Choroidal readaptation to gravity in rats after spaceflight and head-down tilt

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EXPOSURE TO MICROGRAVITY IN SPACE induces a shift of body fluids toward the head. Moreover, symptoms such as headaches, nasal stuffiness, or a sense of fullness in the head suggest that cerebral fluid volume and flow, which partly depend on cerebrospinal fluid (CSF) production, might be affected by microgravity (29). Studies have been performed using ground-based models that simulate weightlessness, such as head-down tilt (HDT) in humans or animals, to determine whether CSF outflow or pressure was modified when a cephalad fluid shift was induced. Results show that 1) a significant increase in total water content measured in the head by magnetic resonance spectroscopy after 4 h of a 6°-HDT in humans (21) or 2) a very slight increase in CSF pressure during the first 30 min of a 45°-HDT in rats (29). No data have been obtained until now on CSF outflow or pressure in humans or animals, after longer simulations during and/or after spaceflight.

CSF is mainly secreted by choroid plexuses from the lateral, third, and fourth ventricles (see Refs. 9, 10, and 23 for reviews). Consequently, it seemed interesting to investigate the choroidal response to exposure to microgravity. During experiments flown on Space Life Sciences-1 (SLS-1), we showed that a 9-day spaceflight significantly increased the number of atrial natriuretic peptide (ANP) binding sites in choroid plexus of rats dissected 5–7 h after landing (14). As ANP is involved in the body fluid adaptation in space (4, 6, 13) and is known to control, at least partly, the choroidal CSF production (8, 28, 30), we proposed to further investigate the choroidal adaptation to spaceflight by studying two points: 1) the impact of weightlessness on choroidal cell structures, and 2) the effects of a readaptation to Earth’s gravity.

The first point was checked during Space Life Sciences-2 (SLS-2) experiments by performing a conventional transmission and scanning electron microscopy (SEM) study with choroid plexus samples obtained from five rats dissected in flight, after 12 days aboard the space shuttle, and comparing the results with choroid plexus dissected from ground control and HDT rats. We showed in a previous report (11) that organization of microvilli and ezrin distribution were altered in choroidal epithelial cells from rats flying in space or suspended in antistathotic position. In both groups, major alterations were noted at the level of structures involved in cell polarity and secretory processes: microvilli were reduced or absent from the apical pole, and large cytoplasmic extensions appeared. Kinocilia tended to disappear from the apical surface. Large amounts of clear vesicles were accumulated in the apical cytoplasm of choroidal cells, demonstrating a partial loss of exocytotic efficiency and suggesting a reduced CSF production. In HDT rats, reduction of apical microvilli was confirmed by modifications in the distribution of ezrin, a cytoskeletal protein involved in the apical organization of choroid plexus.

To investigate the effect of readaptation, the present study was designed to show whether alterations induced in the choroid plexus of rats in microgravity were still obvious or rapidly restored by 6 h after recovery of the orbiter (R+6h; SLS-2, October 1993) or by 2 days after return (R+2d) to Earth [National Institutes of Health-Rodent 1 (NIH-R1) experiment, November 1994]. We also compared HDT rats dissected 6 h after the end of 14-day head-down suspension (S+6h) with...
rats dissected immediately at the end of the HDT period (S) to observe the effects of postflight dissections that begin 4–8 h after landing.

**MATERIALS AND METHODS**

Animals. Sprague-Dawley rats (Rattus norvegicus, Taconic Farms and Centre d’Elevage Dépré, St.-Doulchard, France) were used for spaceflight and suspension experiments, respectively. All space protocols were approved by the Ames Research Center Animal Care and Use Committee. HDT protocols were approved by the ad hoc Committee of the French Space Agency. Animal care and use were in accordance with the guidelines of the National Institutes of Health, Bethesda, MD. One hundred sixty rats were used for this study, including 5 male rats dissected aboard the shuttle (SLS-2 Inflight rats), 38 male rats dissected 6 h after landing (SLS-2 Flight rats); 30 pregnant female rats dissected 2 days after landing (NIH-R1 rats), and 33 male rats dissected for simulated weightlessness experiments (HDT rats) (Table 1). The animals were ~2 mo old, weighing 200–250 g at the beginning of the orbital flights and HDT protocols and weighing 300–400 g at dissection.

