*/*fa*) Zucker rat, have a marked elevation in hypothalamic NPY levels (Beck *et al*., 1990), and up to 50% of the obesity phenotype is ameliorated in the leptin-deficient, *ob*/*ob* mouse when the NPY gene is deleted (Erickson *et al*., 1996); however, the obese Zucker rats have a reduced sensitivity to the orexigenic actions of intrahypothalamic injections of NPY compared with their lean counterparts (McCarthy *et al*., 1991, Brief *et al*., 1992). Here, we tested the hypothesis that the reduced feeding response to NPY in Zucker rats was correlated with a difference in the electrophysiological responsiveness to NPY in the mpPVN of lean vs obese (*fa*/*fa*) female Zucker rats.

Methods

Coronal brain slices containing mpPVN were prepared from the brains of lean or obese female Zucker rats (5–7 weeks old), and from female Sprague–Dawley rats (4–7 weeks old), as described previously (Pronchuk *et al*., 2002). Animals were handled according to a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta. Briefly, animals were decapitated and brains were rapidly removed and transferred into ice-cold artificial cerebrospinal fluid (aCSF) (<4°C), containing (in mM) NaCl 124, KCl 3, $MgSO_4$ 1.3, NaH_2PO_4 10, kynurenic acid 1, which was continuously bubbled with 95% O_2, 5% CO_2 (carbogen), blocked and sectioned (400 μm thick) with a slicer (Slicer HR2, Sigmann Elektronik, Hüffenhardt, Germany), and were

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allowed to equilibrate for at least 1 h at 32°C in aCSF without kynurenic acid. A single slice was then submerged, suspended on a mesh ring, in a constant flow of carbogenated saline (2.5–3 ml min^{-1}, 35±0.5°C), in a recording chamber (volume <1 ml). Recordings were made using the blind whole-cell patch-clamp technique (Blanton *et al*., 1989; Pronchuk *et al*., 2002). Glass patch pipettes (5–6 MΩ) were filled with a K^+ gluconate-based pipette solution containing (in mM), K^+-gluconate 135, KCl 2, HEPES 5, MgATP 5, NaGTP 0.3, BAPTA-K_4 1.1, pH 7.25, 295 mOsm). Pipettes were connected to the headstage of an Axoclamp 2A amplifier (Axon Instruments, Burlingame, CA, U.S.A.) used either in the bridge current clamp or continuous single-electrode voltage-clamp mode.

Medial parvocellular neurons (mpPVN neurons) were identified by their position in the slice and by their electrophysiological properties, as described previously (Cowley *et al*., 1999; Pronchuk *et al*., 2002). In total, we recorded from 108 mpPVN neurons in brain slices of control female Sprague–Dawley rats, 145 mpPVN neurons of female lean Zucker rats and 61 mpPVN neurons of female obese Zucker rats. As has been described elsewhere (Pronchuk *et al*., 2002), these neurons normally rested at between −45 and −55 mV, and had no spontaneous bursting behavior. Neurons were routinely held in voltage clamp at −60 mV, and were only studied further if their holding current and access resistance remained stable in voltage clamp for 10–15 min prior to any other manipulations.

Synaptic potentials were recorded upon electrical stimulation with a fine, monopolar tungsten electrode, which was positioned in the PVN after the neuron was acquired, and repositioned within this region as necessary to elicit optimal synaptic responses. Pairs of stimuli (10–20 V, 100–200 μs, 80 ms interstimulus interval) generally elicited both inward and outward synaptic currents at a holding potential of around −40 mV. Outward currents were sensitive to bicuculline (10 μM), or picrotoxin (50 μM), thus clearly mediated by $GABA_A$ receptors (Pronchuk *et al*., 2002). At least three control records (each the digital average of three successive responses at 10 s intervals) were taken at 5-min intervals to ensure the stability of synaptic responses prior to drug application. Comparison of control IPSC amplitudes in neurons from lean and obese Zucker rats indicated no significant difference in synaptic responses between strains (178.5±8.44 vs 184.7±9.91pA, respectively, $n=43$ for each strain, $P>0.63$). In addition, voltage ramps (swept from −90 to −50 mV over 2 s) applied just prior to drug application and during the peak effect were used to control for alterations in postsynaptic membrane properties caused by the treatment. In some cases, a hyperpolarizing voltage step (−20 mV, 20 ms) was applied after the synaptic response had subsided, for comparison of access resistance in control and in the presence of NPY.

