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Blood Pressure Regulation in ANF-Transgenic Mice: Role of Angiotensin and Vasopressin

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Summary

Transgenic mice overexpressing a transthyretin promoter-ANF structural fusion gene have a life-long reduction in arterial blood pressure compared to nontransgenic littermates. The present study was designed to test the hypothesis that the high plasma level of ANF in the transgenic mice inhibits the renin-angiotensin and/or vasopressin systems, thereby causing the hypotension. Mice were anaesthetized with Inactin and arterial pressure and heart rate were monitored before and during Saralasin infusion and vasopressin V_1 receptor blockade. Effectiveness of the blockade was determined by injection of angiotensin and vasopressin before and during Saralasin and V_1 receptor antagonist administration. Saralasin was associated with hypotension in both transgenic and nontransgenic mice. The decrease in blood pressure was proportionally greater in the transgenic animals. Vasopressin receptor blockade had little effect on blood pressure in either group. Heart rates were not different between the groups during any maneuver. We conclude that the chronic hypotensive effect of ANF overproduction does not involve the inhibition of either renin-angiotensin or vasopressin systems. The data, however, suggest that the renin-angiotensin system may be stimulated in the ANF-transgenic mice.

Key words

Hypotension – Heart rate – Atrial natriuretic factor – Saralasin – Vasopressin V_1 receptor antagonist

Introduction

Atrial natriuretic factor (ANF), in addition to its natriuretic action for which it is named, can have marked hypotensive effects when injected intravenously into experimental animals (Caramelo *et al.* 1986, De Bold *et al.* 1981) and humans (Bussien *et al.* 1986). The reduction in blood pressure (BP) is primarily caused by a fall in cardiac output (Lee and Goldman 1989), but it may include a variety of peripheral vascular mechanisms. For example, by interacting with specific receptors in vascular smooth muscle, ANF elevates levels of cGMP in the cell, causing relaxation of preconstricted vessels (Rapoport *et al.* 1985, Tremblay *et al.* 1985). It may also reduce plasma levels of angiotensin II by inhibiting the release of renin from the juxtaglomerular apparatus (Scheuer *et al.* 1987). Finally, ANF was shown to lower the secretion of vasopressin (Samson 1985), a hormone which under certain conditions may play a significant role in blood pressure maintenance (Cowley and Liard 1988). By antagonizing such vasoconstrictor influences, endogenous ANF may act as a physiological regulator of the cardiovascular system.

The importance of ANF in long-term regulation of blood pressure is demonstrated in the ANF-transgenic mouse. We have reported the establishment of a transgenic mouse strain which expresses the mouse ANF structural gene in hepatocytes under the regulation of the transthyretin (TTR) promoter (Steinhelper *et al.* 1990). These animals have a life-long 10- to 20-fold increase in plasma ANF concentration, compared with nontransgenic littermates. Renal sodium excretion is not different. However, there is a persistent reduction in blood pressure by about 20 mm Hg (Steinhelper *et al.* 1990, Field *et al.* 1991). These results indicate that compensatory mechanisms are sufficient to counteract

the natriuretic action of chronic elevation of plasma ANF. In contrast, the hypotensive effects of the hormone are preserved.

The present experiments were designed with the following rationale. If the lower blood pressure in the transgenic mice is dependent on chronic inhibition of angiotensin and/or vasopressin by ANF, the antagonists to either of these vasoconstrictors should have little further effect on blood pressure. On the other hand, if these mechanisms are disproportionately activated in an attempt to counteract other vascular effects of ANF, the antagonists should cause a greater degree of hypotension than in the nontransgenic controls. The results to be reported indicate that inhibition of the vascular actions of vasopressin had little effect on arterial pressure in either transgenic or nontransgenic mice. An angiotensin antagonist reduced blood pressure in both types of animals, although the resultant hypotension was disproportionately larger in the transgenic group. We conclude, therefore, that the ANF-induced chronic reduction in blood pressure does not depend on the inhibition of angiotensin or vasopressin. On the contrary, the data suggest, that the renin-angiotensin system may be stimulated in the ANF-transgenic mice.