Orbital flight protocols and corresponding controls. Flight rats from two different National Aeronautics and Space Administration (NASA) missions were used for this study. They were housed in cages of two different designs in the shuttle and maintained with protocols unique to each flight.

SLS-2 rats from flight nominal groups were singly housed in cages of the Research Animal Holding Facility (16), from 2 days before launch until the end of the experiment (1, 11). They were launched into orbit from Kennedy Space Center on October 18, 1993, aboard the STS-58 mission. They flew for 14 days before launching (NIH-R1 rats), and 23 male rats dissected for simulated weightlessness experiments (HDT rats) (Table 1). The animals were ~2 mo old, weighing 200–250 g at the beginning of the orbital flights and HDT protocols and weighing 300–400 g at dissection.

NIH-R1 Flight nominal group of pregnant rats was launched into orbit on November 3, 1994, on STS-66 flight. Ten rats were housed five each in two animal-enclosure modules, located in the shuttle middeck. Flight and control rats were maintained on a 12:12-h dark-light cycle, in 25–70% relative humidity and at 24–26°C. They received water and food food bar diet ad libitum. Food, water, consumption, and general activity were registered daily. The rats flew for an 11-day spaceflight, landing on November 14, 1994. After parturition, NIH-R1 dams were dissected 2 days after return to Earth’s gravity (R +2d group).

Several groups of ground control rats were compared with the Flight animals. Twelve SLS-2 and 10 NIH-R1 synchronous ground controls were housed as Flight rats, either in the research animal holding facility or in animal-enclosure modules, respectively. Temperature and humidity profiles were replicated but not noise, acceleration, or vibration profiles. Synchronous control rats were dissected on the same day as the Flight rats for SLS-2 or were delayed 1 day for the NIH-R1 group. To show eventual effects of adaptation to flight cages, these synchronous controls were compared with rats maintained in polycarbonate vivarium cages, with microisolator tops, on either raised-wire floors or wood-shaving bedding. During SLS-2 operations, 14 vivarium control rats were dissected on the day of the launch (L +0). Ten NIH-R1 vivarium controls were dissected at the same time as the NIH-R1 synchronous controls, with the use of identical protocols (Table 1). In addition, five pregnant female Sprague-Dawley rats were dissected without anesthesia, during NIH-R1 procedure validation tests (PVT) performed before flight at NASA Ames Research Center (Moffett Field, CA). These were compared with five isofluorane-anesthetized rats, to check the effects of anesthesia on the choroidal fine structure.

Experimental ground-based models for simulation of weightlessness. The Morey-Holton tail-suspension model (22) was used with an antiorthostatic angle of 30–45°. These HDT experiments were performed as previously described (11). Male rodents were maintained in three successive adaptive periods: a first period, in polycarbonate cages for a 7-day habitation period; a second period, with the suspension

| Table 1. Glutaraldehyde, formaldehyde, and Bouin’s-fixed choroidal or brain samples from flight, head-down suspended, and control animals |
|-------------------|-------------------|-------------------|-------------------|
| Animal Groups     | Flight or HDT     | Glutaraldehyde   | Formaldehyde      | Bouin’s-fixed   |
|                   | Status            | Fixed Samples    | Fixed Samples    | Fixed Samples  |
| Flight animals    | R +6h             | n = 6*           | n = 4            | n = 2          |
| Synchronous controls | R +6h             | n = 6*           | n = 4            | n = 2          |
| Vivarium controls | L +0              | n = 6*           | n = 4            | n = 4          |
| Inflight animals  | L +13             | n = 5*           |                   |                |
| Flight animals    | R +2d             | n = 2*           | n = 4            | n = 4          |
| Synchronous controls | R +2d             | n = 2*           | n = 4            | n = 4          |
| Vivarium controls | R +2d             | n = 2*           | n = 4            | n = 4          |
| HDT experiments   |                   |                   |                   |                |
| Suspended rats (S rats) | S +6h             | n = 4*           | n = 3            | n = 45         |
| Controls          | S +6h             | n = 4*           | n = 3            | n = 45         |
| Procedure validation tests |
| Vivarium controls (A +) | n = 3* | n = 2 |
| Vivarium controls (A –) | n = 3* | n = 2 |
device attached on the tail but maintaining the rats in a typical orthostatic position for the next 7 days, for habituation to the restraint; and a third period, for an antithorostatic suspension performed by lifting up the hind limbs, during 14 additional days.