All agonists and antagonists were dissolved in warmed, carbogenated saline just prior to use and applied *via* the bath. Peptides and antagonists were stored at −20°C as concentrated aliquots until immediately prior to use. Porcine NPY (pNPY) was purchased from Dr S. St.-Pierre (Peptidec Technologies, Pierrefonds, Québec, Canada). The Y_1 receptor-selective antagonist BIBP3226 (Rudolf *et al*., 1994) was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). The Y_5 antagonist Novartis 1 (*trans*-2-nitrobenzene-2-sulfonic acid (4-(2-naphthylmethylamino) methyl) cyclohexyl methyl)amide was a gift of Dr P. Hipskind, Lilly Research Laboratories. All chemicals for solutions were obtained from BDH (Toronto, ON, Canada) and all other chemicals were obtained from Sigma (St Louis).

The effects of NPY on the IPSC are expressed as percent inhibition of the control responses taken immediately prior to each drug application. Data were taken only from cells demonstrating measurable (>10%) NPY effect, with substantial (>50%) reversal upon washout. Data are expressed as means±s.e. Paired *t*-test statistical comparisons were performed, both for the significance of an NPY effect, and to compare the effect of NPY in the absence and in the presence of an antagonist, while unpaired *t*-tests were used to compare effects in the different strains of rat.

Results

Earlier work from our laboratory showed that NPY suppresses a GABAergic response evoked synaptically in a subpopulation of neurons in mpPVN of Sprague–Dawley rats (Cowley *et al*., 1999; Pronchuk *et al*., 2002). In brain slices from Zucker rats, we identified mpPVN neurons exhibiting a similar GABAergic response. Application of NPY (10–500 nM) resulted in a reversible reduction in amplitude of the IPSC evoked in these neurons (Figure 1, left panel), as described previously (Pronchuk *et al*., 2002). At the same time, there were no accompanying changes in the response to either voltage steps or slow voltage ramps with NPY application, again as seen earlier (Pronchuk *et al*., 2002). Comparison of neurons from lean and obese Zucker rats revealed that the IPSC of obese animals was inhibited less strongly by a maximal concentration (500 nM) of NPY (Figure 1, right panel). No changes in postsynaptic properties with NPY application were observed.

A concentration–response relationship to NPY was established in preparations from lean and obese animals (Figure 2). While the concentration–response relationship to NPY determined in lean Zucker rats did not significantly differ in its maximum from that in Sprague–Dawley rats, the maximum response to NPY was significantly reduced in obese Zucker rats (Figure 2). Thus, the inhibitory response to 500 nM NPY in lean Zucker rats was 42.3±2.6% ($n=31$), the response in

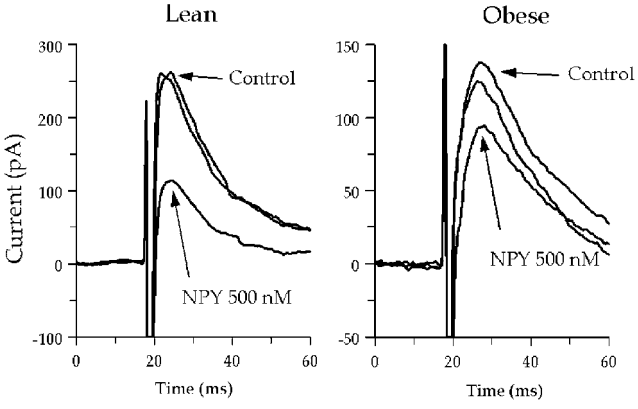

Figure 1 The IPSC in mpPVN neurons is inhibited more by NPY in lean (left panel) than in obese (right panel) female Zucker rats.

