Wednesday, June 11

Session WA2
Room 2
8:30 - 11:30 a.m.

Plant and Animal Gravitational Biology - 2

THE ASYMMETRICAL GROWTH OF OTOLITHS IN FISH IS AFFECTED BY ALTERED GRAVITY AND CAUSES KINETOSIS

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INTRODUCTION

Immediately after having been subjected to microgravity conditions (e.g. in the course of parabolic aircraft flights), many human individuals reveal behavioural abnormalities regarding posture reflexes, ocular counterrolling, nystagmus and others. Given the case that such abnormalities occur, the respective person is apt to suffer space motion sickness (SMS), which is a particular kind of kinetosis-generated nausea including vomiting in the course of a prolonged stay under weightlessness. The basis of SMS (like motion sickness in general) is most likely due to non-matching visual, proprioceptive and vestibular cues in the central nervous system. A classic, exclusively theoretical concept presumes that such mismatches may be based on asymmetrically (from side to side) weighted inner ear utricular otoliths, which may be compensated for at 1g earth gravity by central nervous mechanisms (vestibular compensation), but may be disclosed in weightlessness, thus generating a secondary asymmetry on the level of the brain.

Many individuals of a given batch of larval fish, that are kept at modest hypergravity (3g; centrifuge) during their early ontogenetic development, reveal a kinetotic behaviour (so-called loop-swimming) as soon as they must face 1g conditions like it is observed at the transition from 1g to microgravity. This is possibly due to imprinting-like phenomena at higher g-levels, adjusting the central nervous integration of visual and vestibular cues.

Therefore, larvae after hyper-g experience can serve as model systems to investigate, if SMS might be caused by asymmetrical utricular otoliths.

METHODS

Larval cichlid fish were subjected to hypergravity from hatch until their yolk-sacs were completely resorbed (free swimming stage). After the experiment, the hypergravity animals were divided into a group of motion sick, i.e. looping samples and normally behaving individuals. The functional capacity of otoliths (utricular and saccular otolith, i.e. lapillus and sagitta, respectively; the former is involved in the perception of gravity, whereas the latter plays its role in hearing) was determined by measuring the maximum radius (r_{max}), as it is common in fisheries science when the interesting otoliths are too small to be weighted. Consequently, the absolute differences in r_{max} between the left and right sides (asymmetry) were determined and the two experimental groups were compared with each other and with 1g controls.

RESULTS AND DISCUSSION

Hypergravity reared samples yielded significantly smaller otoliths (regarding absolute r_{max}) than 1g normal earth gravity controls: The sagittae of hypergravity animals were by moderate 14.1 % smaller than those of 1g control animals (Fig. 1a; $p<0.0001$). The lapilli, however, were even by 33.2 % smaller in hyper-g fish than in controls (Fig. 1a; $p<0.0001$).

Calculating in addition the asymmetry, we found that sagittae revealed a tremendously increased asymmetry after hypergravity (Fig. 1b; $p<0.0001$). In contrast, the asymmetry of hyper-g lapilli was significantly decreased (Fig. 1b; $p<0.0001$).

Both the absolute r_{max} and the respective asymmetries of sagittae and lapilli as found in hyper-g animals were not concommittantly seen in any developmental stage (the average r_{max} of hyper-g sagittae, e.g., was seen in stage 18 of normally developing fish, whereas the average r_{max} of hyper-g lapilli was seen in stage 16, and the average asymmetry of hyper-g sagittae was about three times higher than the most prominent asymmetry found during the normal development; the respective data will be available at presentation): Thus, our present findings do not indicate any general effect of altered gravity (i.e. general environmental stress). In fact, they indicate a differential adjustment of lapilli and sagittae in their growth towards the altered gravitational environment. Under increased gravitational conditions, otoliths have a higher physical impact on the sensory epithelia. We propose that the fish compensate the resulting increased bilateral impact on the sensory epithelia by developing much smaller otholits. Since the sagittae will not be used for the perception of gravity in free swimming fish, there will no adaptive mechanism be capable of decreasing any given asymmetry, as it should be the case concerning lapilli.

