Central Nervous System Neuropeptide Y Signaling Modulates VLDL Triglyceride Secretion

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OBJECTIVE—Elevated triglyceride (TG) is the major plasma lipid abnormality in obese and diabetic patients and contributes to cardiovascular morbidity in these disorders. We sought to identify novel mechanisms leading to hypertriglyceridemia. Resistance to negative feedback signals from adipose tissue in key central nervous system (CNS) energy homeostatic circuits contributes to the development of obesity. Because triglycerides both represent the largest energy depot in the body and are elevated in both the plasma and adipose in obesity and diabetes, we hypothesized that the same neural circuits that regulate energy balance also regulate the secretion of TGs into plasma.

RESEARCH DESIGN AND METHODS—In normal fasting rats, the TG secretion rate was estimated by serial blood sampling after intravascular tyloxapol pretreatment. Neuropeptide Y (NPY) signaling in the CNS was modulated by intracerebroventricular injection of NPY, receptor antagonist, and receptor agonist.

RESULTS—A single intracerebroventricular injection of NPY increased TG secretion by 2.5-fold in the absence of food intake, and this was determined to be VLDL by fast performance liquid chromatography (FPLC). This effect was recapitulated by activating NPY signaling in downstream neurons with an NPY-Y5 receptor agonist. An NPY-Y1 receptor antagonist decreased the elevated TGs in the form of VLDL secretion rate by 50% compared with vehicle. Increased TG secretion was due to increased secretion of VLDL particles, rather than secretion of larger particles, because apolipoprotein B100 was elevated in FPLC fractions corresponding to VLDL.

CONCLUSIONS—We find that a key neuropeptide system involved in energy homeostasis in the CNS exerts control over VLDL-TG secretion into the bloodstream. ***Diabetes*** **57:1482–1490, 2008**

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ACC, acetyl CoA carboxylase; apoB100, apolipoprotein B100; apoB48, apolipoprotein ARF-1, ADP-ribosylation factor-1; AUC, area under the curve; B48; CNS, central nervous system; FAS, fatty acid synthase; FFA, free fatty acid; FPLC, fast performance liquid chromatography; MC4R, melanocortin 4 receptor; MTII, melanotan II; MTP, microsomal triglyceride transfer protein; NPY, neuropeptide Y; POMC, proopiomelanocortin; SCD1, stearoyl-CoA desaturase-1; TG, triglyceride; VLDL-TG, elevated TGs in the form of VLDL.

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Obesity confers significant risk of cardiovascular disease. The major plasma lipid abnormality in obesity is elevated triglycerides (TGs) in the form of VLDL (VLDL-TG) and low HDL cholesterol (rev. in 1). Low HDL is a well-appreciated risk factor for coronary heart disease (2), and increasingly it is appreciated that elevated VLDL-TG also confers cardiovascular risk, especially in patients with diabetes or metabolic syndrome (1,3).

VLDL is the major TG-rich lipoprotein and is assembled in hepatocytes. Apolipoprotein B100 (apoB100) is the major structural protein of VLDL and is expressed constitutively in hepatocytes (4,5) where VLDL assembly occurs in a two-step process. In the first step, lipid is added to apoB100 as it is translated into the endoplasmic reticulum lumen by microsomal TG transfer protein (MTP) to form pre-VLDL (6). Next, a larger lipid droplet is added to form mature VLDL (7). The availability of lipid substrate, predominantly circulating free fatty acid (FFA), released from adipose is the key determinant of VLDL production and secretion; in the absence of lipid, apoB is degraded (8–10). In obesity, insulin resistance in peripheral tissues leads to increased FFA levels due to impaired inhibition of lipolysis contributing to the excess VLDL-TG (11–13). Hepatic insulin resistance also contributes to excess VLDL-TG in obesity because several steps in VLDL production are thought to be insulin sensitive (14).

Physiologically, TG represents the largest energy store in the body. Considering that fat mass is tightly regulated by energy-homeostatic circuits and that energy homeostasis is characteristically disrupted in the progression to obesity and hypertriglyceridemia, we hypothesized that central nervous system (CNS) circuits that regulate energy homeostasis may modulate how the liver handles nutrient excess, in the form of VLDL-TG secretion, where a relatively large pool of plasma TG can also be generated.

In weight-stable individuals, caloric intake is matched to expenditure via an endocrine feedback loop in which adiposity signals and nutrients communicate the status of energy stores to the brain (rev. in 15), making the brain ideally suited to coordinate lipid metabolism with metabolic needs. Although numerous brain areas and cell types are involved, the neuropeptide Y (NPY)- and proopiomelanocortin (POMC)-expressing neurons, found in the hypothalamus and elsewhere, function as metabolic sensors and are critical mediators of energy homeostasis (15–17). During energy deficit (such as while fasting), relative activation of NPY neurons occurs at the same time that POMC neurons are suppressed (18), leading to food seeking, energy conservation (19), and if fasting continues long enough, an increased reliance on lipid as an energy source (17). Obesity is also associated with relative elevation of

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NPY tone and reduction of POMC tone due to an impaired ability of these cell types to sense energy excess (20,21), i.e., insulin and leptin resistance in the brain (15). One consequence of impaired CNS sensing of metabolic status in obese subjects is that, like in fasting, the brain receives a signal of relative energy deficit, despite the presence of nutrient excess and enlarged energy stores (15). We hypothesize that this neuroendocrine response to fasting is required for optimal lipid metabolism and contributes to hypertriglyceridemia in obesity.

RESEARCH DESIGNS AND METHODS

Male Long Evans rats (HsdBlu:LE), weighing 250–274 g, were purchased from Harlan (Indianapolis, IN) and maintained on standard rodent chow (LabDiet 5001; 4.5% fat content; LabDiet, Richmond, IN). Studies were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Surgical preparation. Animals were prepared with stereotaxic implantation of a stainless steel guide cannula (22 gauge; Plastics One, Roanoke, VA) into the third cerebral ventricle (22) and a sterile silicon catheter (outer diameter 0.94 mm; internal diameter 0.51 mm) in the carotid artery (23).

