Simulated microgravity impairs aldosterone secretion in rats: possible involvement of adrenomedullin

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Received 19 February 2002; accepted in final form 14 June 2002

Neri, Giuliano, Sergio Bova, Ludwik K. Malendowicz, Giuseppina Mazzocchi, and Gastone G. Nussdorfer. Simulated microgravity impairs aldosterone secretion in rats: possible involvement of adrenomedullin. Am J Physiol Regul Integr Comp Physiol 283: R832–R836, 2002.—The prolonged exposure to microgravity (MG) or simulated MG (SMG) has been reported to cause hypotension, mainly due to reduced vascular contractility, and dysregulation of fluid and electrolyte balance. However, the mechanism(s) involved in these MG- or SMG-induced effects is not yet completely elucidated. Hence, we investigated in the rat the effect of prolonged (15 day) SMG, in the form of hindlimb unweighting, on the renin-angiotensin-aldosterone system (RAAS), as well as on atrial natriuretic peptide (ANP) and adrenomedullin (ADM), two hypotensive peptides that play a major role in the regulation of RAAS activity by inhibiting adrenal aldosterone secretion. SMG caused a mild hypotension in rats, associated with the blockade of body weight gain. Plasma aldosterone concentration and basal and agonist-stimulated aldosterone secretion were decreased, and plasma renin activity was moderately increased. Neither Na⁺ and K⁺ serum concentrations nor ACTH and corticosterone blood levels were significantly affected. Plasma ANP concentration did not display significant alterations, while ADM blood concentration underwent a marked rise. The administration of the ADM-receptor antagonist ADM-(22–52) during the last 3 days of hindlimb unweighting reversed the SMG-induced hypotension and hypaldosteronism. Collectively, these findings allow us to suggest that prolonged SMG impairs RAAS activity in rats, through a mechanism probably involving upregulation of the ADM system. Both hypaldosteronism and increased ADM secretion may contribute to the development of hypotension during prolonged exposure to SMG.

MATERIALS AND METHODS

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THE PROLONGED EXPOSURE to microgravity (MG) produces in astronauts a series of adaptive changes in the cardiovascular system (e.g., impairment of the vasoconstrictor component of the cardiac baroreceptor reflex), referred to as “cardiovascular deconditioning.” On return to Earth such adaptations may cause serious consequences, including severe orthostatic hypotension with postural intolerance and risk of syncope (4). Simulated MG (SMG), in the form of prolonged hindlimb unweighting, has been found to cause hypotension in rats mainly due to reduced vascular contractility, which seems to ensue from both decreased contractile response to vasoconstrictor agents (6, 21, 26) and enhanced vasodilator mechanisms (e.g., overexpression of endothelial NO synthase and increased NO production) (25).

Marked alterations in the regulation of fluid and electrolyte homeostasis have been observed in humans during spaceflight (for review, see Refs. 7, 28). The role of aldosterone in these mechanisms is well known, but conflicting results have been obtained on the effects of MG or SMG on the renin-angiotensin-aldosterone system (RAAS). Increased Na⁺ excretion with the consequent rise in plasma aldosterone concentration (PAC) has been reported in humans and rats (16, 18). No significant alterations in PAC and plasma renin activity (PRA) were observed in cosmonauts and monkeys (5, 11). Conversely, marked rises in PAC coupled with sizeable Na⁺ retention have been more recently found (7, 18).

It therefore seemed worthwhile to reinvestigate the effects of prolonged SMG on RAAS in rats and to ascertain whether this experimental procedure is able to modify the activity of atrial natriuretic peptide (ANP) and adrenomedullin (ADM) systems, which are known not only to exert hypotensive effects but also to play a major role in the regulation of RAAS activity (for review, see Refs. 20, 24).
River (Como, Italy), were kept on a 12:12-h light-dark cycle at 22 ± 2°C, with free access to laboratory pellets and tap water. A group of rats (n = 8) was exposed for 15 days to SMG, according to Delp et al. (6). Briefly, animals were suspended through a tail-swivel harness connected by a hook at the top center of the cage, which allowed free 360° rotation. The front limbs were in contact with the floor, and the hind limbs were elevated 0.5 cm above the floor of the cage, with which the body of the rat formed an angle of 35°. Control rats (n = 8) were kept in single cages without hindlimb suspension. Two other groups of rats were treated as described above; during the last 3 days of the experiment, one half of the animals in each group (n = 4) was given daily intraperitoneal injections of 50 nmol/kg ADM-(22–52) (Sigma Chemical, St. Louis, MO) dissolved in 0.2 ml 0.9% NaCl, and the other half received only the saline vehicle. Body weight and systolic blood pressure (SBP) were recorded at 9:00 and 10:00 A.M. by tail-cuff sphygmomanometry (BP-Recorder; Basile, Commercio, Italy). The animals were decapitated at 11:00 A.M., and their trunk blood was collected with apoprotin (70 mg/ml) and EDTA (1 mg/ml) (Sigma); plasma was separated and stored at −80°C. Adrenal glands were removed and freed of pericapsular fat; the left gland was frozen and kept at −80°C for further studies, and the right one was immediately used for in vitro studies.

