Contrasting effects of systemic and central sibutramine administration on the intake of a palatable diet in the rat

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Keywords: Feeding, food intake, nucleus accumbens, paraventricular nucleus of the hypothalamus, sibutramine
Abstract

Sibutramine hydrochloride monohydrate is the only centrally active weight-modifying agent currently approved by the FDA for long-term use in the treatment of obesity. Systemic sibutramine treatment has been shown to reduce food intake in humans and rodent models in a manner that is consistent with the enhancement of satiety mechanisms. Although it is generally assumed that the hypophagic effects of the drug are mediated by actions within the brain, the locus or loci of these effects remains unclear. These experiments compared the effects of systemic and intracranial injections of sibutramine on the intake of a palatable diet in non-deprived animals. Consistent with prior reports, systemic injections of sibutramine hydrochloride (at 0, 0.5, 1.0, or 3.0 mg/kg sibutramine i.p.) dose-dependently reduced feeding on a high fat/high sucrose diet across a 2-hr feeding session, but did not alter water intake or locomotor activity. In contrast, bilateral injections of sibutramine (at 0.0, 2.0, 4.0 and 10.0 µg/0.5 µl/side) into either the paraventricular nucleus of the hypothalamus (PVN) or the medial nucleus accumbens shell (ACb) significantly and dose-dependently increased food intake of the sweetened fat diet. ACb treatment also modestly inhibited locomotor behavior; intracranial injections had no effect on water consumption. These experiments are the first to suggest that sibutramine treatment may have distinct actions upon separate neural circuits that modulate food intake behavior in the rat.
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In the United States, obesity-related costs are estimated to account for 5-7% of annual medical expenditures, or over $75 billion a year [5]. The only centrally active, FDA-approved drug for long-term weight maintenance is sibutramine (Meridia®, Reductil®), which modestly reduces food consumption when given systemically, and may also attenuate the decline in metabolic activity associated with restricted caloric intake [10, 16, 20, 35]. In humans, long-term therapy on sibutramine significantly increases maintained weight loss at one year or greater when paired with dietary counseling, with reports of between 4.0 kg and 6.2 kg average reductions over that of placebo-treated control subjects [1, 6, 8, 9, 24, 29, 30].

Sibutramine and its active metabolites serve to inhibit serotonin (5-HT) and noradrenaline (NA) reuptake transporters, increasing central 5-HT and NA tone. In rodent models examining food intake and feeding behaviors, systemic sibutramine treatment by oral gravage or by i.p. injection reduces subsequent feeding [7, 13, 20]. The behavioral profile of this reduced food intake suggests a role for the sibutramine in enhancing satiety mechanisms [34]. Consistent with its pharmacological actions at 5-HT and NA transporters, the effects of sibutramine on food intake and thermogenesis are mediated by activation of both serotonin and noradrenaline systems, as its feeding and thermogenic effects can be attenuated by pretreatment with antagonists for andrenergic and serotonergic receptors [7, 13].

Although it is generally assumed that the hypophagic effects of sibutramine treatment are mediated by action within the brain, the locus or loci of this effect remains unclear. Serotonin receptors within both feeding and reward pathways have been shown to regulate food intake. For instance, serotonergic receptor stimulation of the paraventricular nucleus of the
The hypothalamus reduces feeding behavior [18, 19, 31, 33, 36, 37]. Similar feeding reductions can be seen following serotonin receptor stimulation of hindbrain feeding circuitry [11, 17, 32]. Additionally, recent reports that utilized selective 5-HT receptor agonists within the nucleus accumbens shell have demonstrated diverse roles for individual 5-HT receptors upon food intake, with local 5-HT$_4$ and 5-HT$_{1/7}$ receptor stimulation reducing feeding behavior, and 5-HT$_6$ agonism increasing food intake [14, 27]. The hypophagia that is induced by sibutramine may therefore be due to its actions upon one or more of these feeding pathways. This study compared the effects of systemic sibutramine treatment on the 2-hr intake of a palatable sweetened fat diet with that of local sibutramine injections into either the paraventricular nucleus of the hypothalamus (PVN) or the shell of the nucleus accumbens (Acb), both of which heavily express 5-HT and NA receptors and have been shown to modulate food intake in response to energy need and the rewarding properties of a diet, respectively [15, 40].

