Plasma Renin Activity during Exercise in the Dog

Meyer D. Lifschitz, M.D., and Lawrence D. Horwitz, M.D.

SUMMARY Previous workers have suggested that a rise in plasma renin activity (PRA) may mediate some of the hemodynamic changes associated with exercise. To test this hypothesis in nine dogs chronically instrumented for measurement of aortic pressure (catheter) or cardiac output (ascending aorta electromagnetic flow probe) PRA was measured by radioimmunoassay in blood samples drawn before and during running on a level treadmill at 4-8 miles per hour. Exercise caused increases in heart rate from 96 ± 5 (SE) to 186 ± 7 beats/min, cardiac output from 2.8 ± 0.3 to 6.2 ± 0.6 liters/min, and mean aortic pressure from 115 ± 5 to 132 ± 5 mm Hg (P < 0.01). Mean PRA was 6.6 ± 0.7 (SE) ng of angiotensin I/ml per 3 hours before and 7.6 ± 1.2 ng Ang I during exercise, values that are not different statistically. Propranolol reduced PRA at rest from 8.6 ± 1.1 to 5.9 ± 1.1 ng Ang I (P < 0.05), but there was no significant difference between resting and exercise levels, although the increments in heart rate, cardiac output, and mean aortic pressure were reduced. Standing on hindlimbs for 5 minutes did not cause a change in mean aortic pressure or PRA. However, administration of pentolinium reduced mean aortic pressure, and PRA rose from 6.0 ± 1.1 to 9.8 ± 1.5 ng Ang I. Exercise, with or without β-adrenergic blockade, does not cause increased PRA in conscious dogs in which the renin-angiotensin system is normally responsive.

To clarify the relationship of PRA to sympathetic tone during exercise, we obtained renin levels by radioimmunoassay of blood samples drawn from dogs performing running exercise of varying speeds and duration. Simultaneous hemodynamic measurements confirmed the severity of the stress. Studies also were made of the effect on PRA of β-adrenergic blockade with propranolol at rest and during running, of postural change, and of hypotension induced by a ganglionic blocking agent, pentolinium.

Methods

Nine dogs, weighing 15.5-22.7 kg, were trained to run on a level treadmill. After training, in seven dogs a sterile thoracotomy was performed under sodium pentobarbital anesthesia (30 mg/kg, iv). An electromagnetic flow probe was implanted around the proximal portion of the ascending aorta, a solid state pressure transducer (Konigsberg P18) was implanted in the left ventricle, and 18-gauge polyvinyl catheters were implanted in the left internal mammary artery, the left atrium, and the left jugular vein. In two dogs no thoracotomy was performed and surgery was limited to implantation of catheters in the left jugular vein and the aorta via the left carotid artery. These dogs were prepared in this fashion to ensure that the more extensive instrumentation in the remaining dogs did not influence PRA responses. An interval of at least 3 weeks was allowed for recovery from surgery. At the time of study, each dog could exercise at the same levels as had been attained before surgery. Exercise was performed with the treadmill at 0° grade. Resting measurements were obtained with the dog standing quietly on the treadmill. All dogs ran for 3 minutes at 6-8 miles per hour (mph). Four dogs continued to run for a total

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time of 7–10 minutes at this speed. Two dogs also ran at 3–4 mph for 3 minutes. Blood samples for PRA were drawn from the left atrial or arterial catheter before exercise and during exercise at 3 minutes of each run and just prior to the end of the prolonged runs. Hemodynamic measurements were continuously monitored; values reported were obtained immediately before blood samples were drawn.

In five of the dogs exercise also was performed 20 minutes after administration of propranolol (Inderal, Ayerst) (1 mg/kg, iv). In an additional study eight dogs were placed in an upright position, supported by their hindlegs while their front paws were held, for 5 minutes. Blood samples for renin were drawn initially with the dog lying prone and then at the end of the 5 minutes in upright posture. In a fourth set of studies eight dogs lying in a sling were given pentolinium (1.5 mg/kg, iv) to diminish arterial pressure, and blood samples were drawn prior to and 10 minutes after administration.

