Peptides that Regulate Food Intake

Somatostatin alters intake of amino acid-imbalanced diets and taste buds of tongue in rats

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Scalera, Giuseppe. Somatostatin alters intake of amino acid-imbalanced diets and taste buds of tongue in rats. Am J Physiol Regul Integr Comp Physiol 284: R1389–R1398, 2003; 10.1152/ajpregu.00738.2002.—The present studies were designed to evaluate a potential dose-dependent effect of somatostatin (SRIF) administered peripherally on intake of either a low-protein basal diet or threonine-imbalanced diet (THR-IMB), on body weight gain (ΔBW), gut motility, and on the histology of taste buds in rats. SRIF administration had a dual effect related to its concentration, increasing the intake of THR-IMB diet at low concentration and decreasing THR-IMB diet at high concentration. During the light phase, SRIF treatment increased the intake of THR-IMB diet, suggesting that the usual anorectic effect induced by intake of THR-IMB diet was attenuated. High-dosage SRIF decreases gastrointestinal motility, which, in turn, can decrease food intake and ΔBW. The combination of THR-IMB diet regimen and SRIF treatment also induced significant modifications on the taste buds of the tongue. The feeding response to an amino acid-imbalanced diet includes a learned aversion to the diet, and animals may use taste in establishing that aversion. Modifications of taste buds of SRIF-treated rats eating THR-IMB diet might explain the increase of imbalanced diet intake if treated rats perceive this food as less aversive.

RATS REDUCE FOOD INTAKE if they are prefed a low-protein basal (Bas) diet and then switched to a diet with an imbalance or lack of essential amino acids (AAs; see Refs. 24, 27, and 35). It has been shown that somatostatin (SRIF)-containing interneurons are widely distributed in the anterior piriform cortex (APC; see Ref. 10), an area thought to be responsible for the perception and recognition of essential AA levels in the diet (19). The widespread distribution of SRIF in the APC suggests that its role in the response of the APC to AA imbalance may be a target for investigation. Indeed, it has been shown that SRIF injected in the APC at low doses (pmol) increases the intake of the threonine-imbalanced diet (THR-IMB) shortly after injection, whereas larger doses (nmol) of SRIF significantly decreased intake, and the effects of injections appear to be selective for the imbalanced diet (10, 11).

SRIF is a peptide ubiquitous throughout the central, peripheral, and diffuse gastrointestinal nervous system and endocrine pancreas (30, 43). Outside the nervous system, SRIF acts as an autocrine/paracrine regulator. Because SRIF has a multitude of actions on gastrointestinal functions, it appears that this hormone may play a role in the regulation of feeding behaviors in rats and baboons (38, 40, 58) and in somatic growth and body weight gain (ΔBW; see Refs. 7 and 58). A review of the literature reveals that intracerebral, intracerebroventricular, or peripheral (sc and ip) injections of SRIF have equivocal effects on feeding. The data of previous studies appeared to be dependent on the doses, location, and methods of administration of the peptide. SRIF has been reported to increase, decrease, or have no effect on food intake (4, 11, 15, 40, 58, 65). These inconsistencies and discrepancies might be because of peripheral effects secondary to leakage of centrally injected SRIF in the bloodstream (15, 65). Thus the dose-dependent role of SRIF in mediating the anorectic response to AA deficiency (11) might depend on likely secondary peripheral effects. The purpose of the present study was to examine the effects of the administration of SRIF dissolved in Protamine-Zn complex on intake of a Bas diet and on a THR-IMB diet, on ΔBW, gut motility, and the histology of tongue taste buds. Because taste bud cells have a rapid turnover (3–5 days; see Ref. 6), and the development and maintenance of their normal morphological, physiological, and biochemical features depend on axonal transport of proteins in gustatory nerves (47), they might be altered by SRIF treatment and by a Bas diet or AA-imbalanced diet intake. In a previous paper (59), we demonstrated that SRIF treatment interferes with taste preferences and, to a certain extent, with taste bud distribution on the tongue. Moreover, protein nutrition after weaning is important to maintain taste sensitivity in humans (67) and taste preference and morphology of tongue epithelia in rats (1, 45, 46, 48, 49, 50, 62–64). Low-protein or AA-poor diet yields degener-

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Address for reprint requests and other correspondence: G. Scalera, Dip. Scienze Biomediche-Sez. Fisiologia, Via Campi, 287, 41100 Modena, Italy (E-mail: scalera@unimore.it).
eration of both filiform and fungiform papillae of taste buds (49) and decreases serum Zn$^{2+}$ concentration (64). The very short half-life of SRIF (2–3 min) and the observation of rebound at the end of administrations make this substance unsuitable for long-term experiments. When SRIF is dissolved in a solution of Protamine-Zn complex and injected subcutaneously, its concentration remains high and almost constant for a relatively long time (8). Furthermore, because the dark and light phase represent significantly different feeding states in the rat (25, 56), it seemed of interest to compare feeding behavior and $\Delta$BW in the light and dark phases during SRIF treatments.