All experiments were conducted in accordance to NIH animal care guidelines and were approved by the Wake Forest University Animal Care and Use Committee. Twenty-four adult male Sprague-Dawley rats (Harlan, Madison, WI) were acclimated to dual housing in a colony room maintained at ~21 °C with a 12-hr light–dark cycle. Water and standard rat chow (Purina Protab RMH 3000) were available *ad libitum* in the home cage. Two groups of rats underwent standard aseptic procedures to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the paraventricular nucleus of the hypothalamus (with the nose bar level to the interaural plane; -1.5 mm posterior and 0.6 mm lateral to bregma, 6.5 mm ventral to the skull surface) or the nucleus accumbens shell (with the nose bar set at +5.0 mm above the interaural plane; 3.1 mm anterior and 1.0 mm lateral to bregma, 5.0 mm ventral to skull surface). Rats
recovered for one week prior to their first exposure to the feeding chambers and the sweetened fat diet. The systemically treated rats received no surgical procedures.

Feeding chambers were constructed with clear acrylic, with internal dimensions of 42 cm wide, 30.5 cm deep and 33 cm tall. A water bottle was hung at one end of the chamber, and at the opposite end of the chamber a food intake monitor (Med Associates, St. Albans, VT) was filled with a high fat/high sucrose diet containing 278.3 g/kg vitamin free casein, 100.0 g/kg sucrose, 4.2 g/kg DL-methionine, 441.2 g/kg shortening, 77.7 g/kg safflower oil, 26.3 g/kg cellulose, 53.3 g/kg mineral mix, 15.2 g/kg vitamin mix and 3.8 g/kg choline chloride (Kilocaloric value of diet=6.2 kcal/g; Teklad Diets, Madison, WI, USA). Rats maintained on ad libitum rat chow eat this sweetened fat diet when it is presented, and prior reports demonstrate that its intake is sensitive to stimulation of Acb mu-opioid receptors or blockade of Acb muscarinic acetylcholine receptors (increasing or decreasing feeding, respectively) [26, 39, 41]. Infra-red eyebeams were located along the floor at three locations (5 cm above the wire floor) to measure ambulation; four additional IR beams were placed at a height of 16 cm above the floor to index rearing behavior. IR beam interruption was continually recorded by Med-PC software (Med Associates, St. Albans, VT). The weights of the food monitors were recorded at 10-sec intervals throughout each feeding session. A speaker maintained an ambient level of white noise at 65 dB in the experimental room.

All groups of rats received six days of habituation to the feeding chambers and diet prior to pharmacological treatments. Each session consisted of two hours of free access to the sweetened fat diet and water. Drug treatments for the systemically-treated group were modeled after the protocol recently reported by Tallett, Blundell, & Rodgers [34]. During the final three days of habituation to the chambers, each rat received an i.p. injection of 1ml/kg normal saline
30 min prior to the feeding session, to acclimate the rats to the injections prior to starting pharmacological treatments. Following the final day of habituation, each rat (N = 8) was administered one of four IP doses of sibutramine hydrochloride, at 0, 0.5, 1.0, & 3.0 mg/kg in saline, (Tocris Biosciences, Ellisville, MO) across four test sessions, spaced seven days apart to allow wash-out of the drug. All drug doses were administered in a volume of 1 ml/kg. Thirty minutes following the injection, rats were placed into the feeding chambers for a 2-hr feeding session. The order of drug presentation was randomly determined for each rat, with the restriction that all drug doses were represented on each treatment day. On intervening (non-treatment) days, rats were maintained on 2-hr exposure to the palatable diet in the feeding chambers for six days of the week.