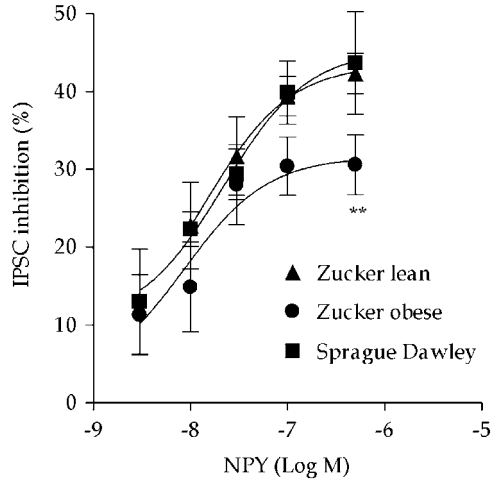

Figure 2 NPY has a lower maximum effect on the IPSC in obese Zucker rats than either in lean Zucker or Sprague–Dawley rats. Data are expressed as the average percent inhibition of the IPSC in response to NPY, which was applied at concentrations between 30 and 500 nM. Each data point represents data from between 7 and 35 neurons. Significance level: $^{**}P<0.0008$.

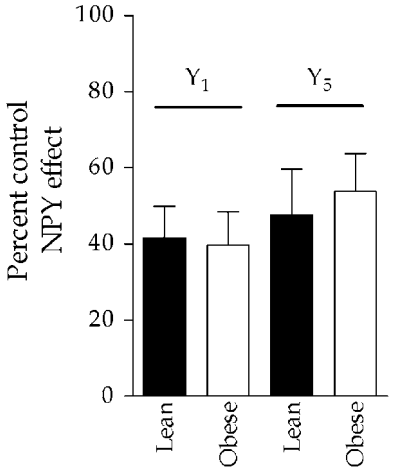

Figure 3 The inhibitory action of 500 nM NPY is reduced equally in lean and obese Zucker rats by the Y_1 receptor antagonist BIBP3226 (500 nM) and by the Y_5 receptor antagonist Novartis 1 (500 nM). Antagonist effects were not significantly different in lean and obese animals ($P>0.9$ for BIBP3226, $P>0.6$ for Novartis 1).

the obese rats was $30.6\pm3.9\%$ ($n=31$, $P<0.003$). Although the maximum response to NPY was diminished in obese Zucker rats, the EC_{50} for NPY ($\log EC_{50}=-8.07\pm0.39$) was not significantly different from that seen in the lean Zucker rats ($\log EC_{50}=-7.85\pm0.12$) or in the control Sprague–Dawley animals ($\log EC_{50}=-7.66\pm0.14$). Thus, while the maximal response to NPY is reduced in mpPVN of obese Zucker rats, the apparent affinity of the receptors is not diminished.

We hypothesized that this reduction in the response to NPY may be due to a reduction in the relative contribution of one or more subtypes of NPY receptors. We tested this by comparing the effects of NPY antagonists against NPY itself in mpPVN of lean and obese Zucker rats. For Y_1 receptors, we first evaluated the response in a neuron to 500 nM NPY, pretreated the neuron with a blocking concentration (500 nM) of the selective Y_1 receptor antagonist BIBP3226, then reapplied NPY to the same neuron in the presence of the antagonist (Pronchuk *et al*., 2002). BIBP3226 reduced the effect of NPY in mpPVN neurons both in lean and obese Zucker rats to 41.53 ± 8.31 ($n=8$) and to 39.69 ± 8.78 ($n=5$), respectively (Figure 3). In similar experiments, we tested the effect of a blocking concentration (500 nM) of the Y_5 selective antagonist Novartis 1 (Pronchuk *et al*., 2002). Novartis 1 suppressed the effect of NPY on the IPSC by about the same amount in lean Zucker rats (to 47.58 ± 12.02 of the control effect) as in the obese rats (to 53.69 ± 10.66 of the control effect; Figure 3).