**GENERAL METHODS**

All experiments reported here were done in conformity with the *Guiding Principles for Research Involving Animals and Human Beings* of the American Physiological Society (2). Twenty-four adult male Wistar rats, ~3 mo old, were housed in individual cages provided with a specially designed cup to eliminate food spillage, which was filled with defined dry powdered diets (AIN93-M purified diet; ICN Pharmaceuticals, Costa Mesa, CA). Distilled water (hereafter referred to as water) and food were offered ad libitum. Rats were kept in a silent room on a 12:12-h light-dark photoperiod (light on at 8:00 AM) and constant temperature ($22 \pm 2{^\circ}C$) and humidity (60%). A dim red light was left on permanently to permit data compilation and treatments during the dark phase. Body weight and food and water intake were measured at the end of both the light (8:00 PM) and dark (8:00 AM) phase. After ~10 days of acclimatization during which rats ate the AIN93-M diet, they were switched to a Bas diet (see below) for 14 days. SRIF-14 (Sigma-Aldrich, St. Louis, MO) was dissolved in the Protamine-Zn complex according to Brazeau et al. (8). Even if two different concentrations of SRIF were used, the volume of solutions or vehicle injected subcutaneously was always the same (0.5 ml/100 g body wt). These doses of SRIF were used because they affected resting serum growth hormone (GH) concentrations in rats (25). The rats were injected subcutaneously (neck region) two times a day for several consecutive days at the beginning of the light (8:00 AM) and dark (8:00 PM) phase. The first treatment started at the beginning of the light phase to synchronize the meal with the light phase. At the same times, food and fluid intake ($\pm 0.1$ g) and body weight ($\pm 1$ g) were measured. Water and food were renewed at the end of the dark phase, and the cup and cylinder were refilled at the end of the light phase. The mean $\Delta$BW (=body wt at beginning of either the dark or light phase – body wt at the end of the respective dark or light phase) was taken as an index of ponderal growth (7).

**Feeding Protocol**

To examine the effects of SRIF on an unbalanced AA diet, two types of diet (besides the AIN93-M purified diet) were used in two different experiments. All three diets had almost the same energetic value. In each experiment, L-threonine was the most limiting AA. Moreover, the two diets contained the same amounts of essential vitamins and minerals, with corn starch and sucrose (2:1, wt/wt) as carbohydrates and corn oil (5%) as the fat source. Diets were the THR-IMB and Bas diets containing only free AAs as the protein source. The threonine-balanced diet composition was the same as that reported by Cummings et al. (Table 1; Ref. 11). Briefly, the Bas diet contained 12.7% (wt/wt) AAs as the protein equivalent, and the growth-limiting AA was threonine. To obtain a THR-IMB diet, a mixture of the essential AAs except threonine (an additional 9.18% of the diet) was added to the Bas diet. To maintain the energetic value of the diet, the amount of carbohydrates was reduced proportionally when AAs were added (11). The store of free AAs and protein of the body is reduced over several days by the intake of Bas, and a decrease in imbalanced diet intake is considered a full expression of the response to an imbalanced diet (27). The THR-IMB diet does not cause food intake depression in animals bearing amygdaloid and prepyriform lesions (44). There are two phases of the feeding responses to the AA-imbalanced diet (THR-IMB). The initial phase consists of a rapid decrease of food intake that depends on the severity of the imbalance and the pretreatment protocol. A later adaptive phase consists of alterations in meal patterns that gradually return to the normal food intake over a period of 1–2 wk (24).

**RESULTS**

All results were expressed as means ± SE. The data collected during each experiment reflected the intake of either Bas or THR-IMB diet and $\Delta$BW for the SRIF treatment and control groups and were collected as grams per 12 h light, grams per 12 h dark phase, and grams per 24-h period. Statistically significant effects indicated by the ANOVA were evaluated by post hoc comparisons (Student-Newman-Keuls or Bonferroni test); $P < 0.05$ was considered statistically significant. Because the results of saline-injected rats and vehicle-injected rats in each experiment did not vary significantly, their data were pooled and considered as one group (saline + vehicle = Con, $n = 12$ rats) for all further statistical analyses. Unless otherwise noted, there were no significant differences between the groups of rats for any of the variables measured at baseline.

