Surgery depresses pulsatile growth hormone release in rats for up to 2 days

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Abstract. In order to study the pulsatile release of rat growth hormone in a stress-free environment, many investigators obtain sequential blood samples from individual rats bearing an indwelling, right atrial cannula. There has been little systematic study of the stressful effects of the cannulation procedure on the pulsatile release of rat growth hormone. We examined the acute and chronic effects of right atrial cannulation on growth hormone release in male rats by monitoring plasma growth hormone concentration at every 2 min. Right atrial cannulation was performed under ether anaesthesia. Blood was collected from the rats for a 2 h period (11:00–13:00 h), either immediately following, or 2, 3 or 7 days following the surgery. When blood was collected 7 days after surgery, growth hormone was released in large bursts. The amplitude of these bursts however, did not differ significantly from the bursts of growth hormone in rats cannulated 3 days prior to blood collection. On the other hand, the bursts of hormone release in rats cannulated immediately or 2 days before blood collection were significantly smaller in amplitude than those in rats cannulated 3 days before collection, but were not significantly different from each other. In many of the rats cannulated immediately before or 2 days before blood collection, pulsatile growth hormone release was completely suppressed. The results of the study suggest that blood sampling from right atrial cannulae to measure the plasma concentration of rat growth hormone should not be carried out until at least 3 days after the cannulation procedure.

The demonstration that rat growth hormone (rGH) release is pulsatile (Martin et al. 1974; Tannenbaum & Martin 1976) ushered in a new era in the study of rGH release. Because of the rhythmic nature of plasma rGH fluctuations, blood samples for the study of rGH release must be obtained sequentially, in a stress-free manner, from individual rats. To solve this problem, many investigators (Tannenbaum & Martin 1976; Eden 1978; Katakami et al. 1981; Shin 1982) collect blood from a cannula implanted into the right atrium of the rat.

However, right atrial cannulation is itself a stressful procedure. There has been little systematic study of the effect of this surgery on rGH release that has been communicated to workers in this field. We examined the acute and chronic effects of right atrial cannulation on rGH release in male rats, by monitoring plasma concentration of rGH at every 2 min. The release profiles of rGH from all rats were quantitated, and compared by standard statistical procedures.

Materials and Methods

Male Sprague-Dawley rats (300–500 g) (Charles River, CD, Canadian Breeding Farm and Laboratories) were acclimatized in a controlled environment with illumination for 14 h daily (06:00–20:00 h) and a temperature of 25 ± 1°C. The rat housing room was equipped with a laminar flow system (Airo Clean Engineering, Inc., Pennsylvania) capable of maintaining a ‘disease-free’ environment. Purina Lab Chow and tap water were supplied ad libitum.

Each rat was anaesthetized with ether, and cannulated as previously described (Shin 1980). The cannula was then filled with heparinized (50 U/ml, from porcine intestinal mucosa, Sigma Chemical Co.) saline and the free end tied off with a thread.

After the surgery, the rats were allowed to recover in single housing cages. These rats were then placed in single sampling cages 1 day before blood collection.
Single sampling cages were inside a wooden box equipped with a one-way observation window. Blood was collected either 7, 3 or 2 days after surgery. One hour before blood collection, polyethylene tubing (PE 60, 0.030 inches inner diameter, 0.040 inches outer diameter, 45 cm in length, Intramedic) was filled with saline and connected to the end of the cannula.

One group of rats was not allowed to recover from the surgery. These rats were cannulated between 09.00 and 11.00 h, then transferred to single sampling cages. Saline-filled polyethylene tubing was connected to the cannula and blood samples were collected immediately.

Blood samples (approximately 70 µl) were collected in heparinized microhaematocrit tubes (Fisher Scientific Co.) at consecutive 2 min intervals. The tubes were sealed with Critoseal and placed on ice. The tubes were then spun at 13000 × g for 4 min, at 4°C, then stored at −20°C.

Triplicate 10 µl plasma samples were assayed with a radioimmunoassay kit kindly provided through the NIADDK Rat Pituitary Hormone Distribution Program. The quantity of rGH was expressed in terms of NIADDK GH RP-1. The coefficients of variation for inter-assay variability and intra-assay variability were 4.4 and 3.8%, respectively. The maximum reliable sensitivity was 50 pg or better.