Calculating the asymmetry of sagittae, we found no statistically significant difference between looping and non-looping fish after hyper-g experience (Fig. 2). Individuals, which showed a motion-sick behaviour after transfer from hypergravity to normal 1g earth gravity, however, exhibited a significantly higher lapillar asymmetry than normally behaving hypergravity samples (Fig. 2; $p<0.05$).

Our data are the first experimentally derived evidence in support of the theoretical concept mentioned in the introduction, according to which kinetoses might be based on asymmetrical (utricular) otoliths.

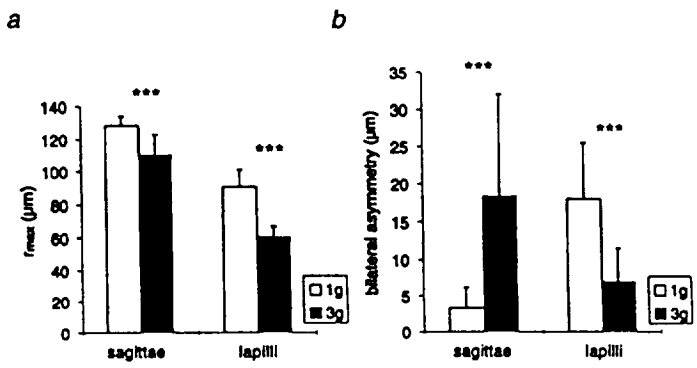

Fig. 1. Effects of altered gravity on the growth of inner ear otoliths of cichlid fish, which had been raised under 3g within a centrifuge, in comparison to 1g controls. sagittae: saccular otoliths (hearing); lapilli: utricular otoliths (perception of gravity). a, Size (maximum radius, r_{max}). ***: $p<0.0001$. b, Asymmetry (absolute bilateral difference in r_{max} between the left and the right side otoliths). ***: $p<0.0001$.

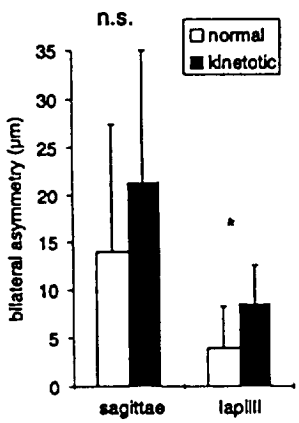

Fig. 2. Otolith asymmetry in kinetotic (loop-swimming) and normal behaving cichlid fish, which had been raised at 3g hypergravity. *: $p<0.05$; n.s.: not significant. sagittae: saccular otoliths (hearing); lapilli: utricular otoliths (perception of gravity).

CONCLUSION

The differential effect of altered gravity on different otoliths (i.e. lapilli and sagittae) implies the existence of a hitherto unknown centrally guided feedback mechanism, which adjusts the functional capacity of otoliths towards the requirements for spatial orientation.

ACKNOWLEDGMENT

This work was financially supported by the German Space Agency DARA (FKZ: 50 WB 9533).

NEUROBIOLOGICAL RESPONSES OF FISH TO ALTERED GRAVITY CONDITIONS: A REVIEW

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INTRODUCTION

An alteration of sensory modalities such as gravity, that serve a fish for orientation, results in a changed behavior: A transfer from normal gravity to microgravity in the course of parabolic aircraft flights and spaceflights, respectively, results in a so-called loop-swimming behaviour in adult fishes. A qualitatively similar loop-swimming- (or somersaulting-) behaviour can also be observed in larval fish during parabolic flights.

Most interestingly, a transfer from a long lasting stay under hyper-gravity (centrifuge) conditions to normal 1g earth gravity results in a loop-swimming-behaviour in larval fish, which could still be observed after five days under 1g conditions.

As a matter of fact, the basis of the "loop-swimming" and related aberrative behaviours might be searched for in the central nervous system, where a signal-transduction from the inner ear related signal internalisation to the signal response (i.e. behaviour) takes place.