Intracerebroventricular infusions. Studies were performed 5–7 days after surgery when food intake and body weight curves returned to presurgery trajectory. All intracerebroventricular compounds were prepared freshly, dissolved in 0.9% normal saline, and administered in a 2-μl volume over 1 min. NPY was from Phoenix Pharmaceuticals (Burlingame, CA), Bachem (King of Prussia, PA), or GenScript (Piscataway, NJ). We assured that the NPY preparation in a given study increased food intake in a separate control group of rats with intracerebroventricular cannula only. GR231118 was from Sigma. BWX-46 was from Tocris (Ellisville, MO). Melanotan II (MTII) was from Phoenix, and SHU9119 was from Bachem. The doses of MTII (1 nmol) and SHU9119 (15 μg) used are pharmacological doses at or above those that maximally attenuate food intake in Long Evans rats (24,25).

Lipid production experiments. For studies with NPY, Y5 receptor agonist, and melanocortin receptor agonist and antagonist, rats were fasted but with free access to water from 6:00 to 10:00 A.M. For the Y1 receptor antagonist, the study was performed after fasting from 6:00 A.M. to 2:00 P.M. A baseline blood sample was drawn through the carotid catheter. VLDL-TG clearance was blocked by an intravenous dosage of 300 mg/kg tyloxapol (26,27). Thirty or 40 min later, intracerebroventricular compounds were injected, and at 20- to 30-min intervals, 200 μl blood was collected in a tube with 2 μl 50 mmol/l EDTA.

Lipid assays. Triglycerides were assayed from serum using a kit from Raichem (catalog no. 84098) or, where glycerol values are shown, a Sigma assay kit (catalog no. TR 0100). The TG secretion rate was determined by the slope of the TG rise over time by linear regression analysis. Cholesterol was assayed from serum with a kit from Raichem (catalog no. 80015). FFA measurements were made by a NEFA-C assay kit from Wako (catalog no. 99-75409).

Hepatic TG content and MTP activity. Approximately 50 mg liver was prepared for MTP activity assay as described previously (Roar Biomedical, New York, NY). One-half of this homogenate was taken for lipid quantification by modified Folch extraction (28).

Plasma hormones. Plasma levels of insulin, glucagon, and leptin were quantified by the Vanderbilt Diabetes Research and Training Center Hormone Assay Core using radioimmunoassay.

Lipoprotein and long-chain CoA fractionation. Fast performance liquid chromatography (FPLC) analysis was performed as described previously (29). Liver long-chain CoA was isolated according to Deutsch et al. (30) and dried. CoA concentration was determined as described previously (31).

Western blots. Twenty-five-microliter aliquots of FPLC fractions from the VLDL peak were probed by Western blot with an antibody that recognizes both apoB100 and apolipoprotein B48 (apoB48) (32) and by standard enhanced chemiluminescence detection systems (Promega and Amersham). Western blots from liver homogenates were probed with the following antibodies: acetyl CoA carboxylase (ACC) and phosphorylated ACC, and fatty acid synthase (FAS) antibodies were purchased from Cell Signaling. MTP antibody was from Larry Swift (33).

RT-PCR was performed with Sybr-Green as previously described with primers from previously published reports: stearoyl-CoA desaturase-1 (SCD1) (34,35); FAS and HSL (35); MTP, 5′-TCACGATAACGGCTGTCAATGTC-3′ and 5′-CCTCCAAGTTGTCCTTTCCTACG-3′; ADP-ribosylation factor-1 (ARF-1), 5′-CGGAACCAGAAGTGAACCAGACC-3′ and 5′-CGTTTGCCACATGAGAG GAAAGC-3′.

Statistical analysis. Student's *t* test (paired or two-sample equal variance, two tailed) was used to compare with the appropriate vehicle-treated group; $P < 0.05$ was considered significant.

RESULTS

CNS NPY signaling modulates TG production. To test whether an acute elevation in NPY signaling in the brain could increase the TG secretion rate in the absence of either obesity or feeding, a single intracerebroventricular injection of recombinant NPY was administered. TG clearance from the bloodstream was blocked by injection of tyloxapol, which inhibits the action of lipoprotein lipase on secreted lipoprotein particles (26). The rate of TG production was estimated by serial quantification of plasma TG after a 4-h fast. We found that intracerebroventricular injection of 1 nmol NPY (t0) after tyloxapol pretreatment resulted in increased accumulation of plasma TG (calculated production rate was 5.3 ± 0.18 mmol TG/h) compared with vehicle-treated animals (2.1 ± 0.1 mmol TG/h, $n = 3$, $P < 0.0001$, Fig. 1*A* and *D*). In a separate, limited dose-response study, we found that 1 nmol NPY maximally increased TG secretion (4-h vehicle, 2.6 ± 0.24; 100 pmol NPY, 4.54 ± 0.15; 1 nmol NPY, 5.06 ± 0.84; 10 nmol NPY, 3.78 ± 0.65 mmol TG/h).

Peripheral NPY does not recapitulate the effect of CNS NPY to increase TG production. NPY is an abundant neurotransmitter in both the central and peripheral nervous system. To localize the observed effect of intracerebroventricular NPY to the CNS, we tested a peripheral injection of the same dose of NPY and found that while intracerebroventricular NPY increased the TG secretion rate (intracerebroventricular NPY, 3.4 ± 0.2 mmol TG/h; vehicle, 2.2 ± 0.2 mmol TG/h, $P < 0.01$), intravascular NPY had no effect compared with vehicle (peripheral NPY, 2.6 ± 0.2 mmol TG/h; vehicle, 2.2 ± 0.2 mmol TG/h, NS).

Intracerebroventricular NPY increases TG production via NPY Y1 and Y5 receptors. NPY Y1 and Y5 are the receptor subtypes most implicated in energy homeostasis in the CNS (36,37). To test whether the effect of NPY to increase TG secretion was a specific, receptor-mediated event, we used a peptide agonist of the NPY Y5 receptor (30 μg BWX-46) and a peptide antagonist of the NPY Y1 receptor (30 μg 1229U91, also known as GR231118) in a similar experimental design. The Y5 receptor agonist given intracerebroventricularly after a 4-h fast increased TG secretion twofold to 4.3 ± 0.4 mmol · l^{1-} · h^{1-} ($n = 6$, $P < 0.0001$ compared with 4-h vehicle-treated animals, 2.1 ± 0.1 mmol · l^{1-} · h^{1-}; Fig. 1*B* and *D*). The Y1 receptor antagonist was given after an 8-h fast (when NPY tone is predicted to be higher) and compared with 8-h vehicle. The Y1 receptor antagonist decreased TG secretion ~50% below that of vehicle control (vehicle, 1.96 ± 0.16; Y1 receptor antagonist, 0.96 ± 0.07 mmol · l^{1-} · h^{1-}; $n = 3$, $P < 0.0001$; Fig. 1*C* and *D*).

