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Somatostatin release as measured by in vivo microdialysis: circadian variation and effect of prolonged food deprivation

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Abstract

In vivo microdialysis was used to determine SRIF release from the hypothalamus in unanesthetized male rats over a period of 24 h and in rats deprived of food for 72 h, in relation to changes in plasma GH levels. Before the experiment, a microdialysis probe was inserted into the anterior pituitary gland of the rats with an indwelling right atrial cannula. Dialysates and blood samples were collected serially, after normal feeding or 72-h deprivation of food. Normal rats implanted with the microdialysis probe showed an episodical pattern of GH secretion at intervals of 3 h. SRIF was secreted in a pulsatile fashion in the dark period in a similar manner to the light period. Mean SRIF pulse amplitude and mean SRIF level were significantly increased in the dark period. There was no significant correlation between the SRIF and GH pulses in the light period. SRIF levels in dialysates obtained from fed rats and food-deprived rats showed a pulsatile pattern. Food deprivation resulted in significant increases in mean SRIF level and mean SRIF pulse amplitude. These results suggest that the existence of circadian rhythm in SRIF release and the increase in SRIF release play an important role in suppressing GH secretion during prolonged food deprivation.

Keywords: Microdialysis; Somatostatin; Growth hormone; Food deprivation; Circadian rhythm; Adenohypophysis

1. Introduction

Growth hormone (GH) is secreted episodically in many species including man. GH secretion is stimulated by growth hormone releasing hormone (GHRH), and suppressed by somatostatin (SRIF) released from the hypothalamus. Although the effects of GHRH and SRIF on the secretion of GH have been studied extensively, the dual control mechanism of these hormones on GH secretion in physiological conditions is still not fully understood owing to a number of technical difficulties. Therefore, the relationship between the release of hypothalamic GHRH and SRIF and GH secretion has often had to be estimated indirectly. Studies using anti-GHRH antibody [36] suggest that the ultradian surges of GH secretion from the hypophysis are due to the episodic release of hypothalamic GHRH. Studies using anti-SRIF antibody [4,26] suggest that SRIF is possibly released episodically into the hypophyseal portal vein to inhibit GH release and induces the GH trough periods. Plotsky and Vale [19] studied the relationship in rats directly by measuring GHRH and SRIF levels in the hypophyseal portal blood. However, care is needed in interpreting these data because the blood was obtained from hypophysectomized rats under anesthesia. Some investigators [7,10] measured SRIF release from the median eminence of unanesthetized, freely moving rats, using the push-pull perfusion method. However, long-term measurement is difficult because this method seriously damages the tissue. Ungerstedt et al. [34] devised a new technique called brain microdialysis to resolve the problem. This technique has since been modified by many investigators, and has started being used for measuring hypothalamic hormones [15,18]. However, it still involves many problems, such as low recovery of the hormones and low sensitivity of the assay system. We previously established a method for measuring SRIF levels in rat adenohypophysis by microdialysis, and reported the effects of various anesthetics on somatostatic release [24]. The present study was undertaken firstly to simultaneously measure intrahypophyseal SRIF levels and plasma GH levels in the peripheral vein in unanesthetized, freely moving rats, secondly to examine the circadian rhythm of hypothalamic SRIF release and finally to assess the effects of prolonged food deprivation on SRIF secretion.

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2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan), weighing 300–400 g, were individually housed in a temperature-controlled room with lights on at 09.00–21.00 h, and given regular rat chow (Oriental Yeast, Tokyo, Japan) and tap water ad libitum.