The 33 HDT rats were assigned to three groups: 1) S rats; 2) S+6 h rats, to reproduce the conditions of the SLS-2 Biospecimen Sharing Programs; and 3) control rats dissected after 28 days in polycarbonate cages, without HDT, to be compared with the 14-day head-down suspended rats (Table 1).

Dissections and postflight or postsuspension procedures for electron microscopy. Because of the cooperative nature of spaceflight experiments and their ground studies, it was necessary to share a limited number of specimens with other investigators. Consequently, it was not possible to fix samples by intravascular perfusions of fixatives during the SLS-2 and NIH-R1 Biospecimen Sharing Programs. To evaluate the effects of this protocol on the fine structure of choroidal cells, 6 HDT rats were anesthetized and intracardially perfused with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.5, before brain removal and choroid plexus dissection (Table 1). By performing these complementary experiments, we demonstrated that very similar ultrastructural results were obtained when choroid plexuses were either dissected then fixed by immersion in glutaraldehyde (in the 3-min period after the decapitation), or fixed in situ by perfusion of the same fixative, then dissected before conventional processing for SEM and transmission electron microscopy (TEM) observations.

Animals from SLS-2 and NIH-R1 flights, HDT experiments, or controls were anesthetized with either methohane (SLS-2) or isoflurane (NIH-R1) or with ethyl-carbamate (HDT). SLS-2 and NIH-R1 rats were bled by cardiac excise before decapitation. Brains from the different groups were removed from the skull after decapitation. Choroid plexus samples dedicated to ultrastructural studies were dissected from brains by removal from the lateral (CPX-1), third (CPX-3), and fourth (CPX-4) ventricles. Choroidal samples from SLS-2 and NIH-R1 Flight, vivarium, and synchronous control animals (Table 1) were fixed by immersion in the fixative for 16 h and similarly processed: all glutaraldehyde-fixed samples were postosmicated and ethanol dehydrated. Pieces of each glutaraldehyde-fixed sample from SLS-2 and HDT experiments were cut in two equal parts. One-half was embedded in LX-112 resin for TEM observations, whereas the other was stored in 70% ethanol before processing for SEM.

Ultrastructural observations. LX-112 embedded pieces of choroidal plexuses from each experimental group were thin sectioned, uranyl and lead contrasted, and observed with a JEOL 1200 transmission electron microscope, with a 70-kV accelerating voltage.

Samples for SEM observations were dehydrated with ethanol, incubated in liquid CO$_2$ at 5°C, and dried with the critical-point method before sputtering. Surfaces of the samples were examined with a JEOL JSM 6300-F field-emission scanning electron microscope, with a 2- to 10-kV accelerating voltage. To reduce charges observed even with a 2-kV accelerating voltage, different sputtering protocols (carbon, gold, platinum, or gold-palladium) were used. The best results were obtained at 8 kV after glow-discharge of the samples and gold-palladium sputtering with a BAL-TEC SCD 050 device. For SEM observations, choroidal samples from SLS-2 Flight rats were compared with samples from SLS-2 Inflight rats (Table 1), treated as previously reported (11).

Immunocytochemical distribution of ezrin and carbonic anhydrase II (CA II). Comparative studies were also performed to check the effects of fixation procedures in immunocytochemical experiments. Three protocols were used: 1) dissected choroid plexus samples, fixed with formaldehyde (SLS-2 and HDT experiments); 2) whole brains, fixed with Bouin’s solution by immersion (SLS-2, NIH-R1, HDT, and PVT experiments); 3) whole brains, fixed with Bouin’s solution by perfusion (HDT experiments only).

