Hemodynamics in Transgenic Mice With Overexpression of Atrial Natriuretic Factor

R. Wayne Barbee, Bret D. Perry, Richard N. Ré, Joseph P. Murgo, Loren J. Field

Abstract  The circulatory effects associated with lifelong plasma atrial natriuretic factor (ANF) elevation were examined by generating transgenic mice, which constitutively express a fusion gene consisting of the transthyretin promoter and the ANF structural gene. These mice have chronically elevated ANF levels as compared with their nontransgenic siblings. Transgenic animals exhibited immunoreactive ANF levels that were nearly fivefold higher than those measured in nontransgenic littermates. Systemic and regional hemodynamics and blood volumes were explored by using modifications of the reference microsphere and dilution techniques. Mean arterial pressure was reduced by 24 mm Hg, associated with a 27% reduction in total heart weight. This chronic reduction in blood pressure was due to a 21% reduction in total peripheral resistance, whereas cardiac output, stroke volume, and heart rate were not significantly altered, despite a 15% elevation in plasma volume. Transgenic mice displayed reductions of 35%, 33%, 32%, and 19% in muscle, skin, brain, and renal vascular resistance, respectively, whereas coronary and splanchnic resistances were not significantly altered. The findings complement earlier data from chronically infused normotensive mammals and suggest that these mice are an excellent model for investigating the effects of lifelong ANF elevation. (Circ Res. 1994;74:747-751.)

Key Words  blood pressure  •  blood volume  •  cardiac output  •  vascular resistance  •  autoregulation  •  atrial natriuretic factor

Atrial natriuretic factor (ANF) is perhaps the best-known peptide in a family of compounds intimately involved in pressure and volume homeostasis.1–3 Although bolus injection or acute infusion of ANF is generally linked with hypotensive and/or natriuretic responses,4,5 the chronic effects of ANF are not clearly understood. Harrison-Bernard et al6 and Parkes et al7 have shown that chronic infusion of ANF to normotensive animals in the pathophysiological range is associated with a reduction in mean arterial pressure (MAP), mediated initially by a fall in cardiac output (CO). This response is followed by a diminished total peripheral resistance (TPR), while CO returns to normal. However, this is not a universal finding.8 Furthermore, these “chronic” studies normally involve only a few days of ANF infusion because of technical and economic limitations. Hemodynamic variables may not reach steady-state values during these infusion periods; some effects of ANF may require much longer to develop.

To overcome these obstacles, a transgenic mouse model with chronically elevated ANF levels was generated.9 In this model, constitutive expression of the murine ANF gene was targeted to hepatocytes with the transthyretin (TTR) promoter. The transgenic mice have elevated ANF levels and reductions in MAP compared with the nontransgenic siblings. These changes occur without modifications in basal urine output or sodium excretion, suggesting a leftward shift in the pressure-natriuresis relation. The systemic and regional hemodynamics, in addition to body fluid measurements, were characterized in this model using previously published modifications of the reference microsphere and dilution techniques.10

Materials and Methods

Animal Care

Adult male C3H/HeJ mice and their nontransgenic sibling controls (maintained in a C3HeB/FeJ background, total n=34) were shipped from the Krannert Institute of Cardiology and allowed at least 2 weeks of acclimatization before use. Animals were provided chow and water ad libitum and were maintained on a 12-hour light/dark cycle. Experiments were approved in advance by the animal care and use committee.

Surgery

Mice were rendered unconscious with ether; anesthesia was then maintained with Avertin (2.5%, 17 μL/g body weight IP). The right carotid and femoral arteries were exposed and cannulated. The right carotid artery was used for injection of microspheres; the femoral artery was used for arterial pressure measurement and blood withdrawal. The carotid cannula (tapered PE-50 tubing) was advanced =1 cm until the left ventricular cavity was catheterized (verified by pressure tracings).

All mice were allowed at least 4 hours of recovery from anesthesia before beginning the experiments. The animals were placed in a minimally restraining enclosure fashioned from a 35-mL plastic syringe holder. To prevent stress-induced changes in systemic hemodynamics, the animals were acclimatized to this environment for at least 4 days (2 hours per day) before surgery and experimentation. The placement of the carotid cannula was confirmed by briefly checking diastolic and pulse pressure to ensure that the catheter tip was in the left ventricle. The carotid cannula was then disconnected, and the femoral catheter was connected to a Statham pressure transducer (model P23Db, Gould, Cleveland, Ohio) for measurement of MAP. The phasic arterial pressure recorded from the
femoral artery was processed by an amplifier (Grass Tachograph) to record heart rate (HR). Stable MAP and HR were monitored for 10 to 15 minutes before beginning the experiment, and measurements of these variables occurred every 30 seconds for the last 5 minutes before microsphere injection.

