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Sibutramine alters the central mechanisms regulating the defended body weight in diet-induced obese rats

BARRY E. LEVIN AND AMBROSE A. DUNN-MEYNELL

Neurology Service, Department of Veterans Affairs New Jersey Health Care System, East Orange 07018; and the Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07103

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Levin, Barry E., and Ambrose A. Dunn-Meynell. Sibutramine alters the central mechanisms regulating the defended body weight in diet-induced obese rats. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R2222–R2228, 2000.—Chronic administration of sibutramine lowers body weight, presumably by altering brain monoamine metabolism. Here the effect of sibutramine on sympathoadrenal function (24-h urine norepinephrine and epinephrine levels) and arcuate nucleus (ARC) neuropeptide Y (NPY) and proopiomelanocortin (POMC) expression was assessed in diet-induced obese rats fed a low-fat diet. Chronic (10 wk) sibutramine [5 mg·kg⁻¹·day⁻¹ ip; rats fed ad libitum and injected with sibutramine (AS)] lowered body weight by 15% but only transiently (3–4 wk) reduced intake compared with vehicle-treated controls [rats fed chow ad libitum and injected with vehicle daily (AV)]. Other rats food restricted (RS) to 90% of the weight of AS rats and then given sibutramine restored their body weights to the level of AS rats when allowed libitum food intake. After reequilibration, RS rats were again energy restricted to reduce their weight to 90% of AS rats, and additional vehicle-treated rats (RV) were restricted to keep their body weights at the level of AS rats for 3 wk more. Terminally, total adipose depot weights and leptin levels paralleled body weights (AV > AS = RV > RS), although AS rats had heavier abdominal and lighter peripheral depots than RV rats of comparable body weights. Sibutramine treatment increased sympathetic activity, attenuated the increased ARC NPY, and decreased POMC mRNA levels induced by energy restriction in RV rats. Thus sibutramine lowered the defended body weight in association with compensatory changes in those central pathways involved in energy homeostasis.

norepinephrine; serotonin; epinephrine; neuropeptide Y; proopiomelanocortin; melanocortin; arcuate nucleus; sympathetic nervous system

OBESITY IS A chronic illness that has proven refractory to most types of interventions. Pharmacotherapy successfully lowers body weight in many individuals, but this amount of weight loss is generally limited to only ~10–15% and requires constant administration to maintain this modest loss (1, 4, 5, 12, 34). Centrally acting drugs such as sibutramine and fenfluramine produce such weight loss. The reduction in body weight

achieved with fenfluramine treatment is defended avidly against both over- and underfeeding, suggesting that some new, lower set point has been established by chronic drug treatment (10, 30). Sibutramine inhibits the reuptake of central norepinephrine and serotonin, whereas fenfluramine evokes the release of serotonin (11, 14). Although both norepinephrine and serotonin receptors are known to be involved in the acute regulation of energy intake (9, 14, 17, 18), it is much less clear how changes in their synaptic availability produced by such drugs interact with other central neurotransmitter or neuropeptide systems to produce long-term weight loss. Moreover, it is unclear why there appears to be a limit to the weight loss achievable by these drugs.

The rat model of diet-induced obesity (DIO) has proven to be a useful one for the study of central mechanisms controlling energy homeostasis (22, 23, 25, 26, 28, 29). We have selectively bred two substrains from the parent outbred strain of Sprague-Dawley rats (27). One substrain reproducibly develops DIO, while the other is obesity resistant when placed on a diet relatively high in energy, fat, and sucrose content (27). The current studies were conducted using rats of the DIO substrain, made obese on such a diet, to test the hypothesis that chronic treatment with sibutramine would lower the defended body weight by interacting with neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus (ARC). These neurons and their peptides play a critical role in the central regulation of energy homeostasis (2, 3, 23, 25, 32). It was postulated that chronic sibutramine would reset the expression of one or both of these neuropeptides to function in an apparently normal fashion at the new, lower body weight.

METHODS

Animals and experimental design. Rats selectively bred for the DIO trait were raised in our vivarium (26). They were fed Purina rat chow (no. 5001) and water ad libitum from weaning to 2 mo of age and were housed at 23–24°C on a 12:12-h light-dark cycle (lights on at 1700). At 2 mo, they were fed for

Address for reprint requests and other correspondence: B. E. Levin, Neurology Service (127C), VA Medical Center, 385 Tremont Ave., E. Orange, NJ 07018-1095 (E-mail: levin@umdnj.edu).

