Cardiovascular Responses of Snakes to Hypergravity

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ABSTRACT

Snakes have provided useful vertebrate models for understanding circulatory adaptation to gravity, attributable to their elongate body shape and evolutionary diversification in terms of ecology and behavior. Recently we have studied cardiovascular responses of snakes to hypergravic acceleration forces produced acutely in the head-to-tail direction (+Gz) on a short-arm centrifuge. Snakes were held in a nearly straight position within a horizontal plastic tube and subjected to a linear force gradient during acceleration. Carotid blood flow provided an integrated measure of cardiovascular performance. Thus, cardiovascular tolerance of snakes to stepwise increments of Gz was measured as the caudal Gz force at which carotid blood flow ceased. Tolerance to increasing Gz varies according to adaptive evolutionary history inferred from the ecology and behavior of species. With respect to data for six species we investigated, multiple regression analysis demonstrates that Gz tolerance correlates with gravitational habitat, independently of body length. Relative to aquatic and non-climbing species, carotid blood flow is better maintained in arboreal or scansorial species, which tolerate hypergravic forces of +2 to +3.5 Gz. Additionally, semi-arboreal rat snakes (Elaphe obsoleta) exhibit plasticity of responses to long-term, intermittent +1.5 Gz stress. Compared to non-acclimated controls, acclimated snakes show greater increases of heart rate during head-up tilt or acceleration, greater sensitivity of arterial pressure to circulating catecholamines, higher blood levels of prostaglandin ratios favorable to maintenance of arterial blood pressure, and medial hypertrophy in major arteries and veins. As in other vertebrates, Gz tolerance of snakes is enhanced by acclimation, high arterial pressure, comparatively large blood volume, and body movements. Vascular studies of snakes suggest the importance to acclimation of local responses involving vascular tissue, in addition to centrally mediated responses to fluid shifts.

INTRODUCTION

Adaptation to gravitational force is a fundamental requirement for life on Earth and is especially important with respect to structuring of musculoskeletal systems and the functional stability of body fluids. Blood circulation, in particular, requires mechanisms for regulation with respect to body size, posture and behavioral activities. Physiological responses to high speed aircraft and to space travel illustrate how organisms become susceptible to shifts or instability of integrated functions when exposed to abnormal acceleration or microgravity (Burton and Smith 1996; Wattenpaugh and Hargens 1996). It is also clear that such organismal responses include facultative adjustments or "acclimation" to prolonged or repeated gravitational disturbance. Presumably, such processes of adjustment are adaptive responses, even though the environment is novel and the transition of G force is abrupt when considered on an evolutionary time scale.

There is considerable interest in evaluating human cardiovascular responses as well as countermeasures to dynamic extremes of gravitational challenge, especially in contexts of aerospace physiology. Investigation of both genetic and physiological plasticity of cardiovascular performance is important for understanding biological adaptation to dynamic gravity environments in general, as well as specific problems related to human health and space exploration. While human investigations have inherent limitations both in terms of scope and design, studies of animals that are naturally subjected to variation of gravitational stress can provide important insights concerning aspects of gravitational adaptation and therefore the design and effectiveness of strategic countermeasures. Yet there are few comparative investigations concerning the cardiovascular performance of vertebrates in contexts of gravitational adaptation (Lillywhite 1995). Interest therefore arises in the circulatory system of vertebrates such as snakes, which because of their elongate shape and ecological diversification, are particularly sensitive to gravitational disturbance (Lillywhite, 1987; 1988). The present paper reviews our recent findings with respect to the cardiovascular tolerance and adjustments of snakes to hypergravity.

SNAKES AND GRAVITY

Gravity disturbs the hydrodynamics of blood circulation when tall or long-bodied animals such as snakes assume upright or vertical postures. Under such circumstances gradients of hydrostatic (=gravitational) pressures promote tendencies for blood to accumulate in dependent tissues, attributable to vascular compliance and pooling (especially in veins) and to excess filtration of plasma (edema). This potentially jeopardizes adequate venous filling of the heart such that arterial blood flows and pressures are seriously reduced in the upper body. Maintenance of cephalic blood flow requires potent cardiovascular adjustments to counteract such effects of gravity and to maintain critical pressures in the anterior arteries. Snakes are variably susceptible to such
hemodynamic problems depending on their size, environment, evolutionary history, and behavioral activities.

