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EFFECTS OF MICROGRAVITY ON LIPOSOME-RECONSTITUTED CARDIAC GAP JUNCTION CHANNELING ACTIVITY

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Effects of microgravity on cardiac gap junction channeling activity were investigated aboard NASA zero-gravity aircraft. Liposome-reconstituted gap junctions were assayed for channel function during free-fall, and the data were compared with channeling at 1 *g*. Control experiments tested for 0 *g* effects on the structural stability of liposomes, and on the enzyme-substrate signalling system of the assay. The results demonstrate that short periods of microgravity do not perturb reconstituted cardiac gap junction channeling activity. © 1989 Academic Press, Inc.

Gap junctions contain proteinaceous channels between cells that permit molecules smaller than approximately 1700-1900 daltons to pass from cell to cell (1). Gap junctions are extensive in eukaryotic organisms, providing the physical basis for coupling between all epithelial cells. They are crucial for control of embryonic development and for normal adult-tissue physiology (2), and are lost when tumor cells metastasize (2). In the heart, gap junction communication between myocardial cells coordinates the contractile activity of individual cells into rhythmic heartbeat (3-5).

As the prospects for frequent and prolonged manned space missions increase, it is increasingly evident that little is known about the effects of microgravity on cellular and subcellular systems. Given the importance of gap junction-mediated communication in cellular activities, it is pertinent that the effects of gravity on gap junction physiology be investigated. Theoretical models demonstrating gravitational effects on the cell nucleus and organelles are unable to predict the influence of microgravity on the behavior of membranes and large integral membrane proteins (6). The present study empirically measures gap junction channeling activity during short episodes of microgravity in order to detect possible gravitational influences on channel function.

A previous study has shown that cardiac gap junction channeling can be assayed by isolating gap junctions from heart muscle and reconstituting the channel proteins into liposomes (7). Functional organization of these integral membrane proteins into hexameric channels, or connexons, was assessed by the ability of appropriately-sized

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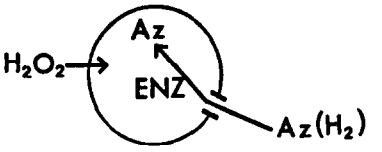

Figure 1. Schematic illustration of the liposome-reconstituted gap junction channeling assay. Microperoxidase (ENZ) is entrapped within liposomes; if channels are present, exogenous Azure substrate (Az(H_2)) will transit the channels to be oxidized by the enzyme and hydrogen peroxide (H_2O_2). Increasing oxidation is thus a monitor of increasing gap junction channeling.

substrate molecules to enter the liposomes to be oxidized by liposome-entrapped microperoxidase (Fig. 1). This model system thus utilizes liposome-reconstituted gap junctions, and detects channeling activity spectrophotometrically by using an enzyme-substrate signalling system (7).

MATERIALS AND METHODS

Microgravity experiments were conducted aboard the NASA zero-gravity aircraft (NASA 930) based out of Ellington Airforce Base at the Johnson Space Center in Houston, Texas. During the 2 hour flight, 33 reduced gravity episodes lasting 20-30 seconds each were produced by free-falling from 34,000 to 26,000 feet. Fifteen different measurements of cardiac gap junction channeling activity were successfully performed during periods of 0 to 0.010 *g*-forces. In addition, four control experiments were conducted in microgravity on the structural stability of liposomes, and on the assay signalling system.

Preparation of Gap Junctions. Previously published methods were used to extract contractile proteins and isolate gap junction membranes from cardiac tissue. Briefly, chicken hearts (Pel-Freeze) were homogenized, filtered, and washed by centrifugation before exposure to a series of high salt (0.6 M KCl) and low salt buffers for extraction of contractile proteins (8). Following salt removal, membranes were solubilized with N-lauroylsarcosine and then gap junctions were isolated by discontinuous sucrose gradient centrifugation according to Manjunath (9).

Gap Junction Reconstitution in Liposomes. Procedures for the reconstitution of cardiac gap junctions into artificial membranes and the entrapment of microperoxidase within liposomes were essentially performed as before (7). Isolated gap junctions, or control non-gap junctional membrane proteins, and FITC-microperoxidase (7) were solubilized in 2% octyl glucoside and dialyzed in the presence of a sphingomyelin-brain phospholipid mixture (10). Following liposome formation, liposomes were seperated from non-entrapped FITC-microperoxidase by centrifugation at 10,000 *g* for 15 minutes.

Reconstituted Channeling Assay. Cardiac gap junction channeling activity was assayed at 1 *g* as previously described (7). Tests conducted at 0 *g* were identical except for the following equipment modifications required to perform experiments in microgravity.

A Gilford Model 2400 spectrophotometer and an Omnigraphic Model 100 strip recorder (Houston Instruments) were mounted on the floor of the aircraft. The investigator was "tied down" with elastic cords next to the equipment. Prior to flight, 100 *ul* of buffer (50 mM Tris, pH 7.5) containing liposomes with gap junctions, or control liposomes with non-gap junctional membrane proteins, or assay controls of 0.15 nmoles of microperoxidase, were loaded by syringe injection into sealed semi-micro plastic cuvettes. The cuvettes had been sealed off at a volume of 600 *ul* with silicone rubber sealant (General Electric) and vented via a 27 gauge needle permanently inserted through the seal.

