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Adrenal function and the incidence of bends after decompression in mice: effect of adrenalectomy, corticosteroids, decompression intensity, and time of day

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Rattner, B. A., and S. P. Gruenau. 1979. Adrenal function and the incidence of bends after decompression in mice: effect of adrenalectomy, corticosteroids, decompression intensity, and time of day. Undersea Biomed. Res. 6(2): 155–166.—The adrenocortical endocrine subsystem has been demonstrated to enhance mammalian tolerance to harsh environmental conditions, including hypoxia and temperature extremes. In a series of factorial experiments, mice were exposed to one of three elevated hydrostatic pressures for 30 min and then decompressed (0.75 atm/s). It was demonstrated that 1) tolerance to decompression does not differ significantly ($P > 0.3$) in surgically intact, sham adrenalectomized, or in adrenalectomized animals; 2) intraperitoneal administration of pharmacologic doses (0.4, 1.0, and 2.0 mg/mouse) of corticosterone or deoxycorticosterone acetate does not significantly enhance ($P > 0.1$) survivorship when compared to vehicle-injected controls; and 3) the incidence of decompression sickness (DS) does not fluctuate with time of day ($P > 0.4$). In a fourth study, the plasma concentration of corticosterone was quantitated in 1) colony control mice, 2) mice exposed to the 1-ATA chamber environment (chamber control), or 3) mice compressed to 3, 5, 7, 9, or 11 ATA and then decompressed. In general, plasma corticosterone in symptom-free mice was elevated approximately threefold ($P < 0.05$) by exposure to the 1-ATA chamber environment and by decompression from 3 to 11 ATA. At 11 ATA, plasma corticosterone levels in decompressed mice exhibiting decompression sickness symptoms were significantly elevated ($P < 0.05$) compared to the levels observed in decompressed symptom-free mice. These studies indicate that adrenocortical function does not enhance tolerance to decompression in mice.

decompression sickness

corticosteroids

adrenal function

The functioning of the hypophyseal-adrenocortical subsystem has been investigated during and after exposure to a variety of environmental conditions, e.g., hypoxia, ionizing radiation, temperature extremes. Adrenalectomy frequently renders experimental animals less tolerant to such conditions, and this effect is usually reversed by the administration of adrenal cor-
ticoids (Selye 1950, 1971). Furthermore, tolerance, adrenocortical secretory responses, and plasma corticosterone concentration are related to the intensity and duration of such stimuli (Yates and Urquhart 1962; Friedman, Ader, Grota, and Larson 1967), and often exhibit a circadian rhythm (Halbert 1964; Zimmermann and Critchlow 1967).

A number of studies employing either experimental animals or humans have described adrenocortical secretory responses to compression and decompression. Several investigations have reported elevations in blood and urine levels of corticosteroids during compression and during or after decompression (Schaefer, Bond, Mazzone, Carey, and Dougherty 1968; MacInnis and Bond 1969; Bennett and Gray 1971; Bitter and Nielsen 1972; Bitter, Hootman, and Nielsen 1973; Leach, Alexander, Fischer, Lamberts, and Johnson 1973; Rostain, Fructus, Ghata, Legrand, Naquet, and Reinberg 1977). Other studies have indicated that blood cortisol is actually reduced during or after hyperbaric exposure (Chouteau 1969; Philp, Ackles, Inwood, Livingstone, Achimatos, Binns-Smith, and Radomski 1972; Martin, Gray, and Nichols 1973). The increased secretion or utilization of adrenal steroids in these studies generally has been associated with a stress reaction.

The present study investigated the function and functioning of the adrenocortical subsystem during hyperbaric exposure and decompression. The overall objectives included 1) determining if the presence or absence of the adrenal glands in mice alters the incidence of decompression sickness; 2) assessing the potential prophylactic action of corticosteroid administration (corticosterone and deoxycorticosterone acetate) on the incidence of decompression sickness; 3) examining the effect of varying the intensity of decompression on plasma corticosterone concentration; 4) establishing if the incidence of decompression-induced decompression sickness fluctuates with time of day.

MATERIALS AND METHODS

In all studies 6-week-old male mice weighing approximately 25–30 g were received and held 7–10 days before the initiation of an experimental replicate. Groups of 20 mice were maintained in clear plastic cages (26 × 46 × 16 cm) at 23 ± 1°C on a 12-h light:12-h dark photoperiod with constant red lighting, and were given access to food and water ad libitum.