Isolated choroid plexuses of 12 SLS-2, 12 NIH-R1, and 9 HDT animals (Table 1) were fixed in 3% formaldehyde freshly prepared from paraformaldehyde powder in phosphate-buffered saline (PBS; tablets, Sigma Chemical, St. Louis, MO). Small pieces of choroidal tissues were infused in buffered 1.2 M sucrose and shipped to France. Thin-frozen sections (200–300 nm) of choroidal tissues were prepared at −75°C by using a Reichert Ultracut with a FC4 cryoequipment, put on polylysine-coated glass slides and immunostained according to Tokuyasu’s method, as previously described for choroidal tissues (24). Frozen sections (60–100 nm) attached to glass slides were postfixed in 3% formaldehyde in PBS.

Whole brains of 8 SLS-2, 12 NIH-R1, 6 HDT, and 4 PVT animals (Table 1) were fixed overnight by immersion in Bouin’s fixative solution, as previously described for HDT experiments (11). Six additional HDT rats (2 S, 2 S +6 h, and 2 control rats) were anesthetized and intracardially perfused with Bouin’s fixative before brain removal to show the effects of fixation on protein distribution. After being washed in water, brains were dehydrated with a progressive ethanol series, incubated in butanol, and embedded in Paraplast Plus (Sigma Chemical). Sections (5–7 μm) containing choroid plexuses from the lateral, third, and fourth ventricles were selected by using a stereotaxic atlas and, after removal of Paraplast by xylene baths, treated with anti-ezrin (2) and anti-CA II (12) antibodies, with the use of conventional immunocytochemical methods.

Frozen or Paraplast sections were thoroughly washed in PBS containing 50 mM NH$_4$Cl and incubated overnight at 4–20°C, with specific antibodies diluted (10–20 μg/ml) in PBS containing 0.2% gelatin and 1% normal goat or rat serum. After thorough washing and incubation with secondary fluorescein- or rhodamine-labeled antibodies, sections were washed and mounted in Moviol 488 (Calbiochem, La Jolla, CA). Anti-ezrin antibodies were used with both frozen and Paraplast sections from 20 SLS-2, 24 NIH-R1, 21 HDT, and 4 PVT animals. Anti-CA II antibodies were used to study Paraplast sections from 8 SLS-2, 12 NIH-R1, 6 HDT, and 4 PVT rats. Immunoreactions were observed with a Zeiss epifluorescence microscope equipped with ×25 and ×40 Plapachromat objectives and interferential filters or were tridimensionally analyzed with Biorad or Leica confocal argon-krypton laser scan microscopes, to obtain data concerning the cellular distribution of proteins and to differentiate the apical from the cytoplasmic labeling.

RESULTS

Fine structure of choroid plexus in ground controls. Comparison of choroid plexuses from different ground control groups was performed by SEM and/or TEM observations. Investigations performed to validate the protocols demonstrated the absence of postmortem alterations in vivarium control groups dissected on the day of launch (SLS-2) or maintained during the flight duration in vivarium cages (NIH-R1) and in HDT control rats. In all these control rats, we observed a typical organization in choroidal cells. “Bare” cells were extremely rare, and apical microvilli, kinocilia, and cytoplasm were typically distributed as previously described by numerous authors (see Refs. 9, 10, and 23 for...
reviews). In choroid plexuses from synchronous control dams dissected at R+2d (NIH-R1), cells without any microvilli were extremely rare, and cellular alterations were very scarce. Conversely, choroid plexuses from SLS-2 synchronous controls dissected at R+6h demonstrated a slightly altered organization of microvilli at the apical pole of some epithelial cells. We noted that microvilli were missing in some scattered cells, whereas they were very abundant, long, bulbous, and homogeneously distributed in other cells. Kinocilia remained typically localized at the apical pole.

Considering the modified pattern noted in microvilli from synchronous controls from SLS-2 experiments (which were dedicated to hematology studies), we tried to explain the abnormalities in this one group. We first demonstrated the absence of effect of both anesthesia and cardiocentesis. During NIH-R1 PVT, we used rats of the same strain and from the same vendor. We did not observe any structural changes with SEM and TEM investigations in anesthetized and nonanesthetized animals submitted to cardiocentesis for blood collection.

