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Regulation of the Myocardial Endothelin System by Angiotensin-II and Losartan

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Summary: Evidence for interactions between the endothelin (ET) and renin–angiotensin systems is plentiful *in vitro*, but few studies have investigated these interactions *in vivo*. In the study reported here, we have investigated the influence of chronic angiotensin-II (A-II) infusion *in vivo* on expression of preproendothelin-1 (PPET-1) and endothelin-A- (ET_A) and endothelin-B- (ET_B) receptor mRNA in the heart. The role of the angiotensin type 1 (AT1)-receptor in mediating the actions of A-II was studied using losartan, the selective AT1-receptor antagonist. Male rats received an infusion of A-II (200 ng/kg/min) or vehicle for 14 days via mini-osmotic pumps; losartan (10 mg/kg/day) was administered in the drinking water. PPET-1 and ET_A- and ET_B-receptor mRNA were detected in

heart sections using nonradioactive antisense *in situ* hybridization. Independent treatments with either A-II or losartan had no significant effect on PPET-1, ET_A- or ET_B-receptor expression. Combined treatment resulted in an increase in PPET-1 mRNA ($p < 0.001$) and ET_B-receptor mRNA expression ($p < 0.01$), while ET_A-receptor mRNA expression was decreased ($p < 0.001$). These results suggest that selective AT1-receptor blockade, in the presence of an elevated plasma A-II concentration, causes upregulation of ET-1 synthesis in the myocardium as well as modification of ET receptor expression. These effects may be mediated via angiotensin type 2 (AT2)-receptors. **Key Words:** Angiotensin-II (A-II)—Endothelin (ET)—AT1-receptor antagonist—Myocardium—ET_A-receptor—ET_B-receptor.

Angiotensin-II (A-II) and endothelin-1 (ET-1) are potent vasoconstrictor peptides that also exert a number of important effects in the myocardium, including regulation of growth. The effects of ET-1 and A-II are mediated by endothelin-A- (ET_A) and endothelin-B- (ET_B) (1) and angiotensin type 1 (AT1)- and angiotensin type 2 (AT2)- (2) receptors respectively. Many studies have shown that the independent effects of ET-1 and A-II on cardiac function and structure are mediated via their respective predominant myocardial receptors, ET_A and AT1 (3). However, it has become increasingly clear that the two systems also act synergistically. A-II increases ET-1 protein and mRNA expression in cultured rat fibroblasts (3), and stimulates cardiomyocyte hypertrophy via ET_A-receptor (4). Increased ET_B-receptor expression in cultured cardiomyocytes by A-II has also been reported (5). Although cell culture experiments predict the potential importance of these interactions, the evidence for interaction between the A-II and the ET sys-

tem in the heart *in vivo* is less conclusive. Recent studies have demonstrated the existence of an intramyocardial ET system. However, no studies have localized or quantified changes in preproendothelin-1 (PPET-1) or ET_A- and ET_B-receptor expression in response to elevated plasma levels of A-II, or assessed whether treatment with losartan, the A-II AT1-receptor antagonist, affects ET gene expression *in vivo*. The hypothesis that we have tested is that A-II initiates changes in ET system expression in the heart *in vivo* and that the effects are mediated by the AT1-receptor.

MATERIALS AND METHODS

Animals and A-II infusion with and without losartan

Adult male, Wistar rats (200–250 g) were anaesthetized (sodium pentobarbital, 60 mg/kg) and implanted with Alzet (Alza Corp., CA, U.S.A.) mini-osmotic pumps for delivery of A-II (200 ng/kg/min) or vehicle. They

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TABLE 1. Effects of angiotensin-II (A-II) with and without losartan on systolic blood pressure (SBP), plasma renin activity (PRA) and plasma aldosterone concentration

	Control	A-II	Losartan	A-II + losartan
SBP (mmHg)	150.00 ± 7.00	191.00 ± 13.00 ^a	130.00 ± 6.00	136.00 ± 8.00
PRA (ng A-I/ ml/h)	0.45 ± 0.06	0.05 ± 0.11 ^a	2.54 ± 0.78 ^a	2.12 ± 0.61 ^a
Aldosterone (nM)	0.42 ± 0.15	1.78 ± 0.77	0.38 ± 0.06	0.65 ± 0.18

^ap < 0.05, n = 8 per group.

were simultaneously given drinking water with or without losartan (Merck Sharp & Dohme, St. Louis, MI, U.S.A., 10 mg/kg/day). The treatments were given for 14 days. Experiments were conducted under licensed approval by the Home Office, London, U.K.

