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CALMODULIN-DEPENDENT PROTEIN KINASE MEDIATES HYPERGRAVITY-INDUCED CHANGES IN F-ACTIN EXPRESSION BY ENDOTHELIAL CELLS,

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INTRODUCTION: A number of basic cellular functions, e.g., electrolyte concentration cell growth rate, glucose utilization, bone formation, response to growth stimulation and exocytosis are modified by microgravity or during spaceflight (1-4). Studies with intact animal during spaceflights have found lipid accumulations within the lumen of the vasculature and degeneration of the vascular wall (5). Capillary alterations with extensive endothelial invaginations were also seen (6). Hemodynamic studies have shown that there is a redistribution of blood from the lower extremities to the upper part of the body; this will alter vascular permeability, resulting in leakage into surrounding tissues (7). These studies indicate that changes in gravity will affect a number of physiological systems, including the vasculature. However, few studies have addressed the effect of microgravity on vascular cell function and metabolism. A major problem with ground based studies is that achieving a true microgravity environment for prolonged period is not possible. On the other hand, increasing gravity (i.e., hypergravity) is easily achieved. Several researchers have shown that hypergravity will increase the proliferation of several different cell limes (e.g., chick embryo fibroblasts) while decreasing cell motility (8) and slowing liver regeneration following partial hepatectomy (9). These studies suggest that hypergravity will alter the behavior of most cells. Several investigators have shown that hypergravity affects the expression of the early response genes (c-fos and c-myc) and the activation of several protein kinases (PK's) in cells (1 0,11). In this study we investigated whether hypergravity alters the expression of f-actin by aortic endothelial cells, and the possible role of protein kinases (calmodulin(II)-dependent and PKA) as mediators of these effects.

EXPERIMENTAL METHODS: BAECs were obtained from NIGMS/Coriell Cell Repository. Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and 1X antibiotics. We assessed F-actin expression in BAECs subjected to centrifugation in the presence or absence of protein kinase inhibitors (PKIs).

F-actin Expression When Subjected to Hypergravity Subconfluent cultures were subjected to hypergravity (centrifuged at 6 and 12 G) for 24,48 and 72 hr. The cultures were rinsed with phosphate buffered saline (PBS) and then fixed with 1%glutaraldehyde/0.1% Triton X-100/2.5% Formalin in PBS for 24 hours. Following fixation, the cells were stained with FITC-phalloidin and examined using phase and fluorescence microscopy. Controls were treated similarly except they were not subjected to centrifugation.

Effectof PKIs on F-actin Expression Under Hypergravity: The PKIs, KT5926 and KT5720, were added, singly, to the medium of subconfluent cultures (2uL PKI/mL) and centrifuged at 6 and 12 G for 24,48, and 72 hr. Cultures were stained and examined for f-actin expression as previously outlined. Controls were treated similarly except they were not subjected to centrifugation.

RESULTS AND DISCUSSION: Figure 1 shows subconfluent BAECs that were subjected to hypergravity (12G). The cells generally showed increased F-actin expression throughout the cell as a function of time under hypergravity. Optimal fluorescence was observed after 48 hr with a slight decline seen after 72 hr. Cells subjected to 6G of hypergravity gave similar results. The controls, however, generally showed a decreased F-actin expression with increasing time of culture, with no fluorescence observed after 72 hr. The fluorescence observed for control cells

was localized to the nuclear region instead of throughout the cell as observed for hypergravity treated cells. There were no other morphological differences seen for either hypergravity-treated or control cells when examined by phase contrast microscopy. In this study, we only examine changes in f-actin. These findings suggest that hypergravity has a direct effect on f-actin levels in endothelial cells but could also affect other forms of actin. The latter possibility will be investigated in future studies.

Subconfluent BAECs subjected to hypergravity in the presence of the PKI - KT5926, which is a specific inhibitor of calmodulin(II)-dependent protein kinase, exhibited increased f-actin expression with increasing time under 6G is shown in Figure 2. Similar results were observed for cells subjected to 12G hypergravity. The fluorescence, observed under either 6 or 12G, was especially noted at cell-to-cell junctions as well as for the nuclear regions. The controls for PKI-KT5926 showed a slight increase in f-actin expression with increasing time under 6G. The protein kinase inhibitor KT5720 did not affect f-actin expression by BAEC under any of the conditions studied. Again, no other morphological changes were observed when examined by phase contrast microscopy. These results indicate that, of the two protein kinases examined, only the calmodulin(II)-dependent protein kinase has a mediator roles for cells subjected to hypergravity. Since calmodulin(II)-dependent protein kinase is activated normally by either increased intracellular Ca^{+2} concentration or inositol-3-phosphate, these may also mediate hypergravity-induced cellular changes. However, with the complexity for signal transduction mechanisms, it is not possible with the present study, to venture farther in possible signaling events.