Fine structure of choroid plexus from SLS-2 Flight animals (Figs. 1 and 2). Choroidal cell surface and fine structure from SLS-2 Flight animals, dissected at R+6h, were clearly altered, as demonstrated by SEM (Fig. 1) and TEM observations (Fig. 2): some choroidal cells had a complete lack of microvilli, whereas other nearby cells expressed more or less differentiated apical microvilli. In CPX-L and CPX-3 cells, microvilli were more reduced than in CPX-4 cells, and a larger number of cells without microvilli was noted. These observations (Fig. 1, c-d) were compared with those obtained from Inflight rats (Fig. 1, a-b) dissected the...
day before shuttle landing. Alterations were similar, except that cell surfaces of CPX-4 cells in SLS-2 Flight rats frequently presented enlarged bulbous and spherical microvilli (see 3 in Fig. 1d), which were not observed in CPX-4 from Inflight rats (Fig. 1b) and were sparse in CPX-L from Flight rats (Fig. 1c). This suggested an early and rapid increase in the apical membrane domain, as soon as 6 h after landing, mainly in the CPX-4 cells. TEM observations (Fig. 2, a-d) confirmed that choroidal structures started to be restored at the apical domain, as shown by the bulbous microvilli obvious in CPX-3 and CPX-4 (Fig. 2, a-b and d). In contrast, microvilli remained very dispersed in CPX-L (Fig. 2c). As in Inflight rats (11), no abnormalities in Flight animals were noted by TEM at the level of cytoplasmic organelles and nucleus.

Fine structure of choroid plexus from HDT rats (Figs. 1 and 3). At the end of a 14-day suspension, we observed by SEM the choroid plexuses of rats (Fig. 1, e and f) dissected 6 h after return to the orthostatic position. We noted that microvilli, in particular in CPX-4, appeared larger and more bulbous. However, bare cells remained apparent, as previously shown in S rats (see figure in Ref. 11). With TEM studies, we noted similar alterations in the fine structure of choroidal cells from Flight and HDT animals dissected 6 h after the end of the exposure to microgravity or antirheostatic restraint. In S+6h rats, the presence of very small microvilli supported by dense cores of microfilaments (Fig. 3), together with the absence of apical clear vesicle accumulations, indicated that a 6-h delay between the end of simulated microgravity and dissection induced the beginning of the restoration in choroidal structures and suggested a restored secretory function.

Fine structure of the choroid plexus from NIH-R1 Flight rats (Fig. 4). TEM observations of choroid plexi...
uses from NIH-R1 Flight animals, dissected at R + 2d, demonstrated a very different pattern in the organization of apical microvilli (Fig. 4). As NIH-R1 rats were pregnant dams, we carefully observed the NIH-R1 ground controls, which were also pregnant, in comparison with male adults (not shown). No alterations were noted in choroid plexus of NIH-R1 control dams when compared with male adults. In NIH-R1 Flight rats, beside sparse bare cells, we noticed that cells were higher, displaying a darker cytoplasm, and rich in mitochondria (Fig. 4a) and that microvilli were generally very high and enlarged, containing a fuzzy cytoplasmic matrix rich in microfilaments and membrane profiles (Fig. 4b), looking like the choroidal pattern described in hypertensive rats (27). After 2 days of readaptation to Earth’s gravity, most of the cells in lateral, third and fourth ventricle choroid plexuses displayed a huge increase of apical membranes, organized in microvilli with a dense cytoskeletal matrix, or myelinlike profiles, both demonstrating a restoration of the apical domain and evoking a restored fluid transport activity.

Ezrin distribution in SLS-2, NIH-R1, and HDT rats (Figs. 5 and 6). Ezrin, a cytoskeletal actin- and membrane-associated protein, is abundantly distributed in the apical microvilli of choroidal cells in mature rats. In vivarium and synchronous control rats, ezrin was typically distributed in all ventricular regions at the apical pole of the choroidal cells as a large, bright, and regular margin between the negative cytoplasm and the ventricular lumen (Figs. 5a and 6a). This apical and homogeneous labeling demonstrated that ezrin could be a good marker for microvilli in choroidal cells.