**Experiment 1**

In a previous paper (58), it has been shown that the chronic and peripheral (sc) SRIF treatment decreased food intake of a normal powdered diet (AIN93-M purified diet), $\Delta$BW, and gut motility in rats; these effects were dose dependent. Recently, Cummings et al. (11) found no effects of SRIF injected centrally to the APC on a “low protein basal diet (Bas) containing 12.7% (wt/wt) amino acids as the protein equivalent, providing ~50% of the amino acid requirements for growth.” These authors showed that low (pmol) doses of SRIF-28 in the APC may increase the intake of THR-IMB, whereas larger doses (nmol) of SRIF-28 significantly decreased THR-IMB intake. These authors concluded that the effects of SRIF injections in the APC, either at low or high doses, on the intake of a THR-IMB diet appeared to be selective for the imbalanced diet, since there were no effects on Bas diet intake at any dose. Unfortunately, this paper did not provide data on the
body weight modification of rats treated with SRIF injected directly in the APC. The aims of the present experiment were to verify if peripheral administration (sc) of SRIF might interfere with the intake of a Bas diet and thus influence ΔBW of rats. The experiment was divided into two consecutive periods. During the first period, rats ate a normal powdered food (AIN93-M purified diet) and drank water for 10 days to allow them to acclimatate to the new environmental and feeding conditions. After this period, rats were fed Bas diet for 10 days. Four groups of rats were established in accordance with the baseline intake of the Bas for the following treatments: a Con group (n = 6; treated with 0.5 ml/100 g body wt saline), a vehicle-injected group (n = 6; treated with 0.5 ml/100 g body wt of the Protamine-Zn complex), a group treated with a low dose of SRIF (SRIF2, n = 6; 2 μg/100 g body wt), and a group with a relatively high dose of SRIF (SRIF8, n = 6; 8 μg/100 g body wt). Because Zn²⁺ used to dissolve SRIF may play a role in supporting, nourishing, and differentiating taste buds (28), it was appropriate to use the following two groups of rats as Con: one injected with saline and the other with Protamine-Zn complex to check that the differences might be ascribed to possible changes in taste preferences (59). During SRIF treatments, rats were fed the Bas diet and drank water ad libitum.

*Fluid intake.* As normal in rats, water intake was greater in the dark than in the light phase, but the relative amounts drank did not differ between groups during baseline or treatment with SRIF. The SRIF treatment did not influence the amount and the light-dark cycle of water intake.

*Food intake.* During the baseline period \[F(5,42) = 141.96, P < 0.0001\] and during the treatment period \[F(5,42) = 154.79, P < 0.0001\], all rats ate more Bas diet during the dark than light phase (Fig. 1, A and C). ANOVA showed significant interactions between Bas diet intake of Con, SRIF2, and SRIF8 rats during the dark phase of treatment \[F(2,21) = 27.49, P < 0.0001;\] Fig. 1C). Indeed, SRIF8 rats ate significantly less Bas diet than Con (t = 7.151, \(P < 0.0001\)) and SRIF2 (t = 5.728, \(P < 0.0001\)); no significant difference was noted between Con and SRIF2 rats. During the light phase of treatment, significant effects emerged at ANOVA \[F(2,21) = 10.95, P < 0.001\], and post hoc comparisons showed that SRIF8 rats ate significantly more Bas diet than Con rats (t = 6.584, \(P < 0.001\)) and SRIF2 rats (t = 4.344, \(P < 0.001\); Fig. 1C).

**Fig. 1.** Food intake (means ± SE) of rats eating a low-protein basal (Bas) diet during the light and the dark phase of either baseline (A) or somatostatin (SRIF; C) treatment. During baseline and SRIF treatment, rats ate more Bas diet during the dark than light phase. During treatment, high-dose SRIF (SRIF8) rats ate less Bas diet than control (Con; \(P < 0.0001\)) and low-dose SRIF (SRIF2) rats (\(*P < 0.0001\) vs. Con and SRIF2). On the contrary, during the light phase, SRIF8 rats ate more than Con and SRIF2 rats (\(**P < 0.001\) vs. Con and SRIF2). During the baseline period, the body weight gain (ΔBW) of all rats decreased during the light but increased during the dark phase (\(P < 0.0001\)); daily ΔBW was almost unchanged in all rats (B). During treatment, the ΔBW of all rats still decreased during the light and increased during the dark (D), but during the dark phase and entire day the ΔBW of SRIF8 rats decreased significantly more than that of Con and SRIF2 rats (\(\bullet P < 0.0001\) vs. Con and SRIF2).

