Acute heat/exercise stress in rats: Effects on fluid and electrolyte regulatory hormones

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Summary. Adult, male rats were exercised in the heat until hyperthermic exhaustion ensued. Plasma aldosterone levels were significantly ($p < 0.001$) elevated after 8 min of exercise and remained increased throughout the exercise and recovery periods. Alternatively, plasma angiotensin I levels were unaffected during exercise, but increased significantly ($p < 0.001$) during the recovery period. These rapid elevations in hormonal levels may be part of a sympathicoadrenal response to heat/exercise stress as well as an adaptational response to maintain plama volume during and following exercise in the heat.

It has been reported by various investigators that exposure of sedentary humans to extreme heat stress is characterized by significant increments in circulating levels of aldosterone, vasopressin, and renin-angiotensin[2-5]. While Finberg et al.[6,7] have reported that both natural and artificial heat acclimatization attenuated the normal heat-induced elevation in plasma renin activity, Davies et al.[8] found no effects of acclimatization on the increments of either plasma renin activity or aldosterone, but these responses were reduced by saline consumption.

Similar responsiveness of these hormones has been noted during exercise at more moderate environmental temperatures. Melin et al.[9] observed significant increments in levels of aldosterone, renin activity, and vasopressin in both trained and untrained men after exercise at 25 °C. The same workers (later) demonstrated that levels of vasopressin and aldosterone were unaffected by training, but that resting levels of plasma renin activity were reduced by the training regimen[10]. Convertino et al.[11] attributed hypervolemic responses to exercise training to significant elevations in plasma renin activity and arginine-vasopressin during exercise. Convertino et el.[12] demonstrated greater exercise-induced than thermal-induced hypervolemia, partially attributable to larger increments in vasopressin responses to exercise.

Thus, while thermal or exercise stress, singularly or in combination, is effective in eliciting increments in circulating levels of these fluid regulatory hormones, much less is known about the responses of these hormones during acute heat/exercise stress, during the development of severe hyperthermia, and following incurrence of hyperthermic injury[13]. By obtaining serial blood samples during the development of potentially fatal rectal temperatures, we were able to characterize further the role of these hormonal responses on the ability to exercise in the heat and on the ability to survive the heat/exercise injury.

Materials and methods. Adult, male rats (325–400 g) were used in all experiments. They were kept in holding rooms in wire-bottomed cages (1 animal/cage) at 22 ± 1 °C with free access to food and water. Automatic fluorescent lighting (on, 06.00–18.00 h) was maintained throughout, and entrained normal diurnal/nocturnal periodicities of body temperature. On the day prior to an experiment each animal was fitted with a permanent indwelling Silastic catheter in the external jugular vein for rapid, convenient blood sampling. Rats usually recovered from the surgery within 2–3 h and no effects on the ability to exercise in the heat were noted.

Prior to experimentation a thermistor was inserted to a depth of 6 cm beyond the anal sphincter for monitoring core temperature (T_{re}); the probe was securely fixed in place so that its position was unaffected by exercise. Following this and immediately prior to commencing exercise in the heat, 0.3 ml blood was removed, hematocrit was measured, and the remaining blood was centrifuged (3–4 °C, 10,000 × g), the heparinized plasma removed, frozen (−30 °C), and stored for subsequent assay. Following removal of the control blood sample (time 0), the rat was quickly transferred to a large stainless steel chamber (35 ± 0.5 °C) and the animal began treadmill exercise (9.14 m/min 0° angle). When T_{re} reached 40 °C, a 2nd blood sample was taken and processed exactly as the first. The rat continued running until hyperthermic exhaustion (T_{re} = 42.5–43 °C) when a 3rd blood sample was removed. The animal was then returned to a moderate environment (22 °C) and allowed to remain sedentary with continual

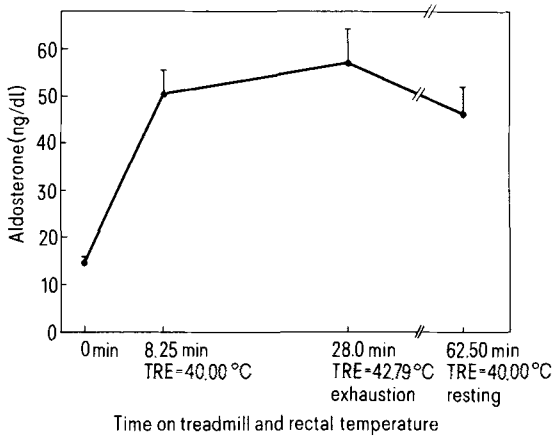

Figure 1. Effects of exercise (9.14 m/min, level treadmill) in the heat (35 °C) on plasma levels of aldosterone. Mean values ± SE of the mean are recorded for an n of 15. Times at which the blood samples were obtained are noted on the abscissa.

