

## EFFECTS OF NEURONAL BLOCKADE OF NORADRENALINE REUPTAKE IN AN EXPERIMENTAL MODEL OF HEART FAILURE

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### ABSTRACT

We investigated neuronal uptake of noradrenaline (NA) at the level of larger vessels (thoracic aorta and vena cava; left renal artery and left renal vein; lateral saphenous artery and lateral saphenous vein and finally central ear artery and marginal ear vein) in a model devised to mimic heart failure. The model presented here is the rabbit coronary ligation model in which myocardial infarction was produced in male New Zealand white rabbits (2.6kg-3.0kg) by ligation of the marginal branch of the left descending coronary artery. The development of chronic heart failure was allowed to proceed over eight weeks. Animals were killed by overdose with pentobarbitone sodium (IV injection). Arteries and veins were carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS). The arterial and venous rings were mounted in 10mL isolated organ baths, bathed in Krebs maintained at 37°C and gassed with 95% O<sub>2</sub> plus 5% CO<sub>2</sub>. The rings were then placed under different resting tensions. They were allowed to equilibrate for 1 hour before the experiments. Initially all tissues were exposed to cumulative concentrations of NA (1nM-300μM). Following complete washout, the preparations were left for 45 minutes to re-equilibrate. After preincubation with cocaine (10μM) for 10-15 minutes to inhibit neuronal uptake of NA, final NA cumulative concentration-response curves (CCRC) were conducted. Arterial plasma noradrenaline is 163% higher in patients with heart failure than in control patients. High plasma noradrenaline correlates directly with the hemodynamic severity of the disease and inversely with survival. Activation of the sympathetic nervous and renin-angiotensin systems may be important in the pathophysiology of heart failure associated with severity of the disease. Elevated levels of circulating noradrenaline in heart failure may result from impaired peripheral reuptake of this catecholamine. Cocaine has generally been used as the prototype drug for inhibition of neuronal uptake of catecholamines. The aim of our study was to investigate the possibility of changing reuptake of noradrenaline by

## Effects of Neuronal Blockade of Noradrenaline Reuptake in Heart Failure

using cocaine in this model of heart failure. In conclusion, effects of cocaine on noradrenaline responses were identical in sham operated compared with coronary ligated rabbits. These results suggest normal neuronal uptake of noradrenaline in this model of heart failure.

*MJIRI, Vol.18, No.3,265-272, 2004.*

**Keywords:** Heart Failure, Cocaine, Noradrenaline, Larger Vessels

### INTRODUCTION

Chronic heart failure is a clinical syndrome characterized by the inability of the heart to provide adequate nutrient supply to metabolically active tissues.<sup>1</sup> Sudden cardiac death claims an estimated 350,000 lives per year in the United States and between 50,000 and 100,000 lives a year in the United Kingdom. There are numerous underlying diagnoses in patients suffering sudden cardiac death. In 75% of cases, the underlying pathology causing heart failure in patients with sudden cardiac death is coronary heart disease.<sup>2</sup> Heart failure has been described as a condition of generalized neurohumoral excitation, characterized by activation of the sympathetic nervous and renin-angiotensin systems, increases in plasma vasopressin concentration, and parasympathetic withdrawal.<sup>3</sup> Elevated plasma noradrenaline resulting from increased noradrenaline release and decreased noradrenaline

reuptake can act as a natural agonist on vascular  $\alpha$ -adrenoceptors, which mediate vasoconstriction via vascular beds.<sup>4</sup> Several animal models of human congestive heart failure (CHF) have been developed in attempts to reproduce these features to study the pathogenic mechanisms involved in this disease. The coronary artery occlusion model of heart failure in the rat has been extensively studied. The model has been validated by the measurement of hemodynamic variables.<sup>5</sup> Rapid ventricular pacing in the dog has been shown to fulfill the clinical, radiographic and haemodynamic definitions of congestive heart failure.<sup>6</sup> In coronary ligation model it has become recognized that collateral flow is the most important determinant of the rate and extent of cell death within an ischemic zone. Collateral flow in the rabbit has been shown to be essentially very poor, similar to the human and pig.<sup>7</sup> Since in 75% of cases, the underlying pathology causing heart failure in patients with sudden cardiac death is coronary

