Regulatory Peptides, 18 (1987) 85–92
Elsevier

RPT 00593

Exercise-induced hyperphagia in the hamster is associated with elevated plasma somatostatin-like immunoreactivity

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(Received 5 December 1986; revised version received 18 February 1987; accepted 26 February 1987)

Summary

Syrian golden hamsters when allowed free access to food and an exercise wheel will run long distances and develop hyperphagia and accelerated linear body growth with high circulating levels of growth hormone and insulin. Somatostatin, a widely distributed brain–gut neurohormonal peptide, modulates nutrient absorption and may regulate food intake. To examine the role of circulating plasma somatostatin-like immunoreactivity (SRIF-LI; pg/ml) in exercise induced hyperphagia 4 groups of animals were studied; an unrestricted exercise group (279.0 ± 107.7, $n = 10$); a sedentary group (121.1 ± 40.8, $n = 8$); a restricted exercise group (107.7 ± 12.4, $n = 6$); and a restricted no exercise group (115.5 ± 45.9, $n = 9$). Thus, the unrestricted exercise group has a significantly elevated SRIF-LI concentration ($P < 0.01$) while there was no difference between the other 3 groups. The elevation of plasma SRIF-LI in the unrestricted exercise group may represent a response to modulate increased nutrient entry in this group or may represent an incompletely effective satiety signal.

Hyperphagia; Accelerated growth; Somatostatin

Introduction

Syrian golden hamsters, allowed free access to food and an exercise wheel, will run long distances at night which induces a syndrome of hyperphagia and accelerated

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linear body growth [1]. These hyperphagic growing animals have high circulating levels of growth hormone due to increases in pulse frequency and amplitude [2] and of insulin [1] which may mediate the accelerated linear growth. When exercising with restricted access to food, linear growth [1] and cellular proliferation of the distal femoral epiphyseal growth zone is blocked [3].

Somatostatin (somatotrophin release inhibiting factor, SRIF), was initially extracted from the hypothalamus and acts within the hypothalamic-pituitary portal venous system as a true hormonal regulator of growth hormone secretion [4,5]. It has since been found to have a far wider tissue distribution and probably serves multiple functions [6,7]. These include: (a) presence in neurons of many areas of the brain, spinal cord and peripheral nervous system where it is a putative peptidergic neurotransmitter/neuromodulator [6,8]; b: widespread distribution throughout the gut [6,8] where it is distributed in the intrinsic autonomic neurons and in mucosal endocrine cells [6,8], where it is believed to act as a paracrine transmitter and, within the visceral portal system, as a hormone [9,10]. SRIF's actions on the gut include suppression of many gut hormones including gastrin, secretin and CCK; reduction of gastric secretion, reduction in pancreatic-exocrine secretion, reduced gut motility, and delayed nutrient absorption [5,11–14]; c: within the pancreas the majority of SRIF is present in the δ cells of the islets [6,8] where it appears to act as a paracrine transmitter or to transmit a signal by intimate cell-to-cell contact via tight junctions. Its action here is to reduce insulin and glucagon secretion [12]. It also enters the portal system where it may act as a hormone [9,10,15].

We examined somatostatin-like immunoreactivity (SRIF-LI) in the general circulation of exercising hyperphagic animals and appropriate controls to test the hypothesis that this state with its striking alteration in nutrient ingestion, growth and activity, may be associated with alterations in circulating SRIF-LI.

Materials and Methods

Female golden hamsters (*Mesocricetus auratus* Waterhouse) over 10 weeks old and weighing 90–100 g (Eagle Laboratory Animals, Farmersburg, IN) were individually housed under conditions of constant temperature (22°C and a 12 h light/dark cycle with continuous access to tap water ad lib. Animals were divided into 4 experimental groups of 10 [1,3]:

(a) Unrestricted exercise group. Animals were allowed free access to an unlimited supply of Purina Chow Formulab 5008 and to a horizontal exercise wheel.

(b) Sedentary group. Animals were allowed free access to unlimited supply of Purina Chow Formulab 5008 without an exercise wheel.

(c) Restricted exercise group. Animals were allowed free access to an exercise wheel but food intake was restricted to the quantity consumed by the sedentary group.

(d) Restricted no exercise group. Animals were not allowed access to the exercise wheel and food intake was restricted to a point where no weight gain occurs.

The disk exerciser was a freely turning perspex disk 23–25 cm in diameter, the revolutions of which could be electrically recorded [1,3].

Animals were weighed daily and the ponderal growth calculated for the experimental period. Animals were sacrificed in random order by decapitation during the second hour of the daylight cycle. Trunk blood was collected into chilled glass tubes containing Trasylol (aprotinin 5000 KIU/ml) and EDTA to yield a 10:1 ratio (4 ml of blood). The plasma was immediately separated by centrifugation and stored in aliquots at −20°C until assay.