Compression-decompression protocol

All pressure exposures were conducted within a Bethlehem model 1836-10 HP chamber (The Bethlehem Corp., Bethlehem, Pa.). The caged mice were placed in the chamber and compressed to 1.3 ATA with oxygen to attain a PO2 of approximately 0.5 ATA; they then were compressed with nitrogen at a rate of approximately 1.8 atm/min. The animals were maintained at the desired pressure for 30 min. Chamber atmosphere PO2 (0.5 ATA) was monitored with a Beckman model F-3 paramagnetic oxygen analyzer (Beckman Instruments, Inc., Fullerton, Calif.) and was adjusted when necessary to maintain the O2 within ±0.2% of the desired level. Carbon dioxide tension was not monitored because previous investigations in this laboratory consistently have demonstrated that Pco2 is less than 0.5% surface equivalent. During the final 60 s, the chamber was vented to elevate the PO2 to approximately 15% for surfacing. The mice were decompressed to 1 ATA at a rate of 0.75 atm/s, removed from the chamber, and observed after 30 min.
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Preliminary investigation

To determine a decompression-death dose-response curve for 7-week-old CFW outbred albino mice (Swiss-Webster descendents; Charles River Breeding Laboratories, Inc., Wilmington, Mass.), groups of 20 animals were decompressed from either 9.0, 9.7, 10.5, 11.2, or 12.0 ATA between 1300 and 1700 h, as previously described. The number of survivors was counted after 30 min. Analysis of variance and linear regression were employed to estimate the lethal dose of decompression at which 25%, 50%, and 75% of the mice succumbed (LD_{25}, LD_{50}, LD_{75}). A similar method was employed to establish such a dose-response curve for 7-week-old Swiss-Webster outbred albino mice (Taconic Farms, Inc., Germantown, N.Y.).

Experiment 1

This experiment was designed to determine if the presence or absence of the adrenal glands alters the incidence of decompression sickness. Subjects were CFW mice obtained surgically intact (control), sham adrenalectomized, or adrenalectomized, from Charles River Breeding Laboratories, Inc. The mice were housed as previously described, with the exception that adrenalectomized animals received 1.0% sodium chloride in place of tap water. Sets of 9 to 12 mice from each of the 3 treatment groups were compressed together in the chamber and then decompressed at the approximate LD_{25}, LD_{50}, and LD_{75} between 1300 and 1700 h. The number of mice surviving and the number of survivors without decompression sickness symptoms were noted after 30 min. (Decompression sickness was characterized by abnormal gait, paralysis, convulsions, and tumbling.) This experimental protocol was replicated on three consecutive days (blocks). This study was conducted and analyzed as a 3 x 3 factorial experiment (3 treatments x 3 decompression doses) with randomized complete blocks.

Experiment 2

This study was conducted to determine if predive corticosterone (B) or deoxycorticosterone acetate (DCA) steroid treatment alters the incidence of decompression-induced death. Swiss-Webster mice, 7 weeks old, were injected intraperitoneally with either 0.1 ml peanut oil (PO) (control group), or 0.4, 1.0, or 2.0 mg of corticosterone in 0.1 ml PO. Sets of 7 mice from each of these 4 treatment groups were compressed together in the chamber 30 min postinjection and then decompressed at the approximate LD_{25}, LD_{50}, and LD_{75} between 1300 and 1700 h. The number of mice surviving after 30 min was determined. This study was conducted and analyzed as a 4 x 3 factorial experiment (4 doses of B x 3 decompression doses) with randomized complete blocks, which was replicated on 3 consecutive days.

The same numbers of mice, steroid doses, decompression doses, and statistical analyses were used to assess the effect of DCA on the incidence of decompression-induced death.

Experiment 3

The objective of this experiment was to determine if the concentration of plasma corticosterone is affected by the magnitude of decompression. Between 1300 and 1700 h, sets of mice (4–16) were removed from the animal colony and immediately killed (1-ATA colony control), placed in the chamber for 30 min (1-ATA chamber control), or placed in the chamber and compressed to either 3, 5, 7, 9, or 11 ATA for 30 min, and then decompressed. Thirty minutes after chamber or hyperbaric exposure, four symptom-free mice were selected at random and killed by decapitation. Blood was collected through funnels into heparinized tubes and cen-
trifuged, and the plasma obtained was frozen at \(-10^\circ\text{C}\). Plasma was also collected from surviving mice that exhibited decompression sickness symptoms in the 11-ATA group. This study was conducted and analyzed as a \(7 \times 3\) randomized complete block experiment, which was replicated on 3 consecutive days.