Thus, the present paper is intended to provide a review on our investigations upon neuroplastic reactivities of fish to altered gravitational environments, comprising behavioural, biochemical, histochemical and electronmicroscopical-cytochemical results, which have been achieved by our working group for some ten years on the search for the understanding of space-sickness in fish in order to contribute to the understanding of the neuronal basis of motion sickness in man.

METHODS

Several developmental stages of cichlid fish *Oreochromis mossambicus* and swordtail fish *Xiphophorus helleri* were subjected to hyper-gravity (3g in laboratory centrifuges), hypo-gravity ($>10^{-2}$g in a fast-rotating clinostat) and to near weightlessness (10^{-4}g aboard the spacelab D-2 mission). After the end of the gravitational relevant experiments, the samples recovered were processed for further behavioural, biochemical, histochemical and electronmicroscopical analyses.

The swimming **behavior** of the animals was qualitatively observed during altered gravity. A quantitative evaluation of the swimming behaviour (quantification of swimming traces) was performed after the experiments by means of a computer-based video analyser.

Subsequently, samples were dissected for further neurobiological investigations: **Biochemically**, the total brain activity of glucose-6-phosphate dehydrogenase (G6PDH, cytosolic pentose phosphate pathway), succinate dehydrogenase (SDH, mitochondrial tricarboxylic cycle), cytochrome oxidase (CO, mitochondrial respiratory chain), cytosolic, plasma membrane located and mitochondrial creatine kinase (CK, creatine phosphoryl circuit), Ca^{2+}/Mg^{2+}-ATPases and sialidase (membrane metabolism) was analysed. For **histochemical analyses**, the reactivity of succinate dehydrogenase was determined in various brain nuclei. On **electronmicroscopical** level, the reactivity of cytochrome oxidase was determined both in a vestibular brain nucleus and in inner ear epithelia. (Details of the methods used will be available at presentation.)

RESULTS AND DISCUSSION

Regarding **behaviour**, *larval fish* did not show any aberrative movements during hyper-gravity. After hyper-gravity as well as under microgravity, many of the animals performed a loop-swimming-behaviour. In the case of *subadult* and *adult fish*, qualitative and quantitative analyses of the behaviour after hyper-gravity experience (no abnormal behaviour observed during hyper-gravity exposure) did not yield a marked effect. These results strongly indicate, that a given gravitational environment results in imprinting-like phenomena on the level of the brain, disclosing an aberrative behaviour only when gravity is reduced.

Biochemically, G6PDH- and SDH- activities in the brains of larvae were significantly increased by hyper-gravity exposure. The cytosolic and membrane-located CK- activity as well as the activity of sialidase was decreased after hyper-gravity, whereas the activity of the mitochondrial membrane-located isoform of CK was slightly increased. Microgravity yielded the opposite effects. The brains of adult fish did not show a significant effect of altered gravity. Altered gravity did not yield any significant effect both on larval and adult fish regarding the activities of CO and Ca^{2+}/Mg^{2+}-ATPases.

In order to gain some insights into the anatomical substratum of the gross-biochemical results, according to which SDH was strongly affected by altered gravity, the light-microscopical, **histochemical approach** was undertaken. Hyper-gravity resulted in an augmented enzyme reactivity within entire brains of larvae (entire sections were densitometrically investigated as a whole and "all over brain" reactivities were calculated) and

within vestibular brain nuclei, whereas non-vestibular nuclei revealed no effect. Weightlessness yielded the opposite results. Corresponding effects (hyper-gravity only) were obtained in the case of adult fish. The effect of altered gravity, however, was much less significant as compared to the circumstances observed in developing fish. The histochemical data strongly indicate, that the gross-biochemical results may represent sum-up effects of vestibulum related brain structures. However, an additional, general impact of altered gravity on the CNS (e.g. via the hormone system) cannot yet be excluded. Since the effect of altered gravity was much more distinct in larval animals than in adults (the range of the neuronal plasticity in larvae is generally higher than that in adults), the alteration of enzyme activities due to altered gravity might represent imprinting-like and adaptational processes