PRA was determined by radioimmunoassay using the procedure of Stockigt et al. Blood was collected and quickly placed in cold tubes containing ethylenediaminetetraacetic acid (EDTA). The plasma was extracted immediately and frozen until analyzed. At the time of analysis, 1 ml of plasma was defrosted in the cold, 8-hydroxyquinolone and dimercaprol were added as converting enzyme and angiotensinase inhibitors, the pH was adjusted to 5.5–5.7 with 1–2 drops of HCl, and the samples were placed in a 37°C shaking water bath. After 3 hours, the generation of angiotensin I was terminated by the addition of water and by boiling. The supernatant fluid was obtained by centrifugation and appropriate dilutions were added to labeled angiotensin I (New England Nuclear Corp.) and a 1:68,000 dilution of rabbit antibody specific for angiotensin I. Results were read from a standard curve of synthetic angiotensin I (Schwarz/Mann), and pools of known amounts of renin and angiotensin I were included in each assay as internal controls. All samples were analyzed in duplicate and results expressed as nanograms of angiotensin I generated per milliliter per 3 hours of incubation (ng Ang I). Angiotensin I generation was linear for 3 hours with this technique.

All hemodynamic measurements were inscribed on a Beckman RM oscillograph and an Ampex FR 1300 tape recorder. Cardiac output was obtained with a Zepeda electromagnetic flowmeter from the aortic probe. The flowmeter is limited in frequency response only by the response of the recorder which was flat to 80 Hz. The solid state transducer used for measurement of left ventricular pressure has a natural frequency in excess of 3,000 Hz. Aortic pressure was measured with a Statham P23Db manometer; this system had a natural frequency of 18 Hz. The maximum first differential of the left ventricular pressure (dP/dtmax) was obtained with an active circuit which was linear to 70 Hz. Hemodynamic data were analyzed by averaging the results of eight consecutive beats to reduce the effects of respiratory variation or atypical beats. Random sampling indicated that a satisfactory steady state had been obtained at the time of each measurement.

Statistical analyses were performed using the paired t-test, with each dog serving as its own control. Mean values are expressed with 1 SE. A P value less than 0.05 was considered significant.

Results

RESPONSIVENESS OF THE RENIN-ANGIOTENSIN SYSTEM TO POSTURAL CHANGE AND DRUG-INDUCED HYPOTENSION

Two interventions were studied to confirm that responsiveness of the renin-angiotensin system was normal. Eight dogs were held by the front paws in a standing position for 5 minutes. The results of PRA measurements before and during standing on the hindlimbs are shown in Figure 1. PRA was 7.1 ± 0.9 ng Ang I before and 8.1 ± 1.3 during standing. This change was not statistically significant.

No hemodynamic measurements were made during these studies of postural change. However, when the expected rise in renin did not occur, random arterial pressure measurements were made in several of these dogs before and during standing. It was noted that these conscious dogs, which were partially conditioned by repeated exercise runs, did not consistently exhibit a fall in blood pressure on assuming the erect position. Accordingly, it was assumed that the stimulus was inadequate to induce renin release. We then studied drug-induced hypotension.

Pentolinium (1.5 mg/kg, iv) was given to eight dogs. Peak aortic or peak left ventricular pressure fell by 17–37 mm Hg in six dogs, rose in one, and was not recorded in another. The results of PRA determinations before and 5 minutes after pentolinium administration are shown in Figure 2 for the six dogs in which blood pressure fell. In these six dogs PRA increased from 6.0 ± 1.1 to 9.8 ± 1.5 ng Ang I (P < 0.05). When the results from all eight dogs are analyzed,
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PRA increased from 5.7 ± 0.8 to 8.4 ± 1.4 ng Ang I (P < 0.05). PRA rose in all those dogs in which a fall in pressure occurred.