2.2. Surgical protocol

Rats were anesthetized by an intraperitoneal (i.p.) injection of 50 mg/kg pentobarbital. By using a stereotaxic device (David Kopf Instruments, USA), a guide cannula was implanted with its tip just dorsal to the anterior pituitary using the coordinates 0.9 mm lateral to the midline, 6.0 mm posterior to the bregma and 8.5 mm ventral to the dura, and fixed in position with dental resin (GC Reparisin, GC Shika, Kyoto). The length of the cannula was predetermined, such that the insertion of the microdialysis probe allowed the entire length of the probe tip (dialysis membrane) to extend beyond the tip of the guide cannula and span the dorsoventral extent of the anterior pituitary tissue. A stainless steel stylet was fixed within the guide cannula and left in place until the time of the experimental sessions.

Three days after the implantation of the guide cannula, some of the rats had a chronic indwelling cannula implanted in the right atrium via the external jugular vein under pentobarbital anesthesia. Catheters exited at the back of the neck, to permit repeated blood sampling from freely moving rats without handling. Cefotetan (20 mg/kg body weight (BW)) was injected after the operation to prevent infection. All experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Tokushima.

2.3. Microdialysis probes and system

Microdialysis experiments were conditioned as we previously described [24]. The microdialysis probes used were commercially available probes (CMA/12, CMA microdialysis, Sweden) with 2 mm polycarbonate membrane. Ringer's solution containing 0.1% bovine serum albumin (BSA) was perfused at a flow rate of 2 ml/min, using a pulse-free microinfusion pump (EP-60, Eicom, Kyoto, Japan) and a 2.5-ml Hamilton gastight syringe. Tubes made of fluoroethylenepropylene (FEP) were connected between the syringe and probe and between the probe and sampling tube. Perfusion was carried out using the push method. To proceed with the microdialysis experiments, the stylet was removed and the microdialysis probe was slowly inserted 12 h prior to the start of sampling and secured to the previously implanted guide cannula under an anesthetic-free, non-immobilized state. During this period, the probe and tubes were continuously perfused.

2.4. Sampling procedure

In the first group of rats with chronic indwelling cannula, one week after the surgery, the collection of microdialysis dialysates was started at 09.00 h. The dialysates were collected every hour for 12 h into collection tubes, and stored at −20°C for subsequent SRIF radioimmunoassay. Simultaneously, blood samples of 0.15 ml were withdrawn via the indwelling right atrial cannula at 30-min intervals during the entire duration of the experiment, and immediately centrifuged. The plasma was stored at −20°C for subsequent rGH assay.

In the second group of rats, in which only the guide cannula was implanted, dialysate was collected one week after the implantation from 09.00 h at 1-h intervals for 24 h into collection tubes, and stored at −20°C for subsequent SRIF assay.

The first and second group of rats were allowed free access to food.

In the third group of rats with chronic indwelling cannula, one week after the surgery, were food-deprived for 72 h before the collection of dialysates and blood samples. The collection of microdialysis dialysates and blood samples were carried out in the same manner as the first group. All samples were stored at −20°C for subsequent assay.

At the end of all experiments, the rats were anesthetized with pentobarbital and their brains were fixed by the infusion of 10% formaldehyde; the probe position was then verified histologically.

2.5. Determination of SRIF and GH levels

SRIF levels were measured by RIA as reported previously [21,22]. The lowest detectable SRIF level was 1.5 pg/tube, and a dose-response was observed in the 1.5–200 pg/tube range. The cross reactivity of SRIF(1–28)-NH2 with the SRIF antibody used in this study was 109.2 ± 4.5% (mean ± S.D.) on a molar basis [9]. Rat GH levels were measured by RIA using materials supplied by NIDDK, NIH(Bethesda, Md.). The intra- and interassay coefficients of variation (CVs) of these two assays were less than 9 and 14%.

2.6. Statistical analysis

To determine if observed fluctuations in dialysate SRIF levels constituted endogeneous pulse, results were analyzed by Cluster analysis method of Veldhuis et al. [35]. At statistic of 4.1 was selected to maintain a maximal false positive rate of 1% or less by using cluster sizes of one or two in the nadir and peak. For analysis of data, the SRIF levels that fell below the level of assay detectability were

assigned as a value equal to the sensitivity of the assay (1.5 pg/tube). The significance of differences between pairs of groups were tested by the unpaired Student's *t*-test. Data are expressed as mean ± S.E.M.