14 wk on a high-energy (HE) diet composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (Research Diets). The HE diet contains 4.47 kcal/g with 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose (28). They were then switched back to chow to simulate the switch to a low-fat diet associated with many weight reduction programs in humans. Body weight and food intake were monitored during the second week back on chow. During this week, rats were placed in metabolic cages for collection of 24-h urine catecholamines, and tail blood was obtained by tail nicking for leptin levels. Rats were randomized by weight into four groups of six rats each and were subjected to a two-phase study (Fig. 1). Food intake and body weight were monitored weekly for the entire 10 wk of the study.

Phase I (weeks 1–7). This phase was designed to test the hypothesis that sibutramine would lower the defended body weight. AV rats were given ad libitum access to chow and were injected daily with saline vehicle (0.5 ml/day ip) 30 min before the onset of the dark cycle; AS rats were given ad libitum chow access and were injected with sibutramine (5 mg·kg⁻¹·day⁻¹ in 0.5 ml saline ip) 30 min before dark onset. Vehicle-treated (RV) and food-restricted (RS) rats had their energy intake restricted to 60% of the AV rats for a period of 3 wk to bring their body weights to 90% of AS rats (Fig. 1). At this time, vehicle injections were begun in RV rats, sibutramine injections were started in RS rats, and groups were allowed ad libitum access to chow. RS rats were allowed to regulate their body weight and food intake ad libitum, whereas RV rats had their intakes restricted to maintain their body weights as close to that of AS rats as possible until week 7. During week 1, rats were placed back in metabolic cages for 24 h to collect urine catecholamines. At the end of weeks 1 and 5, tail blood was collected for analysis of leptin levels.

Phase II (weeks 7–10). This phase was designed to lower the body weight of RS rats to 90% of AS rats while keeping

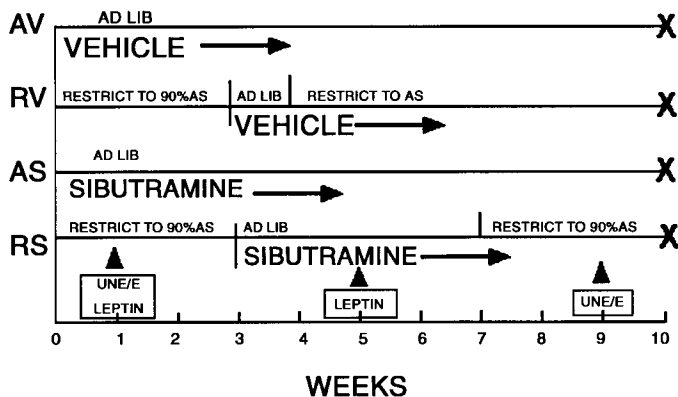


Fig. 1. Time line for experimental procedures for 4 groups of 7 diet-induced obesity (DIO) rats/group. Week 0 is the baseline level beginning with the 2nd wk on chow. **Phase I:** AV rats were allowed ad libitum access to chow and were vehicle injected with saline (0.5 ml/day ip); AS rats were given ad libitum chow access and were injected with sibutramine (5 mg·kg⁻¹·day⁻¹ in 0.5 ml saline ip); RV and RS rats had their intake restricted to 60% of AV rats for 3 wk until their body weights reached 90% of AS rats. They were then begun on saline (RV) or sibutramine (RS) injections and allowed ad libitum access to food. At week 4, energy intake in RV rats was restricted sufficiently to match their weight to that of AS rats for the remainder of the 10-wk experiment. **Phase II:** at week 7, intake of RS rats was restricted to produce a 10% decrease in their body weight compared with AS rats by the end of week 10. Ad lib, ad libitum. UNE/E, 24-h urine norepinephrine and epinephrine levels.

the relative weights of the other groups intact (Fig. 1). This was done to make the rats comparable to those at the end of phase I for assessment of ARC NPY and POMC mRNA. Toward that aim, AV, RV, and AS rats were maintained on their respective treatments whereby AV and AS rats were fed ad libitum chow and RV rats had their intakes restricted to maintain their body weights at the level of AS rats. RS rats had their energy intake restricted further to reduce their body weight to 85% of AS and RV rats by the end of week 10. During week 9, rats were again placed in metabolic cages for urine catecholamine levels. At the end of week 10, ad libitum-fed rats were allowed to eat overnight, and restricted rats were fed at the onset of the dark cycle. Between 0800 and 1100, rats were killed by rapid decapitation. Trunk blood was collected for leptin levels. Brains were quickly removed, frozen on dry ice, and stored at -70°C for assay of NPY and POMC mRNA by in situ hybridization. Fat pads and livers were removed and weighed.