**Experiment 4**

This investigation evaluated the effect of time of day on the number of survivors and symptom-free animals after decompression. A single cage of Swiss-Webster mice (17–18) were removed from the animal colony and placed into the compression chamber in an adjacent room. The lighting in the hallway, chamber room, and chamber was in phase with the lighting schedule of the animal colony. Three groups of mice were compressed and decompressed at the approximate \(LD_{25}\), \(LD_{50}\), and \(LD_{75}\) during a 24-h period between 0300 and 0500, 0700 and 0900, 1100 and 1300, 1500 and 1700, 1900 and 2100, and 2300 and 0100 h. The number of mice surviving and the number of animals with decompression sickness symptoms were determined 30 min after each decompression. This study was conducted and analyzed as a \(6 \times 3\) factorial experiment (6 times of day \( \times 3\) decompression doses) with randomized complete blocks. It was replicated on three occasions, differing only in the initiation time of the replicate, i.e., 0300, 0700, 1500 h.

Two mice from each cage were decapitated immediately before the cage was removed from the animal colony and placed in the compression chamber. The blood was collected and processed as described in Experiment 3. This procedure was conducted to establish that the mice exhibited a circadian corticosterone rhythm.

**Corticosterone radioimmunoassay**

The reagents and protocol available from Radioassay Systems Laboratories, Inc. (Carson, Calif.) were used for the quantitation of plasma corticosterone by radioimmunoassay (RIA). In this assay, 2 \(\mu\)l of unextracted plasma sample in a total volume of 0.5 ml assay buffer is heat denatured. Tritiated corticosterone and corticosterone antiserum are incubated overnight at 4\(^\circ\text{C}\) in the assay tubes. The free and bound corticosterone is separated with charcoal-coated dextran, and the bound steroid is quantitated by scintillation spectrometry. The only modification in the protocol of Radioassay Systems, Inc. was the inclusion of 2 \(\mu\)l of plasma from adrenalectomized mice in the 100% binding, residual, and standard curve tubes to correct for substantial additional nonspecific binding of mouse plasma.

When known quantities of corticosterone (25–1000 pg) were added to diluted plasma from adrenalectomized mice, the quantity of corticosterone estimated by the procedure yielded a regression line with a slope of 0.95 pg estimated/pg added, with a correlation coefficient of 0.96. The slope of this line was not significantly different from 1 \((P > 0.1)\), which indicated that the assay was accurate. The specificity of the antiserum is such that it cross-reacts equally with cortisol; however, this steroid is not present in mouse plasma (Spackman and Riley 1978). Serial dilutions of mouse plasma with high corticosterone content were assayed and yielded a regression line parallel to the standard curve \((P > 0.25, \text{analysis of covariance})\), which further substantiated the specificity of the antiserum. The limit of sensitivity for this RIA was approximately 12.5 pg/assay tube. All samples from any single experiment were analyzed within the same assay run with a precision (coefficient of variation) of about 4.0%. The interassay precision was approximately 7.4%.
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Statistics

The number of mice surviving and the number of mice without visible decompression sickness symptoms (binomial data) in each experimental replicate were converted to percent, arc-sin transformed (Snedecor and Cochran 1967), and compared by two-way analysis of variance. Treatment means were compared by Duncan’s New Multiples Range Test. The RIA data were processed using natural logarithm and logit transformations that employed a weighted linear regression model.

RESULTS

The percent of mice surviving 30 min after rapid nonstop decompression to 1 ATA was inversely related to the initial hydrostatic pressure (Fig. 1). From this relationship the LD_{50}, LD_{50}, and LD_{50} for 7-week-old CFW mice were estimated to be 9.1, 10.3, and 11.5 ATA; for 7-week-old Swiss-Webster mice the respective lethal doses of decompression were estimated to be 10.5, 11.2, and 12 ATA. In Experiments 1, 2, and 4, analysis of variance indicated a significant effect of decompression dose (P < 0.001) such that the percent of mice surviving or symptom-free differed significantly (P < 0.05) for at least one of the decompression doses (see, for example, Fig. 3). No interaction of treatment and decompression dose was detected in Experiments 1, 2, and 4.

In Experiment 1, the percent of mice surviving decompression pooled across depth (see Fig. 2), and the percent of mice without visible bends symptoms in the control group was 42.4% and 35.4%, respectively. These same variables in the sham adrenalectomy group were 47.5% and 42.4% and in the adrenalectomy group were 52.7% and 35.5%. Neither sham adrenalectomy or adrenalectomy significantly altered survivorship or decompression sickness incidence (P > 0.3).

