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THE PITUITARY GROWTH HORMONE CELL IN SPACE

W.C. Hymer
Penn State University

R. Grindeland
NASA Ames Research Center

Issues relating to the effects of microgravity (μG) on pituitary cell function require an understanding of how these cells are thought to be controlled and function both *in vivo* and *in vitro*. Our experimental designs for spaceflight research have been driven by current ground based research.

Ground based research. The mammalian pituitary is a small, well protected gland that is vitally important for the control of proper body function. A rigorous understanding of that control is hindered by heterogeneities, both cellular and molecular, of different hormone subsystems that we believe exist within the pituitary gland. The fact that there are disproportionate numbers of growth hormone (GH)- and prolactin (PRL)-producing cells must certainly reflect the importance of these two protein hormones in body metabolism. Results of intensive research now make it quite clear that these hormones are multifunctional. In the case of GH, for example, the hormone is a) involved in metabolism of fat, carbohydrate and protein and b) has receptors for GH that can be found on numerous tissues such as bone, muscle, liver and cells of the immune system. In light of these diverse biological activities, the idea that a single GH assay might not detect all of them is not surprising. Thus, hormone assays based on immunological techniques, while being easy and relatively inexpensive, may not always yield correct potency estimates (Ellis and Grindeland, 1974). Ongoing work in our laboratories unequivocally demonstrate that GH cells are heterogeneous with regard to the biological activity of the hormone they secrete (Grindeland, R. and W.C. Hymer. Differential Release of Bioreactive to Immunoreactive Growth Hormone from Separated Somatotrophs. Proc. Soc. Exp. Bio. Med. Manuscript submitted.).

In addition to heterogeneity of GH activities and GH cells, heterogeneities also exist within the GH molecules themselves. For example, alternative splicing of the GH mRNA results in two variants that may have different biological activites. Furthermore, post-translational modifications such as phosporylation, proteolytic cleavage, glycosylation and disulfide aggregation are known to occur within the gland (Lewis, 1984). Apart from one recent abstract (Farrington and Hymer, 1988) a positive correlation between these cellular and molecular heterogeneities has yet to be made.

While the foregoing comments relate to GH once released from the cell, it is important to know that sensitive techniques are now available to study GH cells themselves, For example, it is possible to 1) objectively categorize 30,000 cells by flow cytometry to determine percentages of GH cells in a suspension (Hatfield, and Hymer, 1985); 2) obtain information concerning the cells internal structure by laser flow cytometry (Hatfield, and Hymer, 1986a; Hatfield and Hymer, 1986b) 3) quantitatively measure measure hormone release from single cells (Kendall and Hymer, 1987); 4) isolate GH cells and GH cell subpopulations (Snyder et al, 1977); 5) maintain GH cells in culture in either serum-containing or serum-free media (Grindeland et al, 1987) and

finally 6) implant GH cells into the living animal using hollow fiber technology (Hymer et al, 1981) to determine the effects of the secreted GH *in vivo*.

Since the subject of this conference relates to μG effects directly at the cellular level, it is particularly important to understand the rationale and ramifications underlying the hollow fiber procedure. In this technique living cells ($2x10^5$) are injected (1.5 μl) into the lumen of a 10 mm XM-50 fiber and sealed-in using wax on the fiber ends. The fibers are implanted into the brains of hypophysectomized rats in such a way that the fiber courses through the lateral ventricles and is bathed in cerebral spinal fluid. This fluid is rich in a peptide (GRF) that can stimulate the encapsulated GH cells to release hormone from the fiber into the recipient's bloodstream so that the biological consequences of the hormone can be assessed by measurement of the tibial epiphyseal plate width some 10 days post-implantation.

Results of pituitary cell spaceflight research. Three experiments have been done to date; 1) STS-8 (1983); 2) SL-3 (1985) and 3) Cosmos 1887 (1987). In two cases pituitary glands, obtained from male rats that had flown in μG for 7 days (SL-3) or 13 days (Cosmos 1887), were used to prepare cells for subsequent study on Earth. In the other experiment, dispersed cells were maintained in a closed tube containing culture medium and serum at 37 ° C in a middeck locker. On return, the cells were recovered and cultured in fresh serum-containing medium for 6 days to determine what effect exposure to μG might have on the ability of the cell to release GH.

The results of these 3 experiments are summarized in Table 1. Details of each experiment can be found in (Hymer et al, 1987; STS-8), (Grindeland et al, 1987; SL-3) and (Hymer WC, Grindeland R, Krasnov I, Sawchenko P, Victorov I, Vale, W, Motter K and Vasques M. Changes in Pituitary growth hormone cells prepared from rats flown on Cosmos 1887. Manuscript submitted). Clearly, exposure to μG subsequently affected GH release fom the pituitary cell. Since most of our data come from rats that have "flown" in space, it is tempting (at first glance) to attribute the results to physiological effects at the organ/systemic level; for example, changes in fluid shifts, microcirculation, non-specific stress and the like. Closer inspection of our results, however, support the counter hypothesis that exposure to the unique environment of space affects secretory processes directly at the level of the pituitary cell. The arguments are:

- implantation of cells from flight rats into hypophysectomized rats, under conditions where flight cells could be maximally stimulated to release GH, clearly did not. This suggests a "secretory defect" within the flight cell that was maintained on subsequent testing in vivo.

- culture of cells from flight rats consistently showed partial shutdown of GH release.

- continued culture of cells that "flew" in space also showed shutdown of GH release on Earth.

What intracellular mechanisms could account for the effect? Some of the more obvious targets are a) the microtubular system; b) the GH packing system (golgi/secretory granule) and c) plasma membrane receptor defects. Since the fluorescence staining intensity of the GH cell is increased (Table 1), we currently favor the hypothesis that hormone packaging is a likely target.

However, microtubule "motors" drive the granules out of the cell and receptor function is likely to be mechanically coupled to these motors. Future experiments will test these various possibilities.

Since GH controls the function of other systems (bone, muscle, immune) which are themselves affected by μG, our research is relevant to the issue of long-term manned spaceflight.

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TABLE 1

Summary of results of three spaceflight experiments concerned with effects of μG on pituitary growth hormone cell function.

QUESTION	METHOD	FLIGHT	RESULTS
1. Is GH release affected in vitro? (immunoassay)	Cell Culture	STS-8	Yes. Reduced 20 fold
	"	SL-3	Yes. Reduced by ~ 50%.
	"	1887	Yes. Reduced by ~ 30%.
2. Is GH release affected in vitro? (bioassay)	HPLC of culture media-3T3 cell bioassay	SL-3	Yes. Activity of high molecular weight hormone reduced.
	Tibial assay of culture media	1887	Yes. Activity reduced by ~ 50%
3. Is GH release affected in vivo? (bioassay)	Hollow fiber Implantation	SL-3	Yes. Reduced by ~ 50%.
	"	1887	Yes. Reduced by ~ 50%.
4. Is the percentage of GH cells affected?	Laser flow immuno-fluorescence	SL-3	No.
	"	1887	No.
5. Is the size of the GH cell affected?	Laser light scatter	SL-3	No.
	"	1887	No.
6. Is the GH fluorescence staining intensity affected?	Laser flow immuno-fluorescence	SL-3	Yes. Increased about 16%/cell
	"	1887	Yes. Intensity doubled/cell
7. Are the variant forms of GH affected?	Western blotting	SL-3	No.