Concerning the brain, **ultramicroscopically** demonstrated cytochrome oxidase (CO)- reactivity in the vestibular Nucleus magnocellularis was increased after hyper-gravity and slightly decreased after microgravity. This is in complete agreement with the histochemical data. Ultramicroscopically demonstrated CO in the inner ear epithelia yielded the following findings: Weightlessness resulted in a decrease of CO- reactivity within the utricle, but not in the saccule as compared to 1g controls. The utricle is responsible for the internalisation of gravitational information, whereas the saccule of fish is believed in hearing. Interestingly, hyper-gravity had no effect on inner ear epithelia. This is in contrast to the findings on the level of the vestibular brain nuclei. An explanation could be as follows: The CO- reactivity in the inner ear epithelia at 1g seems to be close to the possible maximum activity. Any hyper-g effect thus is likely to have been overlooked. Additionally, it is possible to assume, that hyper-g facilitates the signal transmission of the inner ear, transmitting a respective rate of action potentials towards the vestibular brain nuclei without a concommittantly increased energy production. Further studies are sorely needed to clarify this topic.

CONCLUSION

All data taken together clearly demonstrate, that altered gravity affects developing fish on all levels of organismal organization investigated. The results obtained most possibly reflect adaptational phenomena to the altered gravity environments, i.e. imprinting-like processes. The gross-biochemical results probably resemble sum-up effects of particular vestibulum-related brain centers. However, an additional, general impact of altered gravity, e.g. stress related phenomena, cannot be excluded. The cellular activity of macular cells is not clearly correlated with the acceleration provided in contrast to higher order neuronal cells. This speaks in favour of the assumption, that especially hyper-gravity induces a facilitation of synapses in inner ear epithelia. The aberrative behaviour when long-term provided gravitational forces are reduced suggests, that imprinted brain structures cannot immediately handle with the actual, new input from the inner ears, possibly due to asymmetric otolith weights, as has already been proposed earlier. Our results regarding adult fish suggest that, as compared to developing fish, adult fish do not as heavily respond to altered gravitational forces, possibly due to a partial loss in neuronal plasticity.

Summarized, fish subjected to altered gravity seem to be a valuable model for the investigation of neuronal plasticity in general, and moreover, may provide clues and insights regarding the neuronal basis of kinetosis in man.

ACKNOWLEDGMENT

This work was financially supported by the German Space Agency DARA (FKZ: 50 WB 9533).

AN AGE-DEPENDENT SENSITIVITY OF THE ROLL-INDUCED VESTIBULOOCULAR REFLEX TO HYPERGRAVITY EXPOSURE OF SEVERAL DAYS IN AN AMPHIBIAN (*XENOPUS LAEVIS*)

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INTRODUCTION

Exposure of amphibian tadpoles to altered gravitational forces affected the development of the roll-induced static vestibulo-ocular reflex (*rVOR*). It was shown that for maximal postural changes, the appearence of the rVOR during development is an important milestone for the development of adaptive properties of the underlying neuronal network (Horn, Sebastian, Neurosci Lett 216:25-28, 1996). In*Xenopus* tadpoles with 3g-experience, we have tested the question, whether this observation is also valid if the animals were tested by small roll angles during 1g-readaptation.

METHODS

Hypergravity was induced by centrifugation of the animals and started at different developmental stages, two of them before (stages 6-9 and 33-36), and one of them (stage 45) after stage 42 at which the rVOR appeared for the first time. Duration of 3g was 9 to 12 days. Recordings of the rVOR started 24 hrs after termination of the 3g-period. The tadpoles were rolled either by 15° or by a complete 360° lateral roll around their longitudinal axis. From the latter stimulation procedure, the response amplitude was determined which is the peak-to-peak excursion of the rVOR characteristic recorded during a complete 360° roll.