3. Results

Over the whole duration of the experiment, each rat exhibited the full range of its normal activities, including drinking, feeding, grooming, and sleeping. Dialysate collected from most rats allowed us to measure SRIF concentration. In some rats, the insertion of the dialysis membrane fitted to the tip of the probe into the hypophysis could not be confirmed, and therefore these rats were excluded from the analysis. The dialysate SRIF levels in most of these excluded rats were lower than the detectable limit.

Fig. 1 shows the time course of plasma GH and dialysate SRIF levels of freely moving rats in the first group. Plasma GH levels showed a pulsatile pattern. Cluster analysis revealed periodic secretion of GH, with a pulse frequency of 4 pulses/12 h and a mean period of 3 h. The pulse amplitude averaged 192.7 ± 14.4 ng/ml, and the GH level averaged 42.2 ± 6.0 ng/ml. The SRIF levels in dialysate also showed a pulsatile pattern, with a pulse frequency of 2.0 ± 0.3 pulses/12 h, a mean pulse amplitude of 7.4 ± 0.8 pg/h and a mean SRIF level of 3.9 ± 0.3 pg/h.

Fig. 2 shows changes in dialysate SRIF levels over 24 h for the freely moving rats in the second group. Dialysate SRIF levels continued to show a pulsatile pattern both during the dark (21.00–09.00 h) and the light periods (09.00–21.00 h) of their day. During the light period, the pulse frequency was 1.3 ± 0.2 pulses/12 h, and the pulse amplitude was 8.1 ± 0.8 pg/h, with a mean SRIF level of 4.1 ± 0.3 pg/h, none of which differed significantly from those in the rats which received simultaneous plasma GH measurement. The pulse frequency during the dark period

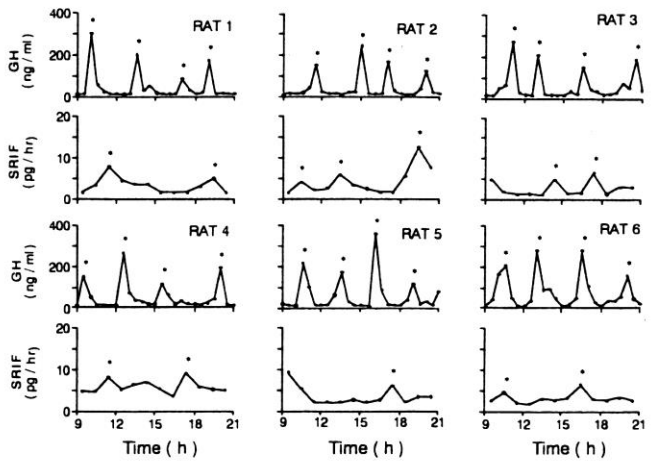

Fig. 1. SRIF levels in dialysate and plasma GH levels in atrial blood obtained from six freely moving fed rats. *, GH and SRIF pulses were defined by Cluster analysis.

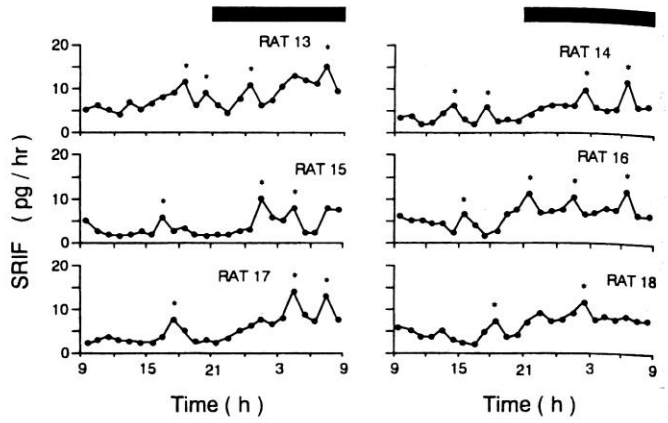

Fig. 2. Circadian variation of SRIF levels in dialysate obtained from six freely moving fed rats. *, SRIF pulses were defined by Cluster analysis. Horizontal bars, periods of darkness.