Upright snakes in terrestrial environments (as in climbing) experience significant gradients of intravascular pressures which must be counteracted to maintain circulatory competence. In aquatic environments, such gradients are counteracted by similar forces in the external water column such that transmural pressures do not change with body position. Thus, because gradients of gravitational pressure within blood vessels are roughly counterbalanced by parallel gradients in the surrounding liquid environment, the distribution of blood volume is not affected by changes of body position or posture. Consequently, strong cardiovascular reflexes that increase peripheral vascular tone as a means of counteracting gravitational disturbance of the circulation (similarly to orthostatic responses of mammals) are not required for cardiovascular performance during posture change in water. Indeed, when fully aquatic snakes are removed from water and tilted head-up in air, arterial pressures diminish at the body center and may fall to zero at head level, reflecting absence of postural adaptations to gravity (Seymour and Lillywhite, 1976; Lillywhite and Pough, 1983).

Systemic arterial pressures vary considerably among species of snakes representing a broad range of habitat and behavioral differences. This observation was first reported by Seymour and Lillywhite (1976) who emphasized the correlation between levels of arterial pressure and the gravitational demands of a species’ habitat. Thus, arterial pressures in arboreal or climbing (=scansorial) species (40-70 mm Hg) are two to three times greater than those of aquatic or non-climbing species (20-30 mm Hg) (Seymour and Lillywhite, 1976; Seymour, 1987). Such differences in levels of arterial pressure are thought to reflect generally greater levels of peripheral resistance in the scansorial species, which regulate arterial pressure effectively and maintain cephalic blood flow during upright posture outside of water (Seymour and Lillywhite, 1976; Lillywhite and Seymour, 1978; Lillywhite and Gallagher, 1985; Lillywhite, 1987; Lillywhite and Donald, 1988; 1994). In contrast, aquatic snakes and strictly ground-dwelling species exhibit far less effective hemodynamic control when subjected to similar conditions (Seymour and Lillywhite, 1976; Lillywhite and Pough, 1983; Lillywhite, 1993a).

Neurally mediated regulation of vascular resistance and capacity is of primary importance to homeostatic responses to gravitational stress. Both systemic and pulmonary arterial pressures of snakes are regulated by neural reflexes, with scansorial species demonstrating superior capability for regulating hemodynamics during posture change (Lillywhite and Donald, 1994). Baroreceptors have been identified in the truncus arteriosus and central arteries, and other mechanoreceptive sites also seem likely. Efferent mechanisms affecting vascular tone are correlated with dense adrenergic innervation of vessels that varies interspecifically as well as regionally within a species (Donald and Lillywhite, 1988; Lillywhite and Donald, 1994; Conklin et al., 1996). Neuropeptides as well as catecholamines are extensively distributed and colocalized in perivascular nerves innervating arteries and veins of snakes. These observations combined with physiological data indicate there is functional specialization within components of the peripheral autonomic system controlling the circulation, especially with regard to regulation of venous capacity (Davies and Donald, 1992; Lillywhite and Donald, 1994). The evolution of dense but variable adrenergic and peptidergic innervation of the heart and vasculature of snakes emphasizes the importance of autonomic reflexes in mediating control of hemodynamics.

Various traits linked to blood pressure and its regulation must co-evolve with the genetically determined level of arterial pressure in response to selection forces that are induced by gravitational stress. Thus, heart mass scales to the 0.95 power in snakes, but only 0.77 to 0.91 in other reptiles which are not as subject to gravitational stress (Seymour 1987). Scansorial species of snakes have heart positions that are closer to the head than are hearts of non-climbing species (Seymour and Lillywhite, 1976; Seymour, 1987). While scansorial species are characterized by comparatively higher levels of arterial pressure, in addition the shorter cervical blood column between the heart and head reduces the requirement for a "safety factor" in blood pressure required to maintain cerebral perfusion at all body angles.