RESULTS

Within 6 to 11 days after termination of the 3g-period, the rVOR induced by a 15° or by a complete 360° roll was similarly affected by the preceeding 3g-exposure. For both the 6/9- and the 33/36-sample, the rVOR of 3g-reared tadpoles did not changed significantly within this readaptation period while the rVOR recorded from the 1g-reared controls did so. In contrast, the rVOR increased significantly in the stage 45-group for both the 3g- and the 1g-groups for the small as well as for the complete roll angle during readaption (complete roll: from 37.1 to 54.4° in the 3g-group, from 50.9 to 70.4° in the 1g-control; 15°-roll: from 4.4 to 7.6° in the 3g-group and from 6.6 to 10.2° in the 1g-control). The only difference of the rVOR induced by the 15° roll to that recorded during a complete roll was its faster 1g-readaptation (15°-roll, last: 3g vs 1g is n.s.; complete roll, last: 3g vs 1g is significant with $p<0.01$).

	15°-Roll						*Complete roll*					
	Stages 6-9		*Stages 33-36*		*Stage 45*		*Stages 6-9*		*Stages 33-36*		*Stage 45*	
g-level	**3g**	1g	**3g**	1g	**3g**	1g	**3g**	1g	**3g**	1g	**3g**	1g
first	**4.5°**	4.7°	**2.8°**	4.9°	**4.4°**	6.6°	**33.4°**	36.7°	**26.1°**	36.9°	**37.1°**	50.9°
last	**3.1°**	8.2°	**1.7°**	8.4°	**7.6°**	10.2°	**33.1°**	48.3°	**35.6°**	55.2°	**54.4°**	70.4°
last/first	**0.68**	1.74	**0.62**	1.73	**1.75**	1.54	**0.99**	1.32	**1.36**	1.50	**1.47**	1.38
period [days]	**6**	6	**11**	11	**10**	10	**6**	6	**11**	11	**10**	10
significances	**n.s.**	<0.01	**n.s.**	<0.01	**<0.01**	<0.01	**n.s.**	<0.01	**n.s.**	<0.01	**<0.01**	<0.01

first (last), median rVOR obtained at the first (last) recording day; *last/first*, ratio between these data; *period [days],* number of days between first and last; *significances*, statistical difference between first and last

CONCLUSION

The developmental stage at which the rVOR appears for the first time is an important milestone in the development of the gravity sensitive system. For small as well as large postural displacements by a passive roll, the rVOR is affected in a characteritic manner by a 9- to 12-days lasting 3g-period. It is likely that there are two components involed: (i) readaptation to 1g is faster the younger the tadpoles were at onset of 3g; this high plasticity is also known from the vestibular compensation. (ii) Slowly developing but long-lasting effective neuroanatomical changes exist, which affect the physiological connections between the central nuclei involved in the control of the rVOR.

Supported by the German Space Agency (DARA), grant no. 50WB9543-7 and the Deutsche Forschungsgemeinschaft (DFG), grant no. Ho664/16-1 to E. Horn.

MECHANICALLY-INDUCED MEMBRANE WOUNDING DURING PARABOLIC FLIGHT.

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INTRODUCTION

Mechanical load induces transient disruptions or "wounds" of the plasma membrane of skeletal muscle cells both *in vivo* and *in vitro*. We have previously shown that such wounding results in the release of fibroblast growth factor (FGF) from the sarcoplasm of both skeletal myofibers and cardiac myocytes *in vivo*. In addition, using a mechanically active tissue culture environment we have shown that there is a linear relationship between the amount of mechanical load placed on differentiated human skeletal myotubes (analogous to myofibers *in vivo*), the degree of membrane wounding and the amount of FGF released into the tissue culture medium. Furthermore, we have also demonstrated that the muscle growth response induced by mechanical load can be specifically inhibited by a site-directed, anti-FGF neutralizing antibody added to the tissue culture medium. Based on these and other results, we have formulated the hypothesis that microgravity exposure may result in the disruption of this mechanically-reactive, signaling pathway within skeletal muscle tissue and postulate that a microgravity-induced reduction in wound-mediated FGF release may contribute to skeletal muscle atrophy. Using the experimental conditions generated aboard NASA's KC-135 parabolic flight aircraft we have investigated whether or not microgravity *per se* alters the membrane wound response of human skeletal muscle cells at the cellular level. We have previously illustrated the utility of mechanically-induced membrane wounding as a highly efficient mechanism for the transfer of genetic material into primary human cells, a process termed "transfection". Transfection efficiency has proven to be an important rate limiting step in several human gene therapy protocols, such as transfection of the dystrophin gene into the muscle cells of Duchenne muscular dystrophy patients. Therefore, we also investigated whether the microgravity environment can be utilized to enhance transfection efficiencies in primary human skeletal myoblasts cultures using two different plasmid DNA constructs.