**Table I.** Comparison of noradrenaline  $pD_2$  in four pairs of arteries and veins of the coronary ligated and sham operated rabbits 8 weeks after operation.

| Vessel           | $pD_2$<br>The 1st CCRC<br>to NA | $pD_2$<br>The 1st CCRC<br>to NA | $pD_2$<br>The 2nd CCRC<br>to NA | $pD_2$<br>The 2nd CCRC<br>to NA |
|------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                  | Sham                            | Ligated                         | Sham +<br>Cocaine               | Ligated+<br>Cocaine             |
| Aorta            | 6.96 ± 0.08                     | 6.72 ± 0.28                     | 6.88 ± 0.09                     | 6.72 ± 0.18                     |
| Vena cava        | 6.23 ± 0.18                     | 5.95 ± 0.15                     | 6.28 ± 0.11                     | 5.89 ± 0.23                     |
| Renal artery     | 6.07 ± 0.08                     | 6.11 ± 0.16                     | 6.28 ± 0.1                      | 6.48 ± 0.2                      |
| Renal vein       | 6.46 ± 0.21                     | 6.29 ± 0.17                     | 6.39 ± 0.12                     | 6.34 ± 0.17                     |
| Saphenous artery | 6.43 ± 0.13                     | 6.72 ± 0.18                     | 7.09 ± 0.06                     | 7.32 ± 0.2                      |
| Saphenous vein   | 6.86 ± 0.16                     | 6.33 ± 0.17                     | 7.24 ± 0.09                     | 7.12 ± 0.17                     |
| Ear artery       | 7.04 ± 0.03                     | 6.65 ± 0.2                      | 6.99 ± 0.11                     | 7.04 ± 0.1                      |
| Ear vein         | 7.8 ± 0.25                      | 7.72 ± 0.13                     | 7.79 ± 0.1                      | 7.57 ± 0.12                     |

$pD_2$  is expressed as the  $-\log$  of the  $EC_{50}$  (concentration producing 50% of the maximum response) of noradrenaline (NA). Initially all tissues were exposed to cumulative concentrations of noradrenaline (1nM-300 $\mu$ M). Following washout, the preparations contracted with noradrenaline cumulatively in the presence of cocaine (1 $\mu$ M) added 10-15min. before to inhibit neuronal uptake of noradrenaline.

Data are expressed as mean  $\pm$  s.e.mean (n=6). Statistical comparisons with controls were carried out using unpaired Student's t test. Effects of cocaine were not significantly different in sham operated compared with coronary ligated rabbits.

