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

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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-h 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.

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 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 adrenergic 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 these effects remain 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 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-h 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

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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-h 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]. Infrared 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-s 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 2 h 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 et al. [34]. During the final three days of habituation to the chambers, each rat received an i.p. injection of 1 ml/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, and 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-h 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-h 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 h 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/0.5 μl/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 h 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-h 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-h session) and drug doses. For groups that had significant drug and/or drug × 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 Fig. 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 × 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 h, 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 h.

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 Fig. 1B, local injections of sibutramine into the PVN dose-dependently increased feeding (drug effect: $F_{3,18} = 4.88$, $p = .012$; drug × 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