METHODS

Reproducible levels of mechanical load were applied to the sarcoplasmic membrane of cultured human skeletal muscle cells using a novel, impact-mediated loading (IML) procedure (Clarke *et al.*, 1994: *Biotechniques* 17:1118-1125) carried out during KC-135 parabolic flight. Wounding levels were assessed by: 1) determining cell survival using a DNA assay and 2) determining the amount of different Mr-sized fluorescent cytoplasmic wound markers (FITC-linked dextrans) present in the cytoplasm of surviving cells using flow cytometry. Transfection efficiencies were calculated using either: 1) cell counting after transfection with the pSVβ plasmid construct encoding for bacterial β-galactosidase or 2) determination of enzyme activity after transfection with the pSVCAT plasmid construct encoding for the acetyl CoA transferase enzyme. The effects of parabolic flight on plasma membrane/intracellular vesicle fusion efficiency was measured after PMA stimulation in differentiated HL-60 cells (i.e. granulocyte-like cells) by the amount of β-glucuronidase enzyme released upon primary granule fusion with the plasma membrane .

RESULTS

Wounding inflicted during microgravity resulted in a significant ($p < 0.004$: n= 31 per condition) reduction in cell survival compared to that observed after wounding inflicted in either the normal or hypergravity phases of parabolic flight (Figure 1). When dextran size was varied from 10 kD to 2 million kD, the degree of membrane wounding, determined by the mean fluorescence value (MFV) of the surviving cells, indicated that the reduction in cell survival observed after wounding in microgravity was not due to an increase in membrane wound size but an inhibition of the membrane wound resealing process (Figure 2).

Figure 1

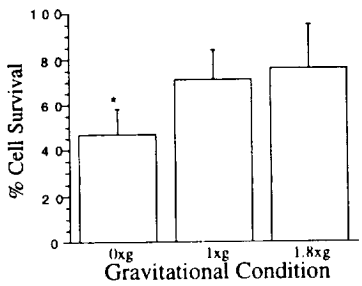
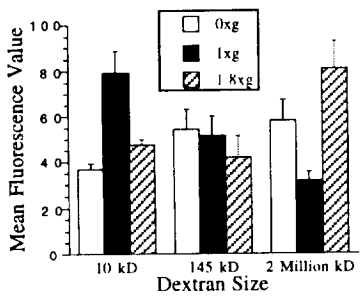


Figure 2



This conclusion was confirmed by the experimental observation that intracellular vesicle fusion with the plasma membrane in PMA-stimulated HL-60 cells, a process essential for resealing of a membrane wound, was significantly ($p < 0.001$; $n = 4$ per condition) inhibited in microgravity but enhanced in hypergravity (Figure 3). No significant amount of β-glucuronidase was released by unstimulated control cells exposed to parabolic flight (Figure 3). Transfection efficiencies of primary human skeletal muscle cells with the pSVβ plasmid construct were significantly (* - $p < 0.01$: n= 20 per condition) enhanced in microgravity and hypergravity compared to normal gravity. When a greater impact pressure (i.e. 40 psi *vs.* 35 psi) was used, the transfection efficiency remained significantly (+ - $p < 0.01$: n= 20 per condition) higher in microgravity than under either normal or hypergravity conditions (Figure 4). Using a quantitative enzyme assay for the product of the pSVCAT plasmid construct, we determined that the membrane active agent PF-68, previously shown to enhance both resealing of membrane wounds and transfection rates in ground-based studies, significantly ($p < 0.05$: n=20 per condition) increased primary human skeletal myocyte transfection in microgravity compared to normal or hypergravity conditions (Figure 5).