Somatostatin-like immunoreactivity (SRIF-LI) was determined in unextracted plasma by a radioimmunoassay using an antiserum (Cape Town 1374) raised in a rabbit to synthetic cyclic SRIF-(1–14) conjugated to whelk hemocyanin by the carbodiimide reaction [16–18]. The antiserum has been shown to be specific for the 6–11 region of SRIF-14. It has 75% cross-reactivity with SRIF-(1–25) and a 60% cross-reactivity with SRIF-(1–28) [18]. [Tyr^1]SRIF was radiolabeled by the chloramine-T technique with ^{125}iodine and the tracer was purified by ion exchange chromatography on CM-52 cellulose to a specific activity of ca. 750 Ci/mol. The standard was synthetic cyclic somatostatin-(1–14) (Ayerst AY 24910, Ayerst Laboratories, Rouses Point, NY). The assay was performed in 50 mM ammonium acetate buffer at a pH of 5.6 [19]. This low pH has been shown to permit the assay of SRIF-LI in rat [10] and human [16] plasma without extraction. Separation of antibody-bound from unbound tracer was achieved by the use of dextran-coated charcoal. The limit of sensitivity (2 standard deviations above assay buffer control tubes) was 2 pg/tube. The intra-assay coefficient of variation was 5% and the interassay coefficient of variation was 12%.

Plasma samples were assayed in triplicate in 3 dilutions to determine parallelism of plasma SRIF-LI with the standard curve. Recovery of standard somatostatin from plasma was determined by the addition of 500 ng/ml of synthetic cyclic SRIF-(1–14) to aliquots of hamster plasma and serially diluting with SRIF-free plasma. Nonspecific binding of the ^{125}I-[Tyr^1]SRIF with hamster plasma resulted in no more than 5% damage to the label as determined by chromatoelectrophoresis. Plasma SRIF-LI concentrations were expressed as pg SRIF-(1–14) equivalents/ml.

Statistical methods

Overall differences between the 4 groups were determined using the Welch statistic for one-way analysis of variance. Multiple pairwise comparisons to determine significance at the $P = 0.01$ level were made using the Bonneferroni correction method [20].

Results

The mean body weights for the 10 animals in each experimental group are shown in Fig. 1 which shows that the exercising group had the typical accelerated growth when compared to the sedentary controls while the exercising restricted and sedentary restricted groups showed minimal growth.

The mean plasma SRIF-LI concentrations for all 4 groups of animals are presented in Table I. The exercised group of animals with free access to food had a highly

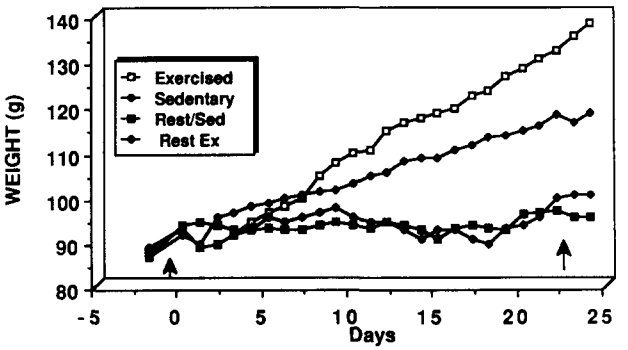

Fig. 1. Changes in weight in the 4 groups of hamsters. The first arrow indicates the start of the experiment and the second arrow the time of sacrifice. Rest, food-restricted; Sed, sedentary; Ex, exercise.

TABLE I

Plasma-SRIF-LI concentrations in the 4 groups of animals (individual data) pg/ml

	Unrestricted exercise	Unrestricted sedentary	Restricted exercise	Restricted sedentary
	302.9	179.7	92.4	178.8
	500.5	155.4	121.6	78.0
	312.9	138.1	91.8	183.0
	161.5	120.7	109.7	112.4
	304.9	137.1	119.3	43.9
	164.5	52.4	111.4	106.2
	346.2	102.3		129.7
	216.1	83.7		127.5
	155.1			79.7
	325.5			
Mean (pg/ml)	279.0*	121.2**	107.7**	115.5**
±S.D.	±107.7	±40.8	±12.4	±45.9
Mean weight (g)	139	118.5	99.5	95

* Unrestricted exercise greater than all 3 other groups, $P < 0.01$.

** No significant differences between unrestricted sedentary restricted exercise and restricted sedentary groups.

significant ($P < 0.01$) elevation of plasma SRIF-LI when compared to be sedentary groups and exercising food-restricted hamsters. Plasma SRIF-LI in the latter 3 groups did not differ significantly. (The F-value for the overall main effect was 13.54.)