### Microsphere Preparation and Reference Sample Microsphere Technique

Radioactive stock microspheres (15±3 μm, 10 mCi/g, or 370 MBq/g; Tracer Microspheres, 3M, St Paul, Minn) labeled with \(^{141}\text{Ce}\) or \(^{89}\text{Sr}\) were mixed and dispensed into segments of silicone medical grade tubing (internal diameter, 0.03 in; outer diameter, 0.065 in; Baxter, McGaw Park, Ill) so that a 3-cm segment contained \(\approx 50,000\) microspheres in 14 μL of isotonic saline containing 0.05% polysorbate-80 (Tween 80). The segment radioactivity was determined before and after injection using a gamma counter (model 5530, Packard, Downers Grove, Ill). The microspheres were dispersed for \(\approx 15\) seconds by mechanical agitation and then injected using \(\approx 100\) μL of donor blood. The reference sample was withdrawn from the femoral artery cannula beginning 2 seconds before microsphere injection and continuing for 10 seconds after microsphere injection for a total of 17 seconds (\(\approx 110\) μL of blood). This volume corresponds to a sample withdrawal rate of \(\approx 0.4\) mL/min. Blood and saline rinses were transferred to a scintillation vial for determination of radioactivity in a gamma counter. CO was calculated (in milliliters per minute) as follows: \(\text{CO} = (\text{SR} \times \text{injected radioactivity})/\text{reference radioactivity}\) where the sampling rate (SR) is expressed as milliliters per minute and injected and reference sample radioactivity are expressed as counts per minute. SR was calculated by the following formula: \(\text{SR} = (\text{change in reference syringe weight} \times 60 \, \text{s/min})/(\text{blood specific gravity} \times \text{withdrawal period})\). Cardiac index (CI, in milliliters per minute per kilogram) was calculated from the quotient of CO and body weight. TPR was expressed as the quotient of MAP and CO and was also corrected for body weight (TPR index=MAP/CI). Just after withdrawal of the reference sample, the placement of the carotid cannula was again confirmed by briefly checking left ventricular pressure.

### Table 1. General Characteristics, Systemic Hemodynamics, and Fluid Volumes in Transgenic and Nontransgenic Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nontransgenic (n=8)</th>
<th>Transgenic (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>34±1</td>
<td>37±2</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>161±5</td>
<td>118±3*</td>
</tr>
<tr>
<td>Plasma IR-ANF, pg/mL</td>
<td>81±16</td>
<td>373±55*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>96±3</td>
<td>72±2*</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>25±1</td>
<td>24±1</td>
</tr>
<tr>
<td>CI, mL.min⁻¹.kg⁻¹</td>
<td>718±20</td>
<td>657±38</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>622±20</td>
<td>665±16</td>
</tr>
<tr>
<td>SV, μL/beat</td>
<td>40±3</td>
<td>36±1</td>
</tr>
<tr>
<td>TPR (MAP/CO), mm Hg.mL⁻¹.min⁻¹</td>
<td>3.9±0.2</td>
<td>3.1±0.1*</td>
</tr>
<tr>
<td>TPRI (MAP/CI)</td>
<td>0.134±0.002</td>
<td>0.113±0.005*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.5±0.9</td>
<td>37.3±0.7*</td>
</tr>
<tr>
<td>Blood volume, mL</td>
<td>2.1±0.1</td>
<td>2.5±0.1*</td>
</tr>
<tr>
<td>Blood volume, mL/kg</td>
<td>61.9±3.2</td>
<td>67.5±2.5</td>
</tr>
<tr>
<td>Plasma volume, mL</td>
<td>1.3±0.06</td>
<td>1.6±0.07*</td>
</tr>
<tr>
<td>Plasma volume, mL/kg</td>
<td>36.8±1.9</td>
<td>42.3±1.4*</td>
</tr>
</tbody>
</table>

IR-ANF indicates immunoreactive atrial natriuretic factor; MAP, mean arterial pressure; CO, cardiac output; CI, cardiac index; bpm, beats per minute; SV, stroke volume; TPR, total peripheral resistance; and TPRI, total peripheral resistance index. Values are mean±SEM.

*\(P<.05\) vs nontransgenic littermates.
Tissue Sampling

After the reference sample and blood volume samples (see below) were withdrawn, animals were killed by exsanguination (collection of ≈1 mL of blood into EDTA-coated syringes for determination of plasma immunoreactive ANF [IR-ANF] levels). If animals showed undue struggling or other signs of excessive anxiety during blood withdrawal, they were quickly anesthetized with sodium pentobarbital (=15 to 25 mg/kg IV).