Figure 3

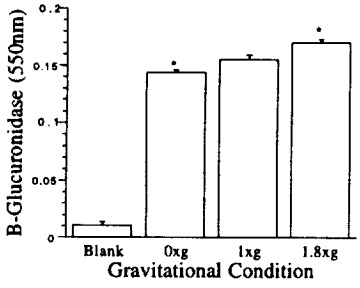


Figure 4

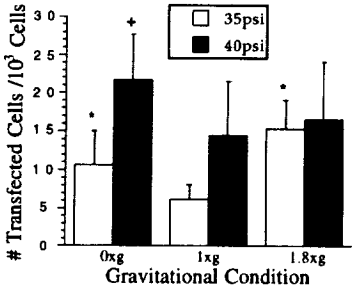
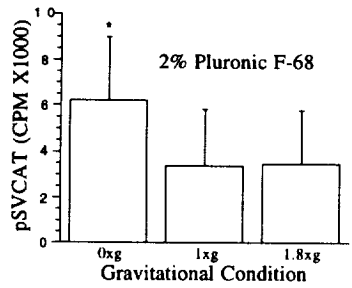


Figure 5



CONCLUSIONS

Our results indicate that microgravity *per se* has an effect upon the resealing process of mechanically-induced plasma membrane wounds. We have previously shown in ground-based experiments that membrane resealing is linked to the physical properties of the plasma membrane, such as membrane fluidity and tensile strength. Alterations in these parameters have also been shown to alter the fusogenic properties of the plasma membrane. The disruption of stimulated primary granule fusion in differentiated HL-60 cells by microgravity, the same intracellular membrane/plasma membrane fusion process required for membrane wound resealing, indicates that microgravity may effect the macromolecular structure of the plasma membrane in some fashion. Such changes in plasma membrane properties may explain why tissue cultured human skeletal muscle cells appear to be more susceptible to plasma membrane damage when mechanical load is imposed during microgravity compared to the levels of damage observed when load is applied under normal or hypergravity conditions..

Although preliminary. these experimental observations have several operational implications. These include the possibility that excessive mechanical load placed on muscle tissue in microgravity may result in greater levels of muscle damage than anticipated from ground-based models. A second implication is that membrane adaptation during space flight may result in membranes which have attenuated function upon return to normal gravity. In addition, during the adaptation period, biological membranes may exhibit abnormal function. These include inhibition of membrane fusion and inhibition of cell-cell signaling associated with membrane-associated channels and receptors. Examples of such processes include neuronal transmitter release at the synapse which could lead directly to alterations in motor neuron activity, cognitive function and peripheral vascular tone. A third implication is that an altered gravity environment (i.e. microgravity or hypergravity) can be utilized to enhance the transfer of genetic material into primary human cells by modulating the effectiveness of membrane wound-mediated transfection.

ERYTHROPOIETIN STIMULATES INCREASED F CELL NUMBERS IN BONE MARROW CULTURES ESTABLISHED IN GRAVITY AND MICROGRAVITY CONDITIONS

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INTRODUCTION

The induced anemia associated with space travel greater than 8 days compromises the oxygenation of the various tissues throughout the body. Davis and his colleagues (1996) report that after eight days of space travel, the number of erythrocytes is reduced by greater than 83% and corpuscular volume is significantly diminished. Mental acuity and physical performance are impaired by the physiological change of tissue hypoxia. Efforts in our laboratory are directed towards inducing the production of fetal hemoglobin (HbF) by the hemopoietic system. Fetal hemoglobin (HbF) has been shown to have a higher affinity for oxygen, and its post natal production is thought to be a countermeasure that improves tissue oxygenation. We are currently testing the efficacy of erythropoietin to induce the differenciation of uncommitted stem cells in bone marrow cultures towards the erythroid line. The derived erythroblasts are called F-cells, because 10 - 25% of their hemoglobin is fetal hemoglobin. Image analysis will be used to quantitate F-cell numbers while protein electrophoresis will be used to measure the variations in fetal hemoglobin expression.

