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CHANGES IN IMMUNOREACTIVE BASIC SOMATOMEDIN IN CONSCIOUS AND ANAESTHETIZED MALE RATS

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Basic somatomedin (B-SM) like insulin-like growth factor-I or somatomedin-C belongs to the basic group of somatomedins. In preliminary studies on the control system for B-SM, we have found that plasma levels of immunoreactive B-SM (IRSM) fluctuate in conscious adult male rats. The peaks average 85% of the local baseline level (minimum of 6 points) and occur with an interpeak interval of 2.1 h. In rats anaesthetized with nembutal (50 mg/kg) very few peaks were found. After approximately 3.4 hr. of anaesthesia, plasma IRSM levels dropped precipitously with a calculated $T_{1/2}$ of 50 minutes. These results suggest that IRSM levels may be related to serum GH levels which occurred 4 hr previously and that part of variation seen in serum levels in conscious rodents may be due to physiological fluctuations.

Somatomedins (SM) mediate the anabolic actions of growth hormone (GH) in cartilage and have insulin-like actions in extra skeletal tissues (1,2,3). As there is also some recent evidence suggesting that the Type I SM, insulin-like growth Factor I (IGF-I) may be responsible for the nitrogen retaining and mineralotropic effects of GH in vivo (4), it may be that SM have a more important metabolic role than previously thought.

The Type I SM are those which have an isoelectric point which is greater than 7.5. IGF-I (5), somatomedin C(SM-C)(6) and basic somatomedin (B-SM)(7) which all belong to the Type I group have marked radioimmunoassayable and physicochemical similarities. Recently IGF-I and SM-C were shown to be virtually identical in structure (8). B-SM has similar N-terminal amino acids but full sequencing is not yet complete.

There are very few studies which have addressed the problem of pulsatility in SM secretion. Minuto et al. (9) found changes in immunoreactive SM-C during sleep but not during wakefulness; however, the plasma samples were integrated over a 1-2 h interval which would obscure any short term variation.

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Other workers have reported rapid changes in SM in patients with a variety of stimuli but SM were measured by bioassay and may have responded to non-SM substances in the sera (10-13). Most recently our laboratory (14; preliminary report) as well as Baxter and his colleagues (15) have reported pulsatility in plasma immunoreactive SM-C measured in chronically catheterized conscious rats. We have now extended these studies on fluctuations of serum immunoreactive B-SM by looking at the effects of anaesthesia.

Methods

Chronic catheterization, conscious rats

Adult male Wistar rats (inbred strain or Canadian Breeding Farms and Laboratories, Montreal) weighing 325-450g were anaesthetized with nembutal(50 mg/kg). Catheters prepared with silastic tubing (Dow Corning #602-155, 0.025" ID x 0.047" OD) were implanted in the right atria via the right jugular vein. The catheters were exteriorized at the back of the neck, filled with a 10% solution of polyvinyl pyrrolidone in heparinized saline and capped. The animals were allowed to recover for 1 week or more, then placed in the test cage equipped with one way glass about 16 hours prior to experimentation. The test environment was the same as the usual environment; lights were on from 7 a.m. to 7 p.m. and food and water were allowed ad libitum. At 9 a.m. on the day of the experiment, the patency of the catheter was checked, and the animals were lightly heparinized (< 250 U/animal). Blood samples (0.5 ml) were removed every 15 minutes for 4 hrs beginning at 10 a.m. or 1 p.m., centrifuged for 1 min in a Beckman microfuge, the erythrocytes resuspended in saline and reinjected. No change in hematocrit was observed over the course of an experiment following this procedure.

In order to determine whether a pulse of B-SM was related to a pulse of GH in the preceding 1-2 hrs, we injected 5 ug GRF (Sigma growth hormone releasing factor fragment 1-40, human synthetic) via the atrial cannula into anaesthetized and conscious rats (N=10). Blood samples (0.2 ml) were removed every 2-3 minutes for up to 2.5 h. Erythrocytes were not reinjected because of the problem of sample contamination with saline when sampling at such frequent intervals.

