Cross-talk between sympathetic neurons and adipocytes in coculture

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White adipose tissue plays an integral role in energy metabolism and is governed by endocrine, autocrine, and neural signals. Neural control of adipose metabolism is mediated by sympathetic neurons that innervate the tissue. To investigate the effects of this innervation, an *ex vivo* system was developed in which 3T3-L1 adipocytes are cocultured with sympathetic neurons isolated from the superior cervical ganglia of newborn rats. In coculture, both adipocytes and neurons exhibit appropriate morphology, express cell-type-specific markers, and modulate key metabolic processes in one another. Lipolysis (stimulated by β-adrenergic agents) and leptin secretion by adipocytes are down-regulated by neurons in coculture, effects apparently mediated by neuropeptide Y (NPY). Secretion of NPY by neurons is up-regulated dramatically by the presence of adipocytes in coculture and appears to be mediated by an adipocyte-derived soluble factor. Insulin, an antilipolytic agent, down-regulates NPY secretion. Our findings suggest that an adipocyte-derived factor(s) up-regulates the secretion of NPY by sympathetic neurons, which, in turn, attenuates lipolytic energy mobilization by adipocytes.

Adipose tissue functions not only as a storage site for triglyceride energy reserves, but also as the source of numerous endocrine (1–3) and paracrine factors (4). The excessive accumulation of adipose tissue as occurs in obesity (5) or too little of this tissue as occurs in lipodystrophy (6) leads to major human health problems that are increasing in frequency. For these reasons, there has been increased interest in understanding how endocrine, paracrine, and neural factors control the metabolism of white adipose tissue (WAT). Least understood are the neural factors that regulate the metabolism of WAT.

The primary role of adipose tissue is to store energy as triglyceride when nutritional fuel is abundant and to mobilize that energy reserve when stimulated appropriately. In WAT, stored triglyceride is mobilized by the hormone-sensitive lipase to release and export free fatty acids and glycerol to provide physiological fuel for other tissues. In contrast, the lipid in brown adipose tissue (BAT) is used predominantly for heat production. Lipolysis is triggered by a variety of hormonal and neural effectors that increase the cAMP level in adipocytes, including catecholamines (epinephrine and norepinephrine) that interact with β-adrenergic receptors (7). While circulating catecholamine (epinephrine) regulates lipolysis in adipose tissue, particularly in the fasted state, catecholamine (notably norepinephrine) also is released from sympathetic neurons that innervate WAT (8).

Sympathetic fibers of the autonomic nervous system innervate both white and brown adipose tissue (9). Histological evidence gained from studies with rodents and pigs has shown that adipose tissue is innervated by the sympathetic neurons and is highly vascularized, with every adipocyte in close proximity to a capillary (10). Early studies (11, 12) suggested that the major innervation in WAT is perivascular around arteries, arterioles, and capillaries, with only about 3% of white adipocytes exhibiting direct innervation by adrenergic nerve terminals. However, these studies did not take into account the possible role of *en passant* sympathetic innervation, whereby the release of neurotransmitters occurs anywhere along the axon (8). There is still controversy as to whether the innervation of WAT affects the adipocytes directly via traditional synapses and/or via *en passant* innervation and/or by targeting blood vessels that indirectly affect adipocytes. Recent anatomical investigations indicate that autonomic innervation of WAT is more extensive than appreciated earlier. Injections of fluorescent neural tract tracers into the sympathetic nervous system (SNS) ganglia demonstrated anterograde labeling of epididymal and inguinal WAT in Siberian hamsters, with staining observed around individual adipocytes (13). Moreover, studies using pseudorabies virus to investigate retrograde labeling from WAT in Siberian hamsters demonstrated labeling of central nervous system nuclei associated with sympathetic outflow (9).

Although isolated adipocytes have been used widely to study the effects of adrenergic and other neural factors on adipocyte metabolism (14, 15), the interaction between neurons and adipocytes cannot be assessed by this approach. To this end, we have developed an *ex vivo* model system in which an established and well-characterized white adipocyte cell line (3T3-L1 adipocytes) can be cocultured with primary sympathetic neurons for long periods of time. Using this coculture system, we demonstrate that the two cell types exhibit appropriate morphology, express cell-type-specific markers, and modulate key metabolic processes in one another.