Fig. 1. Decompression-death dose-response curve for 7-week-old male CFW mice (n = 20 mice).
Intraperitoneal administration of corticosterone (Experiment 2) increased the number of mice surviving decompression by 13–15% (Fig. 4); however, this effect was somewhat variable and was not significant by ANOVA ($P > 0.1$). The administration of deoxycorticosterone acetate increased survivorship by as much as 10% (Fig. 5), but the overall treatment effect was not significant ($P > 0.25$). No differential effect of steroid dose was apparent for either corticosterone or deoxycorticosterone acetate.

Fig. 2. Percent of sham adrenalectomized (adrenex) or adrenalectomized mice (Experiment 1) surviving 30 min after decompression from either the $L_{D_{30}}$, $L_{D_{50}}$, or $L_{D_{90}}$ decompression dose. No significant treatment effect was detected by ANOVA ($P > 0.3; n = 9$ dives); SEM for each treatment ranged from 16.47 to 16.65%.

Fig. 3. Percent of mice surviving after various doses of decompression pooled across treatment (Experiment 1). Bars with different letter superscripts are significantly different ($P < 0.05; n = 3$ dives/decompression dose); SEM for each depth ranged from 21.44 to 28.66%.
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Fig. 4. Percent of mice surviving after decompression that were injected 30 min before compression with either peanut oil vehicle (PO) or corticosterone (B) at doses of 0.4, 1.0, or 2.0 mg/mouse. No significant treatment effect was detected by ANOVA ($P > 0.25$; $n = 9$ dives); SEM for each treatment ranged from 15.52 to 16.61%.

Experiment 3 demonstrated that plasma corticosterone is significantly elevated ($P < 0.05$) by merely the physical manipulations involved in hyperbaric exposure in the absence of pressure and decompression (Fig. 6; compare 1-ATA Colony Control to 1-ATA Chamber Control data). Decompression from 3, 5, 9, and 11 ATA did not significantly alter plasma corticosterone concentration in symptom-free mice compared to the level observed in the 1-ATA chamber control group. Decompression from 7 ATA significantly increased ($P < 0.05$)

Fig. 5. Percent of mice surviving after decompression that were injected 30 min before compression with either peanut oil vehicle (PO) or deoxycorticosterone acetate (DCA) at doses of 0.4, 1.0, or 2.0 mg/mouse. No significant treatment effect was detected by ANOVA ($P > 0.1$; $n = 9$ dives); SEM for each treatment ranged from 15.71 to 16.61%.
plasma corticosterone compared to that for all groups. Regression analysis of variance did not reveal ($P > 0.1$) any relationship between decompression dose and plasma corticosterone concentration. In the 11-ATA group, plasma corticosterone was significantly elevated ($P < 0.05$) in mice with visible decompression sickness symptoms compared to animals decompressed from 11 ATA that were symptom-free (Fig. 7).

The percentage of mice surviving or symptom-free did not differ significantly ($P > 0.4$) with biologic time of day in Experiment 4 (Fig. 8). However, plasma corticosterone concentration from colony mice exhibited a typical circadian pattern, with nocturnal levels dramatically elevated.
DISCUSSION

Adrenalectomy has been demonstrated to decrease resistance to a variety of systemic stressors, yet in the present investigation adrenalectomy did not alter tolerance of mice to decompression. However, extravascular bubbles have been observed in the cortex of decompressed guinea pigs (Gersh and Hawkinson 1944), and such bubbles might impair adrenal function, e.g., hypophyseal adrenocorticotropic hormone delivery to the adrenal cortex, steroid synthesis and secretion, or delivery of corticoids to target tissue, in surgically intact or in sham adrenalectomized animals. To overcome any possible impairment of adrenocortical function due to extravascular bubbles and to assess the ameliorative properties of adrenal corticoids on the incidence of decompression sickness, pharmacologic doses of corticosterone or deoxycorticosterone acetate were administered before compression. These steroid treatments did not significantly reduce decompression sickness incidence, which indicates that the adrenal gland and its cortical secretions do not alter acute tolerance to decompression in mice. It is possible that the anti-inflammatory activity of some adrenal steroids, which may be released after decompression, could serve to reduce decompression sickness symptoms over a longer period of time.