Blood pressure

At the end of the treatment, systolic blood pressure was measured by tail-cuff plethysmography, after which rats were exsanguinated, and blood was collected for measurement of plasma hormones by radioimmunoassay (6). Hearts were cut longitudinally through the septum, fixed in 10% formalin and embedded in paraffin wax.

In situ hybridization and quantification of mRNA

The method for detecting PPET-1 and ET receptor mRNA expression using nonradioactive in situ hybridization in paraffin sections has been described previously (7). Two random consecutive sections from each heart were used to semi-quantify PPET-1, ET_A- and ET_B-receptor mRNA signal (8) using a computerized image analysis system (Zeiss, Imaging Associates, Cambridge, U.K., Kontron 300). Four areas in each right and left ventricle were selected blindly by application of a frame of known area (126 015 μm²).

Statistical analyses

The intra-assay variation for each probe was < 5%, thus data from two sections for each probe were pooled and averaged. Values are expressed as mean ± SEM from eight animals per group. Data were analysed using analysis of variance (ANOVA). Unpaired observations were assessed using Student's *t*-test. A value of p < 0.05 was considered significant.

RESULTS

Blood pressure and plasma hormone measurements

After 2 weeks of treatment, A-II increased blood pressure (Table 1). Losartan blocked the effects of A-II and lowered blood pressure when administered alone. A-II suppressed plasma renin activity (PRA, p < 0.05) but had no significant effect on plasma aldosterone levels (Table 1). Losartan caused an increase in PRA (p < 0.05), with or without A-II infusion (p < 0.05), but had no significant effect on plasma aldosterone.

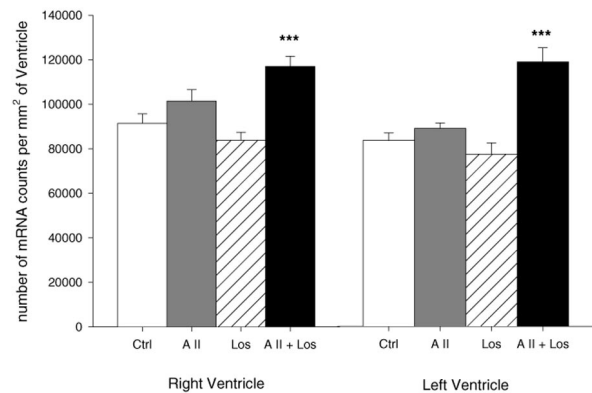


FIG. 1. The effects of angiotensin-II (AII) and/or losartan (Los) on preproendothelin-1 mRNA expression in the left and right ventricle. Control (Ctrl) indicates treatment with AII vehicle and drinking water. Data are expressed as mean ± SEM. ***p < 0.001 vs control, n = 8 per group.

Preproendothelin-1 mRNA expression

In both right and left ventricles, PPET-1 mRNA was distributed ubiquitously in endothelial cells and cardiomyocytes. Treatments with A-II or losartan given alone tended to increase and decrease PPET-1 mRNA levels respectively, although the effects were not significant (Fig. 1). However, concomitant treatment with A-II + losartan caused a clear, significant increase in PPET-1 expression in both right and left ventricles (p < 0.001).

ET_A-receptor mRNA expression

ET_A-receptor mRNA was also expressed abundantly throughout the myocardium. Expression levels in both ventricles were unaffected by treatments with A-II or losartan alone (Fig. 2), but decreased significantly in left and right ventricles when A-II + losartan was given concomitantly (p < 0.001).