For the intracranial infusion groups, we used procedures as previously reported [27]. Briefly, on the final two days of habituation to the feeding chambers, rats received mock infusions to allow acclimation to microinfusion procedures. Experimental treatments began 48 hrs after the last mock infusion. During vehicle and drug infusions, injection cannulas (30 gauge) were lowered into the paraventricular nucleus of the hypothalamus or the shell of the nucleus accumbens and 0.5 µl of solution was delivered (at a rate of 0.32 µl per minute) by a Harvard Apparatus (Holliston, MA) microinfusion pump. Injectors remained in place for an additional minute to allow for diffusion, and rats were then immediately placed in the feeding chambers. Each rat received all four intracranial doses of sibutramine, at 0, 2, 4, or 10 µg/0.5µl/side (0, 6.3, 12.6, or 31.6 nmol/side), in a 10% 2-hydroxypropyl-β-cyclodextrin/saline vehicle solution (Sigma, St. Louis, MO) across multiple treatment days. Each treatment was spaced 48 hrs apart, and the drug order was randomized for each rat. When the experiments were completed, the rats were sacrificed and the location of the injection site was verified utilizing standard histological
procedures. Four rats were excluded from final analysis due to misplacement of cannula tips (N = 3; Acb placements) or due to equipment failure (N = 1; PVN group).

For all experiments, dependent measures included the amount of sweetened fat diet eaten over the 2-hr period, ambulation within the chamber (assessed as the number of complete crossings of the chamber from end to end), the number of rears recorded, and total water intake during the feeding session. Feeding data were analyzed utilizing two-way repeated measures ANOVAs, comparing food intake assessed across time (at 5-min intervals within each 2-hr session) and drug doses. For groups that had significant drug and/or drug x time interaction effects, ANOVAs were run comparing the main effects of drug dose at the time points of 30, 60, 90 and 120 min to further assess the consistency and time course of the drug effects. Locomotor measures and water intake were analyzed with one-way repeated measures ANOVAs with drug dose as the independent variable; Tukey HSD post-hoc analyses were conducted to compare behaviors between vehicle and drug treatments, as appropriate.

Systemic injections of sibutramine hydrochloride potently and dose-dependently reduced food intake on the sweetened high fat diet used here (see Figure 1A). A repeated measures ANOVA comparing food intake across both drug dose and time yielded a significant effect of drug ($F_{3,21} = 11.68$, $p < .01$) and a significant drug X time interaction ($F_{69,483} = 2.37$, $p < .01$). This effect was significant by 30 min into the feeding session ($F_{3,21} = 11.68$, $p < .01$) and remained so until the end of the feeding session ($F_{3,21} = 13.98$, $p < .01$); post-hoc analysis comparing drug doses to vehicle infusion verified a significant decrease in feeding for the 1.0 and 3.0 mg/kg doses when compared to saline injection ($p < .05$ according to Tukey’s HSD). Water intake, although low in all cases, was not affected by drug dose ($F_{3,21} = 2.8$, $p > .05$), suggesting a specific effect of drug treatment on food-directed consumption. Likewise, systemic
sibutramine treatment did not significantly impact ambulation (F_{3,21} = 0.12, p > .05) or rearing behavior (F_{3,21} = 0.07, p > .05). As has been previously reported [34], treatment at 3 mg/kg resulted in a significant weight reduction at 24 hours, averaging 8.2 ± 0.77 g (SEM; data not shown). Rats regained their weight within three days. However, on the day following sibutramine treatment, intake of the sweetened fat diet did not significantly differ as a result of the prior day’s drug treatment, (F_{3,21}= 1.45, p = .26), demonstrating that intake on the palatable diet had recovered within 24 hours.

In contrast to the effects of i.p. injections of sibutramine on feeding behavior, intracranial injections directly into the PVN promoted intake of the high fat/sucrose diet. As can be seen in Figure 1B, local injections of sibutramine into the PVN dose-dependently increased feeding (drug effect: F_{3,18} = 4.88, p = .012; drug X time interaction: F_{69,414} = 3.88, p < .01). Food intake was significantly enhanced by 30 min into the session (F_{3,18} = 5.94, p = .005) and the increase was maintained until the end of the session (F_{3,18} = 3.98, p = .024). The 10.0 µg dose of sibutramine reliably raised food intake compared to vehicle infusions at 30, 60, 90, and 120 min (all p’s < .05 according to Tukey’s HSD). Total water intake was once again unaffected by sibutramine injection; neither were there significant effects of drug treatment on ambulation or rearing (all p-values > 0.1). Despite the 2-hr feeding increase following the 10 µg/side dose, there was no evidence of weight gain or loss during the subsequent 24-hr period.