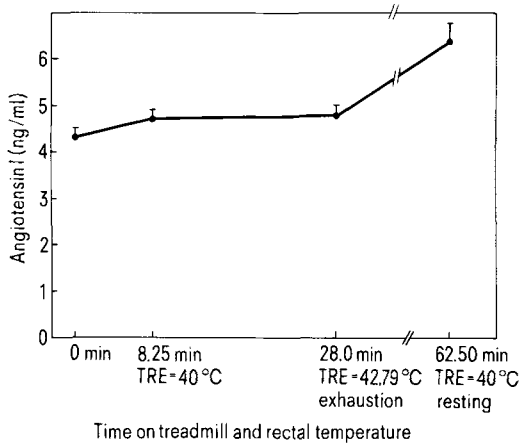

Figure 2. Effects of exercise (9.14 m/min, level treadmill) in the heat (35 °C) on circulating levels of angiotensin I (n = 15, mean ± SE). Times at which blood samples were drawn are noted on the abscissa.

monitoring of T_{re}. When T_{re} fell to 40 °C a final blood sample was obtained. We repeated the entire experimental procedure for 2 separate groups of animals with an n of 15 in each group. Plasma samples of the 1st group were analyzed for plasma angiotensin I while aldosterone was quantitated in the plasma collected from the 2nd group; all experimental procedures were identical between groups.

Aliquots of the frozen plasma were analyzed for aldosterone and angiotensin I. Aldosterone radioimmunoassay test kits were purchased from Damon Diagnostics, Needham, MA; the test kits were manufactured by International CIS, Sorin-Biomedica, Saluggia, Italy. Angiotensin I was analyzed using radioimmunoassay test kits manufactured by New England Nuclear, No. Billerica, MA.

Statistical analyses were performed by a single factor analysis of variance or a paired t-test, where appropriate; critical differences were established by Tukey's-test[14,15]. The null hypothesis was rejected at $p < 0.05$.

Results. Hematocrit ratios in the blood samples taken immediately prior to exercise in the heat ($n = 30$, $x = 46.7$, $SE = 0.4$) were significantly ($p < 0.001$) higher than those of blood samples taken at exhaustion ($n = 30$, $x = 44.1$, $SE = 0.4$). We were unable to discern any consistent relationship between the intensity of the hormonal responses and either the ability to exercise in the heat or the ability to survive the heat/exercise induced, hyperthermic injury.

Figure 1 demonstrates the effects of exercise in the heat on plasma levels of aldosterone. Statistical analysis confirmed what is apparent from this graph: after only 8.25 min (mean time required to achieve $T_{re} = 40$ °C) of exercise in the heat plasma levels of aldosterone rose significantly and remained elevated throughout the exercise and into the recovery period ($p < 0.001$, time 0 vs all other sampling times). However, there were no differences noted in response levels among the latter 3 sampling intervals (50.4, 57.3, and 46.7 ng/dl, respectively; control = 14.9 ng/dl).

Mean plasma levels of angiotensin I are depicted in figure 2. The results indicate that during the actual treadmill run there were no significant changes in circulating angiotensin I levels. However, in the blood sample taken after completion of the treadmill run (recovery period) there did occur a significant ($p < 0.001$) increment in this hormone. This elevation represents an approximate 40% increase over the mean level recorded during the course of the treadmill run.

Discussion. Results of the present investigation confirmed the efficacy of combined heat and exercise stress in eliciting generalized increments in levels of fluid and electrolyte regulatory hormones. Of particular interest was the significant ($p < 0.001$) elevation of plasma aldosterone levels after approximately 8 min of exercise in the heat. We attributed this acute response to heat/exercise stress to increased sympathicoadrenocortical activity. Earlier work has demonstrated that acute exercise stress was effective in inducing elevations in sympathicoadrenocortical activity[16,17]. Likewise, Collins and Weiner[18] reviewed the evidence that acute exposure to extreme heat stress resulted in increments in circulating levels of 17-hydroxycorticosteroids as a result of pituitary-adrenal stimulation. Clearly, the combination of even mild exercise superimposed upon extreme heat stress in naive rats is sufficient to induce an adrenocortical response even within the very brief interval noted in this experiment. Thus, acute increments in circulating levels of aldosterone may be explained by this mechanism. Also lending support to this hypothesis would be the observation that elevations in aldosterone levels temporally preceded effects on angiotensin I. It is generally accepted that, in addition to its role in vasoconstriction, the renin-angiotensin system is involved in the control of aldosterone secretion[19]. Since increments in aldosterone levels preceded responses of angiotensin I, these acute effects on aldosterone levels may be explained in terms of a generalized and acute sympathicoadrenocortical stress response.