ET_B-receptor mRNA expression

ET_B-receptor mRNA, expressed less abundantly in the heart than either PPET-1 or ET_A-receptor mRNA, was unaffected by treatment with A-II or losartan alone (Fig. 3). However, expression in the left and right vent-

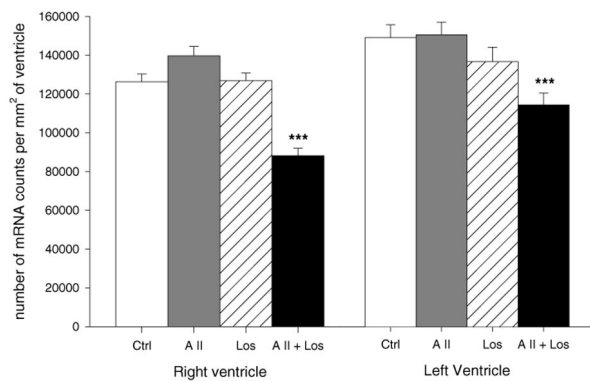


FIG. 2. Effects of angiotensin-II (AII) and/or losartan (Los) on endothelin-A-receptor mRNA expression in right and left ventricles of the rat myocardium. Control (Ctrl) indicates treatment with AII vehicle and drinking water. Data are expressed as mean \pm SEM. *** $p < 0.001$ vs control, $n = 8$ per group.

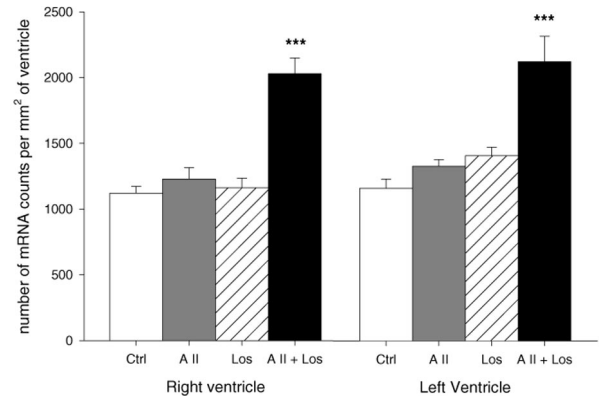


FIG. 3. Effects of angiotensin-II (AII) and/or losartan (Los) on endothelin-B-receptor mRNA expression in right and left ventricles of the rat myocardium. Ctrl, control. *** $p < 0.001$ vs control, $n = 8$ per group.

ricles increased when A-II and losartan were given simultaneously ($p < 0.001$).

DISCUSSION

This study demonstrates that the myocardial ET system gene expression in vivo is modulated by A-II, but only when combined with AT1-receptor blockade. This is despite the effects of A-II on plasma renin activity and blood pressure, and on the proliferation of myocardial fibroblasts, smooth muscle and endothelial cells in the same animals (6). Accordingly, we hypothesize that when AT1-receptors are inhibited, regulation of PPET-1 gene expression by A-II acting through A-II type 2 (AT2) receptors is revealed. Given that administration of A-II alone had no effect and that AT2-receptor expression is low in the heart (9), it is possible that high circulating levels of A-II may be required to stimulate AT2-dependent PPET-1 expression. Alternatively, a phenotypic change in AT2-receptor function may occur when the AT1-receptor is inhibited (9). Clearly studies using a selective AT2-receptor antagonist are now required to confirm whether regulation of PPET-1 expression is dependent on AT2-receptors.

The unexpected finding that A-II given without losartan had no net effect on myocardial PPET-1 expression, contrary to in vitro experiments (3,4), may be explained by variations in tissue responsiveness to A-II in vivo. While increases in ET-1 synthesis in renal and vascular tissue have been reported (10,11), the same dose of A-II as that used in the present study had no effect on ventricular ET-1 synthesis in vivo (10). Taken together, these studies suggest that myocardial PPET-1 expression is not controlled by plasma A-II in the absence of A-II receptor inhibitors.

Concomitant A-II and losartan decreased and increased ET_A - and ET_B -receptor expression respec-

tively, while A-II on its own had no effect on expression. It is possible that ET receptor expression may be co-regulated by both AT1 and AT2 to produce no net effect of A-II alone, but effects are only expressed with the use of an AT receptor antagonist. Alternatively, it may be argued that decreased ET_A -receptor expression is a consequence of elevated PPET-1 expression involving ET_A -receptor downregulation, and that ET_B -receptor expression may be increased to act as a myocardial clearance receptor for increased ET-1.

In conclusion, these results suggest that selective AT1-receptor blockade, in the presence of elevated plasma A-II concentration, causes upregulation of ET-1 synthesis in the myocardium as well as modification of the relative roles of ET_A - and ET_B -receptors mediating its actions. These effects may be caused by A-II acting through AT2-receptors. The results may have important clinical implications for the use of losartan and require further investigation.

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