Blocking nucleus accumbens shell 5-HT and NE reuptake transporters with sibutramine also increased food intake early in the session, although its effects were more transient in nature than PVN infusions (see Figure 1C). A repeated measures ANOVA comparing drug dose across time yielded a significant interaction of drug X time (F_{69,276} = 1.39, p = .035), and a trend towards a significant effect of drug (F_{3,12} = 3.02, p = .072). Follow-up analysis at 30, 60, 90, and
120 minutes yielded a significant drug effect at 30 min, but not the other time points ($F_{3,12} = 10.01, p = .05$). Post-hoc Tukey HSD verified a significant increase in food intake at 30 minutes for the 10 µg dose when compared to the vehicle infusion. Water intake was not affected by nucleus accumbens treatment ($F_{3,12} = 0.22, p < .05$), although there were significant effects of Acb sibutramine on both ambulatory ($F_{3,12} = 3.62, p = .045$) and rearing behaviors ($F_{3,12} = 5.74, p = .011$). Overall, sibutramine treatment tended to modestly inhibit locomotor activity, regardless of dose and not in a dose-dependent manner. Post-hoc analyses using Tukey’s HSD revealed a significant reduction of rearing at the 2.0 µg dose when compared to vehicle. For both rearing and ambulation, all other pairwise comparisons of vehicle to drug doses were non-significant ($p > .05$). There was no evidence of weight gain or loss resulting from Acb drug treatment during the 24-hr period following drug injections.

The goal of the current experiments was to compare the effects of systemic sibutramine treatment with that of intracranial infusions into the PVN and the Acb. Consistent with previous work that has shown that systemic sibutramine treatment reduces food intake and advances the behavioral satiety sequence [34], rats treated with i.p. infusions of sibutramine dose-dependently reduced their intake of the sweetened fat diet across the 2-hr feeding sessions. Surprisingly, direct infusions of sibutramine into the PVN increased, rather than inhibited, food intake. Acb treatment also transiently increased feeding behavior, although not to the extent seen following PVN infusions. Locomotor measures were unaffected by systemic or PVN injections, and the modest inhibition of locomotor activity following Acb injections did not appear to impair consummatory behavior within this paradigm.

The PVN was targeted for these experiments due to its heavy expression of both NA and 5-HT receptors. Prior work, focused upon the role of hypothalamic 5-HT in food intake and
satiety, has demonstrated that 5-HT receptors within the PVN down-regulate feeding behavior, possibly by enhancing satiety mechanisms. For instance, infusing serotonin directly into the medial nuclei of the hypothalamus alters dietary preferences and reduces food intake [3, 19]. Furthermore, medial hypothalamic injections of agonists that selectively activate 5-HT$_{1A}$ or 5-HT$_{2C}$ receptors reduce feeding in rats [21], and stimulation of 5-HT$_{2A/2C}$ receptors of the PVN attenuates food intake elicited by NPY receptor stimulation of the same region [3, 4]. As systemic treatment with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors antagonists has been shown to attenuate sibutramine’s effect on food intake [7, 13], we had hypothesized that the PVN might be a locus of effect for the action of sibutramine. However, sibutramine also blocks the reuptake of NA, and in rat brain tissues it shows a higher capacity to block NA reuptake than that of 5-HT or dopamine [12]. It has long been known that hypothalamic NA infusions increase feeding and gnawing behaviors through the activation of α$_2$ receptors, and that within the hypothalamus this effect is most sensitive within the PVN [18, 38]. The increased feeding observed in the current experiments suggests that, when injected directly into the PVN, sibutramine may enhance feeding through its augmentation of NA α$_2$ receptor activity, an effect not balanced by co-blockade of 5-HT transporters.