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Experimental Procedures

Animals. Pregnant adult female CD IGS (Sprague–Dawley) rats were obtained from Charles River Breeding Laboratories. Shipments were timed such that litters would be embryonic day 15 on the day of arrival at the Johns Hopkins University School of Medicine central animal facility, where they were housed individually. Pregnant rats were allowed to carry their litters to term, with delivery occurring approximately a week after arrival. The number of pups per litter ranged from 10 to 20, and two litters of rats were used per experiment. When rat pups were newborn to 2 days old, they were euthanized by decapitation. Superior cervical ganglia were dissected from each side of the neck. After collection of litters for superior cervical ganglia (SCG) dissection, the adult female rats were euthanized by carbon dioxide. The animal protocol was approved by the Animal Care and Use Committee of the Johns Hopkins University (protocol no. RA98 M533).

Cell Culture. 3T3-L1A preadipocytes were plated onto glass coverslips in 24-well cell culture plates (Falcon) and maintained in 10% calf serum (CS) (Invitrogen) in DMEM (Invitrogen). At 2 days postconfluence, cells were induced to differentiate by using insulin, dexamethasone, and methylisobutylxanthine (16). Neurons from newborn to 2-day-old rat SCG were isolated and dissociated by a combination of mechanical and enzymatic

Abbreviations: GLUT4, glucose transporter 4; MAP2, microtubule-associated protein 2; NPY, neuropeptide Y; SCG, superior cervical ganglion; WAT, white adipose tissue; RT-PCR, reverse transcription–PCR.

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methods (17, 18). After thorough resuspension, the neurons were plated onto 3T3-L1A adipocyte monolayers 3 days after induction of adipocyte differentiation in 10% FBS (Invitrogen) in DMEM supplemented with 200 ng/ml grade II 2.5S nerve growth factor (Roche Molecular Biochemicals, Indianapolis) and 1 μg/ml human insulin (Roche Molecular Biochemicals). The day after plating, cytosine β-D-arabinofuranonucleoside (Ara-C; Sigma) was added to the culture medium at a concentration of 5 μM and kept in the cocultures for the next 6 days to eliminate contamination of the cultures by nonadipocytic, nonneuronal cells. Starting the day after plating, the cocultures were fed every 48 h with 10% FBS/DMEM plus 200 ng/ml nerve growth factor and allowed to proceed for up to 2 weeks before further analysis and fixation.

Immunofluorescence. Indirect immunofluorescence was used to localize microtubule-associated protein 2 (MAP2), synaptophysin, glucose transporter 4 (GLUT4), tyrosine hydroxylase, and NPY in neuron/adipocyte cocultures (19). Cells were fixed in 4% formaldehyde in PBS, permeabilized in 0.075% Triton X-100, and blocked in 2 mg/ml BSA and 0.1% Tween-20 for 1 h at room temperature. Primary antibody incubations were performed for 1 h at room temperature in 2 mg/ml BSA and 0.1% Tween-20 plus antibodies at the following dilutions. Mouse mAbs against MAP2 (Sigma) and tyrosine hydroxylase (Sigma) were used at a dilution of 1:1,000, and mouse mAb against synaptophysin (Roche Molecular Biochemicals) was used at a dilution of 1:10. Rabbit polyclonal antisera against NPY (Sigma) were used at a dilution of 1:1,000, and rabbit polyclonal antisera against GLUT4 (20) were used at a dilution of 1:200. Antigen–antibody complexes were visualized with Alexafluor 488-conjugated goat anti-mouse IgG (Molecular Probes) and Alexafluor 546-conjugated goat anti-rabbit IgG (Molecular Probes). Cells were mounted on slides in ProLong Antifade (Molecular Probes) and visualized with a Zeiss Axioskop microscope (Zeiss) or with a Noran Oz confocal laser scanning microscope with three laser lines (Noran Instruments, Middleton, WI). Digital images were captured by using a Photometrics SenSys camera (Roper Scientific, Trenton, NJ) and analyzed with the assistance of IPLAB 3.5.0 software (Scanalytics, Billerica, MA). For confocal microscopy, data initially were analyzed by using Noran INNOVISION software (Noran) run on a Silicon Graphics workstation (Silicon Graphics, Mountain View, CA). Images were prepared for presentation in CANVAS 5.0 (Deneba Software, Miami) on a Power Macintosh G3 (Apple II+).

Leptin and Norepinephrine ELISAs. Cultured cells were washed twice with serum-free DMEM before overnight incubation in DMEM plus 200 ng/ml 2.5S nerve growth factor (grade II; Roche Molecular Biochemicals). After an 18-h incubation, media were frozen at −80°C until the mouse leptin immunoassays (R & D Systems) or the norepinephrine ELISAs (IBL, Gunma, Japan) were performed.