(2.0 ± 0.3 pulses/12 h) did not differ from that during the light period. However, the mean pulse amplitude (12.9 ± 0.6 pg/h) and the mean SRIF level (8.0 ± 0.4 pg/h) during the dark period were significantly higher than those during the light period ($P < 0.001$, Table 1). The dialysate was collected from two rats (RAT 13 and RAT 14), for an additional 12 h. The SRIF levels in these samples decreased during the next light period and showed a pulsatile pattern, returning to the levels recorded during the previous light period (data not shown).

Fig. 3 shows changes in plasma GH and dialysate SRIF levels in the third group of rats, which were deprived of food for 72 h. The fasted rats showed pulsatile changes in plasma GH levels. Both the mean pulse amplitude (18.4 ± 1.3 ng/ml) and the mean GH level (8.1 ± 0.6 ng/ml) were significantly lower than those in the fed group ($P < 0.001$). During the 72 h period, the fed rats showed an approximately 6.9% weight gain from 339.2 ± 3.0 g to 362.5 ± 3.0 g, while the fasted rats showed about 10.8% weight loss from 340.0 ± 6.2 g to 303.3 ± 6.2 g. The SRIF levels in the dialysate from the fasted rats showed a pulsatile pattern and were higher than those from the fed rats. The mean pulse amplitude (25.4 ± 4.1 pg/h) and the mean SRIF level (16.9 ± 1.4 pg/h) in the fasted group were 3 to 4 times higher than the fed group. The pulse frequency in the fasted group (1.2 ± 0.5 pulses/12 h) did not differ significantly from that in the fed group. The minimum SRIF level in the dialysate was below 1.5 pg/h

Table 1
Circadian variation of SRIF levels in dialysates obtained from freely moving rats

	Light	Dark
Mean level (pg/h)	4.1 ± 0.3	8.0 ± 0.4 [a]
Pulse amplitude (pg/h)	8.1 ± 0.8	12.9 ± 0.6 [a]
Pulse frequency (pulses/12 h)	1.3 ± 0.2	2.0 ± 0.3

Values are mean ± S.E.M. ($n = 6$).
[a] $P < 0.001$ vs. rats in light period.

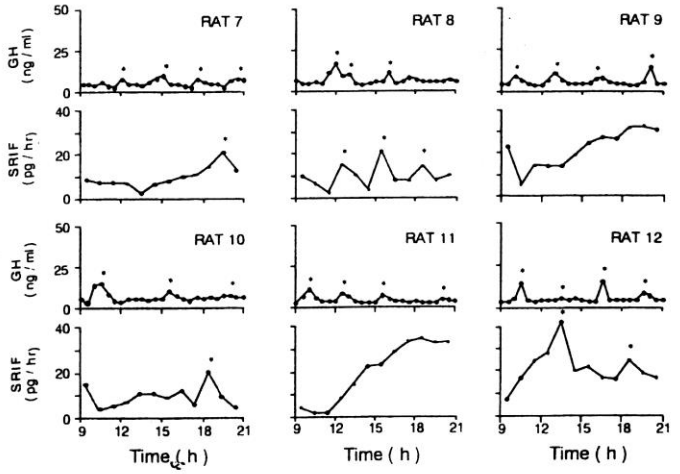

Fig. 3. SRIF levels in dialysate and plasma GH levels in atrial blood obtained from six freely moving rats after 72 h food deprivation. *, GH and SRIF pulses were defined by Cluster analysis. Note that the scales of plasma GH levels are different from those in Fig. 1.

in the fasted group, similar to that in the fed group. In the dark period, both the mean pulse amplitude and the mean SRIF level were significantly lower than in the fasted group ($P < 0.001$).