NPY increases VLDL-TG production. To test whether the increase in TG mediated by CNS NPY signaling was due to increased VLDL-TG secretion, animals were injected with tyloxapol IV and serum samples collected 120 min after intracerebroventricular treatment with NPY (1 nmol), vehicle (saline), or the Y1 receptor antagonist 1229U91 (30 μg). Pooled samples were analyzed by FPLC, and TG content was quantified in the fractions. NPY increased the TG content in the VLDL fractions (fractions 13–23) by 1.6-fold (area under the curve [AUC] 230 μg for vehicle and 370 μg for NPY; Fig. 1*E*), whereas the Y1 receptor antagonist decreased TG in these fractions by

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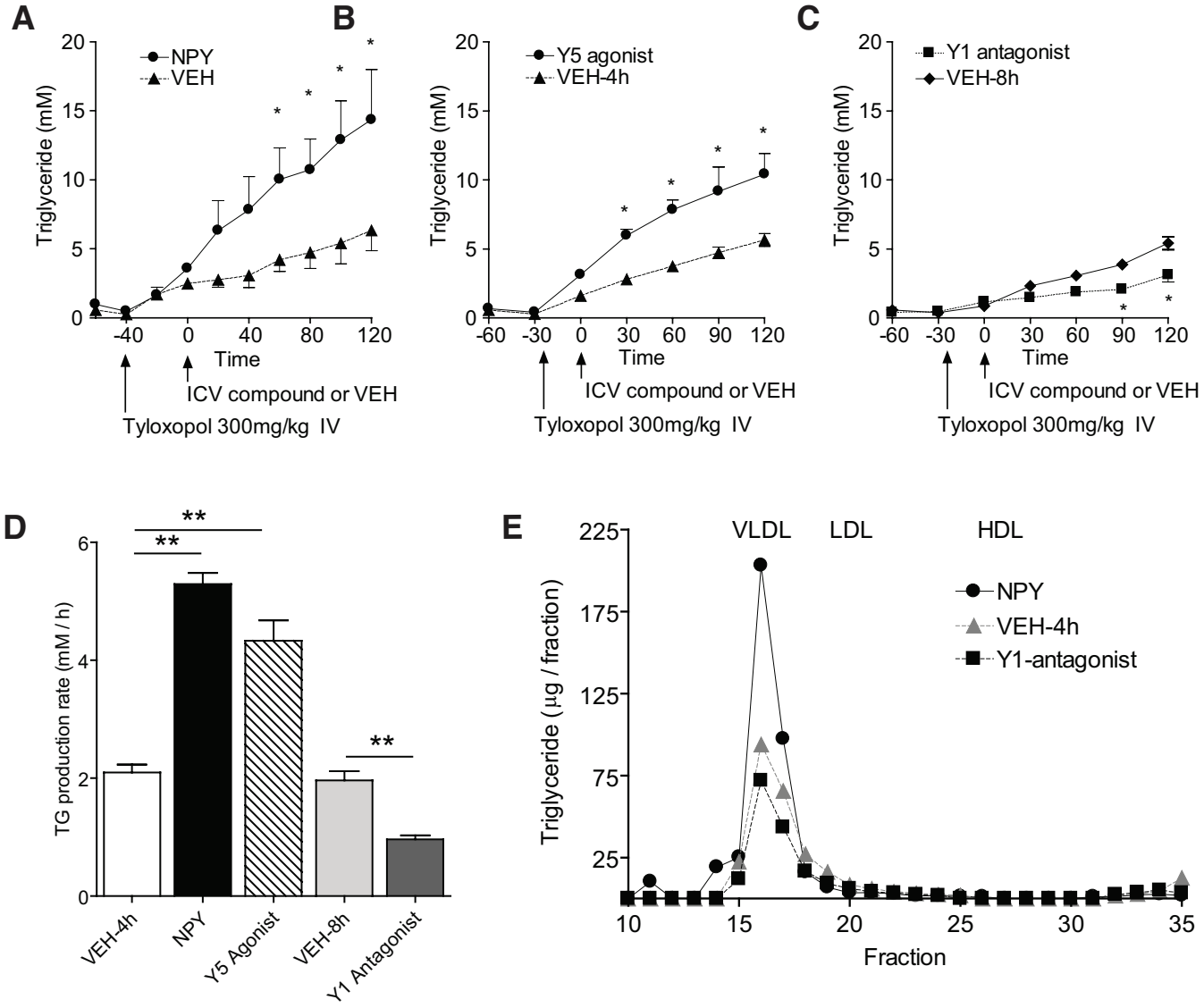

FIG. 1. Intracerebroventricular NPY increases TG production. *A*: Plasma TG levels after an intra-arterial injection of tyloxapol (t-40) and either 1 nmol intracerebroventricular NPY injection ($t = 0$; circles) or saline vehicle (VEH)-4 h (triangles) are shown after a 4-h fast. *B*: Plasma TG levels after intra-arterial injection of tyloxapol (t-30) and intracerebroventricular injection at $t = 0$ of the Y5 receptor specific agonist (BWX-46, 30 μg; circles) or 4-h saline vehicle (triangles). *C*: Plasma TG levels after intracerebroventricular injection of Y1 receptor antagonist (1229U91, 30 μg; squares) or saline VEH-8 h (diamonds) are shown after an 8-h fast. *D*: Changes in TG production rate mediated by CNS NPY signaling calculated from the slope of the rise of the plasma TG line from time of tyloxapol injection. *E*: FPLC was performed from pooled serum samples of animals treated with tyloxapol and 2 h after intracerebroventricular treatment. Samples 14–18 contained VLDL. NPY, circles; VEH, triangles; Y1 receptor antagonist, squares. For all panels, *$P < 0.05$ and **$P < 0.0001$ compared with VEH.

67% (AUC 156 μg; Fig. 1*E*). We confirmed by agarose electrophoresis that 4-h-fasted rats on laboratory chow have nearly undetectable chylomicrons compared with fed rats, and calorimetry in a separate group confirmed that animals were in a postabsorptive state after a 4-h fast (respiratory quotient 0.9 ± 0.0 fed to respiratory quotient 0.8 ± 0.1, 4-h fast), suggesting that chylomicrons do not contribute to the changes in TG observed.