Measurement of Lipolysis by Glycerol-Release Assay. Lipolysis was assessed *in vitro* by using the glycerol-release assay. Cultures (day 8 of coculture/day 11 adipocytes) were incubated in lipolysis medium (3% fatty acid-poor serum albumin in Krebs–Ringer phosphate, pH 7.4) plus the indicated treatment at 37°C for 90 min. The medium then was frozen at −20°C until the enzyme-coupled glycerol assay (Sigma Diagnostics Kit 337) was performed.

NPY RIA. NPY secretion was assayed by using the NPY RIA of Peninsula Laboratories (Belmont, CA). Cultured cells were washed twice with serum-free DMEM before overnight incubation in DMEM plus 200 ng/ml 2.5S nerve growth factor (grade II; Roche Molecular Biochemicals). After an 18-h incubation, media were frozen at −80°C until the RIAs were performed.

RNA Isolation and Reverse Transcription–PCR (RT-PCR). RNA was isolated from rat and mouse tissue samples by means of the acid guanidinium thiocyanate/phenol/chloroform extraction method (21). After isolation, RNA was subjected to DNase I-, RNase-free (Roche Molecular Biochemicals) treatment at 37°C for 15 min (22). For the RT reaction, 2 μg of RNA per sample and 5 μg/μl random hexamer primers were heated at 65°C for 10 min and then quenched on ice. This mixture was combined with 250 μM each of dATP, dTTP, dCTP, and dGTP, 5 mM DTT, 50 mM Tris·HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 18 units of RNAGuard RNase inhibitor (Amersham Pharmacia), and 100 units of SuperScript II RNase H^- reverse transcriptase (Invitrogen). Samples were incubated at 42°C for 90 min in a PTC-200 Peltier Thermal Cycler (MJ Research, Cambridge, MA) and then held at 4°C. After aliquots were taken for immediate use in the PCR, the remainder of the cDNA was stored at −20°C. For PCR, 4 μl of the cDNA from the reverse transcription was combined with 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 1.3 mM $MgCl_2$, 200 μM dNTPs, forward and reverse primers, each at a concentration of 0.1–0.4 mM, and 2.5 units of *Taq* DNA polymerase (Invitrogen). PCR was performed in a PTC-200 Peltier Thermal Cycler. For the β-adrenergic and β-actin RT-PCR, the primer sequences were identical to previously published primers to mouse cDNA sequence and used at a concentration of 125 nM (23). The β-adrenergic and β-actin reaction conditions were as follows: hot start at 95°C for 5 min and then 35 repetitions of a cycle of 94°C for 1 min, 50°C for 2 min, and 72°C for 3 min, followed by extension at 72°C for 10 min and 4°C holding. The Y1, Y2, and Y5 NPY receptor primers were identical to those designed by Goumain (24). The Y4 primers were based on the regions of best homology for the NPY Y4 receptor sequences listed in GenBank and were as follows: forward, 5′-TGGCCTTCTCTGACTTCCTC-3′; reverse, 5′-TGTTGAGAAAGCCATAGATG-3′. The Y6 primers also were based on GenBank Y6 receptors and were as follows: forward, 5′-TTGGTGTGTGTCATGTGCATCC-3′; reverse, 5′-TCGTGGTGGCAGCTCATCAGC-3′. The NPY receptor reaction conditions were as follows: hot start at 95°C for 5 min and then 35 repetitions of a cycle of 94°C for 1 min and 30 sec, 55°C for 1 min and 30 sec, and 72°C for 2 min, followed by extension at 72°C for 10 min and 4°C holding.

Results

A neuronal/adipocyte coculture system was developed by using established protocols for the culture and differentiation of 3T3-L1 preadipocytes into adipocytes (16) and for the primary culture of neurons isolated from the SCG of newborn rats (17, 25).

