Hypothalamic Neuropeptide Y (NPY) Controls Hepatic VLDL-Triglyceride Secretion in Rats via the Sympathetic Nervous System

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Excessive secretion of triglyceride-rich very low-density lipoproteins (VLDL-TG) contributes to diabetic dyslipidemia. Earlier studies have indicated a possible role for the hypothalamus and autonomic nervous system in the regulation of VLDL-TG. In the current study, we investigated whether the autonomic nervous system and hypothalamic neuropeptide Y (NPY) release during fasting regulates hepatic VLDL-TG secretion. We report that, in fasted rats, an intact hypothalamic arcuate nucleus and hepatic sympathetic innervation are necessary to maintain VLDL-TG secretion. Furthermore, the hepatic sympathetic innervation is necessary to mediate the stimulatory effect of intracerebroventricular administration of NPY on VLDL-TG secretion. Since the intracerebroventricular administration of NPY increases VLDL-TG secretion by the liver without affecting lipolysis, its effect on lipid metabolism appears to be selective to the liver. Together, our findings indicate that the increased release of NPY during fasting stimulates the sympathetic nervous system to maintain VLDL-TG secretion at a postprandial level.

The secretion of triglyceride-rich very low-density lipoproteins (VLDL-TG) is increased in type 2 diabetic patients. Licht et al. (2) showed that hypertriglyceridemia in patients with the metabolic syndrome strongly correlates with changes in activity of the autonomic nervous system (ANS). This indicates that besides the availability of free fatty acids (FFAs) and hormones, such as insulin, the ANS might be involved in the regulation of VLDL-TG (3,4). Recently, Stafford et al. (5) reported that central infusion of neuropeptide Y (NPY) increases VLDL-TG. The mechanism of this effect remains unclear. We hypothesized that the ANS mediates the effect of NPY on VLDL-TG secretion and that this mechanism is part of the physiological response during fasting, when lipids become the main energy source. First, NPY neurons in the arcuate nucleus (ARC) of the hypothalamus are activated in response to fasting, and the extracellular availability of NPY in the paraventricular nucleus (PVN) is increased (6–8). Second, Viñuela and Larsen (9) showed that intracerebroventricular administration of NPY activates neurons in the PVN projecting to the sympathetic preganglionic neurons. Third, we and others showed that preautonomic neurons in the PVN are anatomically connected to the liver (10,11). These pharmacological and anatomical data support the concept that NPY neurons in the ARC communicate with peripheral metabolic organs via the ANS. Along this line, the intracerebroventricular administration of NPY induces insulin resistance and prevents the inhibitory effect of hyperinsulinemia on hepatic glucose production via activation of the sympathetic nervous system (SNS) (12–14).

In this study, we tested the hypothesis that during fasting, elevated hypothalamic NPY release regulates hepatic VLDL-TG secretion via autonomic inputs to the liver. We first investigated the importance of the ANS in VLDL-TG secretion during fasting by transecting either the sympathetic or parasympathetic nerves to the liver and measuring VLDL-TG secretion after fasting. Since NPY neurons in the ARC are activated during fasting, we then investigated whether a central NPY infusion alters VLDL-TG metabolism via the SNS. Subsequently, we investigated VLDL-TG secretion after fasting in rats with a chemical lesion of the ARC, a component of the sympathetic outflow circuit to the liver (10). It was shown previously in Siberian hamsters that these ARC-lesioned animals do not show increased NPY immunoreactivity after fasting (15). In the final experiment, we investigated the effect of intracerebroventricular NPY on the availability of substrate for VLDL-TG secretion through lipolysis.

RESEARCH DESIGN AND METHODS

Male Wistar rats weighing 280–310 g (Harlan Nederland, Horst, the Netherlands) were ordered and housed in individual cages with a 12/12 light/dark schedule (lights on at 7:00 a.m.). Standard rodent chow and water were available ad libitum, unless stated otherwise. All procedures were approved by the animal care committee of the Royal Netherlands Academy of Arts and Sciences.