The skin, muscle, brain, heart, kidneys, lungs, stomach, intestines, liver, pancreas, spleen, and testes were removed and blotted dry, weighed, and placed in a gamma counter. We sampled all of the skin and approximately one third of the skeletal muscle, excluding muscles between the ribs and immediately adjacent to major skeletal structures, which were difficult to dissect. We also excluded muscle of the hind limb used for femoral artery catheterization, since we found that this muscle received considerably fewer microspheres than the uncatheterized leg (authors’ unpublished pilot studies). Therefore, the muscle counts are representative of perfused muscle. Organ blood flow as a percentage of CO was calculated as the ratio of organ to injected radioactivity. Adequacy of microsphere mixing was assessed by comparing the blood flow per gram of the left and right kidneys. Animals whose kidney flows differed by ≥15% were eliminated from the study. Adequate trapping of microspheres was evaluated by counting the lung radioactivity. If the percentage of systemic CO to the lungs was ≥5% (excessive arteriovenous shunt), the animal was eliminated from the study. A minimum of 400 trapped microspheres was considered necessary for accurate CO and blood flow determination. Because some splanchnic organs occasionally trapped <400 spheres individually, they were combined (small intestine, large intestine, liver, pancreas, spleen, and stomach) for determination of splanchnic flow. Spillover between channels was corrected with a matrix inversion software package (COMPUSPHERE, Packard).

Blood and Plasma Volumes

Erythrocytes were labeled on the day of the experiment (=0.1 μCi or 3.7 KBg of 51Cr-labeled cells in a volume of 0.1 mL blood) and then injected and flushed with 0.05 mL saline. After allowing 15 minutes for mixing, blood was collected from a cleared arterial catheter into two microhematocrit tubes (Monoject Scientific, Curtin Matheson Scientific) precalibrated to 25 μL each. After centrifugation and recording of hematocrit, the radioactivity of the blood volume (BV) samples and standards was determined. BV was calculated according to the following formula: BV = (injected radioactivity/blood radioactivity concentration) × 0.8, where injected radioactivity is expressed as counts per minute, blood radioactivity concentration is expressed as counts per minute per milliliter, and 0.8 equals the Fm,x ratio, determined in our earlier publication. In these experiments, plasma volume (PV) was calculated as follows: PV = [(1 − (Hct/100)) × BV]/body weight, where Hct is hematocrit.

Additional details regarding surgery and the reference microsphere and dilution techniques are available in a recent publication validating these procedures in mice.

IR-ANF Levels

The radioimmunoassay of IR-ANF was performed as described previously, but with the following modifications. Rat ANF standard (ANF99–126) was purchased from Peninsula Laboratories, Belmont, Calif. The reported ANF values were corrected for percent recovery, which ranged from 74% to 99%, and was quantified on individual samples by the addition of ≈1200 cpm of 125I-ANF (DuPont/New England Nuclear, Boston, Mass) to the columns just before extraction.

Statistics

All variables from conscious transgenic mice and their nontransgenic littermates were compared using two-tailed unpaired t tests with the STATVIEW statistical package (Abacus Concepts, Berkeley, Calif). The level of significance was set at P < .05.

Results

Surgery was performed on 34 mice, and 20 experiments were successful. Of the 14 unsuccessful attempts, three animals died after surgery. Two of these animals expired after ventricular arrhythmias due to improper ventricular catheter placement, and one animal removed his arterial catheter. Of the remaining 11 experimental failures, data from four animals were discarded because of severe bradycardia (≈180 beats per minute), high microsphere counts in the lungs (suggesting an arteriovenous shunt), stroke after microsphere injection, or a nonpatent catheter. The remaining seven unsuccessful experiments were due to inadequate mixing of microspheres (>15% difference of blood flow per gram to the right versus left kidney).