In SLS-2 Flight rats at R + 6h, distribution of ezrin was always reduced and irregular in the apical domain and located at the level of the cell-cell adhesion sites near the junctional complexes (Fig. 5b). The protein was also detected in the cytoplasm of choroidal cells, mainly in the sagittal part of the CPX-4 (Fig. 5c). Such a labeling was never observed in control adult rats. In 1-G-readapted Flight rats from NIH-R1 experiments (R + 2d), we noted that the ezrin distribution in CPX-4 was still irregular and heterogeneous in the apical domain and abundant in the cytoplasm of numerous choroidal cells (Fig. 5d).

Similar results were obtained from SLS-2 and HDT rats studied after immersion or perfusion with Bouin’s fixative and after formaldehyde fixation and thin-frozen sectioning of choroidal tissues. As no samples were available for immunocytochemistry from SLS-2 in-flight dissections, we carefully compared S and S + 6h samples from HDT experiments. No clear differences could be observed in the ezrin distribution of choroid plexus from rats dissected immediately after the end of the head-down tilt or 6 h later (Fig. 6, b and c).

The loss of ezrin at the apical pole, in addition to the strong presence of this protein in the cytoplasm of choroidal cells, mainly in the sagittal region of the CPX-4, which was never obtained in synchronous or vivarium rats, demonstrated that, even after 2 days of readaptation to Earth’s gravity, the distribution of ezrin was not completely restored in choroidal cells.

Distribution of CA II in SLS-2, NIH-R1, and HDT rats (Figs. 7 and 8). CA II is an enzyme distributed in the cytoplasm as well as at the level of membranes in choroid plexus of mature rats. Slight differences were noted in cytoplasmic distribution of CA II when brains were fixed with Bouin’s perfusion or immersion procedures. In perfused brains from HDT experiments, the cytoplasmic pool of CA II was mainly distributed in the basolateral domain of choroidal cells in control rats and near the apical domain in S and S + 6h rats. Perfused brains were not available from SLS-2 and NIH-R1 experiments. Therefore, in the present study, we showed only the choroidal CA II distribution observed in whole brains fixed by immersion within Bouin’s solution. It is important to note, however, that after perfusion of Bouin’s solution, as well as after fixation by immersion, CA II was strongly decreased in the choroidal cytoplasm of SLS-2 Flight rats (Fig. 7) and HDT S and S + 6h rats (Fig. 8), when compared with that of the controls.

CA II was abundantly found in the cytoplasm of choroidal cells in control rats (Figs. 7a and 8a), as demonstrated by the huge immunoreactivity detected.
with the anti-CA II antibodies. In the choroid plexus from SLS-2 flight rats (Fig. 7b) at R + 6h and HDT rats (Fig. 8, b and c), a strong reduction of the cytoplasmic enzyme was observed. Extracellular membrane extensions protruded in the ventricular lumen at the apical pole of the cells. The cytoplasmic immunoreactivity to anti-CA II antibodies reappeared heterogeneously in S16h rats (Fig. 8c). In NIH-R1 rats (R12d), the apical labeling remained obvious, and the cytoplasmic anti-CA II immunoreactivity was still reduced. However, CA II was almost restored in many cells from NIH-R1 dams (cf. Fig. 8c with a and b).

**DISCUSSION**

The results reported here are data obtained from early-readapted rats after 11- and 14-day spaceflights and compare them with early readaptation from a 14-day antiorthostatic hindlimb suspension. The cellular response of choroid plexuses was observed after return to normal 1-G conditions at R + 6h and R + 2d. We compared these results with choroidal features, previously reported in rats dissected aboard a space shuttle (inflight operations of SLS-2 experiments) and rats dissected immediately after a 14-day HDT (11); they revealed that structural organization and protein distribution began to be restored in early readaptation to 1 G after spaceflight or head-down suspension, suggesting a partial restoration of the choroidal functions. In both conditions, partial disappearance of apical microvilli, accumulation of clear vesicles at the apical pole, and cytoplasmic localization of kinocilia, demonstrated 1) a partial loss of cellular polarity, cytoskeleton, and membrane organization; and 2) a partial lack of apical ezrin in HDT rats. Similar effects were observed in most of the choroidal cells in 6-h-readapted rats, except that microvilli were more bulbous in choroid plexus from the fourth ventricle.

