Neuropeptide Y (NPY) in the arcuate nucleus is an orexigenic hormone of which levels are regulated by humoral as well as neural signals. In this study, we examined the regulation of NPY gene expression in the arcuate nucleus in hypothalamic organotypic cultures. Dexamethasone (DEX) (10^{-9} to 10^{-7} M) significantly increased NPY mRNA expression, and the effects were not influenced by coincubation with the sodium channel blocker tetrodotoxin (TTX), indicating that the action of DEX is independent of action potentials. Conversely, insulin (10^{-11} to 10^{-9} M) significantly inhibited NPY expression stimulated by DEX, and the inhibitory action of insulin was abolished in the presence of TTX. Because GABA and its receptors are expressed in the arcuate nucleus and the paraventricular nucleus (PVN) (Kalra and Kalra, 2004), it is possible that GABAergic systems are involved in the insulin action. The GABA_{A} agonist baclofen significantly inhibited NPY expression stimulated by DEX, and the inhibitory action of insulin was completely abolished in the presence of either the GABA_{A} antagonist bicuculline or the GABA_{B} antagonist CGP35348 ( p-3-aminopropyl-p-dihexoxymethyl phosphoric acid). Furthermore, increases in the GABA-synthesizing enzyme glutamic acid decarboxylase 65 (GAD65) mRNA expression preceded decreases in NPY mRNA expression in the arcuate nucleus in the cultures. Experiments in vivo also demonstrated that increases in GAD65 mRNA expression in the arcuate nucleus preceded decreases in NPY mRNA expression in a fasting-refeeding paradigm and that intracerebroventricular injection of insulin increased GAD65 mRNA expression in the arcuate nucleus in fasted rats. These data suggest that insulin inhibits NPY gene expression in the arcuate nucleus through GABAergic systems.

Key words: neuropeptide Y; arcuate nucleus; glucocorticoid; insulin; GABA; GABA receptors

Introduction

The arcuate nucleus in the hypothalamus is supposed to be the integrative site that receives signals from the periphery reflecting energy status and projects to other hypothalamic nuclei, such as the paraventricular nucleus (PVN) (Kalra and Kalra, 2004). Neuropeptide Y (NPY) expressed in the arcuate nucleus is one of the most potent stimulants for food intake in the CNS such that central injection of NPY readily evokes robust feeding in satiated rats (Clark et al., 1984).

Among many signals in the periphery identified so far, insulin has been implicated as one of the key regulators that inhibit food intake (Schwartz et al., 1992a). The central action of insulin on energy balance is supported by findings that centrally injected insulin inhibited food intake (Woods et al., 1979) and that knock-out mice for insulin receptors in the CNS are hyperphagic and obese (Brüning et al., 2000). Several lines of evidence suggest that the action of insulin is mediated via NPY neurons in the arcuate nucleus: (1) insulin receptors are expressed in the arcuate nucleus, and central injection of insulin decreases NPY gene expression (Marks et al., 1990; Sipols et al., 1995; Benoit et al., 2002); (2) decreases in insulin levels induced by streptozotocin injection or food deprivation lead to increases in the NPY gene expression (Schwartz et al., 1992b; Sipols et al., 1995); (3) decreases in insulin receptor expression in the arcuate nucleus by injection of the antisense induced increases in NPY gene expression (Obici et al., 2002); and (4) insulin deficiency induced by streptozotocin did not lead to hyperphagia in NPY knockout mice (Sindelar et al., 2002). Although these data strongly suggest that insulin acts on the NPY neurons in the arcuate nucleus, the detailed mechanisms have remained unclear.