During 1 *g* flight, a cuvette holder containing four cuvettes was loaded into the spectrophotometer and positioned with a manual sample changer. A retractable 3 inch, 19

gauge syringe needle, mounted in the sample chamber lid directly above the light path, was then lowered for insertion into a cuvette, through the seal, where it remained during the course of the reaction. Immediately upon entering microgravity, the reaction was initiated by injecting 500 *ul* of substrate solution (60 *u*M Azure C, 3.6 mM H_2O_2 in 50 mM Tris) into the cuvette. Injection was accomplished by a manually controlled pre-set volume delivery syringe (Cornwall) that was connected by tubing to a reservoir filled with substrate solution. Following injection-initiation, the reaction was monitored for 20 seconds at 612 nm with real-time recording and was expressed as percent change in optical density (OD) over time, where decreasing OD is an indicator of substrate oxidation.

RESULTS AND DISCUSSION

The gap junction channeling assay was conducted at 0 *g* and 1 *g* using either experimental liposomes with reconstituted heart gap junctions, or control liposomes with non-junctional membrane proteins. A comparison of the data from channeling assays performed under both gravitational conditions show essentially identical results (Fig. 2). Liposome-reconstituted gap junctions seemed to function as well at 0 *g* (Fig. 2A, top trace) as they did 1 *g* (Fig. 2B, top trace). In fact, the rate of channeling appears similar in both gravity environments, with substrate oxidation approaching 50% in 20 seconds. Results from control liposomes verify that the rapid oxidation observed requires gap junction channeling. In liposomes formed with non-junctional membrane proteins, substrate oxidation was not detected at 0 *g* (Fig. 2A, bottom trace), a result identical to 1 *g* tests (Fig. 2B, bottom trace). This inaccessibility of exogenous substrate to the entrapped enzyme shows that artificial membrane vesicles remain structurally intact in microgravity, and also demonstrates that not all membrane proteins will reconstitute channeling in this assay. Therefore, oxidation of exogenous substrate can only result from channel-mediated transit through the liposome barrier.

However, these data alone do not prove that gap junction channeling is equivalent at 0 *g* and 1 *g*. Identical channeling rates could have also resulted from accelerated microperoxidase activity and reduced channeling, or from reduced microperoxidase

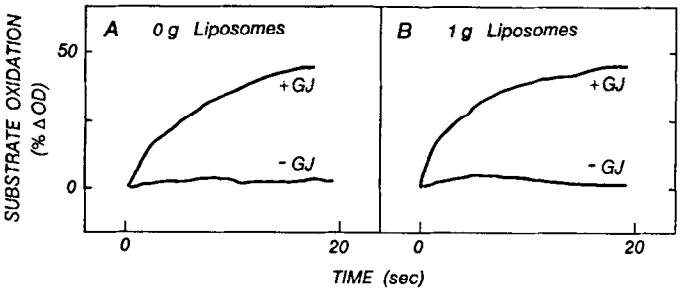

Figure 2. Liposome-reconstituted cardiac gap junction channeling activity over a 20 second interval, monitored by changes in optical density at 612 nm. Substrate oxidation reflects channel transit of Azure C into liposomes containing trapped microperoxidase. **(A)** Channeling activity at 0 *g* in the presence (+GJ) and absence (-GJ) of heart gap junctions. **(B)** Channeling activity during 1 *g* in the presence (+GJ) and absence (- GJ) of heart gap junctions.

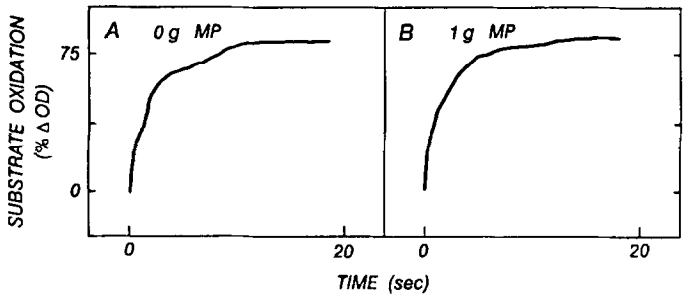

Figure 3. Microperoxidase (MP) oxidation of reduced Azure substrate in the absence of liposomes. At 0 *g* (**A**), the rate of dye oxidation, resulting from H_2O_2/Peroxidase/Azure C complex formation, is similar to that seen at 1 *g* (**B**). In both gravity environments, approximately 80% of substrate was rapidly oxidized.

activity and accelerated channeling. The possibility of microgravity effects on the microperoxidase-Azure C signalling system was addressed in experiments that tested the enzyme-substrate reaction in the absence of liposomes. Data from these assay controls show no differences in enzymatic activity of microperoxidase at 0 *g* and 1 *g* (Fig. 3). This result, along with those from the liposome-reconstituted channeling assay, demonstrates that channeling activity is unaffected by short episodes of microgravity. Thus, the multi-subunit, lipo-protein gap junction channel is shown to maintain functional integrity as the gravitational field is altered.

In summation, the data demonstrate, for the first time, that short periods of microgravity do not perturb heart gap junction channeling function in this liposome-reconstituted model system. The data are of even more general significance in that they suggest that these integral membrane proteins retain their normal macromolecular arrangements as connexons in lipid bilayers when under zero-gravity conditions. Nevertheless, it is important to note that microgravity never exceeded 30 seconds in these experiments. Thus, continuous long-term microgravitational effects on the functional integrity of gap junction proteins remain to be determined.

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