Because of the time required by landing operations, most of the flight samples studied were obtained several hours postflight. Hence, it seemed important to know whether such delays can induce rearrangements and restorations, thereby making it more difficult to understand the real effects of spaceflight. This study allowed us to describe early phases in the recovery processes and to further understand the consequences of microgravity and gravitational readaptation. The SLS-2 experiments provided samples that gave us the opportunity to compare choroidal fine structure of adult rats dissected inflight and postflight. We observed that, as soon as R + 6h, cytoskeleton and membrane organization and cellular polarity started to be restored in choroidal cells, as shown by the reappearance of microvilli with dense cytoskeletal cores. Similar effects were observed 6 h after the end of HDT restraints. Moreover,
after 2 days on Earth (NIH-R1 experiments), microvilli were strongly enlarged, showing a clear increase in apical cytoskeletal and membrane structures, although they had not regained a typical appearance. Overall, these structural restorations confirmed the sensitivity of choroidal cells to gravitational variations.

With immunocytochemical approaches, we observed a modified distribution of ezrin, an actin- and membrane-associated protein involved in the organization of apical microvilli in several cell types (2, 3). These changes, observed at R + 6h and S + 6h, confirmed the previous results obtained by simulating cephalad fluid shift by HDT (11). In both conditions, ezrin distribution was altered at the apical pole, in accordance with the partial loss of choroidal apical microvilli, confirming the structural role of this protein in choroid plexus. Moreover, the presence of large amounts of ezrin in the cytoplasm of CPX-4 cells suggested alterations in cellular processing and apical targeting of this protein during adaptation to actual or simulated microgravity. At early postflight observation, distribution of CA II, an enzyme known to be strongly involved in CSF production (17, 20), was markedly reduced in choroidal cells, showing a reduced enzymatic activity at this time. After 2 days, we noted a very partial restoration of this enzyme, indicating that choroidal function involving this enzyme did not recover its original efficiency, despite the enhanced apical domain.

Both apical and basolateral membrane domains were increased in choroidal cells from NIH-R1 rats dissected at R + 2d. In spontaneously hypertensive rats, as previously reported, this morphological pattern in choroidal cells was correlated with an increased CSF production, an increased Na⁺-K⁺-adenosinetriphosphatase activity (26, 27).

We attempted to understand the choroidal alterations reported in SLS-2 synchronous ground controls, dissected at R + 6h, from the hematology experiment. Two explanations were possible. Rats from the hematology group were anesthetized with methofane and bled by cardiocentesis before decapitation. Anesthesia (15) and hemorrhage (28) as well as spaceflight (4, 6, 8) are known to induce variations in hormonal mechanisms that are involved in the regulation of CSF production (8, 28, 30). We pointed out that anesthesia might induce the changes observed at the apical surface of ground controls and flight animals. However, PVT results reported here suggested that anesthesia was not responsible for such modifications.

Fig. 5. Immunodetection of ezrin in choroidal cells from SLS-2 synchronous (a) and Flight rats (b-c) and from NIH-R1 Flight rats (d), with use of sections of whole brains fixed by immersion in Bouin’s solution. In choroid plexus from control rats (CPX-3, a), ezrin is typically found at apical pole of cells as a large and regular margin between the negative cytoplasm and ventricular lumen (a, ap). In SLS-2 Flight animals (R + 6h), apical labeling was strongly reduced (CPX-3 shown in b) while a bright cytoplasmic reaction (stars) was observed in CPX-4 cells (c). Cytoplasmic immunoreaction (stars) was still observed in CPX-4 cells of NIH-R1 Flight rats (d) fixed at 2 days after return (R + 2d). S, Leptomeningial stroma at basolateral side of choroidal epithelium. Bar = 10 µm (a-d).
A second explanation could be provided by use of cardiocentesis, which led to a 5- to 15-min bleeding before decapitation and dissection of animals. However, we noticed that SLS-2 and NIH-R1 vivarium ground controls, as well as NIH-R1 synchronous ground controls (which received anesthesia and were bled by cardiocentesis), showed very few abnormal microvilli and bare cells. Consequently, it is difficult to understand the origin of modifications observed on the synchronous ground control group of the SLS-2 experiments. We believe that it was likely due to hematology experiment manipulations (1).