In situ hybridization for NPY and POMC mRNA. Brains were processed for in situ hybridization by minor modifications of previously described methods (24, 25, 38). Briefly, the 511-bp probe [derived from the original probe of Higuchi et al. (16)] for NPY and 923-bp probe for POMC (kindly provided by D. Richard) were subcloned into a pBluescript SK(+) vector at an EcoR I site. Radiolabeled cRNA was synthesized in vitro from BamH I linearized plasmids. Sense and antisense probes were transcribed with T3 and T7 promoters, respectively, using [³⁵S]UTP (1,000 Ci/mmol; New England Nuclear). The probes were hydrolyzed in 0.5 M NaHCO₃ for 30 min. Frozen sections of brain were freeze-thawed on gel-coated slides and fixed in 4% paraformaldehyde. They were treated with acetic anhydride for 10 min and dehydrated through six steps of graded ethanol solutions. Prehybridization was carried out at 50°C for 30 min and then hybridized with labeled sense and antisense probes at 50°C overnight. After treatment with RNase A, sections were washed, dehydrated, dried, and exposed to SB-5 X-ray film (Kodak) for 3 days. The resulting autoradiograms were read by a “blinded” observer using computer-assisted densitometry (Drexel). Areal measures were made in the midportion of the ARC, which has been shown to be most affected by metabolic perturbations (32, 35). Readings from the sections with the three largest areas were averaged for comparison among the groups.

Optical density readings were also made within these areas, but the product of optical density times area did not alter the results. Thus results are given as area alone.

Urine catecholamine and plasma leptin levels. Urine was collected in metabolic cages at 12-h intervals over 24 h and was assayed by HPLC with electrochemical detection (22). Tail blood was collected in EGTA-coated capillary tubes, and the plasma was assayed for leptin by RIA (Linco).

Statistics. Data were analyzed by two-way ANOVA (energy intake pattern × drug treatment) at specified time points during the study. Where significant differences were found ($P \leq 0.05$), intergroup differences were assessed by one-way ANOVA followed by post hoc Scheffé’s test for multiple comparisons.

RESULTS

Period I (weeks 1–7). As seen before (28), there was a plateau in the rate of body weight change of DIO AV control rats once they were switched from the HE diet to a low-fat chow diet (Fig. 2). This plateau is generally associated with a transient decrease in food intake in DIO rats lasting ~2 wk (28). However, ad libitum DIO

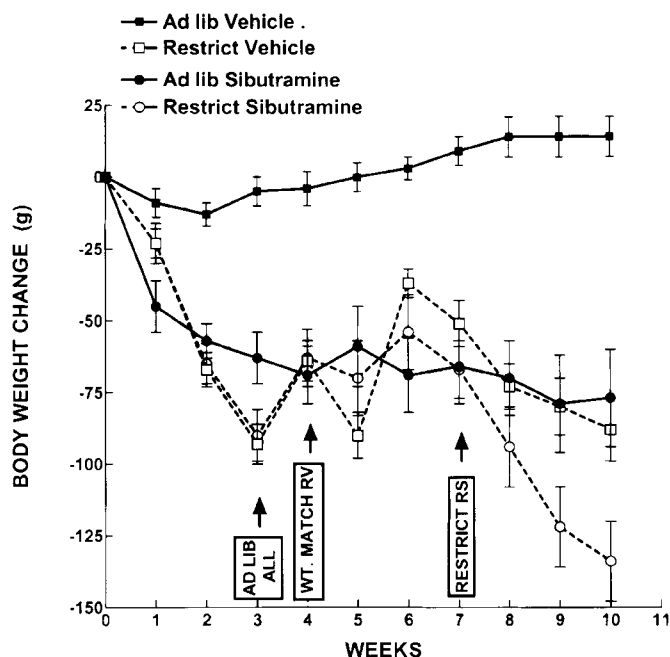


Fig. 2. Change in body weight of rats shown in Fig. 1. AD LIB ALL, rats were placed on chow diet ad libitum at this point; WT MATCH RV, RV rats had their body weights matched to those of AS rats by restricting their food intake; RESTRICT AS, RS rats had their body weights lowered to 90% of AS rats by restricting their food intake. Data are means \pm SE.