The potential roles for 5-HT and NA within the feeding and reward circuitry of the Acb shell are not yet clear, although we show here that blockade of reuptake transporters for both neurotransmitters transiently augment food intake. Previously, it has been shown that unilateral injections of either 5-HT or NA directly into the Acb fail to alter food intake in rats [19, 25]. However, it has been argued that some 5-HT receptors within the meso-accumbens pathway mediate the reinforcing properties of drugs of abuse [23, 28], and recent investigations that have injected selective 5-HT receptor agonists into the ACb have shown that specific 5-HT receptors
differentially impact food intake and locomotor behavior. Specifically, Acb 5-HT₄ receptors have been shown to mediate the anorectic properties of MDMA in the mouse [14]. Additionally, we have recently shown that co-stimulation of 5-HT₁,₇ receptors within the rat ACb reduces food intake and locomotor activity, while activation of 5-HT₆ receptors enhances intake of food in a paradigm identical to that used in the current investigation [27].

It is important to note that sibutramine itself is a relatively weak inhibitor of NA and 5-HT reuptake transporters, and that systemic administration recruits cytochrome P450 enzymes within the liver to metabolize sibutramine into its potent primary and secondary amine metabolites BTS 54 354 and BTS 54 505 [2, 12]. It has been reported that intracerebroventricular injections of either BTS 54 354 or BTS 54 505 inhibit feeding in the mouse [12]. Sibutramine injections directly into the brain by-pass peripheral metabolic activity, and although cytochrome P450 enzymes involved in the metabolism of sibutramine have been shown to be present in brain tissue [22], it is not presumed that peripheral and central injections utilized here resulted in similar enzymatic activity. Thus, it is possible that the contrasting behavioral effects shown following peripheral or central sibutramine injections in these experiments are due to differing concentrations of sibutramine versus its metabolites in the brain regions examined. This explanation for the hyperphagic effects of the intracranial injections seems unlikely, however, as the pharmacological targets of both the parent molecule and its active metabolites are similar, despite differing affinities to the reuptake proteins.

This report is the first to compare the effects of systemic administration of sibutramine hydrochloride on the intake of a palatable diet with that of intracerebral injections into specific brain nuclei known to be involved in the serotonergic regulation of food intake. Neither injection of sibutramine into the PVN, nor into the ACb, mimicked the hypophagic effect of
systemic treatment, suggesting that the actions of sibutramine on feeding behavior are not primarily regulated through its actions directly upon either site. Further examination of the role of sibutramine (and its metabolites) within other hypothalamic and brainstem circuitry may elucidate whether its inhibition of feeding following systemic treatment is the result of action at a specific neural locus, or whether its effects are mediated through more subtle actions across multiple neural pathways that regulate food intake and motivation.
References


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Figure Legend

Figure 1: Effects of sibutramine treatment on the intake of a sweetened fat diet. Systemic injections of 0-3.0 mg/kg sibutramine dose-dependently decreased diet intake in non-deprived rats given access to the palatable diet, without significantly altering water intake or locomotor measures (A). In contrast, infusions of sibutramine into the PVN increased fat diet intake across the 2-hr feeding session (B). Sibutramine injections into the Acb caused a transient, but significant, increase in feeding early in the 2-hr session (C, see text). The locations of the injector tips for animals included in behavioral analyses are shown to the right. Brain drawings were adapted from The Rat Brain in Stereotaxic Coordinates, 4th ed., G. Paxinos and C. Watson, Figures 10, 11, 23, and 26, copyright 1998. *p < 0.05, **p < 0.01 for drug effects; single and double crosses demark p < .05 and p < .01 for drug x time interaction effects, respectively. Stars placed on bars or food intake data points represent significant differences from vehicle treatment for that time point or behavior, as determined by Tukey’s HSD post-hoc analysis.
A. Systemic Sibutramine on High Fat Diet Intake (N = 8)

B. PVN Sibutramine on High Fat Diet Intake (N = 7)

C. Acb Sibutramine on High Fat Diet Intake (N = 5)

Figure 1