Increased Neuropeptide Y Concentrations in Specific Hypothalamic Regions of Streptozocin-Induced Diabetic Rats

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Untreated streptozocin-induced diabetic (STZ-D) rats have previously been shown to have significantly increased hypothalamic concentrations of neuropeptide Y (NPY), a regulatory peptide that powerfully stimulates eating and drinking and inhibits secretion of several pituitary hormones when injected centrally. Tissue NPY concentrations have been measured by radioimmunoassay in selected hypothalamic regions microdissected from fresh, unfixed brain slices to localize diabetes-associated NPY changes precisely within the hypothalamus. Significant (35–200%) increases in NPY concentrations ($P < .01$ vs. matched nondiabetic controls) were found in specific hypothalamic regions between 3 and 14 wk after induction of STZ-D. These regions included the paraventricular and ventromedial nuclei and lateral hypothalamic area, major appetite-regulating areas that are sensitive to the hyperphagic and polydipsic actions of NPY. Increased NPYergic activity in these areas may, at least partly, drive the increased eating and drinking characteristic of STZ-D. NPY concentrations were also increased in the arcuate nucleus and medial preoptic area. Because both of these regions are important in modulating pituitary hormone secretion, local NPY increases may be involved in the impaired secretion of luteinizing hormone, thyroid-stimulating hormone, growth hormone, and prolactin known to occur in STZ-D. Our finding of NPY increases in specific hypothalamic nuclei associated with functional changes found in STZ-D suggests that this peptide may have a role in the altered metabolic and neuroendocrine regulation of the syndrome. *Diabetes* 38:321–27, 1989

Uncontrolled insulin-deficient diabetes in humans and rodents is accompanied by several features suggestive of hypothalamic disturbance, notably hyperphagia, polydipsia, and impaired pituitary hormone secretion (1–7). We have previously investigated the possible role of regulatory peptides in the hypothalamic dysfunction associated with diabetes induced in the rat by the β-cell toxin streptozocin (STZ) (8). Tissue concentrations of several peptides were measured in the hypothalamus, which was excised en bloc and subdivided into central (nucleus-rich) and lateral portions with a reproducible microdissection technique. Of 12 peptides examined, only 1, neuropeptide Y (NPY), has shown consistent changes in the diabetic animals, with significantly higher central and lateral hypothalamic concentrations than in matched nondiabetic controls (8). We have recently found that total hypothalamic NPY mRNA levels are increased fivefold in STZ-induced diabetic (STZ-D) rats, indicating increased NPY synthesis and probably enhanced NPYergic activity (9). Because NPY injected into the hypothalamus or third ventricle is a potent stimulant of eating and drinking (10–14) and impairs secretion of luteinizing hormone (LH), thyroid-stimulating hormone (TSH), growth hormone (GH), and prolactin (15–17), we suggest that increased hypothalamic NPYergic activity may contribute to the hyperphagia, polydipsia, and pituitary dysfunction characteristic of STZ-D.

Because of the great anatomical and functional complexity of the hypothalamus, these observations are difficult to interpret without knowing precisely which hypothalamic regions are involved. Therefore, our aim was to localize NPY changes within the hypothalamus by measuring tissue NPY concentrations in individual hypothalamic areas microdissected from fresh brain (18), including those implicated in regulation of feeding and pituitary secretion. Concentrations of neurotensin and somatostatin, two other major hypothalamic peptides known to have centrally mediated actions on feeding and endocrine function, were also examined (19–22).