rats placed on daily sibutramine (AS; 5 mg/kg ip) began to lose weight during the first week on the drug. By *week 3*, they weighed 90% of AV controls, and their food intake dropped to 76% of AV controls (Fig. 3). At *week 3*, the body weight change of AS rats was 85% of AV rats and thereafter fell gradually to 76% of AV rats by the end of the study at *week 10* (although this decrease did not reach statistical significance). Energy intake in

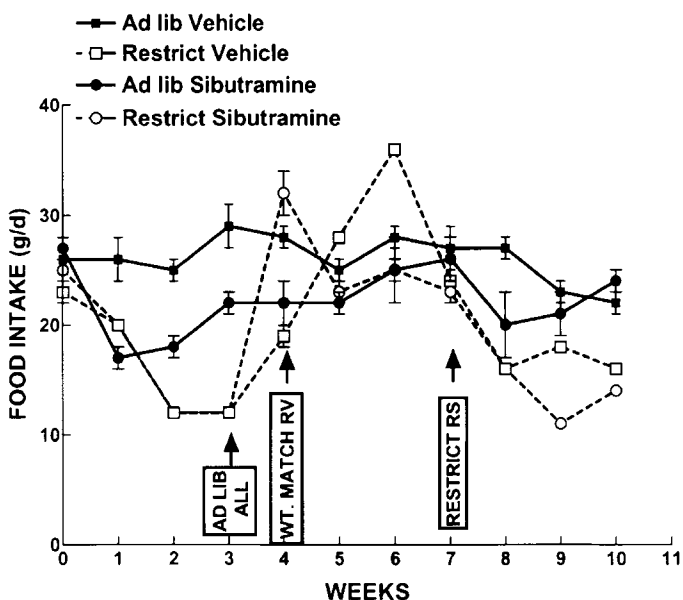


Fig. 3. Food intake (g of chow) during the 10-wk experimental period outlined in the legend of Fig. 1.

AS rats returned to that of AV rats by *week 5* where it remained for the remainder of the study. RV and RS rats were energy restricted to 60% of AV rats. This brought their body weight change to 90% of AS rats by *week 3*. At that point, RV rats were begun on vehicle injections and ad libitum intake while RS rats were begun on sibutramine and ad libitum intake. Despite sibutramine treatment, the body weights of RS rats rose to that of AS rats within 1 wk and remained there until they were again food restricted at *week 7*. This weight gain was associated with a 42% increase in energy intake over AS rats during the first week back on ad libitum intake. Thereafter, their intake matched that of AS rats. Although the body weight gain of RV rats followed the same trajectory as AV rats once they were allowed ad libitum intake, their intake rose more slowly but continued to increase to 128% of AS rats by *week 5* and 163% by *week 6*. This was associated with rather wide swings in their body weights that were finally brought to those of AS rats by restricting their intake to the same intake as AS rats during *week 7*.

Over the first 5 wk, leptin levels fell gradually (Fig. 4). After the first week of restricted intake and/or sibutramine treatment, there was a tendency toward lower plasma leptin levels in AS, RV, and RS rats. However, this did not reach statistical significance. Also, there was not a significant intergroup difference in urine norepinephrine and epinephrine levels during the first week (Fig. 5). By *week 5*, when body weights of AS, RS, and RV rats were all 85% of AV rats, plasma leptin levels in AS and RS rats were 59% of AV rats but were 27% lower than this in RV rats despite their higher food intake than all other groups.