Characterization of the Neuron/Adipocyte Coculture System. Preliminary experiments were conducted to identify the point in the adipocyte differentiation protocol at which to introduce the sympathetic neurons to 3T3-L1 cell monolayers to achieve maximal survival and appropriate morphology and marker gene expression for both cell types. It was determined that day 3 of the differentiation protocol, i.e., the day after removal of the differentiation inducers from the medium, was optimal (results not shown). As illustrated in Fig. 1 *A–C*, after 8 days of coculture extensive, differentiation into adipocytes had occurred as evidenced by the expression of adipocyte markers and the accumulation of cytoplasmic fat droplets, indicators of terminal adipocyte differentiation. Immunofluorescent staining showed that adipocytes in coculture expressed GLUT4 and UCP-2 (Fig. 1 *B* and *C*, respectively), whereas neurons expressed MAP2 (Fig. 1 *B* and *C*) and synaptophysin, tyrosine hydroxylase, and NPY

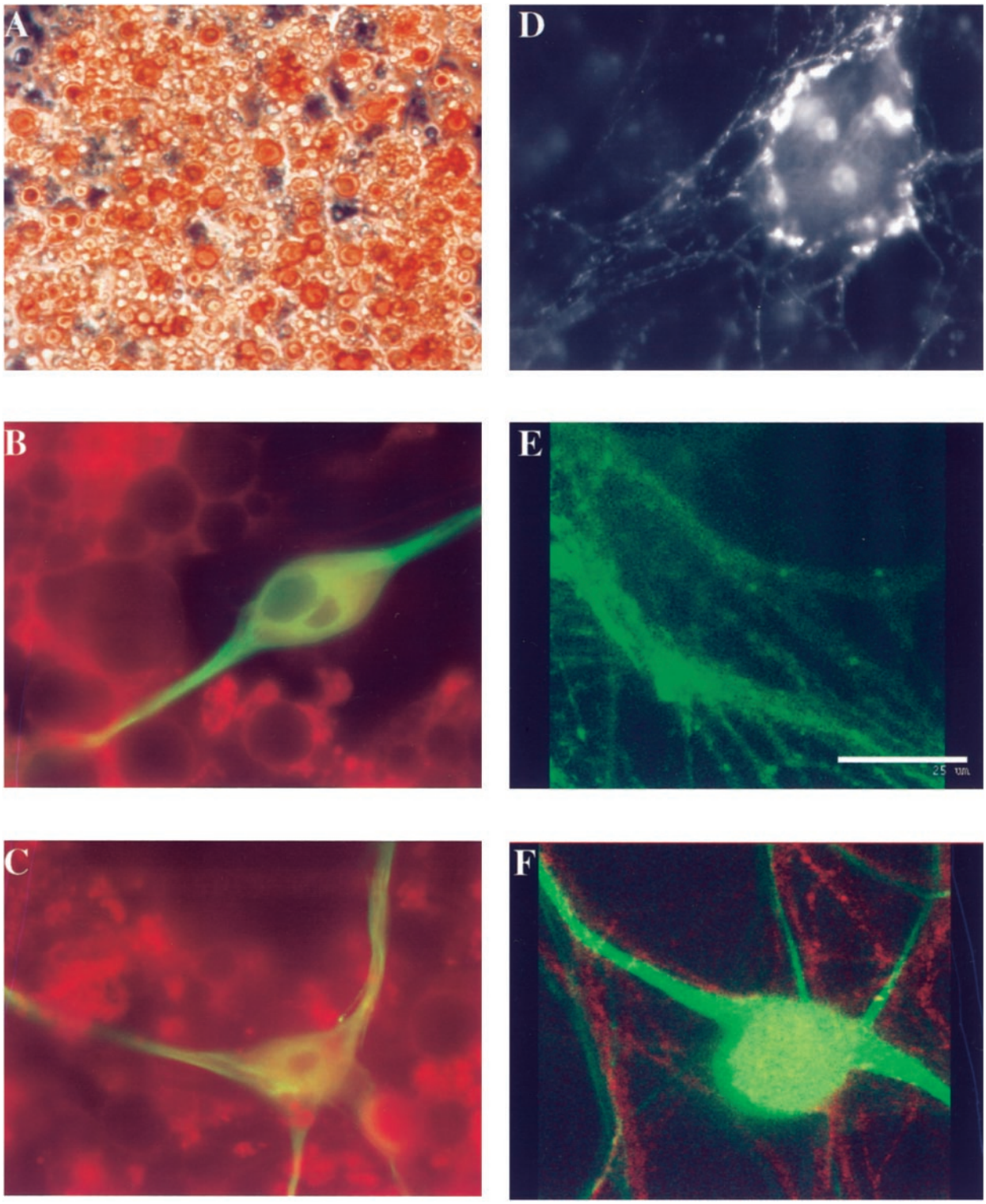

Fig. 1. Microscopy of 7-day SCG and adipocyte cocultures. The 3T3-L1A adipocytes were at day 10 after induction of differentiation on the day of fixation. (*A*) Phase-contrast image of typical coculture stained with oil red O viewed with a ×20 lens. (*B*) MAP2 (green)/GLUT4 (red) immunofluorescent staining of cocultures (×63 oil-immersion lens). (*C*) MAP2 (green)/UCP2 (red) immunofluorescent staining of cocultures (×63 oil-immersion lens). (*D*) Synaptophysin immunofluorescent staining of neurons in coculture (×63 oil-immersion lens). (*E*) Tyrosine hydroxylase immunofluorescent staining of neurons in coculture as seen by confocal microscopy (×100 oil-immersion lens). (*F*) MAP2 (green)/NPY (red) immunofluorescent staining of neurons in coculture as seen by confocal microscopy (×100 oil-immersion lens). MAP2 stains neuronal dendrites and cell bodies, whereas NPY stains neuronal axons primarily.

(Fig. 1 *D*, *E*, and *F*, respectively), characteristic marker proteins for both cell types. Fig. 1 *B–F* shows that the neurons display numerous processes and expression of appropriate markers for dendrites (MAP2) and axons (NPY). On average, each well contained 2×10^5 adipocytes and 10^3 neurons based on cell counts by Coulter counter and cells staining with MAP2,

