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GROWTH HORMONE SECRETION DURING SPACE FLIGHT AND EVALUATION OF THE PHYSIOLOGICAL RESPONSES OF ANIMALS HELD IN THE RESEARCH ANIMAL HOLDING FACILITY

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The spaceflight of the Research Animal Holding Facility (RAHF) on Space Laboratory 3 (SL 3) gave us the opportunity to investigate two of the objectives of the Cooperative Agreement:

1. to evaluate the suitability of the RAHF for housing and maintaining experimental animals during spaceflight; and

2. to determine changes in the secretion of growth hormone during spaceflight.

The other objectives were investigated using ground-based studies:

3. to determine the optimum conditions for creating gravitational force on space flight animals;

4. to identify neural pathways that may play a role in space flight syndrome;

5. to determine the time course of muscle atrophy due hypodynamia and hypokenesia in hindlimb-suspended animals and the role of growth hormone in these processes.

Flight Studies

Evaluation of the RAHF

The RAHF, developed as an semiautomated rodent support facility, was successfully flown for 7 days (April 29-May 6, 1985) as an engineering evaluation test. With exception of escape of particulate matter from the RAHF when waste trays and food casstte were changed and minor problems with the watering and feeding systems, all the environmental control systems performed at nominal values. The payload of 24 male rats (Taconic Farms) returned in good condition. The food consumption and growth of the 12 large (about 400 g) and 12 small (about 250 g) animals were not different from ground control groups. No differences were found in adrenal and liver size and appearance, nor in the levels of tyrosine aminotransferase; all of which are indices of normal unstressed animal. From these observations it can be stated that the RAHF housed animals in a manner equivalent, for biochemical and physiological investigations of weightlessness, to vivarium housed rats in ground-based laboratories. The results of the data derived from the 7-day mission establishes the RAHF as a support system for future animal research in space.

Although the growth and nutrition of flight rats did not differ signicantly from the ground controls, they showed the effects of 7 days in microgravity. On return from the spaceflight, they were flaccid, showing an almost total abscence of muscle tone. There was a marked loss of mass in certain muscles, with greatest loss being found in the antigravity muscles (33% in the soleus).The adductor longus, an antigravity muscle, showed moderate to extensive hemorrhage in the muscle body. Cytological and histochemical studies found a loss of muscle fiber diameter, an almost total abscence of Kreb cycle activity in muscles examined, in the antigravity muscles a replacement of slow twitch fibers by fast twitch fibers and a reduction in the amino acid pool. These changes along with the marked increase in glycogen indicate an altered metabolism due to weightlessness.

Reduced bone mass as the result of space flight was also apparent. The third lumbar verteba had a 13% loss in mass and an apparent greater porosity of the vertebral body. The tibia and humerus showed a marked decrease in bending and tensile strength (up to 36% less). The amount of bone change seen in only 7 days of weightlessness exceeds that seen in ground-based "weightlessness" model models for much longer periods (up to 28 days).

The pituitary gland was of normal size with a 20% increase in growth -hormone producing cells and a decrease in prolactin producing cells. This increase in growth hormone producing cells was not reflected by an increase in circulating hormone as the thymus, an organ sensitive to the concentration of plasma-borne growth hormone, was 20 to 25% smaller than those from ground control animals. Post flight in vivo and in vitro experiments confirmed that changes in secretory patterns had occurred (see below).
The weights of the spleens were 20 to 25% smaller in flight animals and may reflect the dumping of blood on reentry from the mission. Postflight analyses found that the production of interferon by the spleen was lacking or greatly reduced in the flight animals. This and the reduced lymphocyte counts in flight animals

may imply a comprimise to the immune system; that the animals returned in a healthy but altered state, indicates that the defense mechanisms were capable of coping with the immunological challenges met during the 7-day mission. One other significant finding was that the erythropoietic system sensitivity was markedly increased in flight animals when compared to controls.

While food and water consumption were within normal limits, the in-flight patterns of utilization changed. The circadian food rhythm deteriorated after the fourth day of flight with water consumption increasing linearly over the 7-day mission. These along with changing temperature rhythms, decreased temperature during the active phase and a decreased heart rate with decreased difference between the active and resting phases, indicate the effects of weightlessnes on circadian rhytms in rats needs more intensive investigation.

Overall, the RAHF was found to be capable of supporting rodents in spaceflight and the changes of structure and function were so profound that they must be due to the effects of weightlessness and not to the transient stresses of launch and reentry.