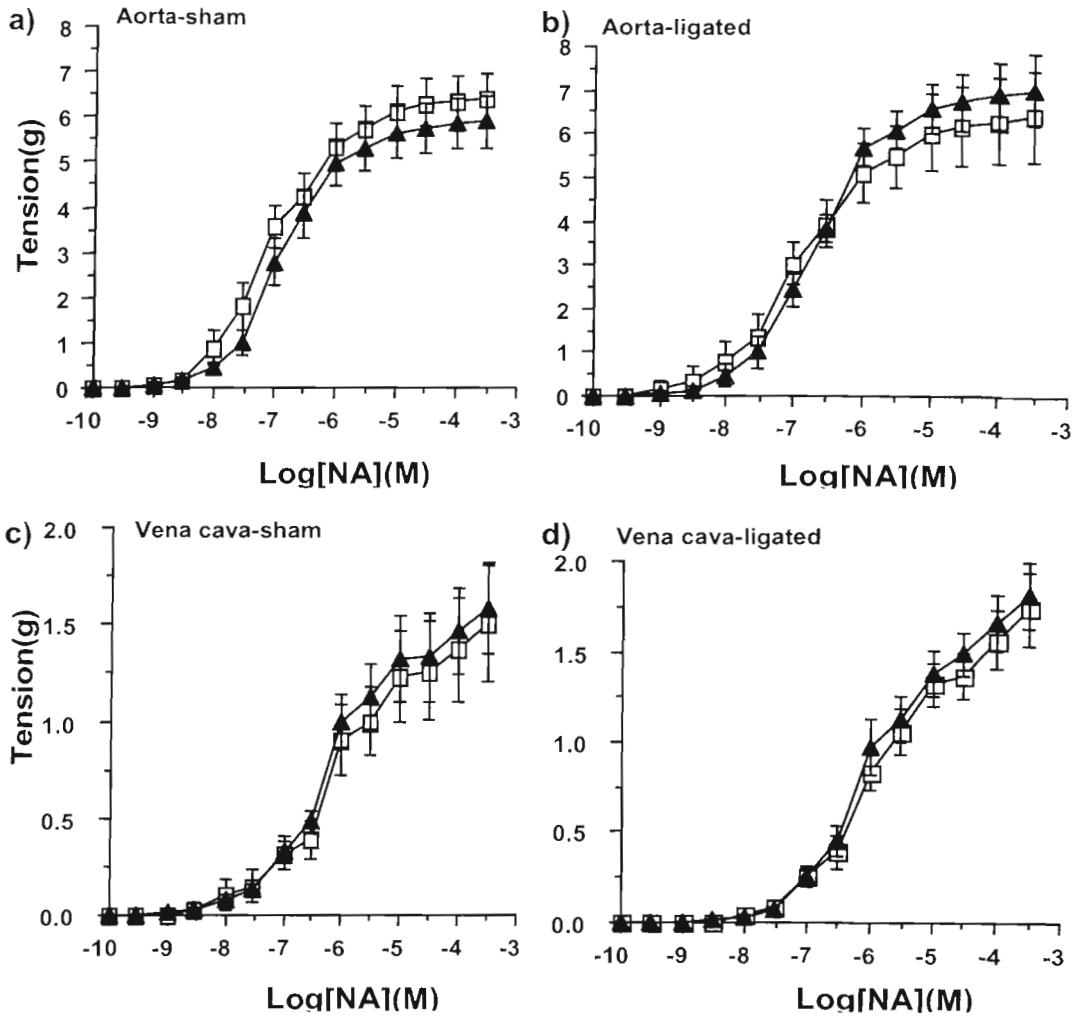


Fig. 1. CCRC to NA and effect of cocaine (1 $\mu$ M) in the isolated aorta and vena cava of the sham operated rabbits with mean ejection fraction of (70.5  $\pm$  2.13) and coronary ligated rabbits with mean ejection fraction of (46.5  $\pm$  4.4) 8 weeks after operation. The CCRC to NA is represented by (□) while (▲) represents CCRC to NA in the presence of (1 $\mu$ M) cocaine. Each point represents mean  $\pm$  s.e.mean (n=6).

heart disease, we employed this model that produces a similar circumstance to coronary heart disease.

It has been shown that plasma noradrenaline is higher in patients with heart failure than in control patients. Elevated levels of circulating noradrenaline in heart failure may result from impaired peripheral reuptake of this catecholamine.<sup>8</sup> Cocaine has generally been used as the prototype drug for inhibition of neuronal uptake of catecholamines. Since cocaine has both a sympathomimetic effect (inhibition of neuronal uptake of noradrenaline) and, at higher concentration, a local anesthetic property (Na<sup>+</sup> channel blockade), caution should be exercised in relation to the concentration of cocaine. We used 1 $\mu$ M cocaine to inhibit neuronal reuptake of noradrenaline and avoid the local anesthetic effect.<sup>9</sup>