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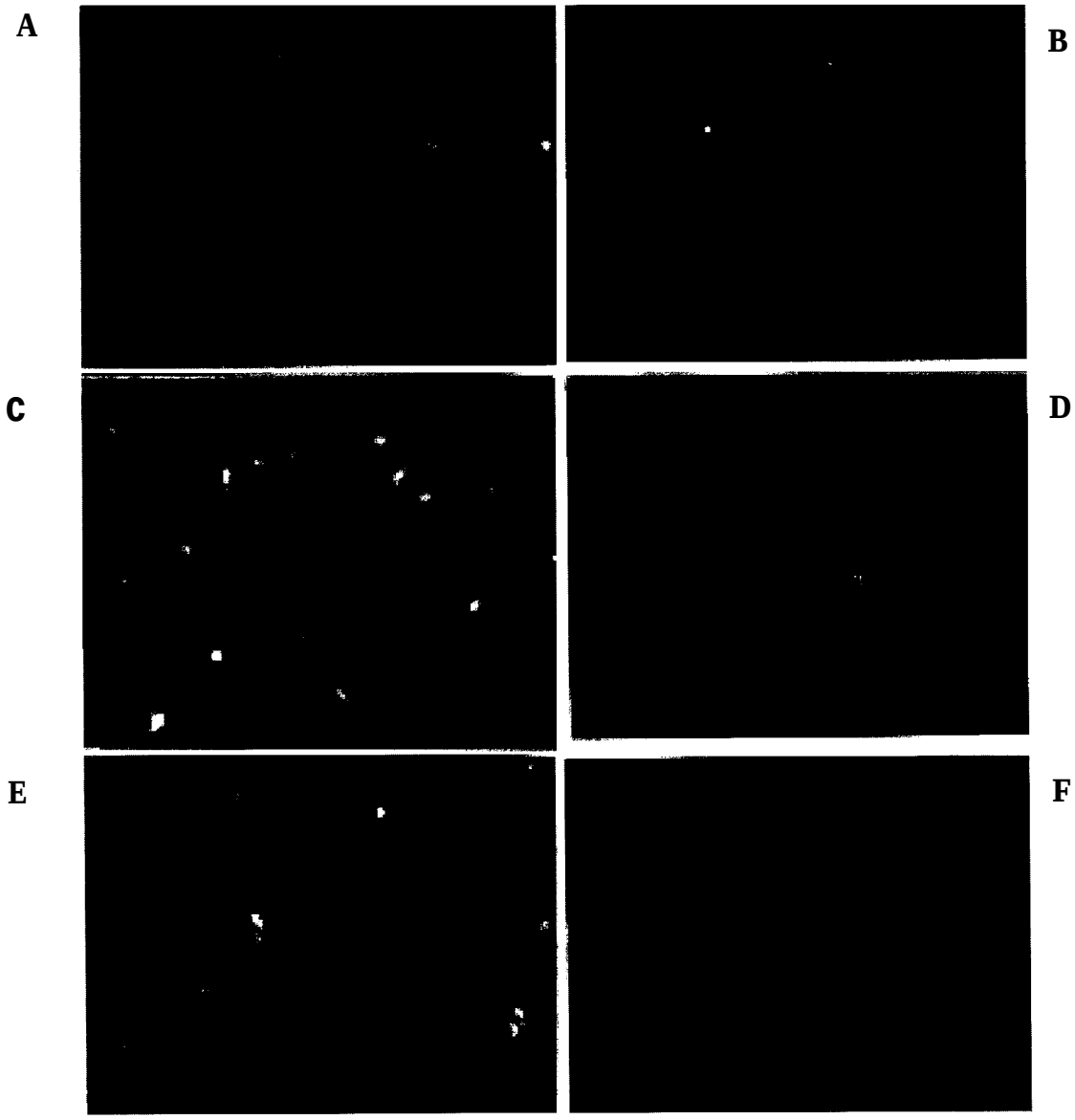

Figure 1. Flourescence micrographs of BAEC after 24, 48 and 72 hr under control (A, C & E) and 12G hypergravity (B, D & F) conditions. Cultures are stained for f-actin using FITC-labeled phalloidin.

