Prolonged Water Immersion

Effects on Blood Pressure Maturation in Normotensive Rats

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The purpose of this experiment was to study the impact of simulated microgravity and of chronic removal of hydrostatic pressure gradients on blood pressure maturation and body growth in rats. A special device was developed in our laboratory to transfer prolonged “dry” water immersion (a technique that has been used for training astronauts under hypogravic conditions) to six Sprague-Dawley test rats (immersion-G group). The time course of heart rate, systolic blood pressure, urinary output, and body weight was monitored from weaning to maturity and then compared with those responses from six sex- and age-matched Sprague-Dawley rats grown in a gravity environment (group G). A downward shift in systolic blood pressure and body weight maturation curves was observed in immersion-G rats from the age of 60 days. Cessation of dry water immersion produced a gradual, significant rise in systolic blood pressure but not in body weight to control values. No marked changes in heart rate and urinary output between G and immersion-G rats were noticed throughout the investigation. Our results provide indirect evidence that an interference in the natural history of blood pressure maturation was introduced by immersion, which dissociated the effects of body weight increase during growth from the effects of ageing per se. It is concluded that the physiological increase in systolic blood pressure during growth is partly gravity-dependent. (Hypertension 1992;19:482-487)

KEY WORDS • blood pressure • urine • immersion • weightlessness • gravity • Sprague-Dawley rats

The growth process is characterized by a progressive increase in body weight, implying not only fine alterations in the chemical and biochemical activity of the living organism but also an increase in the number of its composing cells.1-2 The dynamics of the circulatory system also undergo marked changes3 throughout the period of growth that in terrestrial mammals, is of the “definite” type (i.e., body growth stops when the fusion of cartilaginous plates prevents further elongation of the long bones and species-specific definitive body dimensions are acquired).

A high correlation coefficient was found between the increase in body weight, taken as an index of body growth, and the hemodynamic changes leading to the stable circulatory conditions encountered in adulthood.3 The rate of increase in body weight is followed in a cascade fashion by a gradual increase in both total peripheral resistance and mean arterial pressure (blood pressure maturation curve) and by decreases in heart rate, cardiac output per unit of body weight, central venous pressure, and plasma volume.5 Appropriateness of hemodynamics to body weight and to the degree of maturation suggests that numerous circulatory feedback mechanisms must be activated and operative. Moreover, in spite of widely differing body dimensions and ultimate body weight, remarkable interspecies similarities in the shape of the blood pressure maturation curves have emerged from comparative studies carried out in experimental animals and humans.67 Specifically, the arrest of body growth coincides with a marked decrease or a plateau in the slope of the blood pressure maturation curve.

The impacts of active assumption of upright position on the natural history of circulatory dynamics were also investigated in infants. Suggestive evidence has accumulated that the rise in body weight during growth and the weight of the blood (hydrostatic effect) may be an important, natural stimulus for the remodeling of capacitance and resistance vascular beds through activation of the neurohumoral mechanisms responsible for the control of the dynamics of body fluids, especially for their distribution within the cardiopulmonary and peripheral compartments in a gravity environment.8

However, the question whether the hemodynamic changes occurring from extrauterine life to maturity depend on ageing (time) rather than on growth-related increases in body weight (gravity) is still unsettled. The aim of the present investigation was, therefore, to dissociate the impact of ageing and gravity on the blood pressure maturation curve of developing normotensive rats by exposing the experimental animals to the effects of prolonged simulated microgravity.

The effects of microgravity89 can be studied on Earth by several models such as the free fall phase of parabolic flight in jet aircraft,10 head down11,12 and horizontal bed rest,13-15 and head out water immersion.16-19 The latter
technique, based on the principle of Archimedes, assumes that when a body is placed in a fluid of about its density, the force acting in the opposite direction to gravity is proportional to the mass of the displaced fluid and that the loss of weight of the submerged object is equal to the weight of the fluid displaced by the object itself.\textsuperscript{20,21}

In humans, techniques have been developed that allow water immersion while maintaining the skin dry, so called "dry" water immersion (DWI). Animal models of microgravity simulation have also been developed,\textsuperscript{22} but these are largely confined to acute studies.