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TABLE 1
Characteristics of streptozocin-induced diabetic and nondiabetic control rats

	5 days		3 wk		5 wk		14 wk	
	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 9)	Control (n = 8)	Diabetic (n = 7)	Control (n = 7)
Plasma glucose (mM)	20.9 ± 1.2*	8.0 ± 0.2	25.9 ± 1.1*	8.2 ± 0.3	26.0 ± 0.9	9.5 ± 0.5	25.7 ± 2.1*	8.2 ± 0.5
Plasma insulin (pM)	61.1 ± 0.9†	97.0 ± 4.4	19.3 ± 7.1*	92.8 ± 5.9	63.6 ± 9.5*	116 ± 10.9	36.7 ± 7.7*	73.1 ± 7.9
Initial body weight (g)	164 ± 3	169 ± 3	269 ± 4	279 ± 4	203 ± 8	209 ± 8	202 ± 3	206 ± 2
Final body weight (g)	189 ± 6*	215 ± 1	241 ± 8*	331 ± 8	314 ± 11*	378 ± 11	330 ± 20*	472 ± 11
Weight change from baseline (%)	15 ± 1†	28 ± 2	−11 ± 2*	18 ± 2	55 ± 5*	81 ± 6	61 ± 8*	145 ± 8
Food intake (g · day^{-1} · rat^{-1})	33 ± 4	26 ± 1	41 ± 1*	25 ± 1	52 ± 5*	36 ± 1	46 ± 2*	34 ± 2

Data are means ± SE.
*P < .001, †P < .01, diabetic vs. control rats.

MATERIALS AND METHODS

Animals and experimental design. Groups of 10–12 male Wistar rats initially weighing 211 ± 5 g (mean ± SE) (Charles River UK, Margate, Kent, UK) were given a single injection of STZ (55 mg/kg in citrate buffer; Upjohn, Kalamazoo, MI) into a tail vein while under light ether anesthesia. Control animals, closely matched for age and initial weight, received isotonic sodium citrate solution instead of STZ at the same time (Table 1).

Rats were generally housed in groups of 3–5 but were caged in pairs during measurements of food and water intake, which were performed 1–3 days before they were killed. They were maintained at a constant temperature of 22°C, with a light-dark cycle of 13 and 11 h initiated at 0400. They had free access to water and standard laboratory animal chow (PRD pellets; Labsure, London) throughout the study. Every 1–3 days, they were weighed, and blood glucose concentration was measured in a tail-stick sample by visual reading of Glycemie 1–44 glucose oxidase reagent sticks (Boehringer Mannheim London, Lewes, East Sussex, UK). Only STZ-D rats with blood glucose concentrations consistently >15 mM were defined as diabetic and included in the study. Groups of 7–10 diabetic and control animals were studied at 5 days, 3 wk, 5 wk, and 14 wk after induction of diabetes. The rats were anesthetized by ether inhalation and exsanguinated by cardiac puncture. Diabetic and control animals were examined alternately, and all were killed between 1000 and 1700.

Microdissection methods. The brain was quickly removed and placed on its dorsal surface, and a coronal slice including the hypothalamus was excised by vertical cuts at least 1 mm anterior to the body of the optic chiasm and 1 mm posterior to the mamillary bodies. The cerebral vessels and optic nerves were carefully trimmed away under a binocular dissecting microscope (×15) to facilitate sectioning, and the caudal surface of the slice was mounted on a prechilled metal stage coated with contact adhesive (Super-Glue 3, Loctite UK, Welwyn Garden City, Hertfordshire, UK). The stage was placed on ice for a few minutes to allow the brain to cool and become firmer in consistency and then transferred to the tissue bath (filled with ice-cold isotonic saline) of a vibrating microtome (Microcut HI 200; Polaron, Watford, Hertfordshire, UK). Slices of 50–100 μm were first removed from the top of the slice until the center of the body of the anterior commissure was exposed. With speed and amplitude settings of 1 and 8, respectively, 10–12 slices of 330–500 μm were then cut down to the level of the premammillary nuclei (plate 22 in ref. 23).