4. Discussion

SRIF is secreted from the median eminence endings of the neurons of the periventricular nucleus (PeN) and paraventricular nucleus (PVN) into the hypophyseal portal vein [14], and suppresses the secretion of GH from the pituitary cells [21]. Regarding tissue SRIF levels in rats, Palkovits et al. [17] reported that SRIF was abundant in the median eminence (352.64 ng/mg protein), and Kasting et al. [10] reported pulsatile change in SRIF levels in the median eminence using the push-pull perfusion method. Since the insertion of a probe into the anterior pituitary, the end of the hypophyseal portal vein system, is easy and reliable [12], we have established a method for measuring changes in intrahypophyseal SRIF levels in rats using a microdialysis technique [24]. Meredith and Levine et al. also used microdialysis to examine changes in intrahypophyseal LH-RH levels [15], and reported results similar to data previously yielded by push-pull perfusion of the hypothalamic/median eminence [11]. We also examined changes in peripheral blood GH levels as well as intrahypophyseal SRIF levels over 12 h, using microdialysis. Earlier studies on simultaneous analysis of changes in hypothalamic and hypophyseal hormones included direct measurement of GHRH and SRIF levels in hypophyseal portal vein blood of sheep for 7 h [5,33], and for 6 h [7]. However, in examining hypothalamic SRIF release in rats under various physiological conditions, the duration of measurement was less than 12 h in all previously published studies. In the present study, we continued to collect samples for up to 36 h, with no difficulty in collecting samples, and without changing the behavior of the rats. In the male rats, GH release is characterized by a striking ultradian rhythm, with high-amplitude burst of GH release occurring at 3–4 h intervals [30]. In our study, the rats allowed free access to food showed pulsatile secretion of GH at intervals of 3 h. This indicates that intrahypophyseal microdialysis does not significantly affect the function of the hypothalamus and pituitary involved in the regulation of the ultradian rhythm of GH secretion.

In the present study, fed rats showed pulsatile change in dialysate SRIF levels, with a mean SRIF level of 3.9 ± 0.3 pg/h and a mean peak SRIF level of 7.4 ± 0.8 pg/h. Pulsatile secretion of SRIF has been suggested by experiments using passive immunization [26] and in vivo push-pull perfusion [7,10]. Other experiments using passive immunization [29] and a measurement of hormone concentration in the hypophyseal portal vein [19] suggested that hypothalamic GHRH secretion is synchronous with peaks in GH secretion, and hypothalamic SRIF secretion is synchronous with troughs in GH secretion. These findings were endorsed by the measurement of hypothalamic mRNA which revealed that the expression of GHRH mRNA and that of SRIF mRNA varied in an alternating fashion at the peaks and troughs of the GH secretion [39]. The study of SRIF binding to the arcuate nucleus also revealed that binding activity was higher during peaks in GH secretion than during troughs in GH secretion [28]. In the present study, peaks in SRIF secretion were not clearly synchronized with peaks or troughs in GH secretion measured simultaneously. The measurements of hypophyseal portal vein blood GHRH and SRIF levels and peripheral blood GH levels of unanesthetized sheep [5] demonstrated that 62% of all peaks in GH secretion were identical to the peaks in GHRH secretion, but that the peaks in SRIF secretion did not correlate with peaks or troughs in GH secretion. Taken together, SRIF may not be involved in the generation of GH pulses, but we cannot rule out the possibility that small peaks may not have been detected because the dialysate had to be collected at 1-h intervals because of problems in recovery and RIA sensitivity. We will have to improve our method of microdialysis for detecting GHRH secretion to solve this issue.