Anaesthetized rats

Rats were anaesthetized with nembutal (50 mg/kg) and catheters placed in the right atria. Samples were removed as before for 4-5 hr. In a separate series of experiments a glucose bolus (300 mg) injected 45 min after initiation of sampling was followed by blood samples removed at 3,8, and 15 minutes and every 15 minutes thereafter for 2 hrs.

Diurnal variation - Swiss Webster mice

Adult male Swiss Webster mice (26-30 gm) were maintained in group cages, lights on 6 a.m. to 6 p.m. Mixed arteriovenous blood samples were taken from the tails of mice (N=8) at 2 hr intervals from 6 a.m. to 6 p.m. as it had previously been shown (unpublished observations) that there was no difference in serum levels of immunoreactive SM when samples were taken from the tails of conscious mice or from the brachial arteries of mice sacrificed by cervical dislocation or under ether anaesthesia.

Hormone assays

Levels of immunoreactive basic somatomedin (IRSM) were measured using a radioimmunoassay (RIA) which is highly specific for B-SM (14). All samples

were acidified to a final concentration of 1% formic acid and lyophilized prior to assay in order to measure total IRSM. Standards used were a pooled adult male rat serum (or for mice a pooled adult human serum) assigned a value of 1 U/ml. To determine the effects of heparinized serum or plasma on IRSM levels, 5 male rats were bled by cardiac puncture under nembutal anaesthesia and divided so that a serum sample and a plasma sample were obtained from each rat. Heparin (Sigma, Na salt) was added to individual serum aliquots or a pooled serum aliquot at a concentration of 13 U/ml. All samples were acidified prior to assay for IRSM. Values shown are Mean $\pm$ S.E.M. unless otherwise noted.

Plasma levels of immunoreactive GH were measured in blood samples removed from conscious rats using the rat GH RIA kit from NIADDK and the rat GH standard GH-RP-1.

IRSM Peak Analysis

A peak was defined as a point or group of points greater than 2 standard deviations above the mean local background. The local background was a minimum of 6 points (average of 9 points) about the peak. The absolute magnitude of IRSM (U/ml) was calculated as a % of the mean local background.

Results

Figure 1 shows the change in IRSM levels in sera taken from adult male mice at various times during the day. Values shown are Mean $\pm$S.E.M.; the envelope lines are $\pm$1 S.D. Highest values were from 10 a.m. until 2 p.m. with the lower values occurring at 6 a.m.

Heparin (13 U/ml) either when added to serum or used to anticoagulate whole blood, did not alter the amount of IRSM found in acidified rat serum or plasma. Table 1 shows that IRSM levels in male rats were not different in acidified plasma samples, acidified serum samples or acidified serum samples plus heparin.

Fluctuations in plasma concentrations of IRSM were evident in blood samples taken from conscious, unrestrained male rats (Figure 2). The

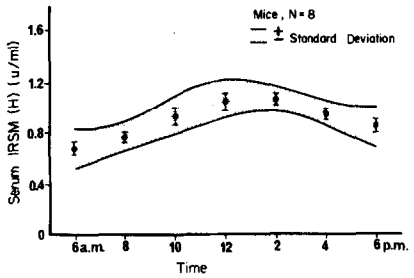

Figure 1. Serum IRSM in mice
Samples were taken at 2 hr intervals from adult Swiss-Webster mice. Values shown are Mean $\pm$S.E.M. from 8 animals; envelope is $\pm^1$ S.D.

Table 1

IRSM U/ml (acidified samples)

Rat #	1	2	3	4	5
Plasma	0.91	0.77	0.97	0.46	0.47
Serum	0.89	0.87	0.86	0.50	0.40
Serum + Heparin	0.78	0.88	0.87	0.55	0.42

IRSM in 5 male rats (400-440 g)

interpeak interval was 2.1 ± 0.27 (N=8) in time course studies of 8 rats. The absolute magnitude of the peak averaged 0.63 ± .08 U/ml (N=16) and the % increase over mean local background was 85 ± 11%. Peaks generally occurred at 10-11, 12:30-13:30, and 15-16 h. IRSM values declined over the 4 hr sampling period in 6 of 8 animals studied particularly towards the end of the experiment.