The consequences of chronic removal of hydrostatic pressure gradients on blood pressure maturation and on body growth were evaluated for the first time in our laboratory by exposing Sprague-Dawley (SD) rats to dry water immersion, a technique currently available for training astronauts under hypogravic conditions.\textsuperscript{23}

Methods

Restraining Devices

To maintain the rats underwater for prolonged periods of time, a simple, economical device was needed that allowed the rats free access to food and drink and provided some form of protection against skin maceration. Furthermore, some form of support had to be provided so that the animal was not obliged to swim since this would result in drowning when the animal became exhausted or fell asleep.

To provide support, we designed an easy to prepare, very durable restraining system that consisted of two stainless steel frames (1 mm thick) and a waterproof polyethylene freezer bag (Figure 1). Each frame had a central rectangular hole (190x140 mm) and external dimensions of 320x180 mm. The frames were separated by 15 mm with three interposed, thermowelded, rectangular bars on their inferior and lateral borders. The latter were conceived and constructed to act as rigid conduits providing entry for air and food. The superior border was constructed to provide support for a standard small animal water dispenser (250 ml) and entry for probes and catheters if required.

The freezer bag (320x220 mm) was wrapped around the frame, providing a container for the test rat. Adhesive tape was used to fix the bag to the top of the frame. The restraining device was then set at an angle of 15° to the bottom of a Plexiglas tank measuring 530x200x170 mm (wall thickness 5 mm) filled with water. Collapse of the bag interfered with neither voluntary movements nor with passive submersion at a 1-3-cm depth below the water surface (Figure 2).

Thermal stresses were carefully avoided by continuously keeping the water temperature thermoneutral (28–30°C) throughout the experiment with a digital thermostat (Regaterm RT 2000, Vittadini, Italy), the latter constituting the only running cost of the system. Preliminary experiments carried out in our laboratory had shown that this temperature induced minimal changes in the heart rate (±10 beats per minute) and no changes in rectal temperature of the rats when the animals were removed from room temperature (19°–21°C) and placed in the water.

The polyethylene bag was changed daily to supply the rats with a clean environment. The rats grown under hypogravic conditions (immersion-G group) were kept nonimmersed for a few seconds once a day (the time required to change the bags). Once the test animals had been reimmersed in the tank, the contents of the plastic bag were collected and centrifuged at 1,000 rpm for 5 minutes (Centrifuge 4225, Apparecchi per Laboratori Chimici, Milan, Italy) to separate excrement from urine. The urinary output was then measured in a graduated test tube.

Validation of the Dry Water Immersion Technique

To assess the adequacy of the DWI technique, we established whether the mechanical force exerted by
water on the rat was somehow interfered with by the plastic freezer bag. The volume of a rat (about 150 ml) was defined by measuring the volume of the water displaced by immersing the test animal in a graduated container. A latex balloon was filled with 150 ml normal saline to simulate the body volume of a rat. The tip of a 7F catheter connected to a Statham strain gauge transducer (P23dB, Gould, Statham Inc., Hato Rey, Puerto Rico) was then placed at the center of the balloon. The latter was attached to the catheter by a silk thread and then immersed at different depths, from 0.5 to 3 cm below the water surface, in a tank. The same procedure was followed after wrapping the balloon with a polyethylene bag.

Since no changes in the pressure recorded inside the latex balloon were observed at the different depths during dry and wet immersion, it was concluded that the polyethylene container did not modify the pressure exerted on a body immersed in a fluid of about its same density.

Protocol

Six normotensive male Charles River SD rats (immersion-G group) were individually housed submerged in our DWI restraining devices for up to 60 days, from the age of 30 days (weaning) to 90 days (maturity) to simulate the lack of hydrostatic pressure gradients. Ultimate cessation of body growth was demonstrated at 90 days of age by a definite plateau in the body growth curve.