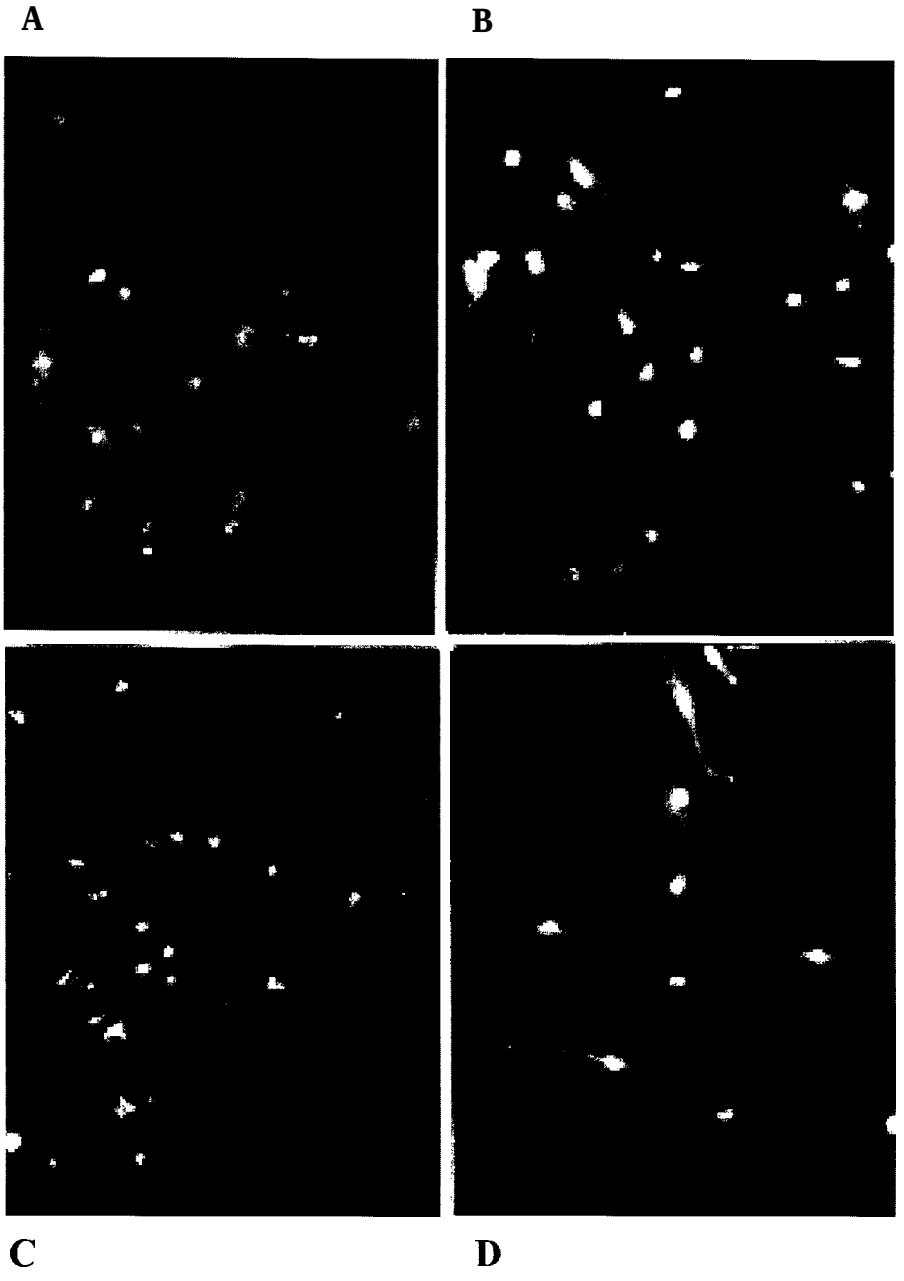

Figure 2. Flourescence micrographs of BAEC treated with PKI KT5926 (for calmodulin(II)-dependent protein kinase) after 24 and 48 hr under control (A & C) and 6G hypergravity (B & D) conditions. Cultures are stained for f-actin using FITC-labeled phalloidin.