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ΔBW. When rats ate Bas diet during the baseline period before SRIF treatment, the ΔBW increased by ~4.5 g at the end of the dark phase, but it decreased by ~2.5 g at the end of the light phase across all groups of rats. Thus the ΔBW increased by ~2.0 g/day in all groups of rats, and no significant differences were noted among all rats (Fig. 1B). During the dark phase of SRIF treatment (Fig. 1D), ANOVA pointed out significant effects [F(2,21) = 8.23, P < 0.001], and post hoc comparisons showed that the ΔBW of SRIF8 rats decreased significantly compared with Con (t = 5.461, P < 0.001) and SRIF2 (t = 4.588, P < 0.001) rats. During the light phase of treatments, the ΔBW decreased in all groups of rats in the same manner. During SRIF treatment [F(2,21) = 15.03, P < 0.0001] the mean ΔBW per day of SRIF8 rats was significantly lower than that of SRIF2 (t = 5.032, P < 0.0001) and Con (t = 4.676, P < 0.001; Fig. 1D) rats. Moreover, only the ΔBW/day of SRIF8 rats was significantly lower than that of the same group (SRIF8) during the baseline period (t = 4.089, P < 0.01). The light-dark comparisons for both baseline [F(5,42) = 507.52, P < 0.0001] and treatment period [F(5,42) = 382.30, P < 0.0001] showed that the ΔBW decreased significantly during the light phase in all rats.

Experiment 2

Rats recovered for ~1 mo after completion of experiment 1. During this period, they were fed the AIN93-M purified diet for 20 days to restore the best condition of body growth and then were switched to the Bas diet for 10 days. The Bas diet reduces the endogenous stores of circulating free AAs and protein to provide a prompt and full expression of the imbalance-induced anorectic response (11, 19, 27). The aim of this trial was to study the effects of chronic and peripheral administration of SRIF on a THR-IMB diet, water intake, and ΔBW. The initial intake of the AA-imbalanced diet is important in determining the depressive effects of such diet on food intake, because animals respond to the imbalanced diet immediately and develop an aversion to the diet during the first day of exposure (35, 36, 41). Three days of access to only a THR-IMB diet after 10 days of Bas diet should have depleted the body content of threonine (23). Thus it is plausible to assume that the threonine body concentration was equal in all rats at the beginning of SRIF treatment. SRIF treatment started on the 4th day of THR-IMB feeding. The groups of rats were the same as those used for experiment 1, and they were injected subcutaneously for seven consecutive days with either vehicle, saline, SRIF2, or SRIF8 solution. During treatments, rats were fed the THR-IMB diet and drank water ad libitum.

Food intake. At the end of the recovery period, the average intake of Bas diet and the ΔBW were very similar to that of experiment 1 before SRIF treatment. Results showed that rats fed the THR-IMB diet significantly reduced intake compared with those on the Bas diet (Con: 30.11%, t = 7.989, P < 0.0001; SRIF2: 30.01%, t = 5.175, P < 0.0001; SRIF8: 29.52%, t = 5.193, P < 0.0001). Also during the light period the consumption of THR-IMB diet was lower than that of the Bas diet, but the differences did not reach significance. As expected, during the baseline period, and during the treatment period, all rats ate more THR-IMB diet during the dark than light phase. Nonsignificant differences were noted between all groups during either the dark or light baseline period (Fig. 2, A and C). During the dark phase of treatment (Fig. 2C), ANOVA and post hoc comparisons showed significant interactions between THR-IMB diet intake of Con, SRIF2, and SRIF8 rats [F(2, 21) = 33.73, P < 0.0001]. Indeed, SRIF8 rats ate significantly less THR-IMB diet than Con (53.17%; t = 5.720, P < 0.0001) and SRIF2 (65.03%; t = 8.115, P < 0.0001) rats. Moreover, during treatment, SRIF2 rats ate significantly more THR-IMB diet than Con rats (25.33%; t = 3.651, P < 0.01). During the light phase of treatment, ANOVA and post hoc comparisons showed significant effects of the treatment [F(2,21) = 124.05, P < 0.0001]. Indeed, as shown in Fig. 2C, SRIF8 rats ate significantly more THR-IMB diet than Con (35.20%; t = 5.062, P < 0.001), but less than SRIF2 (54.28%; t = 14.791, P < 0.0001); SRIF2 rats, in turn, ate more THR-IMB diet than Con rats (70.37%, t = 22.141, P < 0.0001). Interestingly, SRIF treatment increased the intake of THR-IMB diet during the light phase. In particular, SRIF2 rats ate more THR-IMB diet than the baseline intake of Con (69.17%, t = 20.502, P < 0.0001), SRIF2 (67.67%, t = 17.369, P < 0.0001), and SRIF8 (68.27%, t = 17.523, P < 0.0001). SRIF8-treated rats also ate more THR-IMB diet than Con (32.56%, t = 4.412, P < 0.001), SRIF2 (29.28%, t = 3.435, P < 0.01), and SRIF8 (30.59%, t = 3.590, P < 0.001) rats at baseline.