Slices were transferred to petri dishes containing cold isotonic saline and examined unstained against a dark background under a dissecting microscope (×40). Major fiber tracts and nuclear areas were identified from a stereotaxic atlas of the rat brain (23). The areas studied (shown schematically in Fig. 1) were the medial preoptic area (MPO), lateral preoptic area (LPO), anterior hypothalamic area (AHA), paraventricular nucleus (PVN), supraoptic nucleus (SON), ventromedial nucleus (VMH), dorsomedial nucleus (DMH), arcuate nucleus (ARC), and lateral hypothalamic area (LHA). The superior parietal cortex from the most caudal slice was also examined. The LPO and MPO were removed with a fine scalpel blade, whereas tissue from the other areas was punched out with a blunt 19-gauge needle (internal diam ~700 μm). The needle was mounted on a 1-ml plastic tuberculin syringe with the plunger pushed fully home; firm pressure on the plunger compressed its rubber bung sufficiently to expel a small volume of air and the tissue core. All tissue from each area (sampled bilaterally from the appropriate slices) from a single animal was pooled in 500 μl of 0.5 M acetic acid in a screw-capped polypropylene vial that was immersed in boiling water for 10 min to extract the peptides. The extracts were then frozen and kept at −20°C until measurement of peptide and protein concentrations.

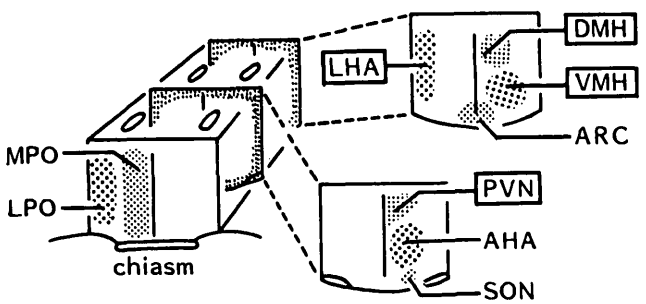

FIG. 1. Schematic coronal sections through rat hypothalamus at different levels. MPO, medial preoptic area; LPO, lateral preoptic area; SON, supraoptic nucleus; AHA, anterior hypothalamic area; PVN, paraventricular nucleus; VMH, ventromedial nucleus; DMH, dorsomedial nucleus; ARC, arcuate nucleus; LHA, lateral hypothalamic area. Boxed abbreviations indicate hypothalamic areas implicated in appetite control.

Assays. Plasma glucose concentration was measured by a glucose oxidase–based autoanalyzer in samples of left ventricular blood collected into fluoride oxalate tubes. Blood for insulin assay was taken into a lithium-heparin tube containing aprotinin (400 KIU/ml blood). The radioimmunoassay (RIA) for plasma insulin employed ^{125}I-labeled porcine insulin (Amersham, Amersham, Buckinghamshire, UK), guinea pig anti-insulin serum (Immunodiagnostics, Washington, Tyne and Wear, UK), and porcine insulin standard; free and antibody-bound label were separated by centrifugation with a dextran-charcoal mixture (24). The detection limit of the insulin assay (95% confidence limits; 24) was 5 pM.

NPY concentrations were measured in 20- to 50-μl aliquots of undiluted extract by RIA with ^{125}I-labeled porcine NPY, porcine NPY standard, and an NH_2-terminal–directed NPY antiserum used at a final dilution of 1:120,000 (25). The sensitivity of the assay was 0.6 fmol/tube. The neurotensin RIA employed bovine neurotensin as standard and label and a COOH-terminal–specific antibody at a final dilution of 1:80,000 (26). Somatostatin (SRIF) was assayed with the native tetradecapeptide as standard, ^{125}I-[Tyr^{11}]SRIF as label, and an antibody (final dilution, 1:240,000) directed against the entire molecule (27). The sensitivity of both neurotensin and SRIF assays was 0.4 fmol/tube. All diabetic and control samples from each time were assayed in duplicate in the same assay. Peptide concentrations were expressed as femtomoles per microgram of protein, protein concentration being measured by the Coomassie blue micromethod (28).

Statistical analyses. All data are presented as means ± SE. Analysis of variance (ANOVA) was used initially to examine the effects of diabetes on hypothalamic concentrations of each of the three peptides. Differences between diabetic and control groups at each time were evaluated by unpaired Student's *t* test or, in the case of plasma insulin levels (which were not normally distributed), by Wilcoxon's test. A significance level of $P < .01$ was selected because of the large number of comparisons tested.