Melanocortin signaling does not modulate the VLDL-TG secretion rate. The melanocortin signaling system is similarly fundamental to energy homeostasis to the NPY signaling system (38). An agonist of the melanocortin 4 receptor (MC4R), 1 nmol intracerebroventricular MTII, however, had no statistically significant effect on TG secretion (1.87 ± 0.07 mmol · l^{1-} · h^{1-}) compared with vehicle (2.3 ± 0.07 mmol · l^{1-} · h^{1-}; $n = 3$ per group, $P = 0.56$; Fig. 2*A* and *B*). Similarly, a MC4R antagonist (15 μg intracerebroventricular SHU9119) did not result in a statistically significant change in TG secretion (2.6 ± 0.16 mmol · l^{1-} · h^{1-}) compared with vehicle (2.2 ± 0.05 mmol · l^{1-} · h^{1-}; $n = 3$, $P = 0.86$; Fig. 2*C* and *D*).

CNS NPY signaling increases secretion of VLDL particles. We next tested whether CNS NPY signaling increases the number of VLDL particles secreted by the liver. These experiments were performed in the absence of tyloxapol, which blocks the clearance of TG from VLDL. Four groups of animals were matched for body weight (Fig. 4*A*) and, as in prior studies, were fasted for the duration of the experiment (Figs. 3 and 4). In this study, NPY or vehicle was given in the postabsorptive state (4-h fast, when NPY tone is predicted to be relatively lower), and the Y1 receptor antagonist or vehicle was given after an 8-h fast (when NPY tone is predicted to be higher). Even in the absence of tyloxapol, NPY treatment (1 nmol

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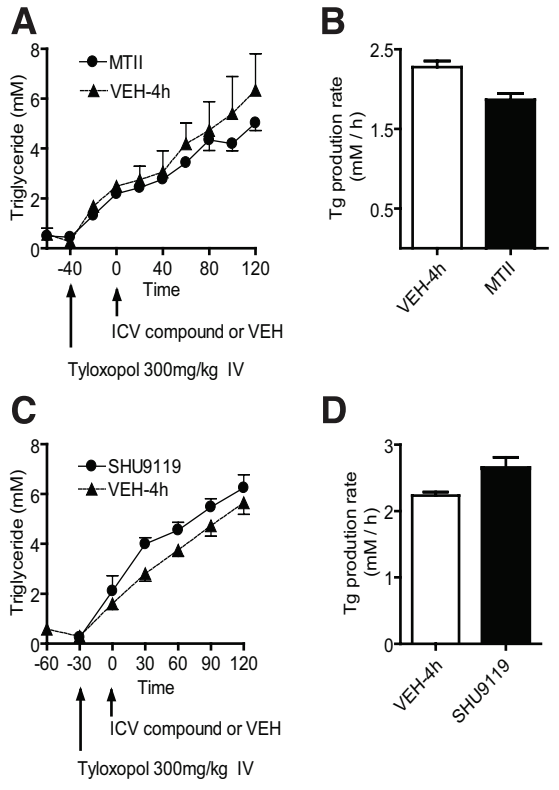

FIG. 2. CNS melanocortin signaling has little effect on TG production. *A*: Plasma TG levels after intra-arterial tyloxapol injection (t-40) in response to intracerebroventricular injection of MTII (1 nmol; circles), compared with vehicle (VEH) (triangles). *B*: Calculated TG production rate in response to MTII. *C*: Plasma TG levels after intra-arterial tyloxapol injection (t-30) in response to intracerebroventricular injection of SHU9119 (15 μg; circles) compared with VEH (triangles). *D*: Calculated TG production rate in response to SHU9119.

intracerebroventricularly) increased plasma TG levels 2.4-fold, from 0.31 ± 0.01 to 0.76 ± 0.06 mmol/l (Fig. 3*A*; n = 3 per group, $P < 0.05$). Fractionation of serum samples by FPLC revealed that intracerebroventricular NPY treatment markedly increased TG in the VLDL fractions (AUC 2.4 ± 0.74 μg for 4-h vehicle compared with 10.5 ± 1.9 μg for NPY, $n = 3$, $P < 0.05$). Because VLDL particles contain one copy of apoB100 or apoB48 (in rodents), analysis of this protein in FPLC fractions corresponding to VLDL provides an estimate of the number of VLDL particles in each fraction (4). Intracerebroventricular NPY treatment caused an increase in the number of apoB100-containing particles compared with vehicle (Fig. 3*C* and *D*). There was only a modest change in apoB48, which in rodents can be liver or intestinally derived, further demonstrating, together with the lipoprotein electrophoresis and calorimetry data, that the majority of change in TG seen was VLDL-TG and not chylomicrons.

Similar experiments performed with the Y1 receptor antagonist reveal that the Y1 antagonist (30 μg intracerebroventricularly compared with 8-h vehicle) did not statistically significantly decrease plasma TG or the AUC for VLDL (Fig. 3*E* and *F*), although in both groups, they were quite low. Western blot analysis for apoB100 from FPLC fractionated serum corresponding to VLDL demonstrated that there was no statistically significant change in plasma VLDL apoB100 compared with vehicle treatment (Fig. 3*G* and *H*). Qualitatively, the antagonist did trend toward lower VLDL-TG.

CNS NPY signaling does not change plasma FFA. Current models suggest that substrate (FFA) delivery to liver contributes to the rate of VLDL-TG production (11–13), although other substrates are also used. We next determined whether CNS NPY signaling, in the absence of tyloxapol, increases FFA, which might be taken up by the liver and thus increase VLDL-TG production and secretion. Neither intracerebroventricular NPY or Y1 antagonist had a detectable effect on FFA levels at 120 min, suggesting that a measurable change in plasma FFA is not required to modulate VLDL-TG production by these agents, at least in this 2-h study (Fig. 4*B*). Glycerol levels were very low in these studies and unchanged with NPY or Y1 receptor antagonist, further suggesting that changes in lipolysis are not required. Insulin is a potent inhibitor of adipose tissue lipolysis, and NPY had no effect on plasma levels, although the Y1 receptor antagonist lowered plasma insulin levels (Fig. 4*C*). Significant changes in glucagon and leptin were not seen between treatment and control groups (Fig. 4*D* and *E*). Despite these findings, definitive proof of the absence for a role of lipolysis will require tracer techniques.