Methods

Generation and molecular analysis of the TTR-ANF transgenic mice have been reported previously (Steinhelper *et al.* 1990). Transgenic and nontransgenic mice were maintained in a C3He/FeJ inbred background (Jackson Laboratories, Bar Harbor, Maine). Male TTR-ANF mice (n=7, average body weight = 32.1±1.1 g) and age-matched nontransgenic littermates (n=7, average body weight = 31.3±0.8 g) were anaesthetized with intraperitoneal injection of Inactin (thiobutabarbital, 100 μg/g body weight), and kept at a body temperature near 38 °C by radiant heat. Following tracheotomy, a jugular vein and carotid artery were cannulated (PE10) for intravenous infusion and for blood pressure measurement, respectively. A solution consisting of 2.25 % bovine serum albumin and 1 % glucose in isotonic saline was infused intravenously at 2 μl/min throughout the experiment to maintain euvolaemia (Field *et al.* 1991). Arterial blood pressure was monitored using a Statham strain gauge connected to a Beckman Dynograph.

After completion of surgery, a 20 min recovery period was allowed before beginning continuous blood pressure monitoring for the next 200 min (see Fig. 1). During a 40 min period of control infusion, a bolus of angiotensin II (ANG II, Ciba-Geigy, 6 ng/g body weight) was injected (at 20 min). From 40 to 80 min an infusion containing Saralasin ([Sar^1,Val^5,Ala^8]-angiotensin II, Sigma, 10 ng/g BW min) was substituted. At 70 min a second dose of ANG II was administered. Control infusion was reinstituted (80–160 min), and arginine vasopressin (AVP, Ferring Ltd., Sweden, 40 nano-IU/g BW) was injected at 100 min. A vasopressin V_1 antagonist (d[CH_2]$_5$Tyr[Me]AVP, 10 ng/g BW) was then injected intravenously (at 120 min), followed by a second dose of AVP 30 min later (at 150 min). During the final 40 min of the experiment (160–200 min) the Saralasin-containing infusion was again added. Both ANG II and AVP were injected at 190 min.

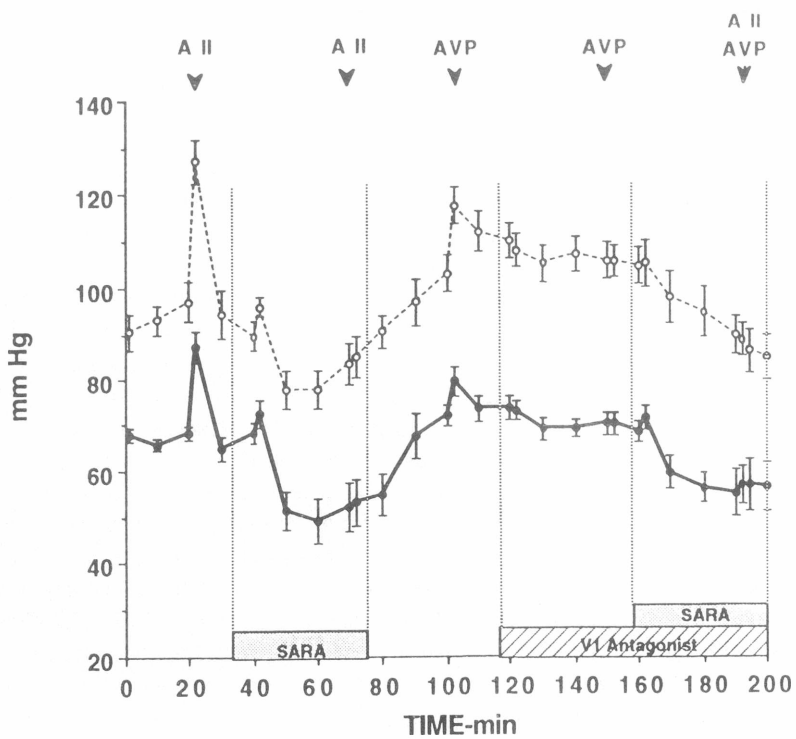

Fig. 1
Average blood pressures vs. time in ANF transgenic (full circles and solid line) and nontransgenic mice (open circles and broken line). Periods of infusion of Saralasin and vasopressin V_1 receptor antagonists are indicated. Intravenous injections of angiotensin II (A II) and arginine vasopressin (AVP) are shown by the arrows.

To assess the effects of the agonists on blood pressure, peak responses to ANG II or AVP were compared to the pre-injection blood pressure in TTR-ANF transgenic and nontransgenic groups. For the antagonists, average blood pressures before and during infusion (Saralasin) or after injection (V_1 antagonist) were compared. Statistical analysis between groups was by one-way ANOVA and by the paired t-test within each group (Sokal and Rohlf 1981). The level of significance was set at 5 %.