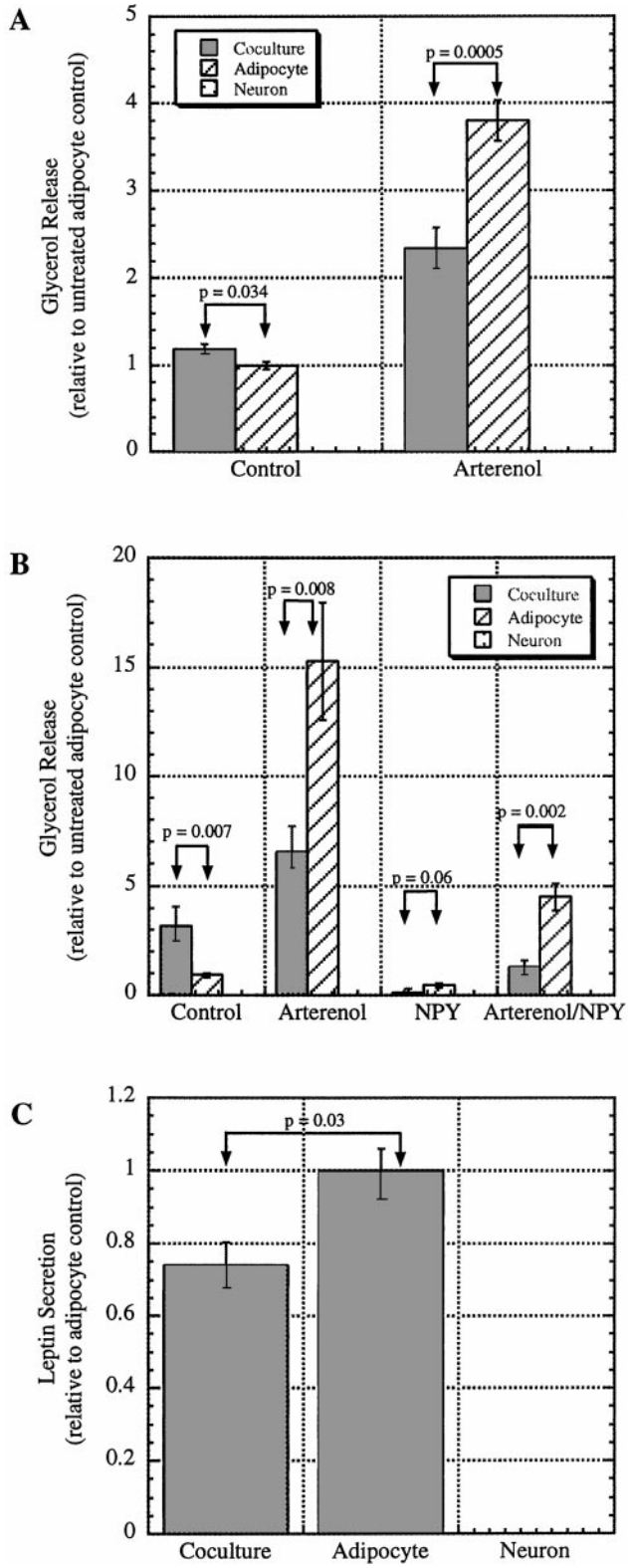

Fig. 2. Effect of neurons on adipocyte metabolism in coculture. Each graph represents a minimum of three separate experiments. (*A*) Effect of neurons on lipolysis (stimulated or not by 100 nM Arterenol) by adipocytes in 7-day cocultures. The 3T3-L1 adipocytes were at day 10 after differentiation on the day of the assay. The *P* values obtained from two-tailed Student's *t* tests comparing cocultures with adipocytes alone are indicated on the graph. (*B*) Effect of NPY on lipolysis (stimulated or not by Arterenol) by adipocytes in 8-day cocultures. The 3T3-L1 adipocytes were at day 11 after differentiation on the day of the assay. The concentrations of reagents added were 100 nM Arterenol and/or 100 nM NPY, as indicated. The mean glycerol concentrationin the control adipocytes was 52 ± 5 μM (equivalent to 16 ± 2 pmol of glycerol per well). The *P* values obtained from two-tailed Student's *t* tests comparing cocultures with adipocytes alone are indicated on the graph. (*C*) Effect of neurons on leptin secretion by adipocytes in coculture. The mean leptin concentration in the medium of adipocytes cultured alone was 450 ± 100 pM after an overnight incubation (equivalent to 140 ± 30 fmol of leptin per well). The *P* values obtained from a two-tailed Student's *t* test comparing cocultures with adipocytes alone are indicated on the graph. The *P* values for neurons versus cocultures or adipocytes alone were less than 10^{-6}.

respectively, sufficient for *en passant* innervation, whereby neurotransmitters are released at any point along the axon (8).

Semiquantitative RT-PCR analysis of RNA isolated from the cocultures revealed that the level of expression of β-adrenergic receptors was similar for cocultures and for adipocytes cultured alone (results not shown). RT-PCR analysis for the various NPY receptor isoforms (Y1, Y2, Y4, Y5, and Y6 receptors) showed that 3T3-L1 adipocytes expressed only the Y5 isoform (results not shown). Cocultures also were analyzed for hormones known to be secreted by adipocytes (≈140 pmol of leptin per well) and by neurons (≈6 pmol of norepinephrine per well and 120 pmol of NPY per well) during an overnight incubation. Adipocytes in coculture exhibit glycerol release responsive to Arterenol (*l*-norepinephrine hydrochloride), a potent β-adrenergic agonist, indicative of lipolysis, a hallmark of adipocyte metabolism (see below).