ΔBW. During baseline THR-IMB diet presentation, in each group of rats, the ΔBW was very small at the end of the dark phase (~0.5 g/12 h). Moreover, it decreased by ~6.5 g at the end of the light phase; thus the daily ΔBW/24 h decreased by ~6.0 g/day in all groups of rats (Fig. 2B). Except SRIF2 rats, which showed a positive ΔBW during the dark phase, Con and SRIF8 rats had a negative ΔBW (Fig. 2D). ANOVA reported significant interactions between the variations of ΔBW. In particular, during the dark phase [F(2,21) = 82.79, P < 0.0001], the ΔBW of SRIF8 rats decreased significantly compared with SRIF2 (t = 17.441, P < 0.0001) and Con (t = 14.313, P < 0.0001) rats. In contrast, during the light phase [F(2,21) = 107.08, P < 0.0001], all groups of rats showed a negative ΔBW. In particular, SRIF2 rats lost less body weight than Con (t = 18.556, P < 0.0001) and SRIF8 (t = 3.253, P < 0.05). The SRIF8 rats showed the same trend, and their ΔBW was lower than that of treated Con rats (t = 14.810, P < 0.0001). When the whole day (24-h) values were considered [F(2,21) = 33.85, P < 0.0001], SRIF8-treated rats had a higher ΔBW loss compared with treated SRIF2 rats (t = 8.114, P < 0.0001) and treated Con rats (t = 5.801, P < 0.001). On the contrary, SRIF2-treated rats lost less ΔBW than treated Con rats (t = 3.568, P < 0.01).
Gut motility. To verify if an imbalanced diet regimen and SRIF treatment might influence the gut motility, mechanograms of jejunal loops at the end of experiments were recorded. The detailed method has been published elsewhere (58). Briefly, at the end of experiments, rats fasted overnight were anesthetized and killed, and the proximal 2 cm of jejunum were isolated and prepared for in vitro mechanograms. The jejunum was mounted in an organ bath through which Krebs solution (37°C, bubbled with O2) flowed continuously; no SRIF was placed in the solution of the organ bath. One end of preparation was fixed to a glass hook, and the other end was tied by a silk thread to the hook of an isotonic tension recorder. Pendular contractions, both spontaneous or stimulated by electrical field stimulation (EFS; 0.1 Hz; 200 ms; 80 V; 10-s interval), were recorded.

The SRIF8 rats had poorer spindle-shape clustering of jejunum pendular contractions than SRIF2 and Con rats. The number of spontaneous spindles per minute \( F(2,21) = 35.16, P < 0.0001 \) or spindles per minute induced by EFS \( F(2,21) = 22.07, P < 0.0001 \) were significantly lower in SRIF8 than SRIF2 and Con rats \( (**P < 0.0001 \text{ vs. Con and SRIF2}). \) During the light phase of treatment, the increase in ABW was too small, but during the light phase and entire day (24 h), ABW decreased significantly in all rats \( (B) \). During the dark phase of the treatment, except the SRIF2 rats that showed a positive ABW, Con and SRIF8 rats had a negative ABW \( (D) \). The ABW of SRIF8 rats decreased significantly compared with SRIF2 and Con rats \( (**P < 0.0001 \text{ vs. Con and SRIF2}). \) During the light phase and whole day (24 h), all groups of rats had a negative ABW. SRIF2 rats lost less body weight than Con rats and SRIF8 rats \( (**P < 0.0001 \text{ vs. Con and SRIF8}). \) The ABW of SRIF8 rats was lower than that of Con and SRIF2 \( (**P < 0.0001 \text{ vs. Con and SRIF2}). \)
post hoc analysis showed that the spontaneous width of the spindle, which represents the isometric force of contraction of jejunal loops (g), was significantly lower in SRIF8 than SRIF2 \((t = 2.945, P < 0.05)\) and Con \((t = 4.787, P < 0.001)\) rats. EFS increased the width of spindles only in SRIF8 rats, but comparisons with SRIF2 and Con rats were not significant (Fig. 3B). The spindle duration \((s)\) of SRIF8 rats was significantly longer than that of SRIF2 and Con rats under both spontaneous \((Ss)\) and EFS conditions \([Ss, F(2, 21) = 49.56, P < 0.0001]\); SRIF8 vs. SRIF2, \(t = 9.095, P < 0.0001\); SRIF8 vs. Con, \(t = 8.557, P < 0.0001\); EFS, \(F(2, 21) = 38.86, P < 0.0001\); SRIF8 vs. SRIF2, \(t = 6.683, P < 0.0001\); SRIF8 vs. Con, \(t = 7.063, P < 0.0001\) \(\text{Fig. 3C}\). The interval between two consecutive spindles \((s)\) was not significantly different between any group of rats, under both Ss and EFS conditions \(\text{Fig. 3D}\).