Several studies that investigated reuptake of noradrenaline in heart failure reported conflicting results in experimental heart failure. Some of them suggested decreased reuptake of noradrenaline effectively increases the concentration of this catecholamine in heart failure.<sup>10</sup> In one experiment conducted in patients with heart failure small arteries showed evidence of an impaired neuronal uptake mechanism, since blockade by cocaine had no effect on noradrenaline induced vasoconstriction in these vessels.<sup>11</sup> Some other reporters found no difference in reuptake of noradrenaline between experimental heart failure and control groups.<sup>12</sup> Therefore, the aim of our study was to investigate the possibility of changing reuptake of noradrenaline by employing cocaine in this model of heart failure.

## Effects of Neuronal Blockade of Noradrenaline Reuptake in Heart Failure

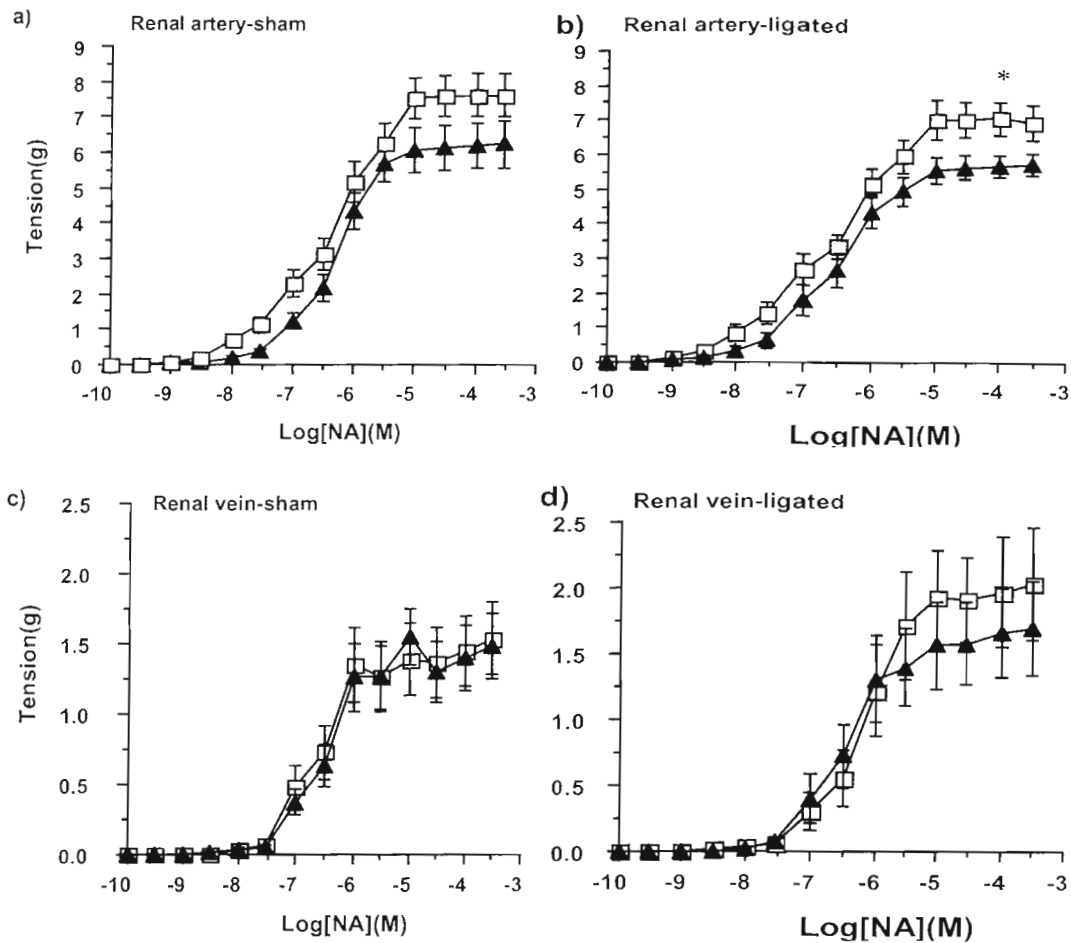


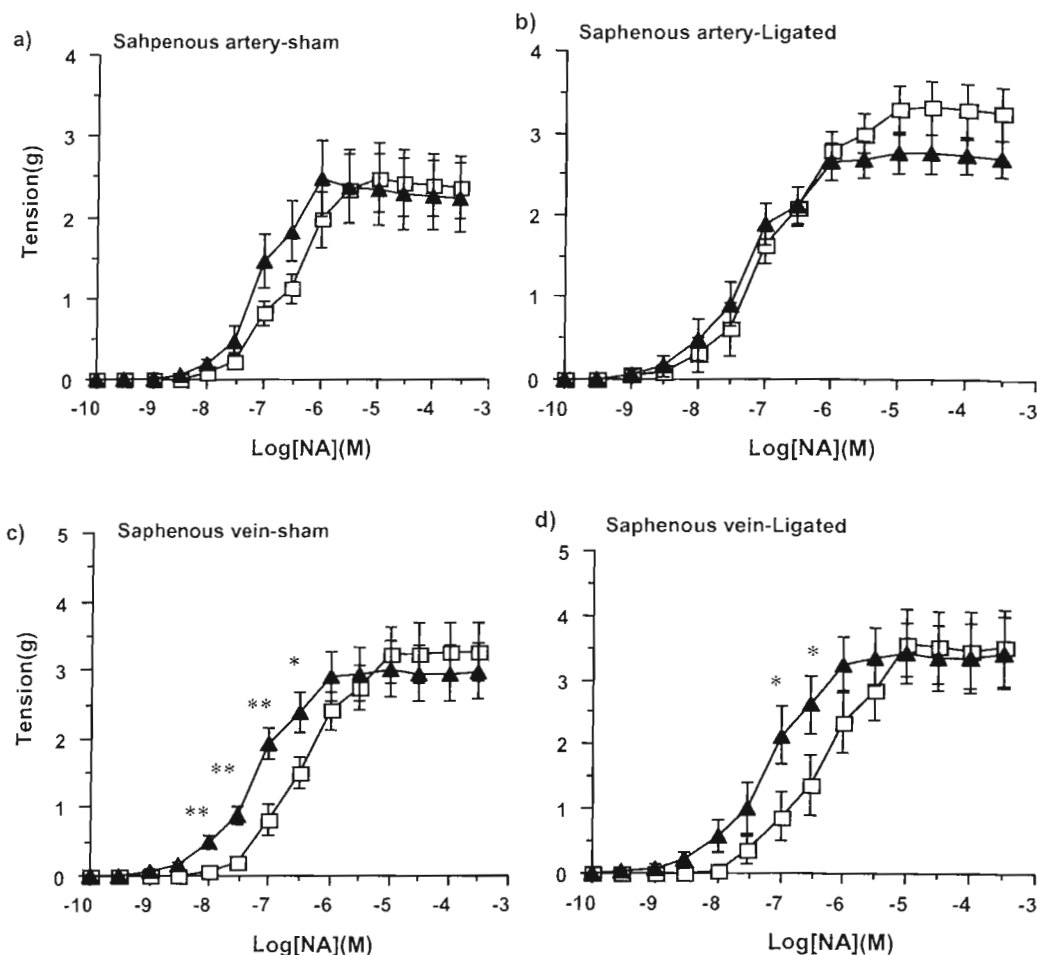
Fig. 2. CCRC to NA and effect of cocaine ( $1\mu\text{M}$ ) in the isolated renal artery and renal vein of the sham operated rabbits with mean ejection fraction of ( $70.5 \pm 2.13$ ) and coronary ligated rabbits with mean ejection fraction of ( $46.5 \pm 4.4$ ) after 8 weeks operation. The CCRC to NA is represented by ( $\square$ ) while ( $\blacktriangle$ ) represents CCRC to NA in presence of ( $1\mu\text{M}$ ) cocaine. Each point represents mean  $\pm$  s.e.mean ( $n=6$ ). Statistically significant differences are represented by \*  $p < 0.05$ , paired Student's t-test.