Other aspects of morphology tending to increase resistance to gravity stress include reduced length of the pulmonary vasculature (hence functional lung segment), short body length, small body girth, proportionally long tail, tight skin and comparatively low compliance of the subcutaneous tissue space (Lillywhite, 1987; Lillywhite, 1993b. These features confer hemodynamic advantages, and gravitational factors might well have contributed to multifaceted forces giving rise to particular body forms (Lillywhite and Henderson, 1993). Substantial evidence suggests a morphological "antigravity suit" is an important adaptive countermeasure to gravitational stresses in snakes. Hence the inability of the non-climbing species to sustain carotid blood flow during upright posture is correlated with looser skin, compliant interstitium, tendencies to blood pooling, and comparatively ineffective vasomotor control of the peripheral vasculature (Lillywhite, 1985a; Lillywhite, 1993b; Lillywhite and Pough, 1983).

The foregoing discussion illustrates how snakes have provided diverse and useful data for elucidating evolutionary adaptation to gravity stresses imposed by natural Earth (+1G) environments. In view of the evolutionary diversification of adaptive responses to gravity demonstrated in this group of vertebrates, it is of interest to examine how cardiovascular function responds to conditions of exaggerated gravity stress.
ACUTE RESPONSES OF SNAKES TO HYPERGRAVIC STRESS

We have studied the cardiovascular and behavioral responses of snakes to hypergravic stresses induced by graded acceleration on a short-arm centrifuge at the NASA Ames Research Center (Lillywhite et al., 1996a). Measurements and observations were conducted with fully conscious snakes surgically fitted with indwelling catheters and blood flow cuffs and restrained within loose-fitting plastic tubes mounted parallel to a radial arm of the centrifuge, with the head of the snake facing the axis of rotation. Snakes were observed during stepwise increments of +0.25 G_z force acting in the head-to-tail direction and measured at the posterior end of the animal. Use of the centrifuge allowed precise gradation of acceleration forces ranging from zero to multiples of normal Earth gravity. At the onset of acceleration, and at each subsequent 0.25 G increase of G_z level, the centrifugal force increased at a rate of approximately 0.02 G/s. In routine experiments, the level of G force was maintained for 10 min before increasing to the next level. Blood velocity and pressure signals were recorded via slip rings on a recorder interfaced with a directional pulsed Doppler flowmeter, a pressure monitor and a computer.

One of the primary goals of our research was to compare species tolerances to gravitational force in terms of overall cardiovascular function and performance. Clearly, the stability of carotid arterial blood flow, which is the principal or exclusive source of cerebral perfusion in snakes, represents an integrated index of cardiovascular performance in response to G_z vectors of gravitational stress. Hence, tolerance to hypergravity was defined as the limiting G_z at which carotid blood flow ceased (in practice, for more than 50% of the exposure time at any given G level).

For descriptive purposes, G_z force was measured at the tail of the animal according to the following rationale. The force gradient associated with graded centrifugal acceleration amplifies hydrostatic pressure gradients and exaggerates stresses that promote blood pooling in dependent (posterior) vasculature. The G_z is the principal vector component of the total G force acting on posterior vasculature, whereas the vertical force of Earth’s gravity contributes increasingly to the total force at anterior locations such as the heart or head (Lillywhite et al., 1996a). Theoretically, anterior G forces are directly relevant to cardiac filling and the functioning of baroreceptors (which are located near the heart; see Lillywhite and Donald, 1994), whereas more posterior forces promote dependent blood pooling and thereby diminish the return of venous blood to the heart. The posterior G force clearly has the major influence because the anterior force is closer to the center of rotation and has a smaller vector component acting parallel to the length of the snake. Only the tolerated posterior force was shown to correlate well with the gravitational adaptedness of species.