Effect of Neurons on Adipocyte Metabolism in Coculture. β-Agonist-stimulated lipolysis and the expression/secretion of leptin are unique characteristics of adipocytes. To assess the effect of coculturing sympathetic neurons with 3T3-L1 adipocytes, these metabolic functions were assessed. As shown in Fig. 2, sympathetic neurons, which do not exhibit lipolysis or express leptin, markedly down-regulate Arterenol-stimulated lipolysis (measured as glycerol release) by cocultured adipocytes. Similar results (not shown) were obtained with CL316,243, another β-agonist that interacts specifically with β_3-adrenergic receptors. Basal lipolysis (in the absence of β-agonist) by adipocytes alone or in coculture was quite variable (compare Fig. 2 *A* with *B*); in most experiments, neurons had little effect on glycerol release. This reason for this variability is unknown. ELISAs for secreted norepinephrine by neurons cocultured with 3T3-L1 adipocytes revealed extremely low levels (≈6 fmol secreted per 18-h incubation) (results not shown). The incubation media for the norepinephrine assays performed on cocultures contained no exogenous ascorbate, which likely contributed to the low levels of norepinephrine secretion observed. The addition of ascorbate to the medium of sympathetic neurons in culture has been shown to induce norepinephrine formation and secretion (26), although its rapid catabolism and formation of a toxic byproduct make its addition to media problematic. In addition, although sympathetic neurons cultured alone are known to secrete norepinephrine, when cocultured with other cell types this capacity is lost (27).

Because NPY, a secretory product of sympathetic neurons, has been reported to affect the β-adrenergic response (28–32), the possibility was considered that NPY may be responsible for the down-regulation of β-agonist-stimulated lipolysis. Therefore, the effect of NPY on lipolysis in mono- and coculture, in the presence and absence of Arterenol, was tested. As shown in Fig. 2*B*, NPY dramatically down-regulates lipolysis in coculture or with adipocytes alone both in the presence and absence of Arterenol. These findings are of particular importance because, as will be shown below, adipocytes promote the secretion of NPY by neurons in coculture.