RESULTS

Metabolic data. These are summarized in Table 1. At death, mean plasma glucose concentrations in each of the diabetic groups exceeded 20 mM. Plasma insulin concentrations in the diabetic rats were significantly lower than in control rats at all times. STZ-D animals had gained significantly less weight than controls at each time and in the 3-wk group had even lost weight compared with their preinjection values. After 1 wk of diabetes, the rats had lost virtually all palpable subcutaneous adipose tissue. Diabetic rats were markedly hyperphagic after 3 wk of diabetes, consuming on average ~50% more food than controls; food intake was increased after 5 days of diabetes, but not significantly so. Postmortem examination showed that the stomach and small intestine were hypertrophied and distended with food in most of the diabetic rats from 3 wk on. Diabetic rats drank nearly three times as much water as controls at all times.

Hypothalamic peptide concentrations. Protein concentrations in tissue extracts from the various hypothalamic areas did not differ significantly between diabetic and control rats at any time.

NPY concentrations (fmol/μg protein) in the nine hypothalamic regions at each of the four times investigated are shown in Fig. 2. In control animals, NPY levels showed considerable regional variation, being highest in the MPO and PVN and lowest in the AHA and SON. This relative distribution is in general agreement with previous studies done with either immunocytochemical methods (29,30) or RIA of microdissected areas (31). ANOVA showed that the presence of diabetes had a highly significant effect on hypothalamic NPY levels ($F = 16.96$; df = 1; $P < .0001$). This was attributable to consistent and significant NPY increases in specific regions of both the central and lateral hypothalamus of diabetic animals at 3, 5, and 14 wk; values at 5 days were similar in the two groups. Within the central hypothalamus, significant NPY increases were seen in the MPO, PVN, VMH, DMH, and ARC. Mean NPY levels in the MPO and PVN were increased in the diabetic rats by between 35 and 145%, with significant differences ($P < .01$) at 3 and 14 wk. Significantly higher NPY concentrations were found in the VMH at 3 and 5 wk ($P < .01$) and in the DMH at 5 wk ($P < .001$). The most