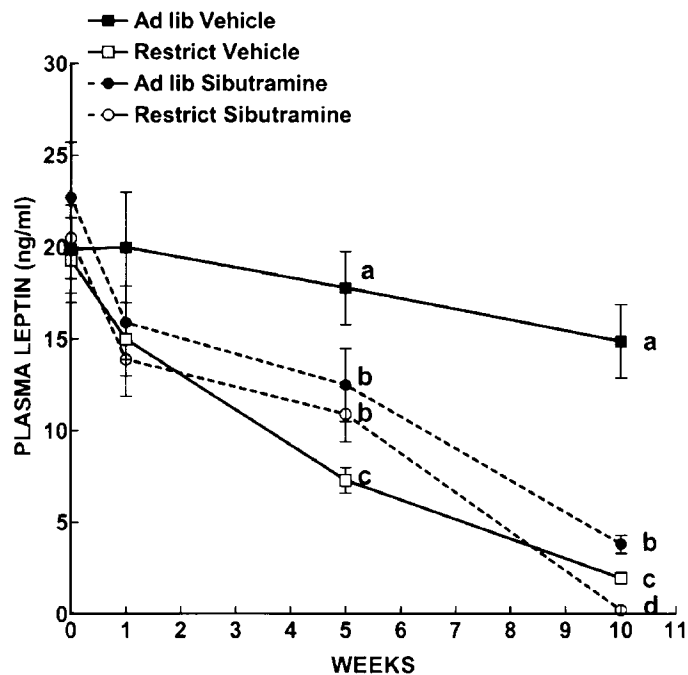


Fig. 4. Tail blood was drawn from rats during *weeks 0, 1, 5, and 10*. Data are means \pm SE. At each period, data points with differing letters differ significantly from each other by post hoc *t*-test after ANOVA showed a significant intergroup difference.

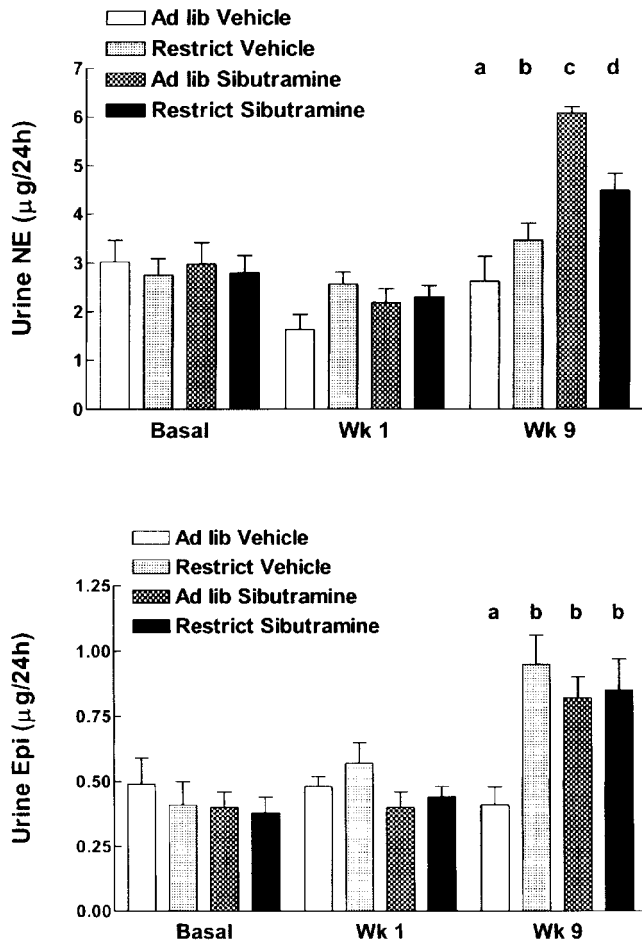


Fig. 5. Urine norepinephrine (NE; A) and epinephrine (Epi; B) levels collected over 24 h during baseline, week 1, and week 9. Data are means ± SE. Bars with differing letters differ significantly from each other by post hoc *t*-test after ANOVA showed a significant intergroup difference.