**Tongue histology.** After 15 days of THR-IMB diet, rats were killed by an overdose of pentobarbital sodium \((150 \text{ mg/kg ip})\), and tongues were removed, fixed in Carnoy solution, and embedded in celloidin and paraffin. Serial cross-sections \((10 \mu\text{m})\) were cut and stained with Heidenhain’s iron hematoxylin to stain the taste buds, and they were then examined under the light microscope. The taste bud density on the tip of the tongue was significantly different in the three groups of rats \([F(2, 21) = 25.49, P < 0.0001]\; Table 1). In particular, it was higher in Con rats than in SRIF2 \((t = 3.029, P < 0.05)\) and SRIF8 \((t = 7.106, P < 0.0001)\) rats and higher in SRIF2 than SRIF8 \((t = 3.531, P < 0.01)\) rats. On the contrary, in the midregion of the tongue, the taste bud density did not change significantly. The number of papillae on the entire tongue decreased according to SRIF dosage \([F(2, 21) = 18.73, P < 0.0001]\). Indeed, the number of papillae was lowest in SRIF8 rats compared with SRIF2 \((t = 3.008, P < 0.01)\) and Con \((t = 6.087, P < 0.0001)\) rats and lower in SRIF2 than Con \((t = 2.623, P < 0.05)\) rats. Moreover, the number of papillae on the tip of the tongue was significantly different depending on treatment \([F(2, 21) = 7.37, P = 0.004]\). Post hoc tests showed that the number of papillae was higher in Con than SRIF8 \((t = 5.055, P < 0.0001)\) and SRIF2 \((t = 3.558, P < 0.01)\) rats, but no significant differences were found between SRIF2 and SRIF8 rats. Both the size of the circumvallata papilla \([\mu\text{m}]; F(2, 21) = 29.34, P < 0.0001]\) and the number of taste buds in the circumvallata papilla \([F(2, 21) = 25.04, P < 0.0001]\) was higher in Con than in SRIF2 \((t = 3.703, P < 0.05)\); taste buds, \(t = 3.872, P < 0.01\) and SRIF8 \((t = 7.650, P < 0.0001)\); taste buds, \(t = 7.064, P < 0.0001\) rats and in SRIF2 than in SRIF8 \((t = 4.743, P < 0.001)\); taste buds, \(t = 3.747, P < 0.01)\) rats.

**DISCUSSION**

The present experiments investigated the effects of SRIF, when its concentration in the body remains relatively high and nearly constant over time \((8)\), on both the intake of a Bas diet, or a THR-IMB diet. Water intake, gut motility in vitro, ΔBW, and topography and structure of taste buds and circumvallata papilla of the tongue were also analyzed. Rats are distinctly nocturnal animals \((56)\), and most of their food intake occurs during the dark phase of the light-dark cycle if food is continuously available \((34)\). Thus, as expected, food intake was significantly higher during the dark than light phase. SRIF treatment did not alter the normal dark-light feeding cycle, but it showed a different effect on Bas intake during both the light and dark phase in a dosage-dependent manner. Indeed, high-dosage SRIF \((8 \mu\text{g/100 g body wt})\) significantly decreased the intake of Bas during the dark phase, whereas low-dosage SRIF \((2 \mu\text{g/100 g body wt})\) did not. On the contrary, during the light phase, the high-dosage SRIF stimulated rats to increase Bas intake, but low dosage was ineffective. Thus peripheral SRIF injection has a photoperiod-sensitive and dosage-dependent effect that facilitates food intake in the light and inhibits it in the dark \((14, 15, 58)\). Two possibilities may explain the increase of food intake during the light. The body fats are lesser available as fuels for metabolism, since SRIF treatment inhibits GH release that is involved in the breakdown of adipose tissue into fuels \((66)\) and may alter the levels of hepatic enzymes \((3, 31)\); or less fats are synthesized during nocturnal feeding; one possibility does not exclude the other. These conditions might be a sufficient stimulus to induce eating in the rat during the light phase to maintain a basal reserve of energy, but this increase in food intake is not sufficient to completely compensate for the energy intake during the night because ΔBW still decreased appreciably during the dark and whole day phase in SRIF8 rats. SRIF treatment influenced ΔBW in rats eating Bas diet in a dose-dependent manner, and its effect was evident during the dark and entire day phase but not during

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**Table 1. Density of taste buds and distribution of papillae on the different parts of the tongue and on the circumvallata papilla**