Figure 3 shows plasma IRSM levels and GH levels in small samples taken at 2-3 minute intervals from an anaesthetized (panel A) and a conscious (panel B) rat subsequent to injection of GRF. All rats exhibited a distinct pulse of GH, which was much higher in the anaesthetized than conscious animals. There were no increases in plasma IRSM over the course of the sampling period in any

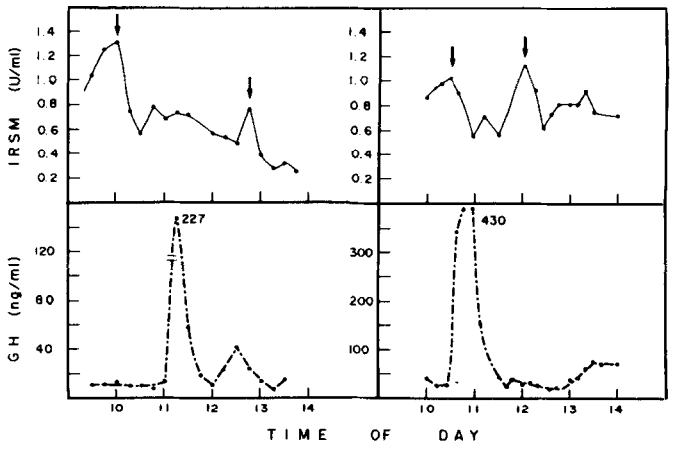

Figure 2. Plasma IRSM and GH in conscious rats
In the upper panels are shown plasma levels of IRSM in samples taken at 15 min intervals from two conscious male rats. The lower panels show plasma GH concentrations in the same animals. The arrows mark IRSM peaks.

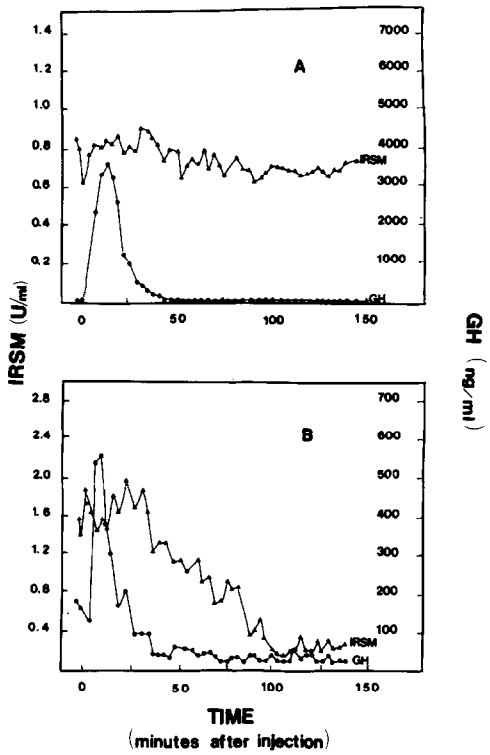

Figure 3. Plasma IRSM and GH after GRF injection
Panel A shows plasma IRSM and GH levels in samples removed at approximately 3 min intervals from an anaesthetized rat injected at t=0 with GRF, while panel B shows a similar experiment in a conscious rat.

of these animals, which were sampled for up to 2.5 h. There is clearly no minute to minute correlation of plasma levels of IRSM and GH.

In anaesthetized rats (Figure 4) plasma IRSM levels showed small fluctuations for the initial 3 hrs of sampling but the levels were more uniform (2 peaks/4 animals, magnitude 33 and 53% of background). After 3.4 $\pm$ 0.2 (N=4) h of anaesthesia the concentration of IRSM in plasma dropped precipitously with a mean slope of 0.01 $\pm$ .002 U/ml min which would give a calculated $T_{1/2}$ of 0.83 h. A glucose bolus had no consistent effect on IRSM levels in anaesthetized rats (N=4) (results not shown). Plasma GH in 6 anaesthetized rats (4-5h) was uniformly low (< 60 ng/ml), although 1 rat showed an unexpected GH peak (> 700 ng/ml) 4h after onset of anaesthesia and which coincided with a marked decrease in IRSM.