Pituitary Function after Space Flight

The putative causes of protein wasting in weightlessness are disuse, decreased frequency of neural impulses, shifts in calcium balance and hormone imbalance. The preeminent role of growth hormone in the regulation of protein anabolism has been shown by restoration of muscle mass and function following degeneration, tenotony, immobilization, hypophysectomy and certain dystrophies. It is well known that the most potent stimulus to growth hormone secretion is physical work; the lack of "work" in the weightless environment could result in diminished secretion of growth hormone. The losses in muscle masses found in the rats flown on SL 3, such as the 33% loss in the soleus of the small rats, indicates significant changes in muscle anabolism which may be due, in part, to changed growth hormone secretion.

To determine the role of growth hormone in the altered metabolism of flight rats, anterior pituitary glands from large and small rats were pooled and trypsinized into two single-cell suspensions. Compared with cell cultures from ground-based control animals, flight cells appeared to contain more intracellular growth hormone but released less hormone over a 6-day culture period. Growth hormone secreting cells (somatotrophs) from large and small, and flight and control rats were implanted into hypophysectomized rats. Both sets of flight cells released only 50% of the growth hormone compared with control cells. Glands from large flight rats contained 44% somatotrophs compared to 37% for controls; small rats showed no difference. There were no striking differences in ultrstructure between flight and control cells, nor were there major differences in immunoactive growth hormone variants. Fractionation of the culture medium indicated that "small" flight cells released 90% less of a high-molecular weight variant rich in growth hormone bioactivity. The results suggest that somatotrophs from rats exposed to microgravity may experience secretory dysfunction.

The results of the pituitary cell study cannot determine the contribution of growth hormone secretory dysfunction to the loss of muscle protein in flight animals. However, it is plausible to assume that the reduction of circulating bioactive hormone is a factor in the lowered rate of protein anabolism during weightlessness, and that amelioration of the defect may be possible if plasma levels of circulating biooactive-growth hormone can be returned to normal levels.

Ground-based Studies

Centrifuge

The planned use of centrifuges on both the Space Shuttle and Space Station to generate a "one gravity" environment requires that studies be carried out on earth to determine the effects of centrifugation on strucutre, physiology, reproduction and development. Other parameters that require evaluation are the gravity-gradients through the body of the organisms and the scaling effects due to the size of the organism generated by the artifical gravity field, and , if possible, distinguish between rotational and g-force effects.

To begin to understand the scaling effects on reproduction, pregnant guinea pigs (b.w. ca. 1200 g) and albino rats (b.w. ca. 300g) were exposed to increasing g-fields up to 1.71 gravity. At the highest level the

survival of guinea pig pups born on the centrifuge was comparable to 1-gravity control born animals. However for rats exposed to the 1.71 gravity -force survival was 60% of controls. Yet at 1.5 gravity, the survival rate of neonates was not significantly different from the 1 gravity controls. The loss of 40% of rats born on the centrifuge with the increase of 0.21 gravity indicates that the threshold for survival of neonates probaly lies close to 1.5 gravity. The normal survival of guinea pig young born on the centrifuge at 1.71 gravity indicates that the threshold for their survival lies above that force. These differences in survival are perhaps due to a scaling effect dependent on the size and maturity of the animal at birth. The guinea pig is more fully developed at birth than the rat and its precocity undoubtedly is a factor in its better survival at the higher g-force.

This does not mean that the exposure to the 1.71 g-force is without effect on the prenatal development of the guinea pig. There is a significant reduction in fetal growth rate with the birth weight significantly less than that of gravity controls. Those born at this elevated g-force level also have a signicantly increased bone to body mass ratio in comparison to non-centrifuged controls, while the muscle weight to body weight ratio was not different from controls. In terms of survival the 1.71 g-force has no effect on survival of the larger animal but does have signicant effects on its birth size and body structure. The implication that can be drawn from these preliminary findings is that data derived from organisms centrifuged as one-gravity "controls" during spaceflight must be evaluated in light of ground-based centrifugation to seperate centrifugation effects from gravity effects.

Nervous System Studies

Vestibular-Ocular Reflex

Vestibular-ocular reflex (VOR) mechanisms were analyzed in the rat. Previous studies had demonstrated specific VOR centers in the superior and medial vestibular nuclei (VN) in cats and monkeys but not in the rat. Utilizing the same horseradish peroxidase (HRP) axon-transport technique on 44 Sprague-Dawley albino rats, vestibular-nuclear patterns of projections to the oculomotor nuclei were found to be comparable to those found in cats and monkeys. This finding suggests that the rat is a suitable subject for VOR research in studies designed to delineate neural components of the space flight syndrome.