In vitro experiments. Adrenals were halved, and each half was cut to obtain thick slices, always containing a core of medullary tissue. Adrenal slices were placed in medium 199 (Difco, Detroit, MI) and Krebs-Ringer bicarbonate buffer with 0.2% glucose containing 5 mg/ml human serum albumin (Sigma). The samples were incubated (4–5 mg/ml) in the presence or absence of 10−8 M ACTH or ANG II (Sigma). The incubation was carried out for 60 min in a shaking bath at 37°C in an atmosphere of 95% air-5% CO2. At the end of the experiment, the incubation media were collected and kept frozen at −80°C until RIA. Protein concentration of the samples was determined by the Lowry method, and basal and agonist-stimulated steroid-hormone secretions were expressed as picomoles per milligram protein.

Electrolyte assay. The serum concentrations of Na+ and K+ were measured by a flame photometer (LKB, Stockholm, Sweden) and are expressed as milliequivalents per liter.

Aldosterone and corticosterone assays. Aldosterone and corticosterone were extracted from plasma and incubation media and purified by high-pressure liquid chromatography (17). Their concentrations were measured by RIA, using the following commercial kits: 1) ALDO-CTK2 (IRE-Sorin, Verceil, Italy): sensitivity, 5 pg/ml; cross-reactivity, 100% (aldosterone) and <0.1% (17-iso-aldosterone and other steroids); intra- and interassay coefficients of variation, 7.1 and 8.2%, respectively; 2) CORTX-RIA (Eurogenetix, Milan, Italy): sensitivity, 25 pg/ml; cross-reactivity, 100% (corticosterone and cortisol), 2% (11-deoxycorticosterone and progesterone), and <0.01% (other steroids); intra- and interassay coefficients of variation, 6.9 and 8.4%, respectively.

ACTH assay. ACTH was extracted from plasma (2), and its concentration was measured by RIA using ACTH Double Antibody kit (Diagnostic Products, Los Angeles, CA): sensitivity, 8 pg/ml; cross-reactivity, 100% [ACTH-(1–24)], 0.3% [α-melanocyte-stimulating hormone (α-MSH)], and <0.001% [other peptides]; intra- and interassay coefficients of variation, 6.3 and 8.1%, respectively.

PRA assay. PRA was measured by RIA of ANG I generated after incubation of plasma, using a kit purchased from Peninsula Laboratories (St. Helens, UK): sensitivity, 6 pg/ml; cross-reactivity, 100% (human, rat, and salmon ANG I) and 0% (ANG II and other peptides); intra- and interassay coefficients of variation, 5.9 and 7.2%, respectively.

ANP assay. ANP concentration in the plasma was measured, without previous extraction, by RIA, using a kit purchased from Peninsula (ANP-rat RIA): sensitivity, 10 pg/ml; cross-reactivity, 100% [ANP(rat), ANP(8–33), αANP(1–28), and atriopeptin III], 60% [ANP(18–28)], and <0.01% [other peptides]; intra- and interassay coefficients of variation, 7.5 and 8.6%, respectively.

ADM assay. Plasma was processed for RIA of rat ADM, as described by Shimokubo et al. (27). ADM was separated and purified by chromatography, using a Sep-Pak C18 cartridge (Waters, Milford, MA), and its concentration in the eluates was determined using a kit purchased from Phoenix Pharmaceutical (Belmont, CA): sensitivity, 65 pg/ml; cross-reactivity: 100% (rat ADM) and 0% (human ADM and other peptides); intra- and interassay coefficients of variation, 6.4 and 7.7%, respectively.

Statistics. Data are expressed as means ± SE (n = 8 or n = 4). Statistical comparison was performed by ANOVA followed by Duncan’s multiple range test.

Fig. 1. Effect of simulated microgravity (SMG) on body weight and systolic blood pressure (SBP) of adult rats. Values are means ± SE (n = 8). **P < 0.01 compared with day 0; *P < 0.01 compared with 15-day control group.

Fig. 2. Effect of SMG on plasma concentrations of aldosterone (PAC) and corticosterone (PBC). Values are means ± SE (n = 8). **P < 0.01 compared with control (C) group.
RESULTS

SMG blocked body weight gain of rats and evoked a mild but significant decrease in SBP (Fig. 1). SMG caused a 50% decrease in PAC (Fig. 2), a 40–50% reduction in both basal and 10⁻⁹ M ACTH- or ANG II-stimulated aldosterone production from adrenal slices (Fig. 3), and a 40% rise in PRA (Fig. 4).

Neither plasma corticosterone concentration (PBC) (Fig. 2) and basal and ACTH-stimulated corticosterone secretion from adrenal slices (Fig. 3) nor ACTH blood level and serum Na⁺ and K⁺ concentrations (Fig. 4) were significantly affected by SMG.

Plasma ADM concentration underwent a 90% increase in SMG-exposed animals, while the blood level of ANP did not display significant changes (Fig. 5). ADM-(22–52) administration reversed the SMG-induced decrease in both SBP and PAC, while it was ineffective in control rats (Fig. 6).