Differences in fine structure and ezrin distribution were reported in choroid plexuses from the forebrain (CPX-L and CPX-3) or brain stem (CPX-4). These regional differences were in accordance with the previous observations performed with SLS-1 rats, which showed that the number of ANP-binding sites was increased in CPX-L and CPX-3 and not significantly modified in CPX-4 (14).

Even though it is not possible to draw definite conclusions without physiological measurements, these morphological features suggest that spaceflight, HDT, and 1-G readaptation impact the choroidal production of CSF. Until now, data concerning changes during choroidal adaptation to environmental conditions were very scarce. However, hormones, environmental adaptations, and diseases seem to modify choroidal structures. Reports have shown that water deprivation or in vitro arginine vasopressin administration induced modifications of apical and basolateral membrane domains on choroid plexus through V₁ vasopressin receptors (19) and that subcutaneous administration of melatonin increased the apical membrane domain by increasing the length of apical microvilli in choroid plexus from lateral ventricles (7). Both results were interpreted as an increased CSF secretory activity. Acute septic ventriculitis was reported to decrease number and size of apical microvilli and, consequently, to reduce CSF production (5).

Indeed, the apical membrane in choroid plexus is characterized by the presence of molecules such as Na⁺-K⁺-adenosinetriphosphatase (25), ANP receptors (18), and G proteins (24), which are involved in fluid transport and/or regulatory processes. Considering the important role played by this membrane domain in these cells (see Refs. 10, 23 for review), we might hypothesize that structural changes at the apical surface of choroidal epithelial cells should involve changes in choroidal functions, as shown in spontaneously hypertensive rats (26, 27). At this moment, if we consider the results reported here, together with the results previously reported on the choroidal ANP receptors (14) and with those that are in progress on the choroidal guanosine 3′,5′-cyclic monophosphate contents (C. Carcenac, S. Herbuté, and J. Gabrion unpublished observations), we can conclude that adaptation to weightlessness involves a choroidal response, hormonally regulated, which presumably impacts the CSF dynamics.
Fig. 7. Immunodetection of carbonic anhydrase II (CA II) in choroidal cells from SLS-2 synchronous control (a), SLS-2 Flight rats (b), and NIH-R1 Flight rats (c), by using sections of whole brains fixed by immersion in Bouin's solution. In SLS-2 synchronous control rats, protein was typically detected at the cytoplasm of epithelial cells, with a discrete positive margin at apical pole between the negative cytoplasm and ventricular lumen (a, long arrows). In choroidal cells from SLS-2 Flight rats (R + 6h), cytoplasmic distribution of CA II is reduced, whereas an intense immunoreaction was observed in apical and ventricular (extracellular) regions (b, open arrows). In NIH-R1 Flight rats fixed at R + 2d, CA II was abundant at apical surface of cells and also in cytoplasm of numerous choroidal cells (c, stars). Bar = 20 µm (a–c).

Fig. 8. Immunodetection of CA II in choroidal cells from HDT control (a), S (b), and S + 6h rats (c) by using sections of whole brains fixed by immersion in Bouin's solution. In CPX-4 choroidal cells from control rats (HDT), protein was well detected at cytoplasm, with a discrete positive margin at apical pole between the negative cytoplasm and ventricular lumen (a, long arrows). In choroidal cells from S rats, cytoplasmic distribution of CA II is clearly reduced and apical positive structures extended in extracellular spaces (b and c, open arrows). In choroidal cells of S + 6h rats, CA II was always abundant at apical surface of cells and in ventricular space. Cytoplasmic reaction (star) is more lightly intense than in S rats (cf. c with b). Bar = 20 µm (a–c).
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