On the other hand, there is compelling evidence to indicate that glucocorticoids enhance feeding and weight gain (Dallman et al., 2004). The arcuate nucleus could also be the site for glucocorticoid action, because glucocorticoid receptor (GR) is expressed in the NPY neurons (Hisano et al., 1988), and peripheral as well as central injection of glucocorticoids increased the NPY expression (Wilding et al., 1993; Zakrzewska et al., 1999). More importantly, NPY gene expression was not increased by insulin deficiency if rats were adrenalectomized (Ponsalle et al., 1992; Strack et al., 1995). These data suggest that insulin and glucocorticoids interact with each other to regulate NPY gene expression, although the exact sites of the interaction are not clear.
To determine how glucocorticoids and insulin regulate NPY gene expression and whether there is any interaction between the two hormones at the level of the arcuate nucleus, we examined the NPY gene expression in the arcuate nucleus in hypothalamic organotypic cultures, which have been shown to maintain the intrinsic properties (Arima et al., 2002; Kuwahara et al., 2003). Our data suggest the possible role of GABA, a predominant inhibitory neurotransmitter in the brain, in mediating the inhibitory action of insulin on NPY gene expression stimulated by glucocorticoids.

Materials and Methods
Slice explant culture procedure. Hypothalamic slice explant cultures were performed as described previously (Arima et al., 2002; Kuwahara et al., 2003). Sprague Dawley pups, 7–9 d old (Chubu Science Materials, Nagoya, Japan) (lights on from 9:00 A.M. to 9:00 P.M.), were killed by decapitation, and hypothalamic tissues were sectioned at 350 μm thickness on a Mcllwain tissue chopper (Mickle Laboratory Engineering, Surrey, UK). Three coronal slices containing arcuate nucleus were separated and placed in HBSS (Invitrogen, Grand Island, NY) enriched with glucose. Selected sections were trimmed dorsally above the top of the third ventricle and laterally from the arcuate nucleus. Explants from individual rats were placed on 0.4 μm Millicell-CM filter inserts (pore size, 0.4 μm; diameter, 30 mm; Millipore, Billerica, MA), and each filter insert was placed in a Petri dish (35 mm) containing 1.1 ml of culture medium. Cultures were performed at 36.5°C in 5% CO2 enriched air under stationary conditions. The standard culture medium was composed of 50% Earle’s MEM (Invitrogen), 25% heat-inactivated horse serum (Invitrogen), HBSS (Invitrogen), 25 U/ml penicillin/streptomycin (Invitrogen), 1 mM L-glutamine (Invitrogen), and 33 mM glucose. The serum-free medium was composed of 75% Earle’s MEM, 25% HBSS, 25 U/ml penicillin/streptomycin (Invitrogen), 1 mM L-glutamine, and 5.5 mM glucose. Cultures were maintained in the standard medium for 15 d so that the slices became thin enough to perform in situ hybridization, and the medium was then changed to defined serum-free medium for an additional 3 d before subjecting slices to different experimental conditions. The standard medium was changed three times a week, and the serum-free medium was changed every 24 h. All experiments were performed on day 18, and the slices were fixed with 4% formaldehyde in PBS for 30 min, washed twice in PBS, mounted onto poly-L-lysine-coated slides, dried, and kept at −80°C until processed for in situ hybridization.

Effects of dexamethasone and insulin on NPY mRNA expression. To examine the time course effects of dexamethasone (DEX) (Sigma, St. Louis, MO) on NPY mRNA expression, slices were incubated with 10−6 to 10−7 M DEX for 4, 12, and 24 h. Control slices were incubated with vehicle (0.1% ethanol) for 24 h. To examine the dose-dependent effects of DEX and insulin on NPY mRNA expression were also examined in the presence whether insulin treatment affected GAD65 mRNA expression, hypothalamic slices were incubated with 10−5 M insulin for 0, 6, or 24 h, whereas 10−5 M DEX was added to medium for 24 h in all groups. To see the changes in GAD65 mRNA expression levels in the NPY neurons, sections were cut into 20 μm thickness as described above, and in situ hybridization for GAD65 and NPY mRNA was performed in adjacent sections.