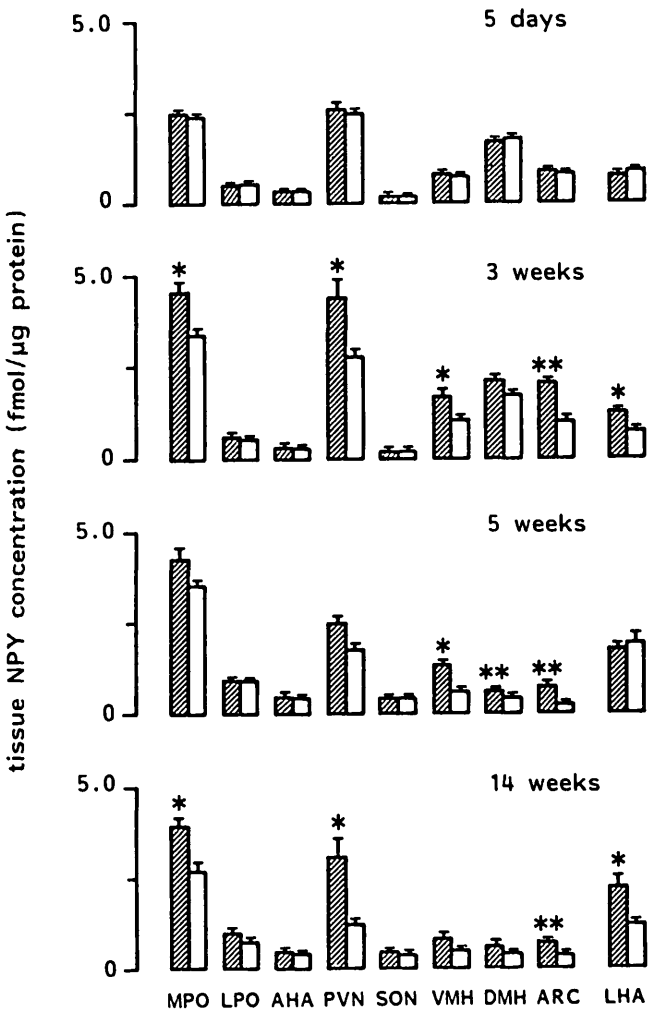

FIG. 2. Tissue neuropeptide Y concentrations (means ± SE) in 9 hypothalamic regions examined, in streptozocin-induced diabetic (*hatched bars*) and control (*open bars*) groups after diabetes induction. MPO, medial preoptic area; LPO, lateral preoptic area; AHA, anterior hypothalamic area; PVN, paraventricular nucleus; SON, supraoptic nucleus; VMH, ventromedial nucleus; DMH, dorsomedial nucleus; ARC, arcuate nucleus; LHA, lateral hypothalamic area. Statistical significance of differences between diabetic and control rats in each region: *$P < .01$, **$P < .001$.

TABLE 2
Neurotensin concentrations in individual hypothalamic regions

Region	5 days		3 wk		5 wk		14 wk	
	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 9)	Control (n = 8)	Diabetic (n = 7)	Control (n = 7)
MPO	1.62 ± 0.07*	1.36 ± 0.06	1.86 ± 0.07	1.83 ± 0.13	1.70 ± 0.06	1.73 ± 0.05	1.43 ± 0.07	1.66 ± 0.10
LPO	0.81 ± 0.04	0.79 ± 0.05	0.82 ± 0.06	0.67 ± 0.04	0.93 ± 0.04	1.00 ± 0.07	0.83 ± 0.04	1.15 ± 0.11
AHA	0.44 ± 0.02	0.49 ± 0.03	0.31 ± 0.02	0.33 ± 0.03	0.39 ± 0.03	0.48 ± 0.02	0.47 ± 0.02	0.43 ± 0.04
PVN	0.66 ± 0.04	0.57 ± 0.03	0.74 ± 0.04	0.75 ± 0.06	0.55 ± 0.03	0.65 ± 0.03	0.43 ± 0.02	0.39 ± 0.04
SON	0.23 ± 0.03	0.18 ± 0.02	0.17 ± 0.02	0.24 ± 0.03	0.14 ± 0.01*	0.20 ± 0.01	0.15 ± 0.03	0.19 ± 0.03
VMH	0.50 ± 0.02	0.39 ± 0.04	0.69 ± 0.09	0.45 ± 0.06	0.46 ± 0.06	0.30 ± 0.01	0.32 ± 0.04	0.27 ± 0.03
DMH	1.11 ± 0.04	1.13 ± 0.08	1.24 ± 0.13	1.06 ± 0.08	1.01 ± 0.05	0.86 ± 0.05	0.77 ± 0.06	0.75 ± 0.04
ARC	0.61 ± 0.10	0.55 ± 0.03	0.41 ± 0.03	0.53 ± 0.05	0.46 ± 0.03	0.60 ± 0.10	0.49 ± 0.07	0.54 ± 0.03
LHA	1.58 ± 0.07	1.29 ± 0.08	1.74 ± 0.13*	1.28 ± 0.05	1.22 ± 0.08	1.33 ± 0.08	1.09 ± 0.07	0.96 ± 0.07

Values are means ± SE in femtomoles per microgram of protein. MPO, medial preoptic area; LPO, lateral preoptic area; AHA, anterior hypothalamic area; PVN, paraventricular nucleus; SON, supraoptic nucleus; VMH, ventromedial nucleus; DMH, dorsomedial nucleus; ARC, arcuate nucleus; LHA, lateral hypothalamic area.
*$P < .01$, diabetic vs. control rats.

striking change occurred in the ARC, with two- to threefold higher levels ($P < .001$) at all three later times. In the LHA, NPY levels were significantly higher ($P < .01$) in the diabetic rats at 3 and 14 wk, the value at 5 wk being close to control. NPY levels in the parietal cortex were relatively low (0.1–0.4 fmol/μg protein) in all animals and did not differ significantly between diabetic and control rats (data not shown).