EXERCISE WITHOUT DRUGS

Exercise was associated with considerable changes in cardiovascular function in this group of dogs (Table 1). During the 3-minute runs at 6-8 mph heart rate increased by 87%, stroke volume increased by 14%, cardiac output increased by 120%, left ventricular systolic pressure increased by 35%, left ventricular dP/dt max increased by 63%, and mean aortic pressure increased by 15%. The prolonged exercise appeared to exhaust the dogs. A slightly higher heart rate (198 ± 11 vs. 184 ± 8 beats/min) was the only consistent hemodynamic change noted between the brief and prolonged efforts at 6-8 mph. The two dogs which ran for 3 minutes at 3-4 mph exhibited hemodynamic changes that were similar to but smaller than those that occurred at the higher speed. In contrast to these changes in hemodynamics, there were no consistent changes in PRA when levels during exercise were compared with those obtained prior to running. PRA was 6.6 ± 0.7 ng Ang I before exercise. 7.6 ± 1.2 ng Ang I after 3 minutes of running at 6-8 mph, and 8.4 ± 1.7 ng Ang I after 7-10 minutes of running at 6-8 mph. Overall, only one of nine dogs exhibited an increase in PRA during exercise (Fig. 3). The two dogs without flow or pressure transducers did not exhibit changes in PRA.

EFFECT OF β-ADRENERGIC BLOCKADE

At rest, administration of propranolol (1 mg/kg, iv) was associated with a significant fall in PRA from 8.6 ± 1.1 to 5.9 ± 1.1 ng Ang I (P < 0.05). Exercise did not result in a significant change from the pre-exercise, post-propranolol PRA level (Fig. 4). The hemodynamic changes after propranolol were similar to those reported previously. As compared with exercise without drugs, propranolol was associated with lower levels of heart rate (~34 beats/min), stroke volume (~3.4 ml/min), and dP/dt max (~1.811 mm Hg/sec).

Discussion

The most striking finding of this study was the lack of response of the renin-angiotensin system to strenuous exercise. This was unexpected, because previous workers had concluded that in man or the rat exercise was associated with consistent increments in PRA. There have been no previous studies of the exercising dog in which PRA measurements were made. It is possible that the dog's mechanisms for regulating PRA are different from those in man. This seems unlikely, however, because many of the studies bearing on the control factors for renin release have been performed in the dog, and where these control factors have been studied in man similar results have been obtained. In addition, the hemodynamic changes reported for the dog during exercise in this study and previously are similar to those which occur in man. Therefore, it would appear that if there is a difference in exercise responses between dog and man, this difference may be limited to the renin-angiotensin system.

Table 1: Hemodynamic Changes with Exercise

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>CO (liters/min)</th>
<th>dP/dt max (mm Hg/sec)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>MAP (mm Hg)</th>
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<tr>
<td>Control</td>
<td>100</td>
<td>2.76</td>
<td>3277</td>
<td>128</td>
<td>3.1</td>
<td>115</td>
</tr>
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<td>6-8 mph</td>
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<td>6.24</td>
<td>5344</td>
<td>167</td>
<td>5.6</td>
<td>132</td>
</tr>
<tr>
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<td>256</td>
<td>3</td>
<td>1.0</td>
<td>11</td>
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<tr>
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Mean changes in nine dogs during brief (3-minute) runs at 6-8 miles per hour (mph) on a level treadmill. X = mean; SE = standard error of each group; P was derived by paired comparison with Student's t-test, using each dog as its own control; HR = heart rate; CO = cardiac output; dP/dt max = maximum first derivative of the left ventricular pressure; LVSP = left ventricular peak systolic pressure; LVEDP = left ventricular end-diastolic pressure; MAP = mean aortic pressure.

Figure 2: Plasma renin activity (••) during control conditions and after pentolinium (1.5 mg/kg) for six dogs whose mean aortic pressure fell after administration of this drug.
Earlier workers in this area measured PRA by means of a bioassay, whereas this study utilized a radio-immunoassay. Although the absolute values obtained with these two approaches may differ, there is a good correlation between them, and at least one laboratory has determined a conversion factor to transform one result into the other. Nevertheless, it is possible that the levels measured by the two assays during exercise may not be the same. In particular, a vasoactive substance liberated into the plasma by exercise could yield a response in the bioassay, but not bind to the antibody. Presumably this substance is not a catecholamine, since the Boucher bioassay technique includes a chromatographic step which excludes these compounds.