Surgery. After 1 week in the facility, rats underwent surgeries according to the different experimental designs. All rats were fitted with an intra-atrial silicone cannula into the right jugular vein and a second silicone cannula into the left carotid artery (16,17). For experiments involving denervation of the liver, hepatic sympathetic or parasympathetic branches were denervated according to the methodology of previous reports (18). A total liver denervation was achieved by cutting the sympathetic and parasympathetic branches to the liver. The effectiveness of the hepatic sympathetic denervation was checked by measurement of norepinephrine content (17). We previously validated our method for selective hepatic parasympathectomy by using retrograde viral tracing (18). For experiments involving acute intracerebroventricular NPY treatment, a stainless steel guide cannula (Plastics One, Roanoke, VA) was implanted into the third ventricle (19). After recovery to presurgery body weight for at least 10 days, rats were connected to an infusion swivel (Instech Laboratories, Plymouth Meeting, PA) 1 day before the experiment for adaption.
**RESULTS**

The Sympathetic nervous system is necessary to regulate VLDL-TG secretion during fasting. We hypothesized that in the fasted state, the ANS is necessary to regulate VLDL-TG secretion. To test this hypothesis, we combined overnight fasting with a selective hepatic denervation (i.e., sham denervation, sympathetic denervation, parasympathetic denervation, or a total denervation). We measured hepatic VLDL-TG secretion after injection of tyloxapol. Tyloxapol inhibits lipoprotein lipase, thereby blocking the uptake of triglycerides by the peripheral tissues. In the absence of chylomicrons carrying triglycerides from the gut, the increase in plasma triglycerides reflects VLDL-TG secretion (20). In the fasted state, VLDL-TG secretion in the selective sympathetically (Sx) denervated rats was significantly lower than in sham and selective parasympathetically (Px) denervated rats (Fig. 1A and B). Importantly, VLDL-TG secretion in Px rats was not significantly different from the sham controls. Moreover, a total hepatic (Tx) denervation did not add to the effect of sympathetic denervation alone (Fig. 1B). The effectiveness of the sympathetic denervation was confirmed by markedly reduced levels of norepinephrine in liver tissue (Fig. 1C). The decrease in VLDL-TG secretion in Sx rats did not result in an increase of liver triglyceride content (P = 0.54).

None of the denervation protocols affected body weight (P = 0.21) or food intake before fasting (P = 0.90) or baseline plasma corticosterone (P = 0.25), insulin (P = 0.27), FFA (P = 0.71), or glucose (P = 0.61) concentrations. These experiments show that an intact hepatic sympathetic innervation is necessary to maintain VLDL-TG secretion during fasting.

**Sympathetic liver denervation prevents the stimulatory effect of NPY on VLDL-TG secretion.** To test our hypothesis that during fasting the increased release of hypothalamic NPY is responsible for stimulating hepatic VLDL-TG secretion via the SNS, we combined the intracerebroventricular infusion of NPY with a selective sympathetic liver denervation. In contrast to the fasted condition in the previous experiment, the rats were instead subjected to the PP condition (4-h fast) to have a low endogenous NPY tone and nearly undetectable chylomicrons in plasma (5). In the PP condition, the sympathetic denervation itself did not change VLDL-TG secretion compared with the sham control.
Infusion of NPY in the third ventricle of the brain in sham-operated rats strongly increased VLDL-TG secretion compared with the vehicle control (4.82 ± 0.35 vs. 3.52 ± 0.28 mmol/L/h) (Figs. 2A and B). However, intracerebroventricular NPY administration in Sx rats no longer resulted in a significant increase of VLDL-TG secretion compared with the vehicle control (4.02 ± 0.14 vs. 3.30 ± 0.22 mmol/L/h). Finally, the NPY-induced VLDL-TG secretion was significantly lower in Sx compared with sham-denervated NPY-infused rats (4.02 ± 0.14 vs. 4.82 ± 0.35 mmol/L/h).

FIG. 1. Sympathetic denervation decreases VLDL-TG secretion in the fasted state. A: Plasma triglyceride levels after intravenous tyloxapol injection in sham and Sx liver-denervated rats. B: Calculated VLDL-TG secretion in sham, Sx, Px, or Tx liver-denervated rats during fasting (one-way ANOVA $P = 0.003$). C: Norepinephrine values in the liver (one-way ANOVA $P < 0.001$). Values are means ± SEM of six to nine rats per group. *$P < 0.05$, **$P < 0.01$.