<table>
<thead>
<tr>
<th>Rat</th>
<th>n</th>
<th>TB Density, n/mm²</th>
<th>No. of Papillae</th>
<th>Entire tongue</th>
<th>Tip of tongue</th>
<th>Midregion of tongue</th>
<th>Size of CP, μm</th>
<th>No. of TBs in CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>2.97 ± 0.35</td>
<td>1.34 ± 0.68</td>
<td>174.07 ± 18.78</td>
<td>98.56 ± 11.43</td>
<td>75.78 ± 16.34</td>
<td>815.41 ± 81.67</td>
<td>386.53 ± 38.27</td>
</tr>
<tr>
<td>SRIF2</td>
<td>6</td>
<td>2.45 ± 0.24</td>
<td>1.27 ± 0.54</td>
<td>145.89 ± 26.49</td>
<td>75.44 ± 22.17</td>
<td>66.31 ± 19.18</td>
<td>731.62 ± 74.51</td>
<td>325.65 ± 55.14</td>
</tr>
<tr>
<td>SRIF8</td>
<td>6</td>
<td>1.75 ± 0.41</td>
<td>0.76 ± 0.33</td>
<td>108.63 ± 21.58</td>
<td>65.71 ± 21.78</td>
<td>46.81 ± 23.79</td>
<td>520.45 ± 68.36</td>
<td>229.43 ± 45.23</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of rats. CP, circumvallata papilla; n/mm², no. of taste buds/mm²; SRIF2, 2 μg/100 g body wt somatostatin (SRIF); SRIF8, 8 μg/100 g body wt SRIF.
the light phase. In this case also, the reduced intake of food and the impairment of AAs or protein metabolism resulting from the altered levels of hepatic enzymes (3, 31) may account for the decrease in ΔBW of SRIF8 rats.

In contrast to data published by Cummings et al. (11), which found a selective action of SRIF injected centrally in APC on the intake of the THR-IMB diet but not on the intake of the Bas diet, results reported here showed that SRIF injected subcutaneously had a dose-dependent and generalized effect on the intake of both THR-IMB and Bas diets. This discrepancy may depend on the methods of administration, dosage, and type of SRIF used. Cummings et al. (11) demonstrated that SRIF injected directly to the rat APC “had a dual effect related to the concentration of the peptide, increasing intake of a diet deficient in AA at pMol concentrations, and decreasing intake at nMol concentrations,” confirming previous published data showing that both SRIF intracerebroventricular injection at picomole concentrations or subcutaneous 2 μg/100 g body wt dosage increased food intake, whereas intracerebroventricular injections at nanomole concentrations or subcutaneous 8 μg/100 g body wt dosage decreased food intake in normal sated rats (15, 58). Results of the experiments reported here confirmed these data. During the light phase, SRIF treatment induced an increase in the intake of THR-IMB diet, but the low dose increased intake more than the high dose. This result is interesting because it shows that the usual anorectic effect resulting from the intake of the THR-IMB diet (19, 27, 29) was attenuated by the SRIF treatment during the light phase. The question of “why does SRIF treatment attenuate the anorectic effect due to imbalanced diet intake” may be answered speculatively. It has been suggested that SRIF may have many inhibiting activities (5), and it has been shown that the intake of palatable sucrose and NaCl solution decreased whereas that of mildly aversive quinine and hydrochloride solution increased significantly in rats treated with SRIF (59). This behavior was explained by a general inhibitory action of SRIF on the central neural system subserving intake of either the palatable or aversive fluids. In other words, inhibition of the gustatory system might decrease the intake of the pleasant solutions (NaCl and sucrose) but enhance that of unpleasant ones (quinine and acid; see Ref. 59). Similarly, the inhibiting activity of SRIF might render less anorectic the intake of an THR-IMB diet; thus rats treated with SRIF ate more imbalanced diet than Con rats. SRIF treatment does not alter the normal dark-light cycle of feeding, but inhibition of the anorectic effect of THR-IMB diet was more evident during the light than dark phase of SRIF treatment. This result is in accordance with that of Feifel and Vaccarino (15) who found that intracerebroventricular SRIF injections significantly increased feeding during the light but not dark photoperiod.

The palatability of a solution or food may determine the amount consumed (12, 13, 42). As a consequence, alterations of taste preferences after SRIF treatment and/or AA-imbalanced diet intake may justify the decrease in food intake. Histological examination of the tongue showed that the combination of THR-IMB diet regimen and SRIF treatment induced significant modifications of the density, distribution, and morphology of taste buds of the tongue. The effect was dosage dependent, since impairment was lower in SRIF2 than SRIF8 rats. SRIF treatment, besides reducing the

Fig. 3. Main parameters (mean ± SE) of spontaneous mechanograms [no. of spontaneous spindles/min (Ss)] or mechanograms induced by electrical field stimulation (EFS) of jejunal loops in vitro. A: Ss were significantly lower in SRIF8 than SRIF2 and Con rats (\( * P < 0.0001 \) vs. Con and SRIF2). EFS increased Ss in all rats but significantly only in SRIF2 and Con rats \( (** P < 0.01 \) vs. SRIF8). B: the spontaneous width of the spindle, which represents the isotonic force of contraction of jejunal loops, was significantly lower in SRIF8 than in SRIF2 and Con rats \( (** P < 0.05 \) vs. Con and SRIF2). EFS significantly increased the width of spindles only in SRIF8 rats. C: spindle duration was significantly longer in SRIF8 rats than SRIF2 and Con rats under both spontaneous (Ss) and EFS conditions \( (** P < 0.0001 \) vs. Con and SRIF2). D: the interval between two consecutive spindles did not differ significantly across any groups of rats, under both Ss and EFS conditions.