In addition to urinary output, measurements of body weight (spring balance), systolic blood pressure, and heart rate (tail-cuff technique, blood pressure recorder 8006, Ligo Basile, Biological Research Apparatus, Varese, Italy) were recorded at 10-day intervals. The recording of systolic blood pressure lasted about 15 minutes in each animal and was carried out from 11 to 12 AM to avoid fluctuations in the blood pressure due to the diurnal rhythms of the rats. During measurement of the systolic blood pressure, the device was kept horizontal at a 0.5-3-cm depth below the water surface, and the rat tail was pulled out through a hole in the upper part of the plastic bag. Once blood pressure was stable, the average of three successive readings was taken as the representative blood pressure value for each rat. After immersion, the fully developed immersion-G rats were transferred to a normal gravity environment, and the time course of the same variables was determined until the rats were 150 days old.

A control group (G) of six normotensive, age-matched male SD rats was tested inside the same restraining devices used for G rats, without undergoing DWI, to the age of 150 days. The devices were likewise set at an angle of 15° to the bottom of the Plexiglas tank at a room temperature ranging from 18° to 21°C but without water surrounding the plastic bag so that hydrostatic pressure gradients in the rats persisted.

Rats from both groups received food (Purina rat chow, Ralston-Purina Co., St. Louis, Mo.) and water ad libitum. The procedures followed were in accordance with our institutional guidelines.

Results

The protocol was well tolerated by all 12 animals. No deaths and no abnormal behavioral patterns, such as head and body shakes, paw tremors, or excessive grooming and shivering, were noticed. The technique enabled the test rats to perform all their basic physiological functions, such as moving (except running at night), breathing, feeding, drinking, sleeping, and resting. No
skin abnormalities were observed at the end of the immersion.

The sequence of changes in the parameters measured throughout the study is outlined in Figure 3. The lack of significant changes in heart rate, systolic blood pressure, urinary output, and body weight between the G and immersion-G rats during the first 30 days of DWI supplied direct evidence that the immersion-G rats were functioning normally and that they were not under any abnormal stress.

At 30 days of age (weaning), before starting DWI, the heart rate, systolic blood pressure, urinary output, and body weight were essentially similar in both rat groups (388±7 versus 385±15 beats per minute; 83±2 versus 85±2 mm Hg; 0.06±0.01 versus 0.06±0.02 ml/24 hr/g; 75±12 versus 80±12 g, respectively). At 60 days of age, DWI induced a significant difference in systolic blood pressure and body weight between immersion-G and G rats (106±4 versus 120±4 mm Hg, p<0.01; and 145±10 versus 180±20 g, p<0.05, respectively), whereas heart rate (372±12 versus 378±15 beats per minute) and urinary output (0.04±0.02 versus 0.05±0.01 ml/24 hr/g) were essentially equal in both animal groups. This trend continued until DWI was stopped, thus demonstrating that the expected physiological rise in systolic blood pressure and body weight, taken as indexes of cardiovascular maturation and development, respectively, was blunted in immersion-G rats compared with G rats during the entire DWI period.

After cessation of DWI, no significant changes in heart rate and urinary output were observed between fully developed G and immersion-G rats. Body weight remained stable, whereas a concomitant, gradual rise in systolic blood pressure was noticed in the DWI rats (Figure 4).

Discussion

Like head down tilt and prolonged bed rest, water immersion is an appropriate model for simulating microgravity on Earth. Since this technique counteracts some of the effects of gravity (i.e., cardiovascular system hydrostatic pressure) it has been exploited to obtain important information relevant to gravitational biology under controlled laboratory conditions. Chronic DWI, a technique simulating exposure to the hypogravic environment used in training astronauts, avoids skin steeping and interferes with neither voluntary movements nor with buoyancy (Figure 5). However, no model of DWI similar to that described for humans has been applied to terrestrial animals.

FIGURE 4. Bar graphs show time course of changes in heart rate (HR), systolic blood pressure (SBP), urinary output (UaV), and body weight (BW) recorded in 12 Sprague-Dawley rats (b, group G, n=6; ■, group immersion-G, n=6) from 90 to 150 days of age.