Neurotensin levels were highest in the MPO, LHA, and DMH and undetectable in the cortex in both diabetic and control rats (Table 2). SRIF concentrations were an order of magnitude greater than those of the other two peptides and were highest in the MPO and ARC (Table 3). Occasional differences ($P < .01$) between diabetic and control rats in neurotensin and SRIF levels in a few isolated hypothalamic areas were revealed by t test. However, no consistent pattern emerged in the diabetic groups, and ANOVA showed no significant effect of diabetes on hypothalamic concentrations of either peptide (neurotensin: $F = 0.97$, df = 1, $P = .34$; SRIF: $F = 1.80$, df = 1, $P = .18$).

DISCUSSION

Study of the hypothalamus in diabetic animals may help to clarify not only the hypothalamic and pituitary dysfunction associated with the disease, but also normal hypothalamic regulatory mechanisms. A readily accessible model of insulin-deficient diabetes is the STZ-D rat, which displays hyperphagia (preferentially for carbohydrate-rich foods; 3), polydipsia and impaired pituitary secretion, with reduced circulating levels of LH (and abolition of its normal pulsatile release), GH, TSH, and prolactin (4–7).

The possible functions of the various peptides found in the hypothalamus are exciting much interest. When injected into specific brain areas, many peptides affect eating and drinking (19,20), glucoregulation and secretion of insulin and glucagon (21,32), and pituitary hormone release (15–17,21,22). NPY is one of the most abundant neuropeptides in the brain and is highly concentrated in functionally important regions of the hypothalamus, including the PVN, ARC, and MPO (29,33–35). NPY exerts powerful behavioral and neuroendocrine actions when injected into the hypothalamus or third ventricle. It is one of the most potent central appetite stimulants known, inducing carbohydrate-selective hyperphagia (the same preference displayed by STZ-D rats; 3) sufficient to cause obesity with long-term administration (10–14). Food-seeking exploratory activity is also enhanced, whereas sexual and other behaviors are suppressed (13,35). Microinjection studies suggest that the hyperphagic action of NPY is mediated specifically by the PVN, VMH, and LHA

TABLE 3
Somatostatin concentrations in individual hypothalamic regions

Region	5 days		3 wk		5 wk		14 wk	
	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 10)	Control (n = 10)	Diabetic (n = 9)	Control (n = 8)	Diabetic (n = 7)	Control (n = 7)
MPO	15.2 ± 0.54	13.5 ± 0.46	18.7 ± 1.19	18.3 ± 1.19	22.3 ± 1.00	21.2 ± 0.90	21.4 ± 1.03	20.6 ± 2.06
LPO	4.39 ± 0.31	4.48 ± 0.19	5.15 ± 0.35	4.53 ± 0.18	7.26 ± 0.56	7.18 ± 0.67	6.79 ± 0.61	6.28 ± 0.69
AHA	3.56 ± 0.14	3.53 ± 0.20	3.77 ± 0.26	3.35 ± 0.20	7.28 ± 0.61*	4.93 ± 0.36	5.79 ± 0.54	6.23 ± 0.73
PVN	9.34 ± 0.46	9.08 ± 0.54	11.3 ± 0.69	9.22 ± 0.57	6.75 ± 0.49	9.05 ± 0.93	5.83 ± 0.47	5.44 ± 0.47
SON	2.69 ± 0.20	2.34 ± 0.17	2.70 ± 0.16	2.75 ± 0.12	3.34 ± 0.20	3.11 ± 0.33	4.12 ± 0.54	3.96 ± 0.45
VMH	8.48 ± 0.23	7.62 ± 0.21	9.94 ± 0.50	9.49 ± 0.31	12.0 ± 0.69	11.4 ± 0.37	13.4 ± 0.69	12.1 ± 0.42
DMH	3.93 ± 0.20	4.29 ± 0.16	4.57 ± 0.31	4.58 ± 0.25	5.21 ± 0.20	4.93 ± 0.20	6.36 ± 0.51	5.52 ± 0.40
ARC	13.2 ± 3.00	9.96 ± 1.30	15.4 ± 1.10	16.1 ± 2.50	28.3 ± 2.24	26.8 ± 3.40	35.4 ± 4.90	25.6 ± 3.30
LHA	4.41 ± 0.27	4.31 ± 0.14	4.92 ± 0.60	3.81 ± 0.44	4.65 ± 0.17	4.72 ± 0.15	4.75 ± 0.08†	4.10 ± 0.10