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number, distribution, and morphology of taste buds and circumvallata papilla, seemed to increase the delay in turnover of taste buds cells. It has been suggested that lower turnover of taste bud cells may explain the inverse relationship between dietary protein content and NaCl intake, even if the short turnover time may be more important for taste detection or acuity than for taste (NaCl) preference. Taste bud modification might modify taste perception of the palatability of food and thus influence food intake, even if changes in taste buds need not necessarily be causally related to the effects on intake of sapid solutions. Although animals make use of olfactory and taste sensations to select a proper diet with regard to AA balance in a choice situation, these senses, although helpful, do not appear indispensable for initial recognition of a deficiency in animals ingesting imbalanced amounts of dietary AAs. Nevertheless, animals use taste cues most effectively in establishing learned aversions involving imbalanced quantities of AAs. The feeding responses to an AA-imbalanced diet appear to involve at least two phases: the initial recognition of AA deficiency and subsequent reduction in food intake with development of a learned aversion to the diet. The parabrachial nucleus is a crucial element in taste aversion learning. Avoidance of an essential AA-deficient or Bas diet reflects a true taste aversion, since lesions of the parabrachial nucleus appear to disrupt the learned aversion for a threonine-deficient diet. The significant modification of taste bud number and morphology of rats treated by SRIF and eating THR-IMB diet might explain the increase on the intake since treated rats might perceive this food as less aversive than Con rats.

During the THR-IMB diet feeding period, all groups lost weight significantly and ΔBW practically became negative during the light and entire day (24 h) period. This result was substantiated by the fact that all groups reduced their average overall energy intake. Also, in this case, SRIF treatment influenced in a dose-dependent manner the ΔBW during either the dark, light, and whole day period. In rats eating THR-IMB diet, loss of satiety has been associated with the general increase in locomotor activity. SRIF injections also may induce hyperthermic events and may increase locomotor activity. Both the effect of THR-IMB diet intake and SRIF treatment may increase waste of energy as heat and may account for the decrease of ΔBW. Because the different diets eaten by rats and SRIF treatment had no reliable effects on fluid intake, suppression of food intake was not likely because of a generalized disruption of behavior or aversive consequence produced by illness or malaise.

Mechanograms of isolated jejunum confirmed previous results and suggested that high-dose SRIF treatment can decrease gastrointestinal motility independent of diet (THR-IMB) assumed lately by rats. Decreased gastrointestinal motility can decrease food intake by prolonging the duration of nutrient contact with the small intestine. Thus SRIF may affect hunger and ingestive behavior since it affects gastrointestinal motility and enzyme secretions. Moreover, SRIF depresses nutrient absorption, gut motility, gut enzymes secretion, and splanchnic blood flow, and these data are concurrent with a reduction of food intake and body weight in SRIF-treated rats, independent of the type of diet eaten by rats.

Conclusions and Perspectives

The data here reported demonstrate that subcutaneous administration of SRIF has a dual effect related to its concentration, increasing intake of a THR-IMB diet at the lowest concentration and decreasing intake at the highest concentration. The significant difference in dose-dependent effects of SRIF strongly concurred with the findings of other authors. This effect may depend on the different activation of the mediators or neurotransmitters involved in feeding behavior as, for example, norepinephrine and serotonin. Norepinephrine and serotonin have been examined rather thoroughly as factors involved in the feeding behavior mediated by APC (19, 21, 24, 26, 29). Intake of AA-imbalanced diets was shown to decrease norepinephrine concentration in the APC and norepinephrine release in the ventromedial hypothalamus. It has also been demonstrated that serotonin-3 receptors mediate the anorexigenic activity of serotonin associated with the intake of an AA-imbalanced diet in the APC. The interactions of SRIF with norepinephrine and serotonin-containing neurons within the APC may modulate the intake of AA-deficient diets. Indeed, activation of the α2-noradrenergic receptors increases the intake of AA-deficient diets (11, 20, 21), but serotonin decreases the intake of those diets activating serotonin-3 receptors (11, 24, 26, 29). In conclusion, SRIF administered peripherally alters intake of an AA-deficient diet and suggests that SRIF may facilitate (at low dosage) or suppress (at high dosage) neural functional circuits, which include serotonin-3 and α2-noradrenergic receptors. Further investigations will clarify the dual effects related to the concentration of SRIF on the intake of either the normal, Bas, or THR-IMB diet. Because this effect may depend on the different activation of mediators or neurotransmitters involved in feeding behavior (i.e., norepinephrine and/or serotonin), considerable work will be necessary to define the potential relationships of SRIF-containing interneurons with other transmitter systems within the neural circuitry playing a role in mechanisms subserving intake and/or the recognition of amino acid-deficient diets.

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