FIGURE 5. Schematic shows restraining device provided the test rats with a uterus-like habitat that made survival in an aquatic environment possible and that simulated microgravity (chronic removal of hydrostatic pressure gradients).
A restraining device was fabricated in our laboratory that allowed us to study some intriguing aspects of the growth process in rats exposed to simulated microgravity.

The precise mechanisms responsible for the blunted increase in body weight observed in rats in DWI are not clear, but they may be hypothetically attributed to interference by the DWI technique with muscle function, bone function, and/or renal function.

It would be reasonable to suppose that the immersed rats, compared with the control rats, were subjected to a lower degree of physical activity that might result in muscular deconditioning, reduced muscular size, and thus reduced body weight. This assumption would be in keeping with the morphological and functional alterations taking place in human skeletal muscles during prolonged inactivity as well as after exposure of rats to weightlessness.24,25 Were this hypothesis true, the body weight of the immersion-G rats should have gradually increased and perhaps have attained the same level as the control group owing to muscular reconditioning after cessation of DWI.26 Although we do not have any reliable index to quantitate and compare the amount of exercise performed by the test rats, one more consideration weighs against the assumption that the attenuated rise in body weight exhibited by the DWI rats was due to a reduction in their muscular size. Indeed, qualitative rather than quantitative differences in the physical activity apparently existed between the two groups of rats. Voluntary movements by the immersed rats (but not the control rats) seemed to be more wasteful of energy because of the higher resistance offered by the surrounding water.

The alternative, attractive hypothesis is that, through decreases in hydrostatic pressure and compressive force on the long bones of the immersed rats, water immersion like prolonged bed rest26,29 might have triggered a rise in calcium urinary excretion and mineral loss,30–33 especially from weight-bearing bones, a reduction in bone density, and changes in bone structure (osteopenia), and thus a blunted increase in body weight.

Last but not least, through the interplay of several mechanisms,34–47 chronic exposure to simulated microgravity and to weightlessness is known to result in body weight loss, consisting mostly of water or changes in the chemical composition of the body.34–46 It is thus also tempting to attribute the blunted increase in body weight in the immersed rats to the interference by the DWI technique with renal physiology and fluid–volume homeostasis. However, owing to the lack of measurements of fluid and sodium intakes, an exhaustive interpretation of urinary data and a conclusive demonstration that the above mechanisms were indeed operative in our test rats cannot be provided.

As for the trend of systolic blood pressure, it is absolutely impossible to define the precise mechanisms governing the changes in arterial pressure maturation observed between the two groups of rats during the study. Of course these changes could have been induced by the interaction of environmental factors, namely, simulated microgravity or other simpler causes including changes in physical activity and caloric intake. The fact that the immersion-G rats after immersion exhibited a marked rise in arterial blood pressure and that body weight persisted in being inappropriate to age also deserve emphasis. This relevant observation implies that a partial dissociation between systolic blood pressure and body weight changes took place at this stage of the study and similarly that under these experimental conditions, body gain cannot be thought of as the sole determinant and predictor of the hemodynamic changes occurring during development. The mechanisms that interfered with the strong link between body weight and hemodynamic remodeling are still unknown. Nevertheless, the present investigation is in keeping with the working hypothesis that the rise of blood pressure with age may be an inevitable consequence of biological growth and maturation. The corollary to this hypothesis is that body weight per se makes an independent contribution to systolic blood pressure that is above and beyond the effect of maturation.

In conclusion, additional studies are required to gain further insight into the mechanisms involved in the persistence of body weight values inappropriate to age after cessation of DWI. Nonetheless, it is tempting to speculate that exposure of the test rats to DWI during a critical stage of their maturation might have irreversibly interacted with the factors determining the ultimate cessation of body growth, namely, the feedback mechanisms governing body weight but not cardiovascular remodeling. Our results suggest that 1) microgravity environment allows dissociation of the effects of body growth and ageing on the systolic blood pressure maturation curve, 2) the physiological rise in systolic blood pressure that takes place throughout the growth process is partly dependent on gravity, and 3) blood pressure maturation is not controlled by the same factors governing body growth.

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