Disuse Effects on Neuromuscular Junctions

Preliminary studies of neuromuscular junctions by both light and electron indicate that after 7 days of hindlimb suspension, the motor endplates of the soleus muscle undergo pathological changes. Light microsopic studies at low power show the spacings between subneural structures to be decreased when compared to control . When seen with high power magnification, the subneural apparatus of the solei from suspended animals appears rounded or markedly decreased in size with irregular borders. The terminal innervation is frequently distroted.

The main changes observed in the electron-microscope images are of abnormal, vacuolated mitochondria within axonal profiles, translucent membrane-bound cavities fill a large portion of the terminal and myelin figures are also found.

These observations, the known changes in muscle structure and function resulting from hindlimb suspension (decreased electrical activity, a continuing loss of mass for up to 28 days of suspension, the change in fibers from slow-twitch to fast-twitch, and reduction in muscle-fiber cross-sectional area) and with the known trophic influences of innervated muscles on motoneurons suggest an interactive responses between the two systems under the experimental conditions. Also, it has also been shown that the morphological and physiological changes found in the soleus due to spaceflight or hindlimb suspension are similar. This suggests that the gound -based disuse model can be used as predicitive of the changes occurring in the neuromuscular junctions due to the effects of spaceflight.

Simulated Weightlessness Studies:

A series of hindlimb suspension studies to model weightlessness were conducted to determine: 1) if growth hormone (GH) concentrations were altered; 2) the time course of changes in GH concentrations and muscle atrophy; 3) if hypophysectomized rats show additional muscle atrophy due to suspension; and 4) if muscle atrophy can be reversed by exogenous GH.

Changes in GH concentrations and the degree of muscle atrophy as the result increased duration of the periods of suspension were measured in male albino rats suspended for 1,2,5 and 7 days. At the end of each suspension period hindlimb muscles, plasma and pituitary glands were collected and the tissues weighed. Varying rates of weight loss were found in the 6 muscles analyzed and at the end of 7 days of suspension all muscles were significantly smaller than controls. Pituitary and plasma GH, measured by bioassay, were decreased 35% after one day of suspension and 50% on successive days. Plasma GH measured by radioimmune assay did not differ from control at any time. Plasma corticosterone was elevated on day 1 but similar to control-rat values at all other times.

To measure the effects of the abscence of GH on the atrophy of hindlimb muscles during suspension, weight-stabilized male male hypophysectomized rats were suspended for 7 days. During this period the body weights of the suspended hypox rats did not differ from the weight at the beginning of the suspension period and the hindlimb muscles showed losses in weight. However, the reduction in muscle mass was attenuated by 1/3-1/2 when compared with intact suspended animals.

To determine if exogenous GH would affect the rate of atrophy of the hindlimb muscles of suspended rats, bovine GH was administered (2-100 microgram injections/day). In the intact rat no attenuation of muscle weight loss was found, in fact, the administration of exogenous GH may inhibit the secretion of endogenous GH. The same dose administered to hypox suspended rats attenuated the the atrophy due to hypophysectomy and/or suspension; in some muscles net growth occurred. The increases in muscle mass were proportionally equal to or greater than the the increases in body mass in reposnse to the exogenous hormone. The responses by 5 of 6 muscles of the suspended hypox rats were 2-5 times more refractory to exogenous GH than the non-suspended hypox control rats.

These studies indicate that in intact rats the depressed concentrations of GH persisted through the 7-day period of suspension and precedes or parallels muscle atrophy, and that the intact rat is refractory to exogenous hormone. On the other hand, hypox rats show increases in both body and muscle weight when exogenous GH is administered. However, the muscles of suspended hypox rats are less sensitive to the administered hormone than their controls. These findings indicate that that altered GH physiology contributes to the muscle atrophy found in the simulated weightlessness resulting from hindlimb suspension in the rat.

From the above conclusion and the fact that flight animals show similar changes in both muscle and GH physiology, it must be postulated that altered GH function is a major contributor to the atrophic changes found in the muscles of rats exposed to the microgravity of the 7-day mission of Space Laboratory 3.

These studies using animals conform to the care and use requirements of NASA/Ames Research Center Handbook 7180-1 and with "Guiding Principles in Animal Care and Use of Animals" approved by the Council of the American Physiological Society.

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