DISCUSSION

Our present results confirm that SMG obtained by hindlimb unweighting induces a mild but significant hypotension in rats. Moreover, they provide evidence that SMG affects RAAS, primarily by impairing zona glomerulosa (ZG) secretory capacity. In fact, PAC de-

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**Fig. 3.** Effect of SMG on basal ACTH (10⁻⁹ M)- and ANG II (10⁻⁹ M)-stimulated aldosterone and corticosterone secretion from adrenal quarters. Values are means ± SE (n = 8). **P < 0.01 compared with the respective baseline value; *P < 0.01 compared with respective control value.

**Fig. 4.** Effect of SMG on Na⁺ and K⁺ serum concentrations, plasma renin activity (PRA), and ACTH plasma concentration. Values are means ± SE (n = 8). *P < 0.05 compared with control group.

**Fig. 5.** Effect of SMG on plasma concentrations of atrial natriuretic peptide (ANP) and adrenomedullin (ADM). Values are means ± SE (n = 8). **P < 0.01 compared with control group.

**Fig. 6.** Effect of ADM-(22–52) on SMG-induced decrease in SBP and PAC. Values are means ± SE (n = 8). **P < 0.01 compared with respective vehicle group; *P < 0.01 compared with respective control group.
crease is coupled with lowered basal and agonist-stimulated aldosterone secretion from adrenal slices obtained from SMG-exposed rats. The resulting decrease in PAC conceivably triggers a negative-feedback mechanism leading to the rise in PRA, which in turn counteracts hypoaldosteronism. Hypoaldosteronism may surely account for the reported MG- and SMG-induced increase in Na⁺ excretion observed in humans and rats (5, 16, 18). However, in our experimental model, SMG-induced hypoaldosteronism is not able to evoke sizeable alterations in the plasma levels of Na⁺ and K⁺. It must be noted that the presently observed hypoaldosteronism is in contrast with the marked PAC rise detected in rats exposed for 24 h to SMG (16). It is conceivable that within 24 h rats have been unable to dampen the acute environmental stress evoked by the experimental condition of hindlimb suspension. Pituitary-adrenal activation is a primary effect of acute stress, and ACTH is one of the main acute stimulators of aldosterone secretion (19). Hindlimb suspension for 15 days evokes, on the contrary, a minimum chronic stress, as indicated by the lack of body weight loss and the absence of sizeable rises in the blood levels of ACTH and PBC: it is conceivable that 15 days is a time long enough for rats to get accustomed to the new experimental environment.

As mentioned in the introduction, compelling evidence indicates that ANP and ADM play a major role in the regulation of fluid and electrolyte homeostasis and aldosterone secretion. ANP is secreted by atrial myocytes, and in addition to its action on kidneys also possesses vasodilatory effects and a potent inhibitory action on RAAS (for review, see Ref. 23). The level of circulating ANP was found to be increased in cosmonauts during flight (11), and MG, although not affecting ANP immunoreactivity in cardiocytes, was apparently able to modify the distribution of ANP-containing neurons in the frog brain (9). However, our present findings appear to rule out the possibility that enhanced production of this peptide may significantly contribute to SMG-induced hypotension and hypoaldosteronism in rats.

ADM, a recently discovered peptide produced by vascular endothelium and adrenal medulla, exerts a potent and long-lasting hypotensive effect by decreasing vascular resistances and by inhibiting aldosterone secretion in vitro (for review, see Refs. 8, 12, 20, 24) and in vivo in two-kidney, one-clip hypertensive rats (14). ADM evokes vasodilation in rats, not only through the activation of adenylate cyclase (24), but also via the stimulation of the NO-cGMP pathway (10). ADM receptors have been detected in human and rat ZG cells (1, 3, 15), in which their activation by ADM leads to inhibition of voltage-gated Ca²⁺ channels (15) and increase in intra-adrenal production of NO, which in turn inhibits aldosterone secretion (22). Our RIA findings show a marked rise in the ADM blood concentration, thereby suggesting that this peptide may be involved in the SMG-induced inhibition of aldosterone secretion in rats. This contention is strongly supported by the demonstration that the administration of high doses of the selective ADM-receptor antagonist ADM-(22–52) (20) reverses the effects of SMG on both SBP and PAC. The ineffectiveness of ADM-(22–52) treatment in control rats rules out the possibility of a nonspecific (ADM receptor independent) action of this ADM fragment.

Although the mechanisms involved in the regulation of ADM expression are largely unknown, alterations in cardiovascular dynamics and hypoxia seem to play a major role (8, 12, 13). Our findings are consistent with the possibility that upregulation of the ADM system may take part in the cardiovascular adaptative changes occurring under MG and SMG conditions. On returning to the normogravitational environment, the exceedingly high activity of ADM system could lead to both hypoaldosteronism and hypotension. Our present results coupled with the availability of selective ADM-receptor antagonists could open new perspectives in the therapy of post-flight disease in astronauts.

We thank A. Coi for help in the search and delivery of bibliographic items and G. Gottardo, P. Roverato, and S. Girardi for excellent technical assistance. This work was supported by ASI (Italian Space Agency) Grant 96–01 to G. G. Nussdorfer.

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