Discussion

If adult golden hamsters in the slow asymptotic phase of growth are given the opportunity to exercise on horizontal exercise disks, they voluntarily engage in high levels of physical activity and re-enter the exponential phase of linear and ponderal

growth throughout their exposure to such exercise. The linear growth is possible as the epiphyseal growth plates remain open in adulthood. This phenomenon is associated with marked hyperphagia and the growth may be inhibited entirely by restricting nutrient intake to the level of sedentary controls [1]. The exercise induced hyperphagia is associated with increased circulating levels of growth hormone and insulin [1] which may act, probably through the mediation of somatomedin, to induce accelerated growth. Increases in circulating growth hormone (GH) are a consequence of a doubling of GH pulse frequencies and amplitude [2]. Exercise-induced facilitation of pulsatile GH release is mediated by release of endogenous opiates which appear to restrain the action of endogenous somatostatin [2]. Which may have been increased in parallel with peripheral blood SRIF-LI.

The ingestion of an increased amount of food in the exercising animals is associated with a striking elevation in the plasma SRIF-LI levels. Exogenously administered SRIF reduces gastrin levels, gastric acid and enzyme output, delays gastric emptying, reduces pancreatic exocrine secretion, reduces gastrointestinal motility and slows nutrient absorption [5,11–14]. SRIF also decreases pancreatic endocrine secretion of insulin and glucagon [12] and gut peptide secretion [12,14]. These effects, coupled with the fact that nutrient ingestion releases gastrointestinal [15,21,22] and pancreatic endogenous SRIF-LI [15,22] has led to the postulate that SRIF-LI acts physiologically to modulate and slow the absorption of nutrients from the gut and modulates the hormonal response to absorbed nutrients [16,17,23–25].

The source of the elevated SRIF-LI in the general circulation of the exercising animals is probably the gut and pancreas which is the site of the largest proportion of SRIF [6–9,18,36] containing tissues and furthermore is a site from which nutrients are known to cause such release [15,17,19,22–25]. The assay utilized measures of SRIF-(1–14) as well as SRIF-(1–25) but the relative proportions of the various subforms of SRIF-LI [17,26] was not further investigated. The SRIF-LI concentrations measured in unextracted plasma by this assay [10,19,34] are higher by a factor of 3 or greater than in techniques utilizing extraction [21,23,24,33]. Nevertheless, the relative concentration in different parts of the circulation and the responses to nutritional and other stimuli are consonant with the findings of others using extraction [10,19,21,23,24,26,37].

The hypothalamic pituitary portal SRIF is unlikely to contribute to the marked increase in SRIF-LI in the general circulation as the degree of dilution of hypothalamic portal blood has been shown to preclude this [9,19]. In addition, the elevated growth hormone levels with evidence that in exercising animals endogenous opiates restrain endogenous somatostatin action on GH release [2] militate against inhibition by this component of circulating SRIF-LI. These concentrations of SRIF-LI are similar to those occurring following meal ingestion in man and other animals [15–17,22,37] and are of a magnitude which has been shown when infused exogenously to reduce nutrient absorption and pancreatic exocrine function [1,5,11–14].

Nutrient ingestion is a major stimulus to SRIF-LI secretion and somatostatin is believed to regulate or modulate nutrient absorption from the gut [22,23,25,35]. In the exercise induced hyperphagic state, increased food ingestion might provide an exaggerated stimulus and thus may explain the striking increase in circulating SRIF-

LI [22,23,25]. Once amino acids and other nutrients are absorbed these also act as a stimulus to SRIF-LI release from the pancreas and gut [15,19,21,23–25]. In addition somatostatin has been shown to induce satiety and thus may serve to modulate nutrient ingestion [29,30,32] in a negative feedback loop. The exercise induced hyperphagia may thus activate counter-regulatory SRIF-LI secretion to modulate the nutrient ingestion (albeit that this satiety signal is at best only modestly effective as evidenced by the ongoing hyperphagia). To determine if the elevated circulatory SRIF-LI levels are the consequence of exercise (by means of the hyperphagia it induces) or to the hyperphagia per se it would be necessary to examine SRIF-LI levels in other hyperphagic models, such as the gold thioglucose mouse, the Zucker rat or, the Ob Ob mouse.

In man [27] and in the horse [28] vigorous exercise has been shown to elevate circulating somatostatin levels, by an, as yet, unknown mechanism. Our experiences would seem to indicate that exercise per se is not responsible for the elevation of plasma SRIF-LI in the hamster since SRIF-LI did not rise in exercised food restricted animals unless restriction of food intake is capable of suppressing exercise induced SRIF-LI secretion. Fasting and starvation do reduce circulating SRIF-LI levels in the rat [10].

In conclusion, we thus believe these studies have demonstrated that the exercise-induced hyperphagia observed in hamsters is associated with the hyperinsulinemia, hypersomatotropinemia and elevation of circulating SRIF-LI which serves to modulate the increased nutrient entry in this state.

Acknowledgements

This work was supported, in part, by the National Science Foundation Grants PCM 78-07626 and PCM 81-06375 to K.T. Borer and NIH Grant AM 27077 and a grant from the Kroc Foundation to A.I. Vinik. We thank Lois Wagner, Joyce Blemly and Lynde Amstutz for skilled technical assistance and for manuscript preparation.

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