### MATERIAL AND METHODS

The model was prepared by M. Hicks and co-workers in the Royal Infirmary, Glasgow.<sup>13</sup> Myocardial infarction was produced in male New Zealand white rabbits (2.6kg-3.0kg) by ligation of the marginal branch of the left descending coronary artery. The development of chronic heart failure was allowed to proceed over eight weeks. Sham operated animals underwent a similar procedure but no ligation was performed. Four pairs of arteries and veins (thoracic aorta and vena cava; left renal artery and left renal vein; lateral saphenous artery and lateral saphenous vein and finally central ear artery and marginal ear vein) of the sham operated with mean ejection fraction of ( $70.5 \pm 2.13$ ) and coronary ligated rabbits with mean ejection fraction of ( $46.5 \pm 4.4$ ), as

determined by echocardiography, were studied. Animals were killed by overdose with pentobarbitone sodium (IV injection). Arteries and veins were carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS). Each preparation was cut transversely into 3-4mm rings and was suspended horizontally by means of two stainless-steel L-shaped hooks carefully passed through the lumen. The upper support was connected by cotton to an isometric transducer while the lower support was connected to a glass tissue holder. The rings of veins and arteries were mounted in a 10mL isolated organ bath, bathed in Krebs, maintained at  $37^\circ\text{C}$  and gassed with 95%  $\text{O}_2$  plus 5%  $\text{CO}_2$ .<sup>14</sup> Isometric contractions were measured by a Grass FT03 transducer connected to a Linseis (TYP 7208) pen recorder.

Blood vessels were used immediately. The rings were



**Fig. 3.** CCRC to NA and effect of cocaine (1 $\mu$ M) in the isolated saphenous artery and saphenous vein of the sham operated rabbits with mean ejection fraction of (70.5  $\pm$  2.13) and coronary ligated rabbits with mean ejection fraction of (46.5  $\pm$  4.4) 8 weeks after operation. The CCRC to NA is represented by (□) while (▲) represents CCRC to NA in presence of (1 $\mu$ M) cocaine. Each point represents mean  $\pm$  s.e.mean (n=6). Statistically significant differences are represented by \*  $p$ <0.05, \*\* 0.001< $p$ <0.01, paired Student's t-test.

then placed under different resting tensions which were determined by contraction to NA (1 $\mu$ M) from some preliminary experiments. They were allowed to equilibrate for 1 hour before the experiments. Initially all tissues were exposed to cumulative concentrations of NA (1nM-300 $\mu$ M). Following complete washout, the preparations were left 45 minutes to re-equilibrate. After preincubation with cocaine (10 $\mu$ M) for 10-15 minutes to inhibit neuronal uptake of NA, final NA cumulative concentration-response curves (CCRC) were conducted cumulatively. Response to each contractile agonist is expressed as absolute tension (g). Statistical analysis of data was performed with paired and unpaired Student's t test. P-values less than 0.05 were assumed significant. All data are given as mean  $\pm$  s.e.mean.

#### Solutions and drugs

The composition of the modified Krebs-Henselite

solution was as follows (in mM): NaCl 118.4, NaHCO<sub>3</sub> 25, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.6, MgSO<sub>4</sub> 0.6, CaCl<sub>2</sub> 2.5 and glucose 11. Na<sub>2</sub>EDTA (23 $\mu$ M) was also included in the Krebs in all experiments to prevent degradative oxidation of noradrenaline and propranolol (1 $\mu$ M) was also included to inhibit  $\beta$ -adrenoceptors. The following compounds were used: (-)-noradrenaline bitrate (Sigma); propranolol HCl (Sigma); cocaine HCl (Mac Carthys). All drugs were dissolved in distilled water. All concentrations of the drugs used are expressed as final concentration in the organ bath.