![Graph](image)

**Fig. 2.**

Weight (mean ± st) of the area under the release profile of rGH between 11.00 and 13.00 h of rats from each experimental treatment. The weight is expressed as a percentage of the greatest mean weight (3-day group). The number in each box indicates the number of rats in each group. Differences between groups are indicated in the text.

The release profile for each rate was plotted as in Shin (1982), and the area under each curve was cut out and weighed. The areas thus measured were compared with Duncan's multiple range test.

**Results**

Investigators (Katakami et al. 1981; Shin 1982) have previously reported that one of the many bursts of rGH release during the day occurs between 11.00 and 13.00 h. We collected blood samples at 2 min intervals during this period of time, to closely examine pulsatile rGH release.

The release profile from a representative rat, obtained from a rat cannulated 7 days before collection is shown in Fig. 1. At 12.00 h, plasma rGH concentration rose abruptly from below 100
ng/ml, to a level approaching 700 ng/ml. Plasma rGH concentration then decreased, to a value below 100 ng/ml, where it remained until the end of the sampling period.

When the weights of the areas under each rGH release profile were plotted (Fig. 2) and compared with Duncan's multiple range test, it was found that the 7-day group did not differ from the 3-day group \( (P > 0.05) \), the 2-day group did not differ from the acute surgery group \( (P > 0.05) \), but the 3-day group was significantly different from the 2-day \( (P < 0.01) \) and the acute surgery \( (P < 0.05) \) groups. The areas obtained from the 7-day group, although greater than those from the acute stress group \( (P < 0.05) \), were not different from those of the 2-day group \( (P > 0.05) \).

**Discussion**

There are few reports describing the effects of stress on pulsatile rGH release. Martin (1974) noticed that pulsatile release of rGH was inhibited for up to 2 h by just opening the cage door. Terry et al. (1976) forced rats to swim in a water bath for 30 min. This stress inhibited pulsatile release of rGH for up to 5 h.

The effects of surgical stress on rGH release was studied by Takahashi et al. (1971) in anaesthetized rats. Femoral catheterization under pentobarbital anaesthesia caused a rapid decrease in plasma rGH that lasted for at least 2 h. However, at the time of Takahashi et al. (1971), investigators were not aware of the pulsatile nature of rGH release. The experimental procedure consisted of obtaining single samples from a number of rats at various time periods.

Eden (1978) sampled blood from rats bearing an indwelling right atrial cannula. Samples were obtained at 30 min intervals, for 3–4 h periods, on each of the 4 days following the surgical procedure. The first samples were obtained 24 h after surgery. The rGH release of 1 day was compared to that of another day by comparing the peak plasma levels of rGH on a given day. Peak levels were low on the first day after the operation, but increased gradually on the second, third and fourth days. Animals were considered healthy only after at least 4 days after surgery.

Given that the half-life of circulating rGH is 5–7 min (Frohman & Bernardis 1970; Strosser & Mialhe 1975; Wallace & Stacy 1975) a sampling frequency of 30 min is much too low to obtain a clear picture of pulsatile release, for 2 reasons: 1) high plasma rGH levels could be missed in the 30 min interval, and 2) peak plasma rGH levels are defined by single points and may not be reliable.

We therefore reexamined the effects of surgical stress on rGH release by monitoring plasma concentration of rGH at every 2 min, a time period much shorter that the half-life of rGH. We also looked at the acute effects of surgery on rGH release. The release profiles of rGH were quantitated by weighing the area under the profile curves.

Our results support the observation of Eden (1978) that surgery dampens the pulsatile release of rGH. Our results indicate that the pulsatile release pattern immediately after surgery is not significantly different from the release pattern 2 days after surgery. However, significantly more hormone is released 3 days after surgery, after which hormone release does not increase significantly (up to 7 days post-surgery).

These results suggest that blood sampling from right atrial cannulae to measure plasma rGH concentration should not be carried out until at least 3 days after the cannulation procedure.

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**References**


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