Period 2 (weeks 7–10). At week 7, RS rats were again food restricted to bring their body weight change to 90% of AS rats by week 10. Thus, at the end of week 10, there were significant differences in body weights among the groups [Table 1 and Fig. 2; $F(3,23) = 10.09$;

$P = 0.001$] that varied as a result of both dietary availability [$F(1,27) = 17.95$; $P = 0.001$] and drug treatment [$F(1,27) = 11.01$; $P = 0.001$]. AS rats weighed 85% of AV rats but had the same energy intake (Fig. 3). To hold RV rats at comparable body weights to AS rats, their intake had to be kept at 23% below that of AS rats. To reduce the body weight of RS rats to 90% of AS rats, their intake had to be held at 40% below that of AS rats. Terminally, there were significant intergroup differences among total fat pad weights [Table 1; $F(3,25) = 31.80$; $P = 0.001$] and plasma leptin levels [Fig. 3; $F(3,25) = 123$; $P = 0.001$]. For both fat pads and leptin levels, these differences were a function of both diet [$F(1,27) = 59.03$; $P = 0.001$; and $F(1,27) = 166$; $P = 0.001$] and drug treatment [$F(1,27) = 30.26$; $P = 0.001$; and $F(1,27) = 100$; $P = 0.001$]. In AS rats, total fat pad weights were 54% and plasma leptin levels were 25% of those in AV rats. In RV rats, fat pad weights tended to be lower, whereas plasma leptin levels were significantly 48% lower than those in AS rats. Total adipose pad weights in RS rats were 81% lower and plasma leptin levels were 95% lower than AS rats (Fig. 4). For abdominal fat depots (retroperitoneal, perirenal, mesenteric), both energy availability [$F(1,27) = 95.73$; $P = 0.001$] and drug treatment [$F(1,27) = 21.23$; $P = 0.001$] were significant factors. Interestingly, AS rats had 87% heavier abdominal but 231% lighter peripheral (inguinal) depots than RV rats of comparable body weights. On the other hand, energy availability [$F(1,27) = 42.43$; $P = 0.001$] and not drug treatment ($P > 0.05$) was the major determinant of liver weights. Despite their 24% lower body weights, AS rats had comparable liver weights to AV rats, whereas the energy-restricted RV and RS animals had 31 and 45% lower liver weights than their respective controls.

Sibutramine treatment appeared to increase sympathetic activity during week 9 [Fig. 5; $F(1,27) = 23.71$; $P = 0.001$]. AS urine norepinephrine levels were 130% greater than those in AV rats. Surprisingly, energy restriction did not affect urine norepinephrine levels in vehicle-treated rats. Although norepinephrine levels in RS rats were 26% lower than those in AS rats, they

Table 1. Terminal body, adipose, and liver weights

	AV	RV	AS	RS
BW _{final} , g	617 ± 18 ^a	506 ± 14 ^b	524 ± 15 ^b	471 ± 8 ^c
Adipose depots, g				
Retroperitoneal	19.4 ± 2.1 ^a	8.71 ± 1.41 ^b	13.0 ± 0.6 ^b	2.32 ± 0.21 ^c
Perirenal	2.81 ± 0.42 ^a	0.91 ± 0.08 ^b	0.95 ± 0.07 ^b	0.42 ± 0.07 ^c
Mesenteric	12.8 ± 1.3 ^a	3.01 ± 0.47 ^b	9.92 ± 0.47 ^c	1.83 ± 0.19 ^b
Inguinal	13.5 ± 2.1 ^a	7.21 ± 0.46 ^b	3.12 ± 0.10 ^c	3.31 ± 0.18 ^c
Total abdominal depots	35.0 ± 3.6 ^a	12.7 ± 1.7 ^b	23.8 ± 1.1 ^c	4.44 ± 0.34 ^d
Total depots	49.1 ± 6.1 ^a	20.1 ± 1.7 ^b	26.7 ± 1.2 ^b	7.7 ± 0.5 ^c
Liver, g	21.3 ± 1.6 ^a	14.6 ± 0.9 ^b	22.1 ± 1.8 ^a	12.1 ± 0.6 ^b

Data are means ± SE. Units are g. Groups of 7 selectively bred diet-induced obesity rats were subjected to 1 of 4 experimental conditions. AV rats were given chow ad libitum and injected with vehicle daily; AS rats were fed chow ad libitum and injected with sibutramine (5 mg/kg ip) daily; RV rats were injected daily with vehicle and had their intake matched to AS rats; RS rats were injected daily with sibutramine and had their intake restricted to bring their body weight to 90% of the AS rats at weeks 3 and 10 (final) of the study. Total abdominal depot weights are the combination of retroperitoneal, perirenal, and mesenteric pads. Data for a given experimental group for a given parameter with differing superscripts differ by $P \leq 0.05$ by post hoc *t*-test after intergroup differences were found by ANOVA.

were still 70% higher than AV rats. Thus both groups of sibutramine-treated rats had higher urine norepinephrine levels than both groups of vehicle-treated rats. On the other hand, AS, RV, and RS rats all had 100–132% higher urine epinephrine levels than AV rats at week 9.