The effect of neurons in coculture on leptin secretion also was

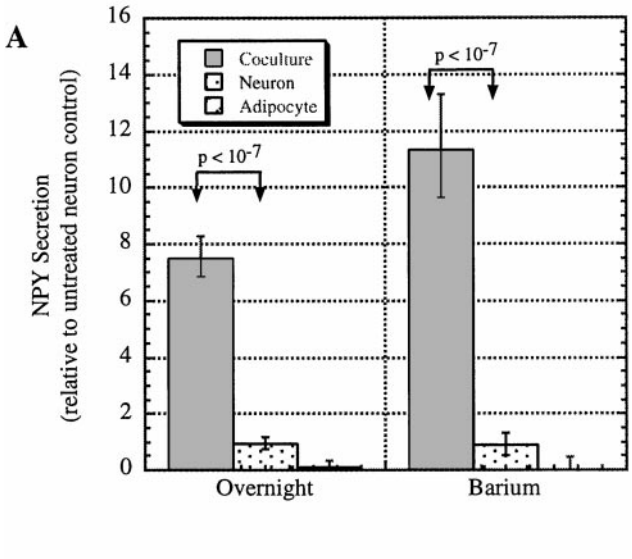

Fig. 3. Effect of adipocytes on sympathetic neurons in coculture. Each graph represents the summary of at least three separate experiments. The mean NPY concentration was 80 ± 30 nM in the media of sympathetic neurons alone after an overnight incubation (equivalent to 24 ± 9 pmol of NPY per well). (*A*) Effect of adipocytes on secretion of NPY by neurons in coculture. Barium chloride was added at 1 mM for a 2-h collection period where indicated. (*B*) A soluble, adipocyte-derived factor increases secretion of NPY by neurons in coculture.

assessed. Consistent with the reported effect of the sympathetic nervous system on leptin secretion *in vivo* (33), neurons caused decreased leptin secretion by adipocytes in coculture (Fig. 2*C*).

Effect of Adipocytes on the Metabolism of Neurons in Coculture. As shown above (Fig. 2*B*), NPY treatment had a profound effect on lipolysis by adipocytes in coculture. Thus, the possibility was considered that adipocytes might affect the production/secretion of NPY by neurons in coculture. As illustrated in Fig. 3*A*, neurons cultured alone secrete only a small amount of NPY. However, when cocultured with adipocytes, neurons increased their secretion of NPY 7.5-fold. To assess the level of cellular as well as secreted NPY, the cells were treated with Ba^{2+} to promote release of cellular NPY. After 1 week in culture, Ba^{2+} treatment increased the release of NPY from neurons in coculture, but not neurons cultured alone, and led to an 11.5-fold increase of secreted plus secretable/cellular NPY relative to that

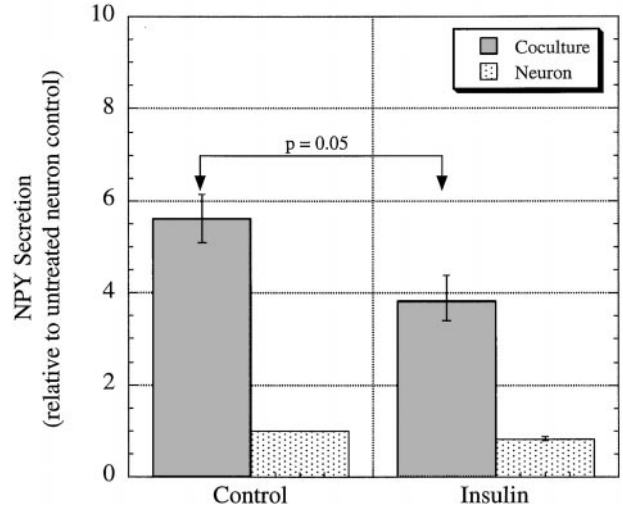

Fig. 4. Effect of insulin on NPY secretion in cocultures.

of neurons alone (Fig. 3*A*). If neurons alone are allowed to proceed in culture for a significantly longer time, their level of basal and Ba^{2+}-stimulated NPY secretion increases (19).

To determine whether the effect of adipocytes on neuronal NPY secretion is due to direct interaction of adipocytes with neurons or to a soluble factor(s) produced by adipocytes, neurons and adipocytes in coculture were maintained in different compartments separated by a membrane barrier. Despite the presence of the membrane barrier, adipocytes provoked a substantial (3.5-fold) increase in the amount of NPY in the culture medium, albeit less than that (7.5-fold) with cocultures without the membrane barrier (Fig. 3*B*). These results indicate that a soluble factor produced by the adipocytes is able to permeate the membrane and cause the neurons to increase secretion of NPY into the medium.