## RESULTS

#### Effect of cocaine treatment

Cocaine (1 $\mu$ M) was used to inhibit neuronal uptake of noradrenaline in the isolated arteries and veins. All the

## Effects of Neuronal Blockade of Noradrenaline Reuptake in Heart Failure

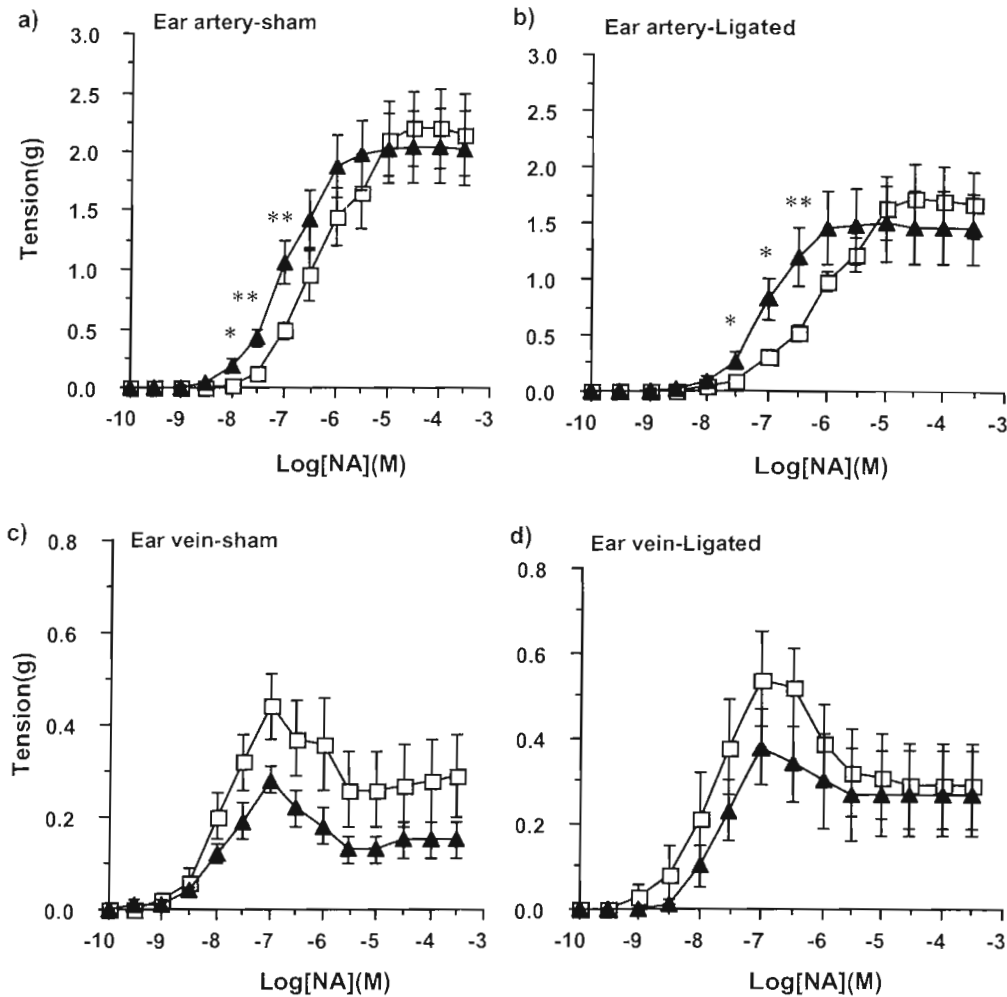


Fig. 4. CCRC to NA and effect of cocaine ( $1\mu\text{M}$ ) in the isolated ear artery and ear vein of the sham operated rabbits with mean ejection fraction of ( $70.5 \pm 2.13$ ) and coronary ligated rabbits with mean ejection fraction of ( $46.5 \pm 4.4$ ) 8 weeks after operation. The CCRC to NA is represented by ( $\square$ ) while ( $\blacktriangle$ ) represents CCRC to NA in presence of ( $1\mu\text{M}$ ) cocaine. Each point represents mean  $\pm$  s.e.mean. ( $n=6$ ). Statistically significant differences are represented by \*  $p < 0.05$ , \*\*  $0.001 < p < 0.01$ , paired Student's t-test.