As expected from tilt studies, carotid blood flow responses to posterior G_z force varied among species of snakes according to specific categorical levels of behavior and ecology (Lillywhite et al., 1996a). Multiple regression analysis showed that gravitational habitat had a significant effect on the G_z tolerance, independent of body length. At the extremes, carotid blood flow decreased in response to increasing G force and approached zero near +1G_z in the aquatic and non-climbing species tested, whereas in scansional species carotid flow was maintained (or even increased) at forces in excess of +2G_z (Fig. 1). The regression model indicated that G_z tolerance = 0.7602 + 0.4604 · habitat rank (where 1 = aquatic; 2 = amphibious; 3 = terrestrial; and 4 = scansional with strong climbing tendencies). Thus, the pattern of G_z tolerances of snakes representing a range of gravitational habitats suggest that cardiovascular adaptation to G_z force is gradational with a spectrum of response characterizing species that are ranked from aquatic to terrestrial and arboreal habits. These and other data appear to reflect heritable characteristics of the cardiovascular system related to sensitivity of these vertebrates to gravity (Lillywhite, 1988). Similarly, studies of birds and mammals suggest there are adaptive patterns of G_z tolerance related to bipedal versus quadrupedal posture (Pertzoff and Britton, 1948; Smith et al. 1979).

The cardiovascular responses of snakes to increasing acceleration force parallel those which are observed during graded, head-up tilt (cf. Lillywhite and Seymour, 1978; Lillywhite, 1987). Heart rates increase linearly during centrifugal acceleration and reach a maximum value at or near the maximum G level that is tolerated. Pulse flow in the carotid artery or dorsal aorta generally decreases in response to increasing G level except during movements of snakes which tend to increase blood flow at lower G levels and create intermittent blood flow at higher G levels when flow becomes null during inactivity (Fig. 2). The reciprocal relation between pulse flow and heart rate is reflected in the tendency for carotid arterial blood flow to be regulated during much of the acceleration protocol. Maximal heart rates measured during acceleration are similar in scansional and in non-scansional species (71.9 ± 11.7 vs. 71.7 ± 15.2 bpm), suggesting that differences in tolerance are strongly dependent on factors related to vascular compliance and peripheral vascular tone (cf. Lillywhite and Seymour, 1978; Lillywhite and Pough, 1983; Lillywhite and Gallagher, 1985).

Crawling behaviors involving undulatory movements transiently increase carotid blood flow and produce momentary positive flow during periods of high-G stress when flow becomes intermittently null if the snake is not active. The importance of such behavioral responses to hemodynamics is also observed in snakes that are stressed by upright posture (Lillywhite, 1985b). Thus, activity of skeletal muscle appears crucially important as a
countermeasure to gravitational impairment of venous cardiac return, similarly to the circumstance in humans (Rowell, 1993).

Tolerant (arboreal) species are able to withstand hypergravic forces of 2–3 times that of Earth g for periods up to 1 h without cessation of carotid blood flow or loss of normal behaviors such as body or head movements and tongue flicking. At present, such data for snakes are not strictly comparable with data from studies of other vertebrates. Some humans in a sitting position can tolerate exposure to +3 Gz for periods up to 1 h, but human subjects typically are exposed to lesser gradients of force produced on larger centrifuges than the one used with snakes (Miller et al. 1959). Tolerance of humans to levels of acceleration force $\geq +3$ Gz are typically measured in minutes or seconds (Burton and Smith 1996), and loss of consciousness will usually occur in subjects if there is a critical reduction of cerebral blood flow for only 5 s (Burton 1959). Snakes probably tolerate reduced cerebral blood flow more effectively than mammals due to a relatively greater tolerance for cerebral hypoxia (Belkin, 1963; Hochachka, 1986).

Finally, the importance of morphological adaptation has been demonstrated by data from an amphibious species of snake (Nerodia rhombifera) that was restrained within a tight-fitting tube (Lillywhite et al., 1996a). Prevention of lower body expansion by the tight tube increased the $G_z$ tolerance level by a full G unit, which is comparable to that of a normal semiarboreal snake that possesses a natural anatomical "antigravity suit". Such data emphasize the importance of morphological adaptation contributing to antigravity mechanisms (see also Hargens et al., 1987; 1988).