Effects of dexamethasone and insulin on NPY mRNA expression. To examine the time course effects of dexamethasone (DEX) (Sigma, St. Louis, MO) on NPY mRNA expression, slices were incubated with 10−7 to 10−8 M DEX for 4, 12, and 24 h. Control slices were incubated with vehicle (0.1% ethanol) for 24 h. To examine the dose-dependent effects of DEX on NPY mRNA expression, slices were incubated with 10−9 to 10−7 M DEX or vehicle for 24 h. To examine the effects of insulin on NPY mRNA expression in the arcuate nucleus of hypothalamic organotypic cultures, slices were incubated with 10−11 to 10−9 M insulin (Humulin R; Eli Lilly Japan, Kobe, Japan) or vehicle (0.9% saline) for 24 h. To examine the possible interaction between insulin and DEX on the regulation of NPY mRNA expression, slices were incubated with 10−11 to 10−9 M insulin or vehicle in the presence of 10−8 M DEX. To see the time course of insulin action, slices were incubated with 10−11 M insulin for 0, 6, 12, or 24 h, whereas 10−8 M DEX was added to medium for 24 h in all groups. The effects of DEX and insulin on NPY mRNA expression were also examined in the presence of the sodium channel blocker tetrodotoxin (TTX) (1 μM; Sankyo, Tokyo, Japan).

Effects of GABA agonists and antagonists and glutamate antagonists on NPY mRNA expression. Slices were incubated with 10−6 to 10−4 M GABAα antagonist bicuculline (10−4 M; Sigma) and GABAβi antagonist CGP35348 (p-aminopropyl-p-diethoxymethyl phosphoric acid) (1 mM; Sigma) in the presence of 10−8 M DEX for 24 h. To assess whether or not the action of insulin was mediated via GABAergic systems, slices were incubated with 10−7 M insulin and 10−5 M DEX in the presence of either bicuculline or CGP35348 for 24 h.

Expression of mRNA for GABA receptors and glutamatergic decarboxylase in hypothalamic slices. To see the distribution of GABA receptors in the hypothalamic slices, the expressions of mRNA for GABAα, GABAβi receptors were first examined in the slices with in situ hybridization using antisense and sense probes. To see whether GABA receptors and glutamatergic decarboxylase (GAD) 65 mRNA were expressed in the arcuate nucleus in the hypothalamic slices, the slices were cut into 20 μm thickness on a cryostat after being fixed with formaldehyde. Whereas dual in situ hybridization was performed for GAD and NPY mRNA, the distribution of the transcripts for GABA receptors was compared with that for NPY in adjacent sections because the expression levels of mRNA for GABA receptors were relatively low in hypothalamic slices. To examine whether insulin treatment affected GAD65 mRNA expression, hypothalamic slices were incubated with 10−9 M insulin for 0, 6, or 24 h, whereas 10−5 M DEX was added to medium for 24 h in all groups. To see the changes in GAD65 mRNA expression levels in the NPY neurons, sections were cut into 20 μm thickness as described above, and in situ hybridization for GAD65 and NPY mRNA was performed in adjacent sections.

Effects of intracerebroventricular injection of insulin on GAD mRNA expression. Seven days before experiments, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) for implantation of a 21 gauge stainless steel cannula stereotaxically into the lateral ventricle. The coordinates of intracerebroventricular operation were 0.8 mm posterior to the bregma, 1.4 mm lateral to the midline, and 4.0 mm below the surface of the skull. After 48 h food deprivation, either 30 mIU of insulin (Humulin R) or vehicle (0.15 M saline) was injected through an intrace- rebroventricular cannula at a volume of 10 μl in 30 s at 9:00 A.M. and 9:30 A.M. The rats were decapitated 2 h after injection, and the brains were immediately removed, frozen on dry ice, and stored at −80°C until sectioning for in situ hybridization. Blood glucose levels were immediately measured after decapitation with a blood glucose monitor (TERUMO, Tokyo, Japan). Blood samples were collected into chilled tubes and separated by centrifugation (3500 rpm, 4°C, 15 min), and serum was stored at −80°C until the insulin determination. Serum insulin levels were measured with an ELISA commercial kit (Shibayagi, Gunma, Japan) using rat insulin as a standard.