Plasma corticosterone concentration increased significantly by chamber exposure in the absence of pressure and provides further evidence that handling, noise, and novel environments evoke adrenocortical secretory responses in rodents (Barrett and Stockman 1963; Spackman and Riley 1978). Bitter and Nielsen (1972) have reported an increase in urinary corticosteroid excretion after a gaseous shift from 1 ATA N₂-O₂ to 1 ATA He-O₂. This finding may be related in part to the noise generated during the helium-oxygen chamber-flushing procedure, since their observations have not been completely substantiated by other studies (Wang and Peter 1977). Unfortunately, Bitter and Nielsen (1972) did not report any comparison between urinary corticosteroid excretion in rats maintained in the colony and then shifted to the chamber.
Varying the decompression dose from 3 to 11 ATA did not elevate corticosterone incrementally when compared to the 1-ATA chamber control group. This would suggest that the plasma concentration of corticosterone in mice is not affected by decompression in a dose-dependent manner. In contrast to these findings, an anticipatory predive rise in plasma cortisol has been reported in man, and increased plasma levels of this corticosteroid have been observed during decompression (Leach et al. 1973). The comparison of the mean plasma corticosterone profile from 11-ATA decompressed symptom-free mice to the concentration observed in animals with decompression symptoms revealed a marked increase and is similar to the decompression sickness-induced rise in urinary 17-hydroxycorticosteroids observed in man (Bennett and Gray 1971).

Diurnal variation in decompression sickness susceptibility has been reported during altitude decompression (Gray 1943; Henry, Jones, Mohney, and Tobias 1943) and hyperbaric decompression (Griffiths 1960; Vann, Grimstad, Nielsen, and Carey 1978). In the present investigation (Experiment 4), it was demonstrated that the incidence of decompression sickness in decompressed mice, which exhibited a predive circadian plasma corticosterone rhythm, did not differ with the time of day. Furthermore, it would appear that several physiologic variables of this nocturnal species that exhibit a biological rhythm, e.g., hormone concentration, body temperature, metabolic rate, etc., do not differentially influence the incidence of decompression sickness in a temporal manner.

In preliminary studies, and subsequently in Experiments 1, 2, and 4, a significant effect of decompression dose on the incidence of decompression sickness was observed. This result supports the hypothesis of a linear depth-dependent relationship between allowable pressure change and the initial nitrogen-oxygen exposure pressure (Berghage, Armstrong, and Conda 1975). It is interesting to note that this linear dose-dependent relationship for two closely related strains of mice (CFW mice were derived from the Swiss-Webster stock) of similar age and weight exhibited somewhat different thresholds for decompression sickness, e.g., L.D.₅₆ of approximately 10.3 ATA versus 11.2 ATA.

Rattner, B. A. and S. P. Gruenau. 1979. Fonction surrenale et incidence de maladies de décompression chez la souris: effets de la surrenalectomie, l’administration de corticostereoides, la vitesse de compression, et l’heure du jour. Undersea Biomed. Res. 6(2): 155–166.—Il est bien connu que le système corticosurrénalien augmente la tolérance pour les conditions environnementales peu favorables, telles que l’hypoxie et les températures extrêmes, chez le mammifère. Dans une série d’expériences factorielles, nous avons exposés trois groupes de souris à trois pressions hydrostatiques élevées pendant 30 min, et puis décomprimés rapidement (0,75 atm/seconde). Ces expériences ont démontré que: 1) Des différences significatives en ce qui concerne la tolérance pour la décompression n’existent pas entre les animaux intacts et ceux qui ont subi une surrenalectomie feinte ou réelle (P > 0,3); 2) l’administration intrapéritonéale en dose pharmacologiques (0,4, 1,0, et 2,0 mg/souris) de la corticostérome ou de l’acétyl de désoxytocortone n’exerce pas d’effet significatif (P > 0,1) sur la survie des animaux traités comparée à celle des témoins injectés de véhicule seulement; et 3) l’incidence de maladie de décompression est sans
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rapport avec l'heure. Dans une quatrième étude nous avons déterminé la concentration plasmatique de la corticostéroïde chez les souris 1) de la colonie du laboratoire (témoins de colonie); 2) souris exposées à la chambre à 1 ATA (témoins de chambre); ou 3) souris comprimées à 3, 5, 7, 9, ou 11 ATA et ensuite décomprimées. En général, la corticostérona plasmatique chez les souris sans symptômes de la maladie de décompression a atteint trois fois sa valeur normale après exposition à la chambre à 1 ATA et après décompression ($P < 0.05$). A 11 ATA, la corticostérona plasmatique des souris symptomatiques était significativement plus élevée ($P < 0.05$) que celle des souris asymptomatiques. Ces résultats montrent que la fonction corticosurrénalienne n'augmente pas la tolérance pour la décompression chez la souris.

maladie de décompression
corticostéroïdes
fonction surrénalienne

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