METHODS

Four milliliters (4ml) of normal human bone marrow was resuspended in 36 ml of IMDM with 10% fetal calf serum, 10% horse serum, and 5ul/ml of penicillin streptomycin. Cell count and viability was obtained by using a hemacytometer and the trypan blue dye exclusion method. Ten mL of this mixture was placed into the 10 ml HARV vessel to the *Synthecon* Bioreactor, while 2 aliquouts of 10 ml of the same were plated into 2 X 75cm^3 tissue culture flasks. The remaining mixture was distributed and plated into 25cm^3 tissue culture flasks, 25cm^3 tissue culture plates, and 6 well tissue culture microtiter plates. Erythropoietin (human recombinant, from Sigma, St. Louis, MO.) was added, at 2U/ml, to half the cultures under gravity conditions and to the culture in the bioreactor. An untreated microgravity cultures were established seven days later. The cultures were maintained in a 5% CO_2 tissue culture incubator at a temperature of 37^{o}C and a humidity > 99%. After one week in culture, three (3) homogeneous, 50 ul aliquouts were removed from each treatment condition and image analysis and protein electrophoresis were conducted. Standard cell counts were also taken.

RESULTS

Provisional results (N=3) show that under microgravity conditions, the three (3) parameters measured showed a decrease in values over intial values at the establishment of the cultures. The total cell count decreased by 86% in the untreated cultures, while total cell counts in the EPO treated, microgravity cultures showed an 82% decrease. A diminished HbF band over baseline was seen for both the EPO treated and untreated cultures in microgravity, as observed on the cellulose acetate blot. (Densitometry of the HbF bands are forthcoming). Using image analysis (Advance Logic Research, Inc., Irvine, CA), the F-Cell count in 5 randomly selected fields showed a decrease of 2.5% and 1.5% in untreated and EPO treated cultures in microgravity, respectively. The count was based on equivalent total cell counts for both culture conditions of microgravity and gravity. Under gravity conditions, the total cell count was increased 39% and 42% in the untreated and EPO treated cultures, respectively. Cellulose acetate blots for the protein electrophoresis, showed a visible increase in HbF in the EPO treated cultures over the untreated cultures. For 5 randomly selected fields, the EPO treated cultures showed increased of 5.5% in the number of F-cells, while the untreated cultures only showed a 1.0% increase in observable, large nucleated F-cells. Other remarkable observations were noticeable cell volume differences in the microgravity and gravity established cultures. The microgravity culture cells visibly appeared to have less cellular volume than those of the gravity established cultures.

CONCLUSION

Our current series of experiments have been to develop a protocol to stimulate uncommitted bone marrow stem cells to differentiated into F-Cells. While microgravity has diminished the proliferation of the total bone marrow cell count, erythropoietin (EPO) appears to significantly increase the F-cell count and fetal hemoglobin expression as measured by protein electrophoresis in cultures under the influence of gravity. Although the total cell count was significantly decreased in the microgravity cultures, EPO treatment appeared to induce a greater number of F-cells than untreated cultures in microgravity cultures. Future studies will be to gather more definitive results from our

erythropoietin studies and to further induce differentiated cultures to express higher fetal hemoglobin. The Extract NXO6999 has been clinically shown to increase blood serum levels of fetal hemoglobin in sickle cell and thalessemia patients (Fadulu, 1995). Development of a prophylactic drug regime, proposed by our research finding, might be effective in inducing an increased production of fetal hemoglobin. Such a development should counterbalance the erythropoietic deficit and ameliorate the anemic conditions experienced by astronauts involved in lengthy space travel. The results of these studies should also improve the anemic conditions and oxygenation deficits experienced by sickle cell and beta thalessemia sufferers.

Acknowledgements:
This research is funded by a National Aeronautics and Space Administration grant "Microgravity and Sickle Cell Anemia" Grant Number: NAG 9-862. Special thanks to Astronaut Ellen Baker, MD. , the technical advisor for this project.

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