Effects of intracerebroventricular injection of insulin on GAD mRNA expression. Seven days before experiments, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) for implantation of a 21 gauge stainless steel cannula stereotaxically into the lateral ventricle. The coordinates of intracerebroventricular operation were 0.8 mm posterior to the bregma, 1.4 mm lateral to the midline, and 4.0 mm below the surface of the skull. After 48 h food deprivation, either 30 mIU of insulin (Humulin R) or vehicle (0.15 M saline) was injected through an intrace- rebroventricular cannula at a volume of 10 μl in 30 s at 9:00 A.M. and 9:30 A.M. The rats were decapitated 2 h after injection, and the brains were immediately removed, frozen on dry ice, and stored at −80°C until sectioning for in situ hybridization. All procedures were performed in accordance with institutional guidelines for animal care at Nagoya University Graduate School of Medicine.

In situ hybridization and quantification. Six sets of sequential coronal sections (12 μm) of brains from in vivo experiments were cut on a cryostat at 2.8 mm caudal from bregma according to the brain atlas of Paxinos and Watson (1998), thaw mounted onto poly-L-lysine-coated slides, and stored at −80°C until hybridization. The RNA probes were generated from the plasmids of NPY (kindly provided by Dr. S. L. Sabol, Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD), GAD65 (kindly provided by Dr. N. Tillakaratne, Department of Physiological Science, University of California, Los Angeles, Los Angeles, CA), GABAβi, GABAα, B3 (kindly provided by Dr. K. Kobayashi, Department of Molecular Genetics, Fukushima Medical University School of Medicine, Fukushima, Japan), and GABAβi.
R2 (kindly provided by K. Kaufmann, Novartis Institute for Biomedical Research, Novartis Pharma AG, Basel, Switzerland) containing 511 bp, 2.3 kb, 960 bp, and 720 bp cDNA, respectively. High specific probes were synthesized using 55 μCi [35S]UTP and 171 μCi [35S]CTP (PerkinElmer Life Science, Natick, MA). Riboprobe Combination System (Promega, Madison, WI), 15 U of Rnasin, 1 μg of linearized template, and 15 U of T3 or T7 RNA polymerase. After 60 min incubation at 42°C, the cDNA template was digested with DNAase for 30 min at 37°C. Radiolabeled RNA products were purified using quick-spin columns (Roche Diagnostics, Indianapolis, IN), precipitated with ethanol, and resuspended in 100 μl of 10 mM Tris-HCl, pH 7.5, containing 20 mM DTT. Digoxigenin-labeled antisense RNA probes from NPY cDNA templates were prepared by using digoxigenin-UTP (Roche Diagnostics).

Prehybridization, hybridization, and posthybridization procedures were performed as described previously (Kuwahara et al., 2003). In brief, after thawing at room temperature, sections were fixed in 4% formaldehyde in PBS for 5 min and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine and 0.9% NaCl, pH 8, for 10 min at room temperature. Sections were then dehydrated in 70, 80, 95, and 100% ethanol, delipidated in chloroform, and hybridized overnight at 55°C with 2 × 106 cpmp of 32P-labeled probes in 90 μl of hybridization buffer (50% formamide, 200 mM NaCl, 2.5 mM EDTA, 10% dextran sulfate, 250 μg/ml yeast RNA, 50 μM dithiothreitol, and 1× Denhardt’s solution). At the end of incubation, sections were subjected to consecutive washes in 4× SSC for 15 min at room temperature and 50% formamide/250 μM NaCl containing dithiothreitol for 15 min at 60°C. After treatment with RNAase A (20 μg/ml) for 30 min at 37°C, sections were washed with 2× SSC, 1× SSC, and 0.5× SSC for 5 min at room temperature, followed by 0.1× SSC containing dithiothreitol for 15 min at 50°C, 0.1× SSC to cool at room temperature, and 70% ethanol for 15 s. To visualize digoxigenin staining, sections were twice rinsed in buffer 1 (100 mM Tris-HCl and 150 mM NaCl, pH 7.5) for 5 min, followed by 30 min in buffer 1 containing 3% normal goat serum and 0.3% Triton X-100, before overnight incubation with alkaline phosphatase-conjugated anti-digoxigenin antiserum (Roche Diagnostics; 1:2000 in 3% normal goat serum/0.3% Triton X-100 in buffer 1). Sections were consecutively rinsed in buffer 1 for 10 min, in buffer 2 (100 mM Tris-HCl, 100 mM NaCl, and 50 mM MgCl2, pH 9.5) for 5 min, and incubated for 30 min at 37°C in the dark in buffer 2 containing 0.34 mg/ml nitroblue toluidinium salt (Roche Diagnostics). Sections were rinsed four times in buffer 2, dipped briefly in distilled water and 70% ethanol, and air dried.