Results

The patterns of blood pressure changes throughout the experiment are shown in Fig. 1. As expected, the transgenic group consistently had a significantly lower blood pressure, compared to the nontransgenic group. Both groups had a shortlasting increase in blood pressure after the first ANG II injection, and an initial blood pressure reduction during Saralasin infusion. The effectiveness of angiotensin blockade was demonstrated by the lack of response to the second ANG II injection. After the control infusion had been reinstituted, both groups showed a shortlasting increase in blood pressure with injection of AVP. Following the injection of the vasopressin V_1 receptor antagonist there were slight reductions in blood pressure in both transgenic and nontransgenic groups. No response to the second dose of AVP was seen in either group, indicating complete blockade of the pressor effect of this hormone. Finally, superimposition of Saralasin infusion after V_1 antagonist administration again caused hypotension in both types of animals. As expected, ANG II did not affect blood pressure during this period. Furthermore, the lack of an AVP effect demonstrated the persistence of V_1 receptor blockade.

Table 1
Average blood pressure (mm Hg) in TTR-ANF transgenic and in nontransgenic mice before and during agonist and antagonist administration

	ANG II		Saralasin		AVP		V_1 Antagonist		V_1 Antagonist + Saralasin	
	Before	During	Before	During	Before	During	Before	During	Before	During
Time (min)	20	22	30–40	50–80	100	102	110–120	130–160	130–160	170–200
TTR-ANF (n=7)	68 ±1	87* ±3	67 ±2	52* ±4	72 ±2	80* ±3	73 ±3	69 ±2	69 ±2	57* ±4
Nontransgenic (n=7)	97 ±4	127* ±5	92 ±4	82* ±4	103 ±4	118* ±4	111 ±4	106* ±4	106 ±4	92* ±5

** Significant difference ($p<0.05$) between "Before" and "During" values in each group*

Absolute values of blood pressure during various maneuvers are presented in Table 1. At any given time, blood pressure in the TTR-ANF group was markedly lower than in the control group ($p<0.001$). Equimolar angiotensin injections caused statistically significant increases of blood pressure by 19±4 and 30±4 mm Hg for TTR-ANF and nontransgenic groups, respectively. Saralasin infusions were associated with significant blood pressure decreases of 15±4 and 10±3 mm Hg. Vasopressin administration significantly raised arterial pressure by 8±2 mm Hg in the transgenic group and by 15±2 mm Hg in the control group. V_1 receptor blockade alone resulted in modest blood pressure decreases of 4±1 and 5±1 mm Hg (Fig. 1 and Table 1). These changes were statistically significant only in the nontransgenic group. V_1 receptor blockade did not prevent significant Saralasin-induced hypotension (−12±3 and −14±2 mm Hg, respectively) during the final phase of the experiment (Fig. 1 and Table 1).

To determine whether the changes in blood pressure due to the agonists and antagonists were proportional in each group, absolute increases or decreases were divided by baseline values, and expressed as percentage changes (Table 2). The fractional increases in blood pressure due to angiotensin and vasopressin injections were the same in both groups. Similarly, the fractional decrease during Saralasin infusions, although twice as great in the transgenic compared to the nontransgenic group, was not statistically different ($p=0.06$). No significant differences were observed during the remaining phases of the protocol.

Table 2
Fractional changes in blood pressure (%) with agonist and antagonist administration in TTR-ANF transgenic and in nontransgenic mice

	ANG II	Saralasin	AVP	V_1 Antag	V_1 Antag Saralasin
TTR-ANF (n=7)	21 ±4	−22 ±5	11 ±3	−6 ±3	−18 ±5
Nontransg. (n=7)	23 ±3	−10 ±3	14 ±2	−5 ±2	−14 ±2

The heart rates during the experiment are shown in Fig. 2. There were no statistically significant differences between groups with any maneuver. The injections of angiotensin and vasopressin were not associated with significant changes in heart rate in either group. Saralasin infusion did not alter heart rate, although there was a significant rise in both transgenic and nontransgenic groups after the infusion had been discontinued. Since this rise paralleled the rise in mean arterial pressure (Fig. 1), it may have been due to activation of the catecholaminergic system during and after the period of Saralasin infusion. Further rises in heart rate were observed after administration of the V_1 antagonist.