CNS NPY signaling reduces hepatic TG and does not alter long-chain fatty acyl CoA content. In the absence of evidence that NPY activates lipolysis to increase VLDL-TG production, we measured hepatic TG content to determine whether changes in VLDL-TG production rate result in measurable changes in the amount of stored TG. NPY induced a small but not statistically significant decrease of total tissue TG (vehicle, 14.4 ± 3.6 μg/mg; NPY, 12.1 ± 0.9 μg/mg tissue; Fig. 4*G*). After 120 min, there were no significant changes in hepatic tissue levels of apoB100 or apoB48 detectable by Western blot (Fig. 4*F*). ApoB100 is made in the liver and, if lipid is not loaded by MTP, becomes targeted for degradation (9,39). No statistically significant changes in hepatic MTP activity were noted 2 h after treatment with intracerebroventricular NPY or the Y1 receptor antagonist (Fig. 4*H*).

Collectively, these data suggest that NPY activates hepatic VLDL-TG not by increasing substrate delivery but potentially by increasing loading of stored or newly synthesized hepatic TG onto apoB and thus stabilizing apoB100 for secretion. We found that NPY had no effect on the distribution of long-chain fatty acyl CoA in liver and no change in oleate CoA (18:1) concentration or the "saturation index," which has been shown to impact VLDL-TG production (34) (n = 3–4 per group, NS; Fig. 4*I*).

Effects of CNS NPY signaling on enzymes involved in TG biosynthesis and VLDL assembly. Western blot analysis was performed from liver samples of six individual animals that were obtained 2 h after treatment with intracerebroventricular VEH 4 h (Fig. 5*A*, left lanes) or intracerebroventricular NPY (Fig. 5*A*, right lanes). A modest, but not statistically significant reduction in ACC was noted (4-h vehicle, 3.58 ± 0.52; NPY, 2.75 ± 0.39), and intracerebroventricular NPY did not alter expression levels of FAS, MTP, or the phosphorylation state of ACC as detectable by Western blot analysis at 2 h (Fig. 5*A*).

To determine whether CNS NPY influenced mRNA levels of enzymes that are key control points in TG metabolism, we performed real-time RT-PCR on cDNA prepared from liver samples in the above experiment. We found statistically significant increases in relative mRNA levels for proteins involved in the assembly of VLDL, MTP (4-h vehicle, 1.0 ± 0.23; NPY, 1.79 ± 0.25; Fig. 5*B*), ARF-1

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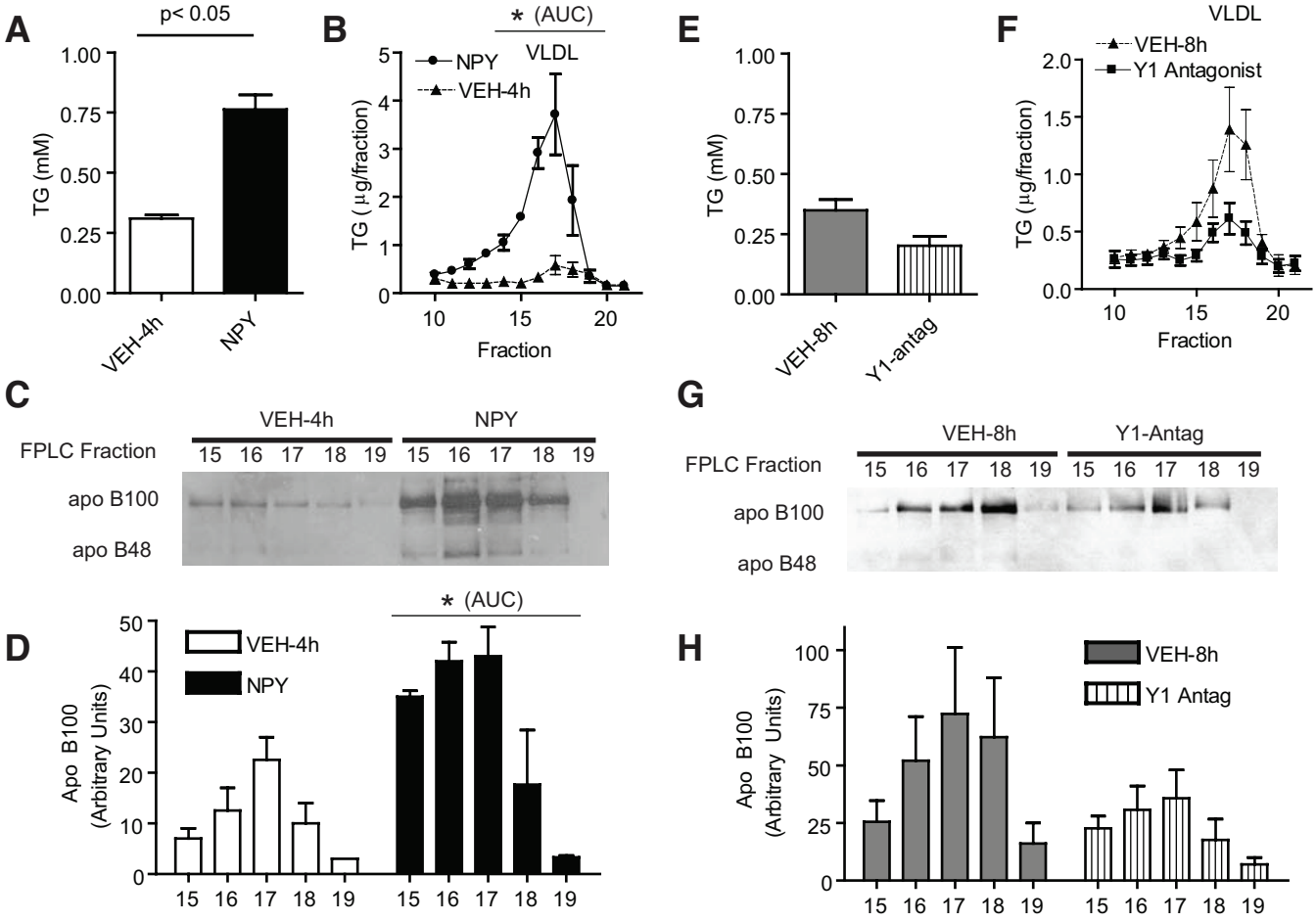