Sections were exposed to Kodak BioMax MR films (Eastman Kodak, Rochester, NY) for various periods yielding appropriate signal intensities. The optimal densities (ODs) of the autoradiographs were quantified by measurements of the integrated OD (OD × area) of the film images. As for the hypothalamic cultures, the total sum of OD signals of NPY mRNA in the bilateral arcuate nuclei in the three hypothalamic explants from each rat was used for the analysis. In each culture, control explants were involved, and the presence of DEX (Decavel and van den Pol, 1992), we examined whether the stimulation of GABA receptors or blockade of glutamate receptors could mimic the insulin action. Incubation with a GABAγ agonist muscimol (10−6 to 10−4 M) for 24 h did not significantly affect NPY mRNA expression in the arcuate nucleus in either the presence (Fig. 2c) or absence (data not shown) of DEX. However, similar to insulin action, incubation with the GABAγ agonist baclofen (10−6 to 10−4 M) significantly decreased NPY expression stimulated by DEX (Fig. 2b), although it did not affect NPY expression in the absence of DEX (Fig. 2a). Incubation with antagonists of NMDA glutamate receptors (AP-5) or non-NMDA glutamate receptors (CNQX) did not affect NPY mRNA expression in the arcuate nucleus significantly in the absence (data not shown) or presence (Fig. 2d) of DEX. To further examine the possible role of GABAergic systems in the regulation of NPY gene expression in the arcuate nucleus, effects of GABA antagonists on NPY mRNA expression were examined. Incubation with the GABAγ antagonist bicuculline (10−5 M) robustly increased NPY mRNA expression in the presence of DEX in the arcuate nucleus (Fig. 2e), suggesting that NPY mRNA expression is tonically inhibited by GABAγ receptor-mediated signaling. Conversely, incubation with the GABAγ antagonist CGP35348 (5 × 10−4 M) did not affect NPY mRNA expression significantly in the presence of DEX (Fig. 2f). Insulin did not significantly decrease NPY expression stimulated by DEX in the presence of either bicuculline (Fig. 2e) or CGP35348 (Fig. 2f).
Expression of GABA receptors and GAD mRNA

The in situ hybridization with antisense probes demonstrated that the mRNA for GABA<sub>A</sub> and GABA<sub>B</sub> receptors were expressed throughout the hypothalamic slices, whereas no visible signals were detected with sense probes (Fig. 3a–d). Analyses of the emulsion-dipped slides showed that the distribution of GABA<sub>A</sub> and GABA<sub>B</sub> receptors overlapped that of NPY mRNA expression in the arcuate nucleus (Fig. 3e–h), suggesting that GABA receptors are expressed in the NPY neurons in the hypothalamic cultures as in vivo (Blasquez et al., 1994; Backberg et al., 2003). The dual in situ hybridization demonstrated that 87% (521 of 597 counted) of cells labeled with digoxigenin NPY in the arcuate nucleus expressed GAD65 mRNA in the organotypic cultures (Fig. 4a). The GAD65 mRNA levels in the arcuate NPY neurons were significantly increased by insulin treatment as early as 6 h and remained elevated for 24 h in the organotypic cultures (Fig. 4b). Representative photographs demonstrating changes in NPY and GAD65 mRNA are shown in Figure 4c.