**RESPONSES OF SNAKES TO CHRONIC, INTERMITTENT HYPERGRAVITY**

The plasticity of cardiovascular responses to hypergravity was further studied in semi-arboreal rat snakes (Elaphe obsoleta) examined for changes of structure and function in response to long-term, intermittent $+1.5$ Gz produced by acceleration on a short-arm centrifuge (Lillywhite et al., 1996b). Routinely, snakes were centrifuged for 1 h daily, except for weekends and
occasional periods required for food digestion or skin shedding. The mean number of centrifuge-days was 47 ± 5, achieved over a period that varied from 67 to 89 days in different animals. As in the acute studies, centrifugal acceleration subjected snakes to a linear force gradient with the maximal force being experienced at the tail. We tested the hypothesis that periodic exposure of snakes to the hypergravic force would result in "acclimation" of the cardiovascular response.
Compared with non-centrifuged controls, \( G_z \), "acclimated" snakes showed trends for larger mean magnitudes of \( G_z \) tolerance, greater maximal heart rates, greater increases in heart rate accompanied by a lesser drop of arterial pressure during head-up tilt or acceleration, greater pressor responses and greater threshold sensitivity to exogenously administered catecholamines. These trends of data are in expected directions assuming acclimation has altered functions to improve performance during \( G_z \) stress. When the \( G_z \) tolerances of snakes were adjusted for effects of body size and related variables, the difference between the mean values of control and acclimated snakes (2.37 and 2.84 \( G_z \), respectively) corresponded closely to the 0.5 \( G \) difference between the acclimation \( G \) (1.5) and Earth gravity (1.0). Thus, as in mammals (Burton and Smith, 1996), repeated exposure of snakes to increased acceleration stress appears to enhance the \( G_z \) tolerance. Regression analysis indicated that \( G_z \) tolerance of rat snakes correlates with shorter body length, higher arterial pressures, comparatively higher blood volume, and comparatively lower maximal heart rate, in addition to acclimation. These characters tend to be associated with high \( G \) tolerance in vertebrates generally (Burton and Smith, 1996).

As in all snakes subjected to graded acceleration on the centrifuge, heart rates varied inversely with stroke flow suggesting that increasing cardiac rate compensates for decreasing stroke volume incurred by increasing \( G \) force. This relationship, and the plateau of heart rate at or near the maximal \( G_z \) tolerated, indicates the importance of cardiac rate to blood flow during \( G_z \) stress on the circulation. In the acclimated snakes, however, higher \( G_z \) tolerance might be associated with a lower maximal heart rate because of compensating factors of blood volume and arterial pressure. The present data do not allow an evaluation of the relative contribution of peripheral resistance to maintenance of arterial pressure and blood flow. However, modulation of peripheral resistance generally contributes more importantly to arterial blood pressure regulation related to orthostatic tolerance than does heart rate in a variety of vertebrates, including snakes (Lillywhit and Seymour, 1978; Lillywhite and Gallagher, 1985; Nolan and Bentley, 1978; Rowell, 1993).

With respect to vascular resistance, preliminary assessment of vascular morphology indicates there is significant hypertrophy of medial smooth muscle in both the arteries and veins of acclimated snakes, whereas capillary structure appears not to differ from that of control animals (unpublished data). It is noteworthy that \( G_z \)-acclimated snakes exhibited blood plasma levels of prostaglandin \( F_{2\alpha} \) (PGF\(_{2\alpha}\)) three times greater than that in controls, while plasma levels of prostaglandin \( E_{2} \) (PGE\(_{2}\)) were one-half that of controls (Lillywhite et al., 1996b). The higher ratios of PGF to PGE in acclimated snakes appear significant in that PGF\(_{2\alpha}\) facilitates hormonally and neurally induced vasoconstriction, whereas PGE\(_{2}\) has the opposite effect (MCGiff et al., 1976). The dramatic differences in blood levels of prostaglandins between acclimated and controls snakes almost certainly reflects differences in mechanisms that might contribute importantly to regulation of blood pressure and blood flow. PGF\(_{2\alpha}\) is synthesized in the vascular wall and might be a principal modulator affecting actions of vasoactive peptides and pressor responses mediated by the autonomic nervous system. Prostaglandins of the E series inhibit not only neurogenic vasoconstriction, but also that due to angiotensin II and to circulating catecholamines (MCGiff, 1981). Thus, in acclimated snakes deficient formation of a vasodilator substance such as PGE\(_{2}\) in response to periodically increased vascular wall tension might render vasculature increasingly sensitive to the action of pressor hormones or neurotransmitters, thereby possibly explaining the lower threshold sensitivity and greater pressor responses seen in acclimated compared with control snakes.