FIG. 2. Hepatic Sx prevents the stimulatory effect of the intracerebroventricular (ICV) administration of NPY on VLDL-TG secretion compared with hepatic sham-denervated animals. A: Plasma triglyceride levels after tyloxapol injection and intracerebroventricular infusion of NPY (1 μg/μL) in sham and Sx liver-denervated rats compared with intracerebroventricular vehicle infusion in sham and Sx liver-denervated rats. B: Calculated VLDL-TG secretion rates in the sham and Sx groups after intracerebroventricular infusion of vehicle (□) or NPY (■) (two-way ANOVA NPY $P = 0.001$). C: Liver norepinephrine levels in the different groups (two-way ANOVA Denervation $P < 0.001$). D–F: Relative gene expression in the liver is shown for ARF-1 (two-way ANOVA NPY × Denervation $P = 0.046$), CPT1a (two-way ANOVA NPY × Denervation $P = 0.046$), and ACC1 (two-way ANOVA NPY × Denervation $P = 0.216$). Values are means ± SEM of seven to nine rats per group. *$P < 0.05$, **$P < 0.01$. 

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mmol/L/h) (Fig. 2B). The marked decrease in liver norepinephrine levels confirmed a successful selective sympathetic liver denervation (Fig. 2C). There were no significant differences in body weight ($P = 0.28$) or food intake ($P = 0.45$) in the night before the experiment. During the experiment, we observed no differences in plasma corticosterone ($\text{Time} \times \text{Treatment} P = 0.85$) or insulin ($\text{Time} \times \text{Treatment} P = 0.24$) between the four groups. To further dissect the metabolic pathways in the liver by which central NPY controls VLDL-TG secretion, we analyzed gene expression of key hepatic enzymes. NPY infusion increased mRNA levels of ADP-ribosylation factor ($\text{ARF-1}$)–only in sham-operated rats (Fig. 2D). The mRNA levels of other genes involved in VLDL secretion, including apolipoprotein B (ApoB) and microsomal triglyceride transfer protein ($\text{MTTP}$), were not modified by NPY treatment. NPY infusion decreased carnitine palmitoyltransferase 1 $\alpha$ ($\text{CPT1a}$) mRNA levels in the sham-denervated rats but not in the Sx groups (Fig. 2E). We found no differences in expression of genes promoting lipogenesis, including acetyl-coenzyme A carboxylase alpha ($\text{ACC1}$) (Fig. 2F), acetyl-coenzyme A carboxylase beta ($\text{ACC2}$), fatty acid synthase (FAS), stearoyl-CoA desaturase 1 ($\text{SCD1}$), peroxisome proliferator–activator receptor $\gamma$ ($\text{PPAR} \gamma$), and sterol regulatory element–binding transcription factor 1c ($\text{SREBP1c}$). Western blot analysis revealed no changes in total ACC protein or phosphorylated ACC–to–ACC protein ratio after NPY infusion (data not shown).

**Nutritional status has clear effects on genes regulating hepatic lipid metabolism.** With regard to nutritional status, the previous experiments show the following: 1) during fasting conditions, a sympathetic liver denervation lowers VLDL-TG secretion compared with sham-denervated animals; 2) in the PP condition, Sx denervated rats do not show a lower VLDL-TG secretion compared with sham-denervated animals; and 3) comparing experiments 1 and 2 shows that in intact animals, VLDL-TG secretion is not significantly different between PP and fasted sham animals. Together, these results clearly indicate that only during fasting conditions is the SNS necessary to stimulate VLDL-TG secretion in order to maintain VLDL-TG secretion at a PP level. We therefore compared expression levels of key genes involved in lipid metabolism, between the sham and Sx denervated rats in the fasted and the PP experiments, to determine which pathways are regulated by the SNS after fasting (Table 1). Lower baseline concentrations of plasma glucose and insulin and higher baseline plasma concentrations of FFA illustrate the clear metabolic differences between the fasted and PP rats (Table 1). Fasted rats show decreased mRNA expression of genes involved in lipogenesis, including $\text{ACC1}$, $\text{ACC2}$, FAS, $\text{SCD1}$, and PPAR$\gamma$, and increased mRNA expression of a gene promoting oxidation, CPT1a (Table 1). $\text{ACC1}$ ($P = 0.057$) and $\text{MTTP}$ ($P = 0.084$) showed a trend toward an interaction effect in the multivariate model including fasting and denervation. Western blot analysis revealed no changes in total ACC protein or the phosphorylated ACC–to–ACC protein ratio between the Sx and sham groups (data not shown). Thus, although several genes involved in hepatic lipid metabolism are clearly affected by the nutritional status, analysis of mRNA expression of the fasted and PP denervated rats did not reveal the molecular pathways involved in lower VLDL-TG secretion in the Sx rats during fasting.