Fasting-refeeding experiments

The changes in NPY and GAD65 mRNA expression in the arcuate nucleus, serum insulin levels, blood glucose levels, and cumula-
tive food intake in a fasting-refeeding paradigm are shown in Figure 5. The serum insulin levels were significantly decreased by food deprivation for 48 h (24.8 ± 10.1 pm vs control rats with ad libitum feeding, 608.1 ± 72.5 pm; p < 0.01) but started to increase after refeeding and remained elevated for 24 h (Fig. 5c). The blood glucose levels were also significantly decreased by food deprivation for 48 h (5.2 ± 0.3 mM vs control rats with ad libitum feeding, 6.9 ± 0.2 mM; p < 0.01) but increased after refeeding and remained elevated for 24 h (Fig. 5d). The NPY mRNA expression in the arcuate nucleus was significantly increased after food deprivation (580.8 ± 68.7 vs control rats with ad libitum feeding, 100.0 ± 32.4 arbitrary units; p < 0.01) (Fig. 5a). After refeeding, the expression levels of NPY mRNA were gradually decreased, and the differences reached statistical significance at 24 h compared with the values before refeeding (Fig. 5a). Expression levels of GAD65 mRNA were not affected by food deprivation but significantly increased 2 h after refeeding and remained elevated for 24 h (Fig. 5b). There were no significant differences in GAD65 mRNA levels between rats decapitated at 9:00 A.M., 11:00 A.M., and 3:00 P.M. without refeeding (data not shown). Representative photographs showing changes in NPY and GAD65 mRNA expression in the arcuate nucleus in vivo are shown in Figure 5f.

Effects of intracerebroventricular injection of insulin on GAD mRNA expression
To see whether insulin could increase GAD65 expression in the arcuate nucleus in vivo, effects of intracerebroventricular injection of insulin on GAD65 mRNA were examined in rats fasted for 48 h without refeeding. The levels of GAD65 mRNA expression were significantly increased in rats injected with insulin compared with those in rats injected with vehicle (191.9 ± 22.9 vs control rats, 100.0 ± 13.1 arbitrary units; p < 0.01).

Discussion
In this study, we first examined the regulation of NPY gene expression in hypothalamic organotypic cultures. Our in vitro data showed that (1) DEX increased NPY gene expression independently of action potentials, (2) insulin inhibited the NPY gene expression only in the presence of DEX, (3) the action of insulin on NPY gene expression was dependent on action potentials, and (4) GABAergic systems are likely to be involved in the inhibitory action of insulin on NPY gene expression. The possible role of GABAergic systems in mediating insulin action was further supported by the time course changes in GAD65 and NPY mRNA expression in the arcuate nucleus in the fasting-refeeding paradigm, as well as by the effects of central administration of insulin on GAD mRNA expression in vivo.

DEX is the synthetic glucocorticoid that binds to not only GR but also mineral corticoid receptors (MRs) in vitro (Reul et al., 2000). Because both GR and MR are expressed in the mediobasal hypothalamus in vivo (Reul et al., 2000), it is possible that the stimulatory effects of DEX on NPY mRNA expression in the present study might be mediated via not only GR but also MR, although a previous study demonstrated that NPY mRNA ex-
pression in the arcuate nucleus was increased by the central administration of GR, but not MR, agonists in vivo (White et al., 1994). The dose of DEX used in this study is similar to that measured in humans after injection of a clinically used amount of DEX (Osathanondh et al., 1977) and equivalent to physiological ranges of plasma glucocorticoid concentration. The doses of insulin used are within physiological ranges of the serum concentration, as shown in Figure 5c. Circulating insulin has been suggested to enter the brain via a transport system (Baura et al., 1993), and insulin concentrations in rat brain are shown to be 30–40% of plasma levels (Yalow and Eng, 1983). Thus, the doses of DEX and insulin used in this study are appropriate, and the findings in the hypothalamic organotypic cultures could well be physiologically relevant.