Values are means ± SE in femtomoles per microgram of protein. Abbreviations as in Table 2.
*$P < .01$; †$P < .001$, diabetic vs. control rats.

(13); the likely importance of PVN NPYergic activity in the physiological control of feeding is emphasized by the recent demonstration in normal rats of increased NPY concentrations in the PVN after food deprivation and by reverse changes after refeeding (36). NPY injection into the same hypothalamic regions also stimulates drinking (14), and its administration into the supraoptic nucleus provokes vasopressin secretion (37). Intracerebroventricular (ICV) NPY injection causes acute insulin release (38). NPY has particularly striking inhibitory effects on pituitary secretion; its injection into the third ventricle blocks GH (15,17), TSH, and prolactin (16,17) release. Its effects on LH apparently depend critically on the sex steroid background of the animal, but ICV NPY injection has been found to inhibit LH secretion in rats of either sex (15,16) and to disorganize LH pulsatility in ovariectomized females (35). Although these experiments are unphysiological, the potent and specific actions of NPY and our previous finding of major changes in hypothalamic NPY concentrations in STZ-D rats suggest a genuine role in hypothalamic regulation.

We examined changes in NPY concentration associated with STZ-D in microdissected individual hypothalamic regions, including those implicated in the regulation of eating, drinking, and pituitary function. In diabetic animals, regional hypothalamic NPY concentrations were significantly increased after the first period (5 days). This change was not attributable to nonspecific effects of diabetes, such as variations in tissue protein or water content, and there were no parallel changes in neurotensin or SRIF. NPY increases were restricted to certain regions of the central hypothalamus (MPO, PVN, VMH, DMH, and ARC) and to the LHA, giving further evidence of specificity. These results extend our immunocytochemical findings of increased immunostaining of NPY-immunoreactive (IR) fibers in the central hypothalamus, particularly the PVN (8,39). Previously, intense NPY immunoreactivity was observed in morphologically abnormal cell bodies in the SON, which is normally devoid of visible NPY-IR perikarya (8); a concomitant decrease in NPY-IR fibers in the SON presumably accounts for the unchanged overall concentrations in the microdissected nucleus.

The fivefold elevation in NPY mRNA that we have recently observed in STZ-D rat hypothalamuses strongly suggests increased hypothalamic NPYergic activity, which may have considerable functional importance in the specific areas showing increased NPY concentrations (9). The VMH, LHA, PVN, and DMH are all associated with appetite regulation (40). The potency and carbohydrate specificity of NPY-induced hyperphagia and its mediation by the PVN, VMH, and LHA (all regions showing increased NPY levels) suggest that the hyperphagia of STZ-D may be driven, at least in part, by increased NPYergic activity in these areas. The increased drinking that accompanies NPY injection into these same regions suggests NPYergic involvement in the polydipsia of STZ-D. The DMH, closely associated both anatomically and functionally with the VMH but apparently not yet tested for its NPY sensitivity, could also help mediate NPY-induced hyperphagia and polydipsia.