Arcuate NPY and POMC mRNA expression. There was a main effect of food intake restriction that was associated with increased ARC NPY mRNA expression [$F(1,27) = 22.29$; $P = 0.001$]. However, this effect was significant by post hoc testing only in vehicle-treated animals where RV levels were 46% higher than AV levels (Fig. 6). Similarly, energy restriction lowered the expression of ARC POMC mRNA [$F(1,27) = 4.18$; $P = 0.05$]. Again, this effect was significant only in vehicle-treated rats where RV levels were 26% lower than AV levels.

DISCUSSION

In rats selectively bred to express the DIO trait, chronic sibutramine treatment reset the defended body weight at ~76–85% of controls. Although energy intake was suppressed over the first 4 wk on sibutramine treatment, it was equal to controls after this time. Weight loss induced by energy restriction is usually accompanied by reduced sympathetic activity (26) and ARC POMC levels (3, 20) and by elevated NPY levels (3, 23, 25). For unclear reasons, sympathetic activity (24-h urine norepinephrine levels) was not reduced in restricted vehicle-injected control rats. However, the expected changes in terminal ARC POMC and NPY mRNA expression did occur. On the other hand, sibutramine-treated rats had elevated sympathetic activity after weight loss associated with chronic treatment. They also had no significant change in ARC NPY or POMC mRNA expression, even when their spontaneous 15–25% weight loss on drugs was compounded by an additional 10% weight loss produced by restricting their intake. This is particularly interesting because the additional restriction-induced weight loss was not

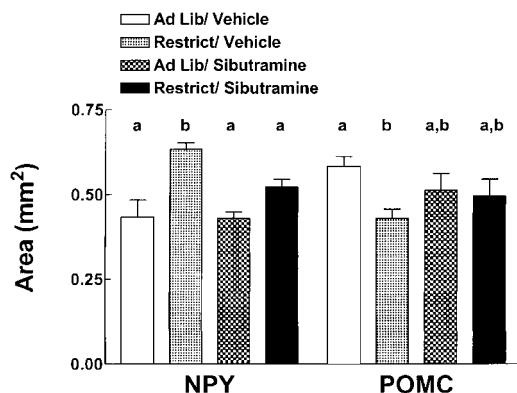


Fig. 6. Arcuate nucleus neuropeptide Y (NPY) and proopiomelanocortin (POMC) mRNA expression by in situ hybridization. Data are means + SE of the 3 largest areas (mm^2) taken through the rostromedial arcuate nucleus. Bars with differing letters differ significantly from each other by post hoc *t*-test after ANOVA showed a significant intergroup difference.

defended in sibutramine-treated rats, despite their failure to increase NPY or decrease POMC expression significantly. This suggests that sibutramine not only “reset” these central pathways to function at a new, lower level but that it additionally dampened their responsiveness to further reductions in body energy stores. Despite this dampening of the central energy homeostasis pathway function, sibutramine-treated rats would not defend the additional, restriction-induced body weight loss. It is worth pointing out that there was a nonsignificant tendency for ARC NPY expression to be increased in these restricted, sibutramine-treated rats. This suggests that other energy homeostasis systems in the brain or periphery are engaged in the defense of the lower body weight set by sibutramine when energy restriction lowers energy stores below that defended level. If so, these other systems would be logical therapeutic targets if loss of further body weight was the desired goal.