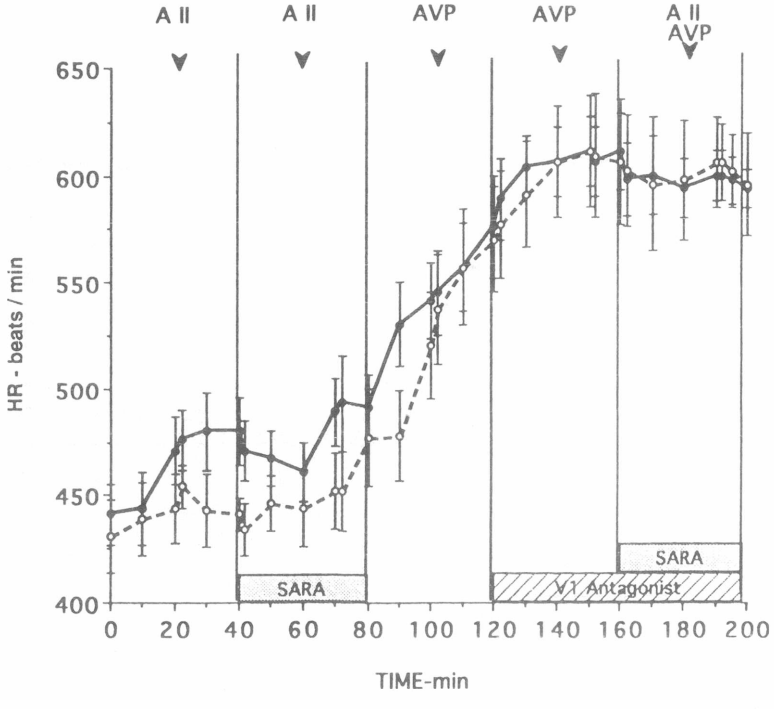

Fig. 2
Average heart rates vs. time in ANF transgenic (full circles and solid line) and nontransgenic mice (open circles and broken line). Periods of infusion of Saralasin and vasopressin V_1 receptor antagonists are indicated. Intravenous injections of angiotensin II (A II) and arginine (AVP) are shown by the arrows.

Discussion

The results indicate that the striking hypotension in ANF transgenic mice, compared to nontransgenic animals was not dependent on inhibition of either angiotensin or vasopressin. If either hormone had been functionally eliminated, there should have been no further reduction in blood pressure after either Saralasin or vasopressin V_1 antagonist in the transgenic group. However, both transgenic and nontransgenic animals showed similar reductions of blood pressure after administration of either Saralasin or V_1 antagonist. It may be concluded, therefore, that the chronic reduction in blood pressure associated with the elevation of ANF was not related to depressed levels of angiotensin or vasopressin.

The proportionately greater reduction of blood pressure in transgenic mice during Saralasin infusion, although not statistically significant, suggests that in these animals the renin-angiotensin system may be activated rather than inhibited. Such activation would be logical as an attempt to compensate for the ANF-induced hypotension. Although plasma renin activity in transgenic mice was not significantly elevated compared to normal controls (Field *et al.* 1991), such a finding does not preclude up-regulation of angiotensin

receptors. To our knowledge there are, as yet, no studies indicating increased receptor density or sensitivity during chronic hypotension. However, a precedent for such regulation is given by the data showing effects of sodium intake on angiotensin receptors (Aguilera *et al.* 1980). Our data, therefore, do not provide support for a functional reciprocity between the ANF and ANG II systems, at least in the long-term regulation. They are also in agreement with short-term experiments showing no effect of angiotensin blockade on the vascular ANF response (Hansell and Ulfendahl 1987).

In contrast to the marked hypotensive effects of Saralasin, blockade of the vascular actions of vasopressin had little effect on blood pressure in either ANF transgenic or nontransgenic mice. Although vasopressin is a potent peripheral vasoconstrictor in most species (Cowley 1982), its effects on blood pressure are buffered by reflex reduction of cardiac output and/or efferent sympathetic outflow (Cowley and Liard 1988). Our data are also compatible with such compensatory mechanisms in the anaesthetized mouse. In addition, they indicate that chronic elevation of plasma ANF concentrations did not interfere with vasopressin-induced direct vasoconstrictor and indirect reflex effects on the circulation. The experiments therefore provide no support for the hypothesis that the reduced blood pressure in transgenic mice is due to the inhibition of cardiovascular vasopressin effects. Conversely, there is also no evidence for compensatory activation of the vasopressin system.

The state of the sympathetic nervous system was not assessed directly in these studies. However, plasma epinephrine and norepinephrine levels in ANF transgenic mice are not different from those in normal controls (Field *et al.* 1991). Although not conclusive, the lack of significant difference in heart rates between the two groups observed here also argues against a differential involvement of sympathetic mechanisms in the ANF-induced hypotension. Apart from the suggestion of a functional activation of the renin-angiotensin system, the present results indicate that the ANF transgenic mice sustain their life-long reduction in blood pressure without major signs of cardiovascular stress. This raises the fascinating question why mice (and men) normally maintain much higher levels of blood pressure.

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