The elevated levels of PGF\(_{2\alpha}\) (as well as its stable metabolite, 13, 14-dihydro-15-keto PGF\(_{2\alpha}\)) were observed in acclimated snakes several days following the conclusion of centrifugation, suggesting either greatly reduced rates of PGF metabolism relative to mammals, or persistence of an acclimation effect that augments prostaglandin release mechanisms in renal or in vascular tissues. In vitro studies of blood vessels suggest that hormones as well as local factors mediate vascular responses in snakes, but further studies are required to assess their importance in gravitational contexts (Conklin et al., 1996).

It is conceivable that data for prostaglandins are related to levels of cortisol and corticosterone which are increased in acclimated compared with control snakes (Lillywhite et al., 1996b). Mineralocorticoids can affect prostaglandin synthesis which, in turn, can affect vascular permeability as well as the effects discussed above (McGiff, 1981). Higher levels of corticosterone in acclimated snakes also suggests that physiological stress is induced by the chronic, intermittent exposure to hypergravic force (Guillote et al., 1995).

Snakes subjected to intermittent 1.5 \( G_z \) acceleration exhibit reduced blood plasma levels of inorganic phosphate and calcium (Lillywhite et al., 1996b). Reductions of total plasma calcium are particularly dramatic, averaging about 50%, and possibly result from impaired digestive function, enhanced utilization by bone in response to increased load stimulation (Morey-Holton et al., 1996), and/or alterations of endocrine function related to stress and decline of mass (-11%) during the acclimation period.

**WHAT HAVE WE LEARNED?**

The total picture of cardiovascular function presented by data from snakes demonstrates plasticity of cardiovascular responses to gravitational stresses in
elongated vertebrates that are naturally challenged by the interactions of gravity and posture. As with many aspects of physiology, there is a margin of safety by which the cardiovascular system responds to a gravitational challenge beyond the usual "loading" factor normally encountered in the animal’s environment.

Two aspects of adjustment to dynamic gravity loading are importantly interrelated. First, there is the genetically fixed response of a species that can be characterized by a mean and variance based on sampling from a representative population. Variation of cardiovascular structure and function among snake species appears well correlated with gravitational histories of the respective taxa. Second, there is plasticity of structure and function that confers adjustment of responses to prolonged changes of gravity stress within the bounds determined by the genetic structure. We have demonstrated aspects of acclimation to hyper-gravity, but the nature of acclimation responses to microgravity remain unknown. Based on inference from numerous studies of other physiological systems and stressors, the capability for cardiovascular adjustment to dynamic G forces should be reversibly bidirectional. In evolutionary terms, adaptive adjustments to reduced G force are well illustrated by cardiovascular character states of aquatic snakes which are derived in phylogeny from terrestrial ancestors (Lillywhite and Pough, 1983).

Indeed, data from snakes reflect the importance of evolutionary response to gravitational history, with variation of the gravitational "load" exposure correlating strongly with adaptive variation of cardiovascular performance. These and other comparative data (e.g., Hargens et al., 1987) support in principle the viewpoint that cardiovascular responses of humans are adapted with respect to upright posture (Gauer and Thron, 1965). Thus, perturbations such as microgravity associated with space travel produce responses in humans by which regulatory systems are thought to return functions generally toward orthostatically associated setpoints (Charles and Lathers, 1991; Watenpaugh and Hargens, 1996). Acclimation to the reduced G force therefore might be expected to readjust the cardiovascular setpoints such that "deconditioning" is required before the cardiovascular system again operates optimally during resumption of orthostatic challenges in normal Earth gravity. Vascular studies of snakes suggest the importance to acclimation of local responses involving vascular tissue, in addition to centrally mediated responses to fluid shifts. Future studies should address deficiencies in understanding the mechanisms of adjustments to chronic gravity stress, especially with reference to blood vessels and vascular tissues.

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REFERENCES


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