FIG. 3. Effects of CNS NPY signaling on substrate availability, glucoregulatory hormones, and liver lipids in the absence of tyloxapol ($n = 6$ for all groups, all panels). For all panels, in the absence of tyloxapol, NPY (black bars) was given after a 4-h fast compared with vehicle (VEH) (4 h, open bars), Y1 receptor antagonist was given after a 8-h fast (striped bars) compared with VEH (8 h; gray bars), and samples were collected 2 h after intracerebroventricular injection. *A*: Body weights were matched between groups. *B*: Plasma FFA levels. *C*: Plasma insulin levels. *D*: Plasma glucagon levels. *E*: Plasma leptin levels. *F*: Hepatic apoB100 and apoB48 content. *G*: Hepatic TG and cholesterol content. *H*: MTP activity assayed from liver samples. *I*: Hepatic long-chain fatty acyl CoA content.

(4-h vehicle, 1.0 ± 0.03; NPY, 1.38 ± 0.07; Fig. 5*C*), and SCD1 (4-h vehicle, 1.0 ± 0.24; NPY, 5.2 ± 1.0; Fig. 5*D*). We found a reduction in mRNA for the enzyme FAS involved in de novo fatty acid synthesis (4-h vehicle, 1.38 ± 0.24; NPY, 0.63 ± 0.14; Fig. 5*E*).

NPY increases cholesterol in the VLDL fraction. TG-rich VLDL and cholesterol-rich LDL and HDL particles are metabolically interrelated (40–42). In the absence of tyloxapol, neither NPY nor the Y1 receptor antagonist induced any changes in total plasma cholesterol (Fig. 6*A*, NS); however, NPY treatment increased the cholesterol content of the VLDL fraction twofold (Fig. 6*B*, AUC of fractions 13–23 of 70-fraction format was 2.35 ± 0.6 μg for 4-h vehicle compared with 4.87 ± 1 μg for NPY, $P < 0.05$). There was no change in cholesterol in the LDL fraction. NPY had no effect on cholesterol content in the HDL fraction (Fig. 6*B*, AUC of fractions 43–60 of the 70-fraction format was 35.2 ± 2.4 μg for 4-h vehicle and 39.8 ± 2.5 μg for NPY, $P = 0.12$) but appeared to cause a shift to increase small HDL cholesterol, because cholesterol came off the column at later fractions, suggesting smaller particle size. The Y1 receptor antagonist had little effect on cholesterol content of the lipoprotein fractions (Fig. 6*C*).

DISCUSSION

A brain-hepatic circuit has been demonstrated to be important for the regulation of peripheral metabolism, including glucose homeostasis and, more recently, TG metabolism (34,35,43). Although current models of TG production, particularly in the context of obesity, suggest that the VLDL-TG secretion rate is largely determined by the rate of substrate (FFA) delivery to the liver and hepatic insulin sensitivity (11–13), we hypothesized that CNS circuits involved in energy homeostasis are well situated to coordinate lipid production and/or secretion with other aspects of metabolism. CNS NPY signaling is thought to contribute to several aspects of obesity pathogenesis (17), including resistin-mediated hepatic insulin resistance (44). Furthermore, exogenous administration of NPY to a lean animal can drive the development of obesity and metabolic syndrome (45,46).

We observed that intracerebroventricular injection of NPY rapidly increased TG secretion (Fig. 1*A*) in the setting in which neither fat mass nor food intake was increased. Local concentrations of NPY in relevant brain areas are not known, and a single injection of NPY intracerebroventricular could conceivably result in elevated concentra-

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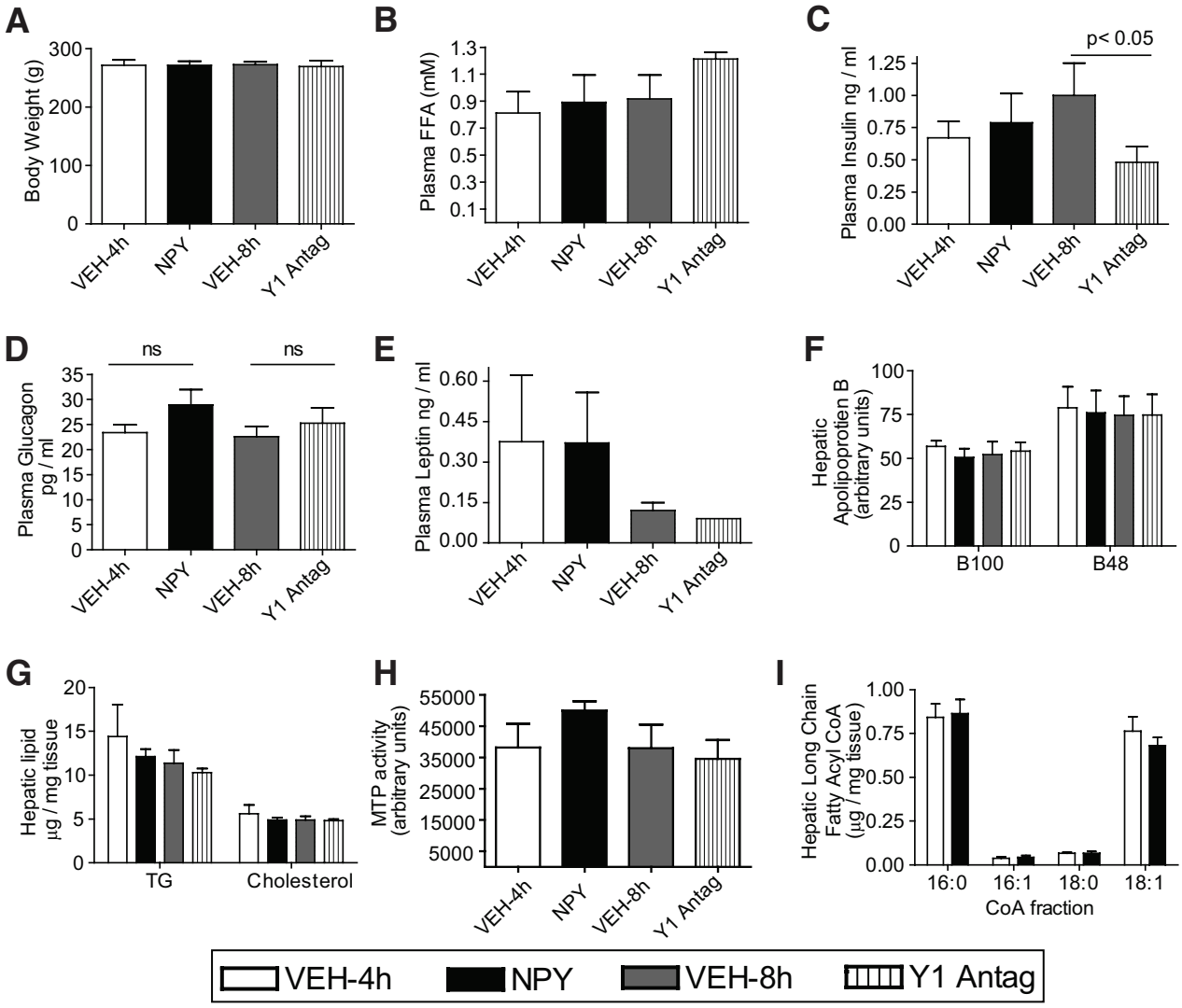