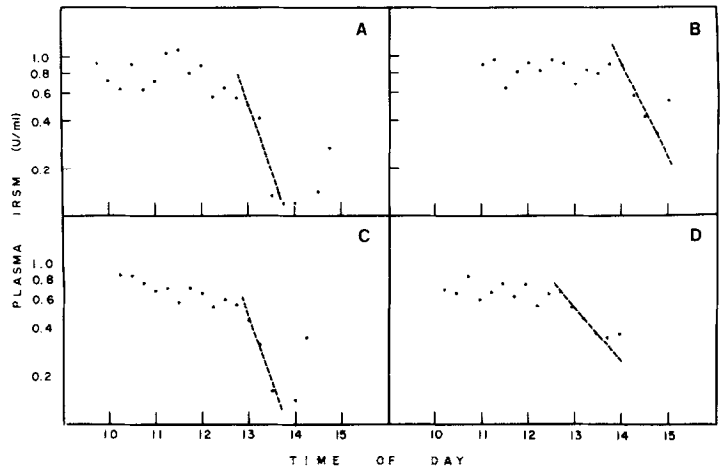

Figure 4. Plasma IRSM in anaesthetized rats
Each panel shows plasma IRSM concentrations in samples taken at 15 min intervals from four anaesthetized male rats. The dotted line marks the notable decrease in IRSM which occurred after long anaesthesia. Note that the Y axis is a log scale.

Discussion

The fluctuations we have found in plasma or serum levels of IRSM in conscious male rats (15) are similiar to those of Baxter et al. (16). They found SM-C peaks at 1.93 ± 0.47 h intervals and a peak/trough ratio of 1.21 ± .29/ 0.60. Our results show IRSM peaks at 2.1 ± 0.27 h intervals and with a magnitude of 85 ± 11% of background.

The effects of anaesthesia on IRSM appear to be complex. There were virtually no IRSM peaks and approximately 3.4 h after the onset of anaesthesia plasma IRSM fell precipitously. Baxter suggests the SM-C peaks correlate with GH pulses which occur 1 hr earlier. As GH serum levels are low in anaesthetized rats, this would explain the cessation of IRSM peaks in anaesthetized animals but not the precipitous fall. We suggest, if GH pulses occur with a 3 hr period (17), that IRSM levels could be dependent upon GH levels occurring 4h previously and the fall results from the expected GH pulse blocked by anaesthesia. Our results with the injection of GRF provide more evidence that IRSM is not correlated with GH pulses which occur in the preceding 1-2 h. However, as IRSM fluctuations occurred with a faster frequency than GH pulses, there may be other influences on IRSM concentrations and the precipitous fall

in IRSM may well be due to other effects of anaesthesia. It should be noted here that perfusion of tissue near the median eminence in rats revealed immunoreactive somatostatin pulses in plasma with an interpeak interval of approximately 1 hr (18). Thus there is similarly no exact correspondence between pulses of the controlling and the controlled hormones: in one case somatostatin and GH, and in the other GH and SM.

The fall in plasma IRSM which occurred in anaesthetized animals had an average $T_{1/2}$ of 0.01 $\pm$ .002 U/ml min which implies that a fall of from 1 U/ml to 0.50 U/ml would take 50 minutes. This is much faster than the suggested 2-6 hrs for protein bound SM (19-21). As our plasma samples are acid extracted to measure total SM (14) but the % of free SM is not known, it may be that much of the change we see is due to degradation of the free component, with only small changes in the protein bound IRSM. The previous studies which showed changes in bioassayable SM may have measured more of the free hormone, or as Hill suggested (12), may have actually measured changes in an inhibitor of the bioassay system. Alternatively the decrease in IRSM could result from sequestration of the hormone or alteration of the size of the fluid compartment sampled.

In summary, we have confirmed that small plasma IRSM fluctuations occur in conscious rats and extended these observations to show what occurs in anaesthetized rats. Further studies must be undertaken to clarify the relationship between GH and IRSM and to determine what other influences may alter IRSM serum levels.

Our results showing a diurnal variation in the concentration of IRSM in serum taken from mice are very similar to those of D'Ercole et al. (22), although in the latter study, sera were not acidified. Thus we have shown that total IRSM may vary in mice depending upon the timing of the samples taken. These studies together with the results in rats emphasize the difficulties in using the rodent model for studies of IRSM.

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