tissues tested from sham, only in ear artery and saphenous vein was the second CCRC to noradrenaline in the presence of cocaine, shifted to the left indicating increased sensitivity to noradrenaline. In the ear artery cocaine allowed recovery to a value not unexpectedly different from the first test, but in saphenous vein the test in cocaine exceeded even in value that of the first test. These effects (and non-effect) of cocaine were reduced similarly in both sham and ligated rabbits (Table I and Figures 1, 2, 3, 4).

### DISCUSSION

Cocaine has been shown to activate the sympathetic system resulting from inhibition of noradrenaline uptake at the sympathetic nerve terminal.<sup>15</sup> Arterial plasma

noradrenaline is 163% higher in patients with heart failure than in control patients. High plasma noradrenaline correlates directly with the hemodynamic severity of the disease and inversely with survival.<sup>11</sup> Elevated levels of circulating noradrenaline in congestive heart failure may result from impaired peripheral reuptake of this catecholamine.<sup>8</sup> Our results in the present experiment showed that neuronal uptake of noradrenaline plays no significant role in aorta, renal artery, renal vein, vena cava and ear vein of the rabbit. In spite of clear evidence for a substantial effect of cocaine on noradrenaline uptake in a variety of tissues,<sup>16</sup> in our experiment cocaine treatment ( $1\mu\text{M}$ ) had only a very modest effect on development of mechanical responses to exogenous noradrenaline in ear artery, saphenous vein or artery.

Cocaine has generally been used as the prototype drug for inhibition of neuronal uptake of catecholamines. Furchgott and co-workers showed that a maximum potentiation of the response to noradrenaline in rabbit aorta and guinea pig and cat left atria could be obtained with 10-30  $\mu$ M cocaine.<sup>15</sup> Since catecholamine neuronal uptake is the major means of terminating sympathetic neural transmission, the adrenergic response is potentiated by cocaine. Inhibition of neuronal uptake by cocaine can have minor effects on responses to exogenous noradrenaline in blood vessels, particularly when those blood vessels have a network of sympathetic neuroeffector complexes that is small in relation to the muscular layer.<sup>16</sup> These results suggest that the density of sympathetic innervations of mentioned preparations are significantly less than that of ear artery and saphenous vein. It appears that among arteries and veins that were studied, ear artery and saphenous vein have significant innervations, which can influence sensitivity to noradrenaline. Also in humans cocaine can inhibit noradrenaline uptake at the sympathetic nerve peripheral circulation. It has been shown that intravascular administration of cocaine increased vascular resistance in human. In human coronary arteries cocaine administration by various methods produced vasoconstriction and induced myocardial ischemia that all related to increase of noradrenaline in peripheral blood vessels.<sup>17,18</sup>

In the present experiment, effects of cocaine on noradrenaline responses were identical in sham operated compared with coronary ligated rabbits. Activation of the sympathetic nervous and renin-angiotensin systems may be important in the pathophysiology of heart failure associated with severity of the disease. It has been supposed that excess activation of the sympathetic system may contribute to the reduction of peripheral reuptake of noradrenaline in heart failure.<sup>19</sup> This finding indicates no impairment of noradrenaline neuronal reuptake in this model of heart failure but the mechanisms behind the potentiated effects of the sympathetic system in heart failure need further investigation.

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## Effects of Neuronal Blockade of Noradrenaline Reuptake in Heart Failure

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