Discussion and conclusions

Previous work from our laboratory has suggested that NPY alters the $GABA_A$ receptor-mediated inhibiton of mpPVN neurons *via* a presynaptic mechanism (Cowley *et al*., 1999; Pronchuk *et al*., 2002), similar to the presynaptic actions of NPY reported on glutamatergic inputs in the hippocampus (McQuiston & Colmers, 1992; 1996). Our results indicate that NPY has the same actions, with the same potency, within the mpPVN of lean female rats, of either Sprague–Dawley or Zucker strains. These results are the same as those obtained earlier with male Sprague–Dawley rats (Pronchuk *et al*., 2002), suggesting little or no difference in the mechanisms by which NPY acts in the PVN of normally leptin-sensitive animals. In obese Zucker rats, by contrast, the maximal actions of NPY are significantly reduced, compared with those seen in their lean littermates. However, there is no evidence that the proportion of the NPY effect mediated by either Y_1 or Y_5 receptors is altered in the obese rats. Coupled with the observation that the EC_{50} for the actions of NPY is essentially unchanged in the obese rats compared with the lean rats, the present evidence is consistent with a reduction in NPY efficacy in the obese rats.

Normally, leptin suppresses NPY expression in the arcuate nucleus (Schwartz *et al*., 1996; Wang *et al*., 1997) and NPY release in the PVN (Wang *et al*., 1997). Thus, in the obese Zucker rats whose leptin receptor is functionally impaired (Phillips *et al*., 1996) reducing their sensitivity to leptin (Cusin *et al*., 1996), NPY synthesis is elevated in their arcuate nucleus (Beck *et al*., 1990; 1993; Pesonen *et al*., 1992) and the content and release of NPY in their paraventricular nuclei is significantly increased (McKibbin *et al*., 1991; Dryden *et al*., 1995; Stricker-Krongrad *et al*., 1997). Despite this, the feeding response of obese Zucker rats to NPY injection was either not seen at all (Brief *et al*., 1992) or had a much higher threshold compared with lean ones (McCarty *et al*., 1991; Stricker-Krongrad *et al*., 1994).

If the response to NPY we observed in mpPVN is indeed related to the feeding response induced by NPY injection into the PVN, then our present data are consistent with a reduction in sensitivity to exogenous application of NPY. An explanation consistent with the available data is that, in obese Zucker rats, there is a reduction in the number of receptors mediating the presynaptic actions of NPY.

The total number of hypothalamic NPY receptors in obese Zucker rats is reduced (McCarty *et al*., 1991; Widdowson, 1997). There is evidence for a contribution of both Y_1 and Y_5 receptors in the feeding response to NPY (Kanatani *et al*., 1996; Cabrele *et al*., 2000), and we provide evidence both here and earlier (Pronchuk *et al*., 2002) that both these receptors

mediate in part the presynaptic actions of NPY in the mpPVN. By contrast, Y_2 receptors are most likely autoreceptors, suppressing the release of NPY from arcuate nucleus neurons, and conditional knockout of Y_2 receptors in the hypothalamus results in weight gain (Sainsbury *et al*., 2002); indeed, there is evidence *in vitro* that Y_2 receptor activation suppresses arcuate nucleus NPY neuron activity *in vitro* and suppresses food intake *in vivo* (Batterham *et al*., 2002); thus, it appears most unlikely that Y_2 receptors mediate the increases in food intake caused by NPY. By using previously-established blocking concentrations of the Y_1 and Y_5 antagonists, we were here unable to demonstrate significant differences in the proportion of the receptor subtypes contributing to the NPY response in the obese and lean Zucker rats. Although the relative contributions of Y_1 and Y_5 receptors do not appear to change, it is conceivable that they might be equally affected by prolonged exposure to the elevated levels of NPY seen in the obese animals as these receptors are known to especially desensitize, and thus potentially downregulate (Berglund *et al*., 2003). However, the present experiments do not rule out the possibility of an alteration in receptor–effector coupling in the obese animals, in addition to a change in receptor number. Irrespective of the mechanism, the reduction in NPY action seen in the mpPVN of the obese rats could clearly contribute to the paradoxical insensitivity of their feeding response to NPY.