FIG. 4. NPY enhances VLDL particle secretion. *A–D*: In the absence of tyloxapol, NPY (black bars) was given after a 4-h fast compared with vehicle (VEH) (4 h, open bars), and samples were collected 2 h after intracerebroventricular injection. *A*: Fasting plasma TG levels. *B*: TG content of FPLC fractions corresponding to VLDL, fractions 10–22 for NPY (circles) compared with VEH-4 h (triangles). *$P < 0.05$ for AUC. *C*: Western blot analysis of apoB100 and apoB48 from FPLC fractions corresponding to VLDL. *D*: Quantification of apoB100 from Western blot, *$P < 0.05$ for AUC. *E–H*: In the absence of tyloxapol, Y1 receptor antagonist given after an 8-h fast (striped bars) compared with VEH (8 h; gray bars), and samples were collected 2 h after intracerebroventricular injection. *E*: Fasting plasma TG levels. *F*: TG content of FPLC fractions corresponding to VLDL, fractions 10–22 for VEH-8 h (triangles) compared with Y1 antagonist (triangles). *G*: Western blot analysis of apoB100 and apoB48 from FPLC fractions corresponding to VLDL. *H*: Quantification of apoB100 from Western blot.

tions and, therefore, nonspecific or off-target signaling events in the CNS. The fact that an NPY Y1 receptor antagonist lowers TG production and Y5 receptor agonist increases TG production, however, enhances the likelihood that we have identified an endogenous, NPY-specific pathway that influences how the liver controls TG secretion (Fig. 1*B–D*). The observation that an NPY receptor antagonist lowers VLDL secretion in a normal, lean, fasting rat by 50% suggests the concept that endogenous NPY tone in the CNS contributes to determining the "basal" rate of VLDL-TG secretion (Fig. 1*C*). The finding that agonists and antagonists of the related melanocortin signaling pathway did not significantly alter TG production adds to the specificity of these findings (Fig. 2), although these compounds were only tested at high doses.

Because FFA availability contributes to VLDL-TG production (11,12,47), the finding that intracerebroventricular NPY increased plasma TG levels nearly twofold while plasma FFA and glycerol levels were unchanged (Fig. 3*A* and 3*B*), as were plasma insulin levels (Fig. 4*C*), suggests that a measurable change in plasma FFA levels was not required. Tracer studies will be required to ultimately rule out an effect on lipolysis, however.

The increase in plasma TG mediated by CNS NPY signaling may, therefore, be due to mobilization of stored hepatic TG. In the absence of available lipid being mobilized onto apoB, the protein is targeted for degradation (9). In hepatocytes, MTP facilitates the addition of TG to the immature VLDL particle (48); this process stabilizes apoB, which can then be secreted into the plasma as part of VLDL instead of degraded. Our data suggest that CNS NPY signaling promotes lipid loading and thus stabilization of apoB100 in VLDL (Fig. 3). We found a 1.7-fold increase in MTP mRNA at 2 h (Fig. 5*B*) but were unable to see changes in protein level (Fig. 5*A*). MTP activity was not statistically increased after NPY, although substrate "flux" through MTP may not be directly related to activity as assayed by this commercially available kit (Fig. 4*H*). A second matu-

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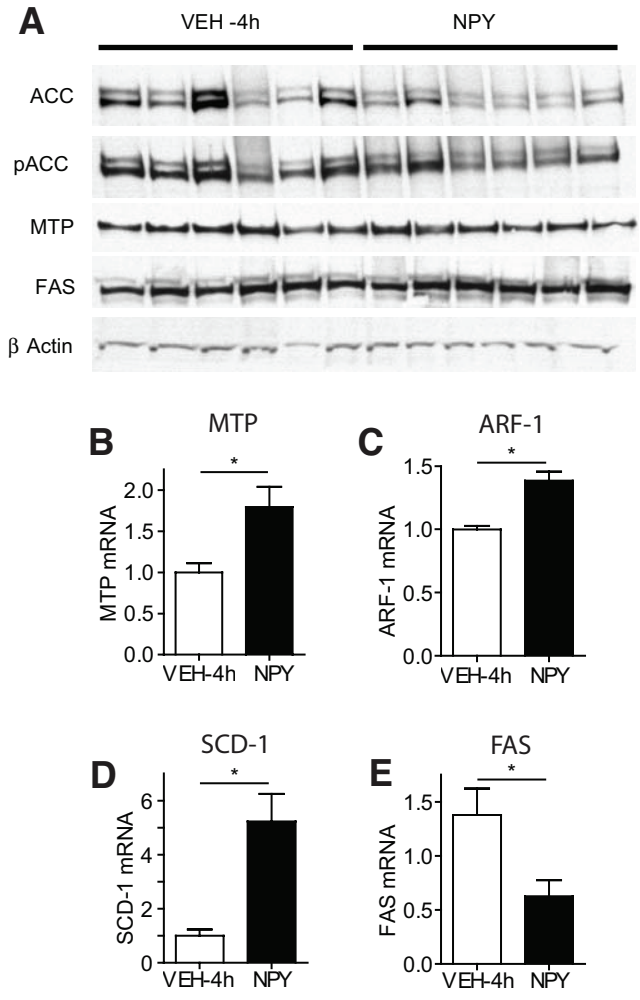