**Rats with a lesioned arcuate nucleus cannot maintain VLDL-TG secretion during fasting.** We subsequently investigated whether rats with a lesion of the ARC, an important component of the sympathetic outflow circuit to the liver (10), can maintain VLDL-TG secretion at a PP level during fasting. Adequate MSG treatment was shown by a pronounced decrease of NPY immunoreactive fibers in the ARC and PVN (Fig. 3A–D) in adult rats. First, comparing MSG-treated sham liver-denervated rats in the PP and fasted state revealed a significant decrease in baseline plasma triglyceride concentration (Table 2) and a 42% decrease in VLDL-TG secretion (Fig. 3E). But in the fasted MSG-treated rats, a sympathetic liver denervation did not result in an additional significant decrease in VLDL-TG secretion as shown in the first experiment in rats with an intact ARC. The MSG-treated rats in the different groups did not differ in body weight on the day before the experiment or in baseline plasma corticosterone levels (Table 2). Norepinephrine values were significantly lower in the MSG Sx group ($P < 0.05$). The fasted condition was clearly reflected in the significantly decreased plasma glucose concentrations compared with the

| Table 1 |
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| **Comparison of baseline plasma parameters and gene expression in liver tissue between fasted and PP rats with a sham or Sx denervation** |

|          | Fasted sham | Fasted Sx | PP sham | PP Sx |
|----------|-------------|-----------|---------|-------|
| Glucose (mmol/L) | 4.1 ± 0.1 | 3.9 ± 0.2 | 5.8 ± 0.2 | 5.5 ± 0.4 |
| FFA (mmol/L) | 0.78 ± 0.08 | 0.72 ± 0.03 | 0.47 ± 0.05 | 0.41 ± 0.05 |
| Insulin (ng/mL) | 0.22 ± 0.05 | 0.33 ± 0.09 | 1.57 ± 0.22 | 1.57 ± 0.09 |
| $\text{ACC1}$ mRNA | 0.07 ± 0.01 | 0.03 ± 0.01 | 0.09 ± 0.02 | 0.11 ± 0.01 |
| $\text{ACC2}$ mRNA | 0.03 ± 0.01 | 0.05 ± 0.01 | 0.11 ± 0.02 | 0.15 ± 0.04 |
| ApoB mRNA | 0.40 ± 0.07 | 0.52 ± 0.06 | 0.44 ± 0.08 | 0.56 ± 0.06 |
| $\text{ARF-1}$ mRNA | 2.12 ± 0.33 | 2.13 ± 0.32 | 2.14 ± 0.32 | 1.95 ± 0.23 |
| $\text{CPT1a}$ mRNA | 1.25 ± 0.20 | 1.14 ± 0.11 | 0.72 ± 0.08 | 0.71 ± 0.13 |
| FAS mRNA | 1.59 ± 0.61 | 2.07 ± 0.44 | 10.83 ± 1.53 | 10.74 ± 1.10 |
| $\text{MTTP}$ mRNA | 0.63 ± 0.07 | 0.79 ± 0.02 | 0.74 ± 0.06 | 0.69 ± 0.05 |
| PPAR$\gamma$ mRNA | 0.32 ± 0.04 | 0.36 ± 0.07 | 0.43 ± 0.04 | 0.49 ± 0.06 |
| $\text{SCD1}$ mRNA | 0.25 ± 0.07 | 0.41 ± 0.13 | 6.05 ± 1.95 | 5.72 ± 1.89 |
| $\text{SREBP1c}$ mRNA | 0.13 ± 0.04 | 0.26 ± 0.07 | 0.24 ± 0.04 | 0.30 ± 0.13 |