It has been shown that NPY gene promoter contains glucocorticoid-responsive elements and that dexamethasone increased the transcription of transfected as well as endogenous NPY gene expression in cell lines (Higuchi et al., 1988; Misaki et al., 1992). It has also been demonstrated that NPY immunoreactivities are increased by incubation with dexamethasone in rat fetal brain cells (Barnea et al., 1991) and dispersed hypothalamic neurons (Corder et al., 1988). In this study, we used the organotypic cultures from the rat mediobasal hypothalamus and showed that DEX increased NPY gene expression independently of action potentials in the arcuate nucleus, suggesting the direct action of glucocorticoids on NPY gene transcription and/or mRNA stability. However, it should be noted that explants from the mediobasal hypothalamus also include other hypothalamic nuclei, such as dorsomedial nucleus, ventromedial nucleus, and PVN, and that signals analyzed with films could contain the transcripts in these nuclei because they were reported to express NPY mRNA in vivo (Morris, 1989).

There is cumulative evidence that the inhibitory action of insulin on food intake is mediated via NPY neurons, although the detailed mechanisms have not been clear. In this study, we clearly demonstrated that insulin could act at the level of the hypothalamus to decrease NPY gene expression. Consistent with in vivo data that NPY expression was not increased by insulin deficiency in adrenalectomized rats (Ponsalle et al., 1992; Strack et al., 1995), our data also showed that the inhibitory action of insulin was only evident in the presence of DEX. Thus, it is suggested that glucocorticoids and insulin interact with each other at the level of the arcuate nucleus and regulate NPY gene expression, although our data do not exclude the other sites for possible interaction between glucocorticoids and insulin in vivo.

Our data showed that GABA_B agonists inhibited DEX-stimulated NPY mRNA expression and that inhibitory action of insulin was completely abolished in the presence of GABA_B antagonists. The GABA_B receptors belong to a family of G-protein-coupled receptors and cAMP responsive element binding protein-2, a transcriptional factor acting either positively or negatively at cAMP responsive element (CRE) (Hai and Hartman, 2001), has been implicated in mediating the signaling (White et al., 2000; Vernon et al., 2001). Food deprivation was reported to induce CRE-mediated gene induction in the arcuate NPY neurons (Shimizu-Albergine et al., 2001), and, given the similarity between insulin and GABA_B agonist action on NPY expression, it is possible that insulin increased GABA release, which in turn acted on GABA_B receptors to suppress NPY gene expression through CRE-mediated mechanisms.

Experiments with GABA_A antagonists suggest that NPY ex-
pression is tonically suppressed through GABA$_\alpha$ receptors. Previous study suggested that corticotropin-releasing hormone and vasopressin in the PVN are also tonically suppressed through GABA$_\alpha$ receptors (Cole and Sawchenko, 2002), and it is demonstrated that such tonic inhibition was well maintained in the hypothalamic organotypic cultures over 2–3 weeks (Bali and Kovacs, 2003; Bartanusz et al., 2004) as in the present study, although it is still possible that synaptic connections were remodeled during culture. Because GABA$_\alpha$ antagonist did not decrease NPY gene expression significantly in the present study, increases in NPY gene expression stimulated by DEX in the arcuate nucleus in organotypic cultures. The effects of insulin were dependent on neural transmission, and GABAergic systems are likely to be involved in the insulin action on NPY neurons.

In conclusion, our data showed that insulin inhibited NPY gene expression stimulated by DEX in the arcuate nucleus in organotypic cultures. The effects of insulin were dependent on neural transmission, and GABAergic systems are likely to be involved in the insulin action on NPY neurons.

References