In summary, we report that the actions of NPY are reduced at the IPSC in the mpPVN of fatty Zucker rats compared with either lean Zucker rats or Sprague–Dawley rats. The reduction is due to a reduction in efficacy, and does not involve a change in the relative contribution of either Y_1 or Y_5 receptors to the overall effect of NPY. The effect is consistent with either an overall reduction in receptor number or possibly a change in receptor–effector coupling. The observed reduction in the effects of NPY in the mpPVN of the obese Zucker rats is consistent with the reported reduction in NPY's ability to induce feeding in this strain.

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References

BATTERHAM, R.L., COWLEY, M.A., SMALL, C.J., HERZOG, H., COHEN, M.A., DAKIN, C.L., WREN, A.M., BRYNES, A.E., LOW, M.J., GHATEI, M.A., CONE, R.D. & BLOOM, S.R. (2002). Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature,* **418,** 650–654.

BECK, B., BURLET, A., BAZIN, R., NICOLAS, J.P. & BURLET, C. (1993). Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to their overeating. *J. Nutr.,* **123,** 1168–1172.

BECK, B., BURLET, A., NICOLAS, J.P. & BURLET, C. (1990). Hyperphagia in obesity is associated with a central peptidergic dysregulation in rats. *J. Nutr.,* **120,** 806–811.

BERGLUND, M.M., SCHOBER, D.A., STATNICK, M.A., MCDONALD, P.H. & GEHLERT, D.R. (2003). The use of bioluminescence resonance energy transfer 2 to study neuropeptide Y receptor agonist-induced beta-arrestin 2 interaction. *J. Pharmacol. Exp. Ther.,* **306,** 147–156.

BLANTON, M.G., LO TURCO, J.J. & KRIEGSTEIN, A.R. (1989). Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Meth.,* **30,** 203–210.

BRIEF, D.J., SIPOLS, A.J. & WOODS, S.C. (1992). Intraventricular neuropeptide Y injections stimulate food intake in lean but not obese Zucker rats. *Physiol. Behav.,* **51,** 1105–1110.

CABRELE, C., LANGER, M., BADER, R., WIELAND, H.A., DOODS, H.N., ZERBE, O. & BECK-SICKINGER, A.G. (2000). The first selective agonist for the neuropeptide Y Y5 receptor increases food intake in rats. *J. Biol. Chem.,* **275,** 36043–36048.

CLARK, J.T., KALRA, P.S., CROWLEY, W.R. & KALRA, S.P. (1984). Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology,* **115,** 427–429.

COWLEY, M.A., PRONCHUK, N., FAN, W., DINULESCU, D.M., COLMERS, W.F. & CONE, R.D. (1999). Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron,* **24,** 155–163.

CUSIN, I., ROHNER-JEANRENAUD, F., STRICKER-KRONGRAD, A. & JEANRENAUD, B. (1996). The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. Reduced sensitivity compared with lean animals. *Diabetes,* **45,** 1446–1450.

DRYDEN, S., PICKAVANCE, L., FRANKISH, H.M. & WILLIAMS, G. (1995). Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. *Brain. Res.,* **690,** 85–188.

ERICKSON, J.C., HOLLOPETER, G. & PALMITER, R.D. (1996). Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science,* **274,** 1704–1707.

KANATANI, A., ISHIHARA, A., ASAHI, S., TANAKA, T., OZAKI, S. & IHARA, M. (1996). Potent neuropeptide Y Y1 receptor antagonist, 1229U91: blockade of neuropeptide Y-induced and physiological food intake. *Endocrinology,* **137,** 3177–3182.

LEVINE, A.S. & MORLEY, J.E. (1984). Neuropeptide Y: a potent inducer of consummatory behavior in rats. *Peptides,* **5,** 1025–1029.

MCCARTY, H.D., MCKIBBIN, P.E., HOLLOWAY, B., MAYERS, R. & WILLIAMS, G. (1991). Hypothalamic neuropeptide Y receptor characteristics and NPY-induced feeding responses in lean and obese Zucker rats. *Life Sci.,* **49,** 1491–1497.