Data are means ± SEM of seven to nine animals per group. In a multivariate analysis including nutritional status and sympathetic denervation, significant differences were observed between the fasted and PP groups in plasma glucose ($P < 0.001$), insulin ($P < 0.001$), FFA ($P < 0.001$), and gene expression levels of $\text{ACC1}$ ($P < 0.01$), $\text{ACC2}$ ($P < 0.01$), FAS ($P < 0.001$), $\text{SCD1}$ ($P < 0.001$), PPAR$\gamma$ ($P < 0.05$), and $\text{CPT1a}$ ($P < 0.01$). $\text{ACC1}$ ($P = 0.057$) and $\text{MTTP}$ ($P = 0.084$) showed a trend toward an interaction effect in the multivariate model including fasting and denervation.
Central NPY does not affect lipolysis. Finally, we investigated whether the increased VLDL-TG secretion after central NPY infusion could be due to increased substrate availability of FFAs through lipolysis. We investigated the effect of intracerebroventricularly administered NPY on lipolysis with a double stable isotope technique measuring endogenous glucose production and lipolysis simultaneously. The stable isotopes [1,1,2,3,3-d5]glycerol and [6,6-2H2]glucose were used (Fig. 4A). No significant effects of central NPY on lipolysis, assessed either by glycerol appearance or plasma FFA levels, could be observed (Figs. 4B and D). On the other hand, a clear effect of central NPY on endogenous glucose production was observed (Fig. 4C). Plasma triglyceride and glucose concentrations showed a trend toward an increase (Fig. 4E and F). We observed no significant differences in plasma corticosterone (Time × Treatment \( P = 0.13 \)), glucagon (Time × Treatment \( P = 0.81 \)), or insulin (Fig. 4G) concentrations between the groups.

DISCUSSION

In recent years, the important role of the central nervous system in controlling liver metabolism has become more and more evident. A number of insightful experiments have considerably increased our understanding of the mechanisms of the hypothalamus to control glucose metabolism through the ANS (12–14,17,18). Our current experiments show that an intact hepatic sympathetic innervation and arcuate nucleus are also necessary to maintain VLDL-TG secretion during fasting. In agreement, a central infusion of NPY cannot increase VLDL-TG secretion in Sx liver-denervated rats. Together, these data indicate that the increased release of hypothalamic NPY during fasting maintains hepatic VLDL-TG secretion via the sympathetic input to the liver. This mechanism could be of physiological importance during fasting, when lipids are the main energy source. However, this mechanism could play a pathophysiological role in conditions characterized by a constant high activity of NPY, as found in animal models of obesity and hypertriglyceridemia (6,8,29). Recent data in humans support this hypothesis, as high sympathetic activity and low parasympathetic activity significantly correlate with components of the metabolic syndrome, including hypertriglyceridemia (2). Additionally, genetic screening revealed a novel polymorphism in the NPY1-5 gene to be associated with reduced serum triglyceride levels in a severely obese cohort (30).

The notion that the autonomic innervation of the liver is not only important for the control of carbohydrate metabolism but also for hepatic lipid metabolism is supported by the findings of a previous study applying phenol to the liver (31). In our study, we used microsurgery to selectively denervate the sympathetic and parasympathetic inputs to the liver and dissect the separate roles of these antagonistic

PP rats (Table 2). With our sample size, we observed no significant effect of fasting on plasma insulin concentrations as seen in nontreated animals. However, MSG-treated animals were clearly hyperinsulinemic and showed larger variance in plasma insulin levels compared with nontreated animals (Tables 1 and 2). These data show that fasted MSG rats cannot maintain VLDL-TG secretion to a PP level. Furthermore, in fasted MSG-treated rats, sympathetic denervation does not affect VLDL-TG secretion compared with fasted sham denervated rats.