The mechanisms by which sibutramine lowers the defended body weight are not well defined. A similar lowering of the defended body weight has been demonstrated with the serotonin-releasing agent fenfluramine (11). Sibutramine is both a norepinephrine and serotonin reuptake blocker (11, 31). It reduces food intake acutely (17, 18, 33) and weight gain chronically (1, 4, 7, 12, 34). It is unclear what the chronic effect of either fenfluramine (5, 21, 36) or sibutramine might be on brain serotonin or norepinephrine turnover, since available studies differ dramatically in both their designs and results. Furthermore, it is unclear what effect any alterations in synaptic availability of either neurotransmitter might have on ARC NPY or POMC expression. In general, there appears to be an inverse relationship between serotonin or norepinephrine availability and the expression of ARC NPY and POMC mRNA (2, 9, 19), but many of these data were gathered in relatively acute experiments and/or under circumstances where turnover of the neurotransmitters was not even measured (2, 9). Furthermore, virtually nothing is known about the effect of chronic administration of drugs like sibutramine on the synaptic release of these transmitters, how such alterations might affect actual synaptic release of NPY and POMC, or how this might affect postsynaptic NPY or melanocortin receptors. These are really the most important issues to be considered. All that can be said here is that chronic sibutramine administration clearly dampened the normal upregulation of ARC NPY and downregulation of ARC POMC mRNA expression in the face of reduced carcass energy stores.

The fact that sympathetic activity was persistently elevated in sibutramine-treated rats is in keeping with other rat (6, 14) studies showing that sibutramine exerts a sympathetically mediated thermogenic effect. This could be secondary to the norepinephrine reuptake blocking properties of sibutramine. However, results in humans have been more variable in this regard (13, 15, 34). Nevertheless, increased thermogenesis would help explain the persistent lowering of body weight in the absence of lowered energy intake in

our rats. Because neither energy expenditure nor full analysis of carcass energy content was carried out here, it is not certain that the concomitant maintenance of a lowered body weight and energy intake was not simply due to a reduction in the metabolically active lean body mass. The finding of elevated urine epinephrine levels in all experimentally manipulated groups (AS, RS, RV) at 9 wk suggests that there was some element of chronic stress involved in either drug treatment or involuntary restriction of energy intake.

Sibutramine-treated rats appeared to preferentially lose carcass fat over lean body mass. This finding was supported by the finding of reduced fat pad mass and leptin levels with no change in liver weights. However, this hypothesis cannot be supported fully in the absence of full carcass composition studies. Actually, the fact that ad libitum-fed, sibutramine-treated rats had comparable fat pad weights but considerably higher liver weights compared with vehicle-treated rats restricted to the same weight suggests that sibutramine might have had an actual sparing effect on lean body mass. The higher plasma leptin levels in the sibutramine-treated rats vs. comparable-weight but energy-restricted control rats further suggest that sibutramine blunted the leptin-lowering effect of starvation (26, 29). Similarly, sibutramine treatment also dampened the starvation-induced elevation of ARC NPY and lowering of POMC expression seen in restricted control rats. The combination of sibutramine and food restriction had an additive effect on lowering fat pad and liver weights as well as plasma leptin levels. Again, this suggests that such energy restriction in sibutramine-treated rats had dropped them below their reset, defended body weights. Finally, although sibutramine treatment lowered total fat pad mass comparably to restricted controls, this was primarily due to loss of peripheral adipose tissue as their abdominal pad weights were significantly higher than restricted controls. This preferential sparing of abdominal fat is just the opposite of what is seen in sibutramine-treated humans undergoing weight reduction (37).

In summary, chronic administration of the norepinephrine and serotonin reuptake blocker sibutramine appears to lower the defended body weight of rats with DIO. This is similar to findings reported with the serotonin-releasing agent fenfluramine (10, 30). The lowering of the defended body weight was associated with an apparent resetting of ARC NPY and POMC expression, suggesting that this might be a potential mechanism by which sibutramine acts. Thus, despite reduced carcass energy stores, sibutramine-treated rats showed a persistent increase in sympathetic activity and a failure to elevate ARC NPY or depress POMC mRNA expression, as was seen in vehicle-treated rats chronically weight reduced to the same body weight by energy intake restriction. It is interesting that the level of reduction of body weight was ~15%. This is qualitatively similar to the "basement effect" of both sibutramine (1, 4, 12, 34) and fenfluramine (8) reported in human studies. Sibutramine-treated rats would not defend an even lower body weight brought about by the

combination of energy intake restriction and sibutramine treatment. Thus further weight loss, above that effected by sibutramine treatment, is likely to require additional treatment modalities. Specifically, the current studies predict that drugs that target other neuropeptides involved in energy homeostasis and/or that have an additional effect on either NPY or melanocortin systems will be required to produce additional weight loss in obese individuals.

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