FIG. 5. Effect of CNS NPY signaling on proteins involved in TG synthesis and metabolism. *A*: Western blot analysis from liver samples of six individual animals that were obtained 2 h after treatment with intracerebroventricular vehicle (VEH)-4 h (left lanes) or intracerebroventricular NPY (right lanes). Total ACC, phosphorylated-ACC, MTP, FAS, and β-actin as a loading control. Intracerebroventricular NPY did not alter expression levels of these proteins at 2 h. *B–E*: Real-time RT-PCR for MTP (*B*), ARF-1 (*C*), SCD-1 (*D*), and FAS (*E*). For all panels, *$P < 0.05$ compared with VEH.

ration step, thought to be regulated in part by ARF-1 and phospholipase D (7), adds a larger lipid droplet to form mature VLDL, and we found an increase in ARF-1 mRNA levels (Fig. 5*C*). Unfortunately, "maturation" inhibitors are toxic in vivo and were not tested.

Recently, Lam et al. (34) demonstrated that intracerebroventricular glucose decreases VLDL production, in part by decreasing SCD-1 activity, and thereby oleate-CoA (18:1) availability, which is known to stimulate VLDL-TG production (40). We found no significant changes in hepatic palmitate-CoA (16:0), palmitoleate-CoA (16:1), stearate-CoA (18:0), or oleate-CoA (18:1) (Fig. 4*I*) between NPY and vehicle-treated groups, but we did find a large increase in SCD-1 mRNA (Fig. 5*D*), suggesting that SCD-1 activation may be a contributor to the VLDL-TG changes seen, even though changes in long-chain fatty acyl CoA saturation were not observed. The modest reductions in ACC and FAS mRNA suggest that CNS NPY signaling may promote VLDL secretion while inhibiting de novo fatty acid synthesis. This combined mechanism might serve to protect the liver from TG accumulation in the settings in which NPY is increased (such as the high FFA flux seen in fasting).

In the dyslipidemia associated with the development of obesity, total cholesterol levels often remain normal, but a decreased HDL concentration is common. This may be mechanistically linked to the increase in VLDL-TG, because TG-enriched HDL undergoes lipolysis to become smaller and more rapidly cleared from the circulation (49). In our studies, although central NPY signaling did not change total cholesterol levels, it did increase cholesterol in the VLDL fraction and generated a small shift in HDL toward smaller particles (Fig. 6*B*). Thus a single dose of NPY recapitulates, to some extent, obesity dyslipidemia in a lean, fasting animal.

To our knowledge, the only other study addressing the role of CNS NPY signaling on TG metabolism is reported by van den Hoek et al. (43), who showed that in the context of hyperinsulinemia, intracerebroventricular NPY resulted in impaired suppression of VLDL-TG by insulin. Coupled with evidence that brain glucose metabolism modulates VLDL-TG production (34), evidence that resistin induces hepatic insulin resistance via NPY (44), and the data presented herein, a compelling argument for a role of the CNS in lipid metabolism can be made. As mentioned, peripheral NPY signaling potently influences adipose metabolism, and as discovered more recently, CNS melano-

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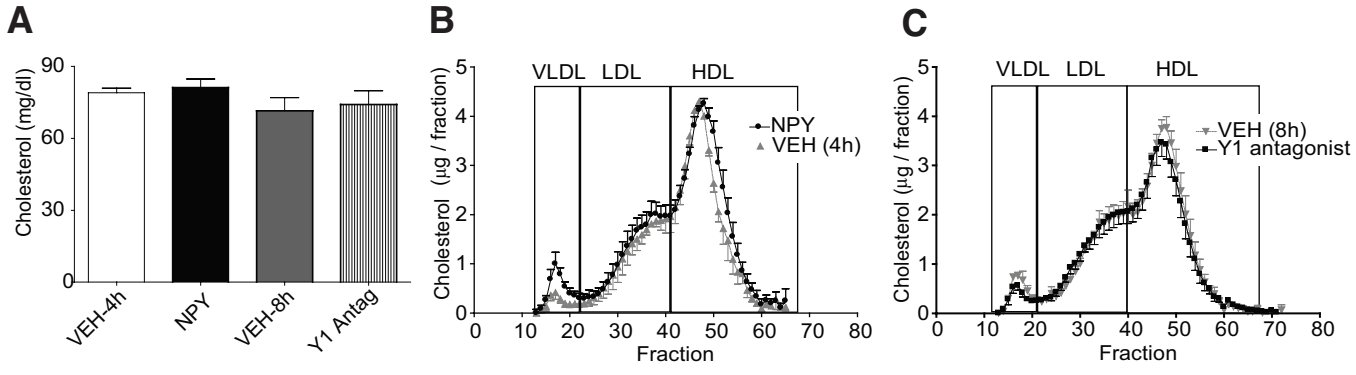

FIG. 6. Effect of NPY signaling on the cholesterol content of lipoprotein fractions determined by FPLC. For all panels, in the absence of tyloxapol, NPY was given after a 4-h fast compared with vehicle (VEH)-4 h, Y1 receptor antagonist was given after an 8-h fast compared with VEH 8 h, and samples were collected 2 h after intracerebroventricular injection. *A*: Total cholesterol ($n = 6$). *B* and *C*: Samples collected 2 h after intracerebroventricular injections were subject to FPLC. Data are means ± SE for cholesterol concentration, $n = 3–6$. *B*: NPY (black circles) compared with VEH-4 h (gray triangles). *C*: The Y1 receptor antagonist (black squares) compared with VEH-8 h (gray upside-down triangles).

cortin signaling also influences lipid homeostasis, particularly in liver and adipose beds (35).

Thus, mounting evidence indicates that energy-homeostatic neurons found in various brain areas are sensitive metabolic sensors that are ideally suited to integrate changes in energy availability with changes in energy demand. NPY-expressing neurons are one such example; its neuropeptide product signals through numerous receptors, including the NPY-Y1 and Y5 receptors, and its signaling output exerts an additional degree of control on VLDL-TG production, perhaps by influencing how the liver handles nutrient and energy flux. Nonetheless, in the context of obesity, where CNS defects in the control of energy homeostasis are increasingly recognized, a common mechanism contributing to both obesity and obesity dyslipidemia is attractive.

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