We considered the possibility that these dogs did not have an intact renin-releasing or synthetic mechanism. However, PRA did change significantly with two maneuvers. The acute administration of propranolol lowered PRA from 8.6 ± 1.1 to 5.9 ± 1.1 ng Ang I. Propranolol may well act to lower renin by inhibiting its release from the juxtaglomerular apparatus. In addition, these dogs responded to pentolinium, a ganglionic blocking agent which lowered blood pressure and, presumably, renal perfusion pressure, and caused an increase in PRA from 6.0 ± 1.1 to 9.8 ± 1.5 ng Ang I. Thus, although they did not respond to exercise or upright posture with a change in PRA, the dogs did respond to propranolol and pentolinium with appropriate changes in PRA.

The level of exercise attained was sufficient to induce high levels of sympathetic tone. Although it might be questioned if the 3-minute exercise period was sufficiently long to allow time for the renin-angiotensin system to respond, the 7- to 10-minute period should have been quite adequate, because responses occur in less time with other interventions, such as postural hypotension.

While not demonstrated in this study, more sustained exercise or higher peak exercise loads might lead to hemodynamic adjustments (renal or regional vasoconstriction in other vascular beds) or hormonal adjustments which, although not directly related to exercise, could reflect a compromised cardiovascular system. These circulatory adjustments to severe stress might then be the factor(s) which have previously led to increases in PRA with exercise. This formulation is consistent with results of previous studies in man which showed no increase in PRA with exercise until near-maximal levels of exercise were achieved and with a recent study which demonstrated little increase in PRA with exercise but a large increase shortly after exercise. Since the dogs in our study were trained and partially conditioned, it is conceivable that they could exercise to a maximum degree without stressing their circulatory system to the same degree as a less well conditioned man. In this light the rise in PRA in association with exercise would not be related to the exercise stimulus, per se, but rather to some secondary effect of prolonged or severe exercise such as a diminished intravascular volume or an increased level of circulating catecholamines.

Whatever the reason for the variance between these results and those of some previous studies, it would appear that exercise in the dog is associated with high levels of sympathetic stimulation and marked hemodynamic alterations, but not with an increase in PRA. With blockade of β-adrenergic receptors, both the resting and exercise PRA levels were reduced, but there were no apparent differences between rest and exercise. There is an apparent paradox in such information; on the one hand, reduced sympathetic tone was associated with a reduction in PRA, whereas, on the other, a physiological setting with an elevated sympathetic tone was not accompanied by a rise in PRA.

A possible explanation for these findings is that the level of sympathetic stimulation is only one of several factors...
affecting rates of renin release. Other possible factors include changes in renal blood flow or perfusion pressure, the concentration of sodium or other electrolytes in the juxtaglomerular apparatus, or release of antidiuretic hormone or angiotensin. During exercise, alterations in certain of these factors may neutralize the effect of the high level of sympathetic stimulation. In fact, mean aortic pressure and left ventricular systolic pressure were elevated in these dogs during exercise, as has been previously observed. This increase in systemic blood pressure would be expected to increase renal perfusion pressure and it is possible that this increase in renal perfusion pressure exerted a sufficient inhibitory influence on renal release of renin to balance the effect of the increased sympathetic stimulation. Indeed, the relative importance of sympathetic stimulation as a mediator of renin release in physiological conditions may not be great. In conclusion, it has been shown that exercise in the dog is associated with a considerable increment in cardiovascular parameters such as heart rate, cardiac output, and dP/dt max, but no increase in PRA. It is suggested that the increase in PRA demonstrated in previous studies may not be due to the exercise phenomenon itself, but rather to some secondary compensatory phenomenon.

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References

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