**TABLE 2**
Comparison of baseline parameters between the MSG groups PP sham and fasted sham, Sx and Px denervated rats

| Parameter                  | PP MSG sham | Fasted MSG sham | Fasted MSG Sx | Fasted MSG Px | ANOVA  |
|----------------------------|-------------|-----------------|---------------|--------------|--------|
| Glucose (mmol/L)           | 6.1 ± 0.1   | 5.0 ± 0.2*      | 4.9 ± 0.2*    | 4.9 ± 0.2*   | <0.001 |
| Triglycerides (mmol/L)     | 2.26 ± 0.42 | 1.18 ± 0.20*    | 1.38 ± 0.23†  | 1.05 ± 0.19* | 0.016  |
| Corticosterone (ng/mL)     | 61 ± 21     | 53 ± 24         | 32 ± 9        | 100 ± 51     | 0.493  |
| Insulin (ng/mL)            | 2.67 ± 0.48 | 1.77 ± 0.38     | 1.58 ± 0.64   | 1.66 ± 0.43  | 0.381  |
| Body weight (g)            | 331 ± 3     | 332 ± 2         | 331 ± 3       | 334 ± 4      | 0.930  |

Data are means ± SEM of six to eight animals per group. *P < 0.01, †P < 0.05 compared with sham PP.
branches of the ANS. We conclude that during fasting specifically, the SNS is important in the regulation of VLDL-TG metabolism. Our results also extend those of the study of Stafford et al. (5) showing that intracerebroventricular NPY increases VLDL-TG secretion in the PP condition and intracerebroventricular infusion of the Y1 antagonist decreases VLDL-TG secretion during fasting.

The MSG model induces a destruction of 80–90% of the neurons in the ARC, while sparing glial cells or axons passing through the nucleus (22,32). It was shown by others that during fasting the increased NPY immunoreactivity in the ARC and PVN does not occur in the MSG model (15). We are, however, aware that the neurotoxic lesion is not restricted to NPY neurons but affects other neurons in the ARC as well (22). In spite of the caveats of the MSG model, the data from our experiments in MSG animals support a role for the arcuate nucleus and possibly others that during fasting the increased NPY immunoreactivity in the ARC and PVN does not occur in the MSG model (15). We are, however, aware that the neurotoxic lesion is not restricted to NPY neurons but affects other neurons in the ARC as well (22). In spite of the caveats of the MSG model, the data from our experiments in MSG animals support a role for the arcuate nucleus and possibly others that during fasting the increased NPY immunoreactivity in the ARC and PVN does not occur in the MSG model (15).

The molecular mechanism through which the activation of the SNS after intracerebroventricular NPY regulates VLDL-TG secretion probably includes an increased VLDL-TG assembly and decreased β-oxidation. The second step in VLDL assembly is dependent of ARF-1, which was increased in our NPY-infused rats. This may reflect an increased production of mature VLDL particles (37). We also observed decreased mRNA levels of CPT1α, indicating decreased oxidation of FFAs in the liver. When oxidation is inhibited, more substrate can be guided to the alternative route, i.e., resulting in a higher VLDL-TG secretion. Therefore, both of the above-mentioned mechanisms may contribute to increased VLDL-TG secretion during the intracerebroventricular administration of NPY. Surprisingly, the effects of exogenous NPY on oxidation were contrary to changes occurring during fasting (when endogenous NPY levels are high), when increased oxidation was observed. Our results point to the possibility that the central nervous system stimulates the liver to maintain VLDL-TG secretion in competition with the peripheral effects of fasting. This is in accordance with our observation that VLDL-TG secretion does not increase after fasting in spite of the fact that NPY levels are physiologically high.

We show that central NPY has no effect on lipolysis and therefore does not contribute to increased VLDL-TG.
secretion. Others have shown that NPY inhibits lipolysis in vitro and after systemic administration, which possibly reflects a peripheral effect of NPY derived from the autonomic nerve endings on adipocytes (38–40).

In summary, we provide evidence that the activation of NPY neurons in the hypothalamus has a stimulatory role on hepatic VLDL-TG secretion through the SNS. We believe our data are of importance in understanding the physiological and pathophysiological role of the central nervous system in controlling lipid metabolism.

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E.B. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. L.P. researched data and reviewed and edited the manuscript, M.T.A., E.Fo., A.J.B., J.K., and A.A. contributed to discussion and reviewed and edited the manuscript. E.Fl. and A.K. contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. E.B. and A.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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