In view of the well-known pleiotropic role of insulin in energy metabolism of adipocytes and the central nervous system (34), we tested the possibility that insulin might affect NPY secretion by sympathetic neurons in coculture. Insulin, like leptin, is known to down-regulate NPY in hypothalamic neurons (35). As shown in Fig. 4, insulin significantly down-regulated the secretion of NPY by neurons in coculture, but had no effect on neurons cultured in isolation.

Discussion

In this paper, we describe an *ex vivo* system with which the interaction of sympathetic neurons with adipocytes can be investigated under the controlled conditions of cell coculture. This coculture system combines two well-characterized cell culture models, the established 3T3-L1 preadipocyte cell line that can be induced to differentiate into adipocytes (16) and primary sympathetic neurons isolated from superior SCG of newborn rats (17, 25). In coculture, both adipocytes and sympathetic neurons exhibited cell-type-specific characteristics consistent with their respective phenotypes and metabolic properties *in vivo*. The sympathetic neurons expressed MAP2 as well as NPY, synaptophysin and tyrosine hydroxylase, whereas the adipocytes (which secrete leptin and undergo hormone-stimulated lipolysis) expressed GLUT4 and UCP2 (Fig. 1). Evidence also was obtained for coculture-dependent cross-talk between the two cell types (Figs. 2–4).

Several findings have emerged from these studies. It was found that adipocytes in coculture provoke a 7.5-fold up-regulation of the expression/secretion of NPY by the sympathetic neurons (Fig. 3*A*). RNase protection analyses showed that the level of

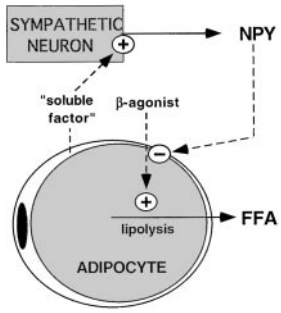

Fig. 5. Schema of cross-talk between neurons and adipocytes. FFA, free fatty acids.

NPY mRNA is ~12-fold higher in neurons cocultured with adipocytes than in neurons cultured alone (results not shown). Thus, both expression of NPY message (and presumably NPY protein) and NPY secretion are increased to about the same extent in coculture with adipocytes. Although the mechanism by which adipocytes cause this increase in NPY has not yet been determined, the increase in NPY secretion appears to be mediated, at least in part, by a soluble factor produced by the adipocytes (see Schema, Fig. 5). This is suggested by the finding that a membrane barrier separating the two cell types did not prevent the up-regulation of NPY secretion (Fig. 3*B*). The somewhat lower level of NPY secretion by neurons in coculture with the membrane barrier is consistent with a proximity effect or instability, i.e., diminishing concentration of the soluble factor with increasing distance between the adipocytes and the neurons. It is likely that the factor is short-lived because conditioned medium from adipocytes cultured alone had no effect on NPY secretion (results not shown). Also of interest is that insulin, which is known to suppress NPY secretion centrally in the hypothalamus (35), lowers NPY secretion by sympathetic neurons in coculture (Fig. 4).

It was also found that β-agonist-stimulated (i.e., Arterenol-stimulated) lipolysis by adipocytes is down-regulated markedly by coculture with sympathetic neurons (Fig. 2 *A* and *B*; see also Fig. 5). It is likely that this down-regulation is a result of NPY being secreted by the neurons because, when added to adipocytes either in coculture or alone, NPY strongly inhibits lipolysis (Fig. 2*B*). The counterregulatory effects of NPY and β-agonists (illustrated in Fig. 5) are consistent with the finding that leptin secretion by adipocytes is also down-regulated by coculture with sympathetic neurons (Fig. 2*C*). The central/hypothalamic action of leptin is known to promote peripheral sympathetic activity (33), which, in turn, would be expected to cause decreased leptin secretion through negative feedback.

Taken together, these findings suggest that NPY secreted by sympathetic neurons innervating adipose tissue serves as a counterregulatory mechanism to attenuate the β-adrenergic response (as illustrated in Fig. 5). We propose that under certain conditions, as yet not identified, adipocytes have the capacity to up-regulate NPY secretion mediated by the adipocyte-derived soluble factor, which, in turn, suppresses β-agonist-stimulated lipolysis. Further investigations are necessary to understand how NPY secretion by sympathetic neurons and the expression of the soluble factor are regulated. The coculture system should be useful for these and other studies involving cross-talk between adipocytes and the sympathetic neurons that innervate WAT.

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