MCKIBBIN, P.E., COTTON, S.J., MCMILLAN, S., HOLLOWAY, B., MAYERS, R., MCCARTY, H.D. & WILLIAMS, G. (1991). Altered neuropeptide Y concentrations in specific hypothalamic regions of obese (fa/fa) Zucker rats. Possible relationship to obesity and neuroendocrine disturbances. *Diabetes,* **40,** 1423–1429.

MCQUISTON, A.R. & COLMERS, W.F. (1992). Neuropeptide Y does not alter NMDA conductances in CA3 pyramidal neurons: a slice-patch study. *Neurosci. Lett.,* **138,** 261–264.

MCQUISTON, A.R. & COLMERS, W.F. (1996). Neuropeptide Y inhibits the frequency of spontaneous, but not miniature, excitatory postsynaptic potentials in CA3 pyramidal cells of rat hippocampus. *J. Neurophysiol.,* **76,** 3159–3168.

PESONEN, U., HUPPONEN, R., ROURU, J. & KOULU, M. (1992). Hypothalamic neuropeptide expression after food restriction in Zucker rats: evidence of persistent neuropeptide Y gene activation. *Brain Res. Mol. Brain Res.,* **16,** 255–260.

PHILLIPS, M.S., LIU, Q., HAMMOND, H.A., DUGAN, V., HEY, P.J., CASKEY, C.T. & HESS, J.F. (1996). Leptin receptor missense mutation in the fatty Zucker rat. *Nat. Genet.,* **13,** 18–19.

PRONCHUK, N., BECK-SICKINGER, A.G. & COLMERS, W.F. (2002). Multiple NPY receptors inhibit GABA(A) synaptic responses of rat medial parvocellular effector neurons in the hypothalamic paraventricular nucleus. *Endocrinology,* **143,** 535–543.

RUDOLF, K., EBERLEIN, W., ENGEL, W., WIELAND, H.A., WILLIM, K.D., ENTZEROTH, M., WIENEN, W., BECK-SICKINGER, A.G. & DOODS, H.N. (1994). The first highly potent and selective non-peptide neuropeptide Y Y1 receptor antagonist: BIBP3226. *Eur. J. Pharmacol.,* **271,** R11–R13.

SAINSBURY, A., SCHWARTZER, C., COUZENS, M., FETISSIV, S., FURTINGER, S., JENKINS, A., COX, H.M., SPERK, G., HÖKFELT, T. & HERZOG, H. (2002). Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knock-out mice. *Proc. Natl. Acad. Sci. U.S.A.,* **99,** 8938–8943.

SCHWARTZ, M.W., SEELY, R.I., CAMPFIELD, L.A., BURN, P. & BASKIN, D.G. (1996). Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest.,* **98,** 1101–1106.

STANLEY, B. & LEIBOWITZ, S. (1985). Neuropeptide Y injected into the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc. Natl. Acad. Sci. U.S.A.,* **82,** 3940–3943.

STRICKER-KRONGRAD, A., KOZAK, R., BURLET, C., NICOLAS, J.P. & BECK, B. (1997). Physiological regulation of hypothalamic neuropeptide Y release in lean and obese rats. *Am. J. Physiol.,* **273,** R2112–R2116.

STRICKER-KRONGRAD, A., MAX, J.P., MUSSE, N., NICOLAS, J.P., BURLET, C. & BECK, B. (1994). Increased threshold concentration of neuropeptide Y for a stimulatory effect on food intake in obese Zucker rats-changes in the microstructure of the feeding behavior. *Brain Res.,* **660,** 162–166.

WANG, Q., BING, C., AL-BARAZANIJ, K., MOSSAKOWASKA, D.E., WANG, X.M., BAY, D.L., NEVILLE, W.A., TADDAYTON, M., PICKAVANCE, L., DRYDEN, S., THOMAS, M.E., MCHALE, M.T., GLOYER, I.S., WILSON, S., BUCKINGAM, R., ARCH, J.R., TRAYHURN, P. & WILLIAMS, G. (1997). Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes,* **46,** 335–341.

WIDDOWSON, P.S. (1997). Regionally selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to Y5 'feeding receptor'. *Brain Res.,* **758,** 17–25.

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