The pituitary impairment of STZ-D may also be related to increased hypothalamic NPY, especially in the ARC and MPO. Inhibition of pituitary secretion after ICV NPY injection is probably mediated by hypothalamic regions adjacent to the third ventricle (15–17). One possible site is the ARC, which is closely related to the median eminence and pituitary stalk and contains dopaminergic neurons suggested to mediate the inhibition of LH, TSH, and prolactin release induced by ICV NPY injection (16,17). NPY could block GH secretion by inhibiting neurons containing GH-releasing factor in the anterolateral ARC, an area rich in NPY-IR terminals (17,42). LH secretion could also be affected by NPY increases in the MPO, which contains a high density of sex-steroid receptors and LH-releasing hormone-IR neurons, suggesting an integrative role in regulating gonadotropin secretion (35). The constellation of pituitary abnormalities associated with STZ-D is therefore explicable by increased NPYergic activity in the ARC and possibly the MPO.

Other central actions of NPY, such as stimulation of insulin secretion via an unknown site (38) and increased vasopressin release from the SON (37), are of interest in the context of insulin-deficient diabetes because by opposing insulin deprivation and diuresis, both would serve homeostatic functions.

We have recently identified increased central hypothalamic NPY concentrations in BB/E rats with diabetes caused by autoimmune β-cell failure, suggesting that this is a common consequence of insulin-deficient diabetes (43). Like STZ-D rats, BB/E rats have hyperphagia, polydipsia, and impaired GH secretion (44,45). In insulin-deficient rats, hyperphagia (especially with a preference for carbohydrate) is an appropriate compensatory response to glucose losses in the urine (which may account for a substantial part of a rat's normal energy intake) and weight loss due to unrestrained catabolism and the failure of glucose to enter cells. In nondiabetic rodents, significant (~20%) weight loss caused by food restriction is also associated with increased hypothalamic NPY levels, specifically in the PVN of normal Wistar rats (36) and in the central hypothalamus of obese Zucker rats (46). By driving hyperphagia, increased NPYergic activity in specific hypothalamic appetite-regulating areas may therefore have an important homeostatic role in restoring body weight after losses because of either uncontrolled diabetes or starvation. Indeed, NPY could act on the hypothalamus in an integrated fashion as an antistarvation peptide. Increased NPYergic activity, triggered by weight loss, might stimulate active seeking of food at the expense of other activities (13,35), encourage eating, and favor substrate storage by stimulating insulin secretion (38), an important factor in the development of obesity after lesions of the VMH (47). Weight loss may be sensed centrally through a reduction in circulating insulin levels, which occurs not only in insulin-deficient diabetes but also in starvation. Plasma insulin levels are closely related to the degree of body adiposity and have been postulated to signal this to the brain (48); hypothalamic NPYergic neurons may therefore complete a chain of events acting to conserve body weight and composition against starvation. Pituitary dysfunction may be an unwanted side effect of increased hypothalamic NPYergic activation, although suppression of counterregulatory hormones (GH and thyroid hormones) might also favor anabolism and therefore survival in a starving animal (6).

Our observations point to a broad integrative role of the hypothalamic NPYergic system in the control of energy bal-

ance and body composition and in hypothalamopituitary function. A similar action of NPY in humans might have important therapeutic implications for the management of diabetes and other human diseases. Abnormal NPYergic activity analogous to that suggested in STZ-D rats may explain the impaired gonadotropin secretion, ovulatory failure, and infertility that affect a proportion of diabetic women and are refractory to intensified insulin treatment (49). However, it is the possible use of specific peptidergic agonists and antagonists to modify eating behavior that could find wide application in the treatment of diseases such as anorexia nervosa, bulimia, obesity, and non-insulin-dependent diabetes. This is particularly relevant to non-insulin-dependent diabetes, in which dietary noncompliance and failure to lose weight are the main obstacles to successful treatment. Such patients are common in affluent Western countries; a safe and effective appetite suppressant could benefit perhaps 50% of diabetic patients in the United Kingdom and North America.

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