Representative pressure (mean and pulsatile) and HR (mean only) tracings for transgenic and nontransgenic mice are shown in the Figure. The average MAP in these particular nontransgenic and transgenic mice before microsphere injection was 98 and 70 mm Hg, respectively, whereas HR averaged 594 and 674 beats per minute, respectively. The brief increase in MAP just before microsphere injection represents an artifact due to catheter clamping with hemostats just before disconnection from the transducer for withdrawal of the reference sample. Likewise, the large variations in the mean HR signal during microsphere injection represent an artifact due to the loss of an input signal to the tachograph amplifier used to determine HR. Portions of each tracing were recorded at a slow paper speed (≈10 mm/min; each major tic mark represents 1 minute) or a high paper speed (≈10 or 50 mm/s; each tic mark represents 1 second). After microsphere injection via the ventricular catheter, left ventricular pressure was recorded to verify the location of the catheter tip in both nontransgenic and transgenic mice. Overall characteristics, central hemodynamics, and fluid volumes are shown in Table 1. Heart weight was reduced ≈27% in the transgenic mice. Plasma levels of IR-ANF were elevated ≈4.6-fold in the TTR-ANF mice compared with control mice. MAP was 24 mm Hg (≈25%) lower in the transgenic mice when compared with the nontransgenic littermates. Neither CO nor CI was significantly different between the two groups, whereas TPR and the TPR index were reduced ≈21% and 16%, respectively, in the TTR-ANF mice. HR was not significantly different in the two groups of mice. Hematocrit was reduced, whereas plasma volume was significantly elevated in the transgenic mice compared with the nontransgenic littermates. However, the increase in blood volume indexed to body weight was not significant in the transgenic mice.

Regional hemodynamics are displayed in Table 2. Regional vascular resistance was reduced in the muscle, skin, brain, and renal vasculatures by 35%, 33%, 32%, and 19%, respectively. When corrected for the mass of sampled tissue, the decreases were similar (28%, 29%, 33%, and 19% for muscle, skin, brain, and renal vasculatures, respectively). Muscle blood flow (in milliliters per minute and percent CO) indicated a tendency toward higher values in the transgenic mice (P = .1 and
Coronary blood flow, measured as percent CO or milliliters per minute, tended to be depressed in the transgenic mice when compared with the nontransgenic littermates; however, these differences were not significant (P = .09). Furthermore, splanchnic blood flow (expressed as blood flow per gram) was significantly decreased in the transgenic mice when compared with the nontransgenic littermates. Splanchnic and coronary vascular resistances were not different between the two groups of mice, regardless of the units compared.

### Discussion

The reduction in MAP observed in the transgenic mice in the present study is similar to what has been previously reported for this model. The 27% reduction in total heart weight may be in part due to this reduction in afterload. The reduction in MAP was not associated with changes in CO but was instead due to a 21% reduction in TPR of the transgenic mice. This reduction in TPR after chronic elevation of plasma IR-ANF supports earlier studies that demonstrated a shift from reduced CO to attenuated TPR in sustaining the hypotensive effect of ANF. This shift in hemodynamics is likely due to whole-body autoregulation.

Contributing to the reduced TPR were reductions in muscle, skin, brain, and renal vascular resistance. Splanchnic vascular resistance was not affected in the transgenic mice, in accordance with the poor autoregulatory ability of the mesenteric circulation. The lack of significant reductions in splanchnic resistance was reflected in a significant decrease in splanchnic blood flow when expressed as blood flow per gram. However, the coronary vascular bed also failed to vasodilate in the transgenic mice, despite the vigorous autoregulatory response of this organ. This is probably not due to failed autoregulation. Rather, the decrease in coronary blood flow (19 mL/min per gram in nontransgenic mice versus 12 mL/min per gram in the transgenic littermates) may reflect in part the reduced work load of the heart in the TTR-ANF mice. Alternatively, ANF expression throughout ontogeny may have compromised the myocardial autoregulatory reserve. Additional potential influences of elevated ANF on the coronary vasculature and on myocardial contractility cannot be ascertained from these findings.

An unusual finding was the decreased hematocrit and increased plasma volume in the TTR-ANF mice when compared with their nontransgenic siblings. This response to lifelong ANF elevation is opposite the effect observed during acute ANF infusion. However, the finding does support our previous observations of a significant fall in plasma protein concentration and slight decrease in hematocrit with chronic fivefold to sixfold ANF elevations in rats.

Previously, no alterations in hematocrit were observed in the TTR-ANF mice. There are several possible causes for these differences. Hematocrit and plasma volume measurements were obtained from conscious mice in the present study, as opposed to anesthetized mice in a previous study. In addition, the mice used in the present study were appreciably older and slightly heavier than those previously examined. Additionally, the transgenic mice from an earlier study exhibited a nearly twofold elevation of plasma aldosterone compared with the nontransgenic siblings (1.37 versus 0.74 ng/mL). The changes in hematocrit and plasma volume observed in the present study may be an age-related phenomenon in response to elevated aldosterone levels in these mice.
The TTR-ANF transgenic mouse experiences lifelong reductions in afterload due to decreases in TPR. Nearly every vascular bed exhibits diminished resistance except the splanchnic and coronary circulations. CO is unaltered despite increases in plasma volume. Differences between this model and chronic infusion of ANF in rats can be ascribed primarily to the time period of ANF elevation. These mice remain an excellent model for examining the long-term effects of ANF elevation.

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