In an earlier experiment in which we were attempting to evaluate the role of fluid regulatory hormones in the ability of sedentary rats to survive a 35 °C environment, we observed no changes in circulating aldosterone levels after 6 h of heat exposure ($\bar{x} = 16.74$ ng/dl, control; $\bar{x} = 14.45$ ng/dl, 6 h heat). This lack of effects of sedentary heat exposure on plasma aldosterone levels indicates that the acute effects noted in the present experiments may be due proportionally more to exercise than heat stress.

The significant increment in circulating angiotensin I levels following the heat/exercise protocol may be related to the loss of total body water occurring during the run. Usually, in a heat/exercise protocol as the one currently used, we have observed a mean body weight (water) loss of 8–9 g. Evidently, the duration of the heat/exercise regimen is insufficient for effecting changes in angiotensin I levels. However, during the recovery period the increase in angiotensin I levels may be an early adaptive response to the loss of body water occurring as a result of both heat and exercise induced dehydration. In accord with this suggestion is the observation that from the end of the run to the time when the recovery ($T_{re} = 40$ °C, resting) blood sample is taken, mean hematocrit level has increased from 44.1 to 46.5 during a mean time interval of approximately 34 min.

Ordinarily, the current regimen, including mild exercise, intense heat, and short duration of treadmill run, effects a slight hemodilution when post-run hematocrits are compared with pre-run hematocrits[20]. Indeed, in the present study, also, hematocrit ratios decreased significantly (46.7-44.1) during the time of treadmill exercise. In a short duration experiment as this, these variations in hematocrit value are unlikely to be related to changes in red cell mass and are more likely associated with increments in circulating plasma volume[21,22] subsequent to the diffusion of interstitial fluid into the vascular space.

Another factor which should be considered in evaluating the present data is the effect of combined exercise and heat stress on the metabolic clearance rates of these hormones. For example, in a controlled hyperthermia study Collins et al.[23] reported that the metabolic clearance rate of aldosterone was reduced by 26% when body temperature was raised by 1.08 °C. Earlier, Rowell et al.[24] had demonstrated large decrements in estimated hepatic blood flow, particularly during periods of strenuous physical activity. Thus, it is probable that the combined effects of heat exposure and exercise reduce even further the metabolic clearance rate of these hormones, and may contribute to the acute and significant elevations generally observed in these experiments.

We have concluded from these studies that a regimen of exercise in a hot environment elicited significant increments in circulating levels of both hormones. The responses of these hormones may be partially responsible for the maintenance of plasma volume generally observed under these conditions. Additionally, the rapidity of the aldosterone response to exercise in the heat indicates adrenocortical stimulation.

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in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.
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Angiogenic factor in vitreous from diabetic retinopathy

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Summary. Vitreous from patients with proliferative diabetic retinopathy contains an angiogenic substance which stimulates the proliferation of blood vessels on the chick chorioallantoic membrane, whereas vitreous from non-diabetics who do not have a proliferative retinopathy does not.

The development of retinal neovascularization in proliferative diabetic retinopathy poses a major clinical problem. The frequent bleeding from newly formed vessels into the vitreous cavity[3,4] results in impaired vision and loss of vitreous integrity, and frequently surgical removal becomes necessary. The underlying mechanism of retinal neovascularization is unknown. However, areas of ischaemia are commonly associated with neovascularization and it has been suggested that such areas generate diffusible angiogenic factors[5]. Recent work by Glaser and co-workers[6,7] has shown a relationship between the occurrence of proliferative diabetic retinopathy and the presence of angiogenic

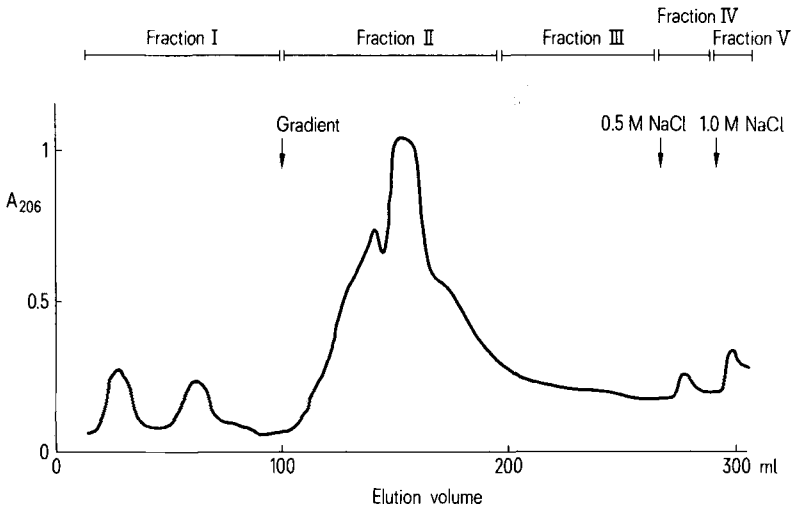

Figure 1. DEAE elution profile from a human diabetic vitreous.