When microdialysis was carried out for 24 h, a pulsatile pattern in dialysate SRIF levels were seen both during the dark period and the light period. The mean pulse amplitude and the mean SRIF levels were, however, markedly higher during the dark period than during the light period, indicating a circadian variation of SRIF release. GH secretion in humans usually shows circadian rhythm, while GH secretion in rats shows ultradian rhythm rather than circadian rhythm [30]. However, thyrotropin (TSH) shows circadian rhythm with an increase during the daytime in rats [20] and SRIF suppresses the secretion of TSH [4]. This suggests that the secretion of SRIF is elevated at night. Although some investigators have reported that hypothalamic SRIF mRNA expression showed no circadian variation [25],

hypothalamic SRIF contents during the light period were higher than those during the dark period, resembling the changes in SRIF contents in the suprachiasmatic nucleus [6]. The suprachiasmatic nucleus is thought to serve as the highest center for the control of circadian rhythm [13]. When this nucleus is destroyed in rats, the secretion of GH does not synchronize with the light/dark cycle, although it continues to show an ultradian rhythm [38]. Rhesus monkeys are reported to show elevation in cerebrospinal fluid SRIF levels during the dark period [1]. The results of our study also indicate the presence of circadian rhythm in the secretion of SRIF from the hypothalamus. It therefore seems likely that the secretion of GH in rats is affected by circadian rhythms of SRIF secretion.

We demonstrated that hypothalamic SRIF secretion was increased, but GH secretion was reduced in food-deprived rats. It has been reported that nutritional conditions affect GH secretion in various ways. Healthy adult men show increases in the pulse frequency, pulse amplitude and 24 h total amount of GH secretion when they fast for 5 days [8]. When rats are fasted for a long period of time, their GH secretion is markedly suppressed [27]. When the hypophyseal GH content of fasted rats is measured by RIA, the content is higher than in normal rats [32].

When fasted rats are treated with anti-SRIF antibody, the suppressed GH secretion recovers [27]. Furthermore, it was reported that GHRH-induced GH release was attenuated in fasted rats [37]. We therefore think it probable that the suppressed GHRH-induced GH secretion observed in fasted rats reflects enhanced endogenous SRIF secretion. On the other hand, Tannenbaum et al. [31] measured right atrial venous blood SRIF levels in fasted rats and found the levels to be higher than those in normal controls. They suggested that increased SRIF levels in the peripheral blood after fasting originate from the pancreas, and digestive system, while no such changes were seen in the central nervous system including the hypothalamus [32]. However, SRIF secretion from cultured pancreatic cells from fasted rats was smaller [23]. In addition, Mounier et al. [16] speculate that the SRIF, which suppresses GH secretion following fasting, originates from the hypothalamus, on the basis of findings in rats whose hypothalamus was artificially destroyed to eliminate the influence of hypothalamic SRIF. The effective SRIF level to suppress GH secretion from cultured anterior pituitary cells was reported to be 1 nM [2]. Since the relative recovery rate of the dialysis membrane of our probe was $6.1 \pm 0.5\%$ in vitro as reported previously [24], the average SRIF level in the pituitary extracellular fluid is estimated to be about 0.34 nM in the light period, about 0.66 nM in the dark period and about 1.40 nM during food deprivation. Although the application of the recovery rate in vitro to an in vivo situation requires great care, we may calculate that the SRIF levels were more than 1 nM in the period during food deprivation.

It was recently reported that fasting did not cause any change in the hypothalamic GHRH and SRIF contents and prepro-SRIF m RNA levels in rats, while it reduced the hypothalamic prepro-GHRH mRNA levels [3]. It seems possible, therefore, that reduced synthesis of GHRH is also associated with a reduction in GH secretion. These findings suggest that the suppression of GH secretion represents a reduced release of GH rather than a reduced synthesis of GH in the hypophysis, by enhanced SRIF secretion and/or reduced GHRH secretion in food-deprived rats.

In the present study, by using pituitary microdialysis, we demonstrated that in addition to the presence of circadian rhythm, SRIF is secreted in a pulsatile fashion and is markedly increased by food deprivation.

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