Changes in the Circulating Endothelin and Atrial Natriuretic Peptide Levels during Coronary Artery Bypass Surgery

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SUMMARY

To evaluate the behavior of circulating endothelin and atrial natriuretic peptide (ANP) during coronary artery bypass graft (CABG) surgery, blood samples from patients with coronary artery disease (n=8) were investigated before, during and after operation. Plasma levels of endothelin and ANP were determined using the radioimmunoassay method. Baseline plasma levels were compared to those of normal volunteers (n=6). Left ventricular function at rest and as a response to isometric exercise was evaluated using radionuclide ventriculography before and after coronary bypass surgery.

The mean endothelin value was found to be within normal limits, however the mean ANP value was slightly higher than control. Patients had significantly improved left ventricular systolic and diastolic function after surgery. The mean endothelin level was higher than initial values immediately after extra-corporeal circulation and returned to initial values in two hours. However, ANP values were increased and remained higher than initial values. Baseline endothelin values were negatively correlated with systolic function parameters, whereas endothelin and heart rate had a positive correlation before extra-corporeal circulation.

Coronary artery bypass graft surgery may cause an increase in the circulating endothelin level either due to endothelial injury or due to myocardial ischemia and hypothermia. Following surgery, increased endothelin levels returned to normal values immediately. (Jpn Heart J **34**: 693–706, 1993)

Key Words:

Endothelin　Atrial natriuretic peptide　Coronary artery bypass graft surgery (CABG-surgery)　Ventricular function　Radioimmunoassay　Radionuclide ventriculography

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VASCULAR endothelium within the coronary arteries is known to release vasodilator and vasoconstrictor agents that regulate coronary blood flow and indirectly affect cardiac contractility. Among the biologically active agents released by vascular endothelium is a recently described group of peptide autocoids known as endothelins.[1] At least three isoforms of endothelin have since been described, each with 21 amino acids and four cysteine residues forming two disulfide bonds. Human vascular endothelial cells function as endocrine and/or paracrine cells for endothelin secretion. Endothelin produces potent and protracted contraction of venous and arterial smooth muscle.[2),3] The precise cellular mechanism of action of endothelin is not known, however, it has been suggested that endothelin might induce its biological actions either by facilitating the intracellular influx of Ca through voltage dependent dihydropyridine sensitive Ca^{2+} channels or by specific receptors.[1),4] The presence of 125 I-endothelin binding sites has been demonstrated in human coronary vessels using *in vitro* autography.[5] This binding is associated mainly with the smooth muscle with some binding to perivascular structures. Additionally, it has been shown that cardiac myocytes have endothelin-1 binding receptor sites.[6] There is recent evidence that endothelin has both positive inotropic[7)-9] and chronotropic[10] effects on the heart.

In addition to its direct action on vascular endothelium, it has been suggested that endothelin may contribute indirectly to the regulation of regional and systemic vascular resistance by modifying the secretion of other neurohumoral mediators, including renin-angiotensin,[11),12] aldosterone[13] and atrial natriuretic peptide.[14)-17] Various clinical conditions have been reported to be associated with an increase in the blood and tissue levels of endothelin such as essential hypertension,[18)-22] myocardial infarction,[23] angina pectoris,[18),25] pulmonary hypertension,[26] heart failure,[27),28] diabetes mellitus[29] and sepsis.[30] In addition, an increase in plasma endothelin has been reported in response to physiological stimuli, such as postural change and the cold pressor test.

Atrial natriuretic factor (ANP), a peptide with potent diuretic, natriuretic and vasorelaxant activity, is released in response to either atrial pressure or to atrial distension.[31] ANP is synthesized by and secreted from the atria of mammals and its production by the atria is increased by neuroendocrine stimuli and by endothelin.[14)-17]

The aim of the study was to assess changes in circulating endothelin and ANP levels during coronary artery bypass surgery (CABG) and to determine the relationship of these parameters with heart rate, blood pressure and renin-aldosterone levels in normotensive patients with an improved left ventricular function after operation as assessed by radionuclide ventriculography (RV).

Materials and Methods

Subjects

The investigation conforms with the principles outlined in the Declaration of Helsinki. The study group consisted of 8 patients with coronary artery disease diagnosed according to coronary arteriogram results who underwent CABG (one female, 7 males, age range: 47–63 years). All patients were normotensive and free of any other systemic and/or metabolic disease. They received only a beta-blocker before surgery. Their left ventricular functions were assessed using radionuclide ventriculography.

All patients underwent CABG in selective conditions. Following median sternotomy, aortic and bicaval cannulations were performed. A Shiley Membrane 2000 oxygenator was used. During extracorporeal circulation, 28°C systemic hypothermia, hemodilution (Hct 28–30%), topical cardiac hypothermia and cold K^+ cardioplegia (St. Thomas No : 2) were used.

Five blood samples were taken before CABG to determine baseline values, before the beginning of extra-corporeal circulation, immediately following and two hours after extra-corporeal circulation and 5 days after surgery, respectively. Changes in heart rate, and systolic and diastolic blood pressures were recorded during the 2 hours following extra-corporeal circulation.

RV was performed 18–24 hours before surgery and 5–6 days after the operation. The imaging protocol was repeated at rest and then during the hand-grip testing to evaluate the response to isometric exercise.

Control group

The control group consisted of 6 healthy volunteers selected from among the medical staff of the hospital and 8 patients with essential hypertension. Their baseline endothelin and atrial natriuretic peptide values were compared to those of the patient group. Radionuclide ventriculography parameters of 15 healthy subjects were compared to the patients' parameters.

Radionuclide ventriculography

Imaging protocol: Autologous red blood cells were labeled with 750–1000 MBq 99mTc-pertechnetate according to *in vivo* labeling methods. Left ventricular function was assessed in a 30–40 degrees left anterior oblique projection in order to separate the right and left ventricles and with 15 degrees of caudal tilt to improve separation between the left atrium and ventricle. The data were collected onto a computer system in 32 frames triggered from the R wave of a single channel ECG monitor. Five million counts were collected to achieve high counting statistics. The time activity curve (TAC) for the left ventricle ROI was obtained using the automatic thresholding technique and background subtraction. The influence of noise was minimized by smoothing the data temporally and spatially. Fourier analysis was then performed on the smoothed data. Phase and amplitude values were derived from the first harmonic of the Fourier transform using the R-R interval as the base frequency. The data were displayed as a color-coded phase map. The data were also presented as a modified phase histogram plotting amplitude against phase. Fourier analysis of TAC with 3 harmonics was performed to derive the systolic and diastolic function parameters. The ejection fraction (EF%), peak filling rate (PFR EDC/sec), peak ejection rate (PER EDC/sec), time to endsystole (TES msec), time to peak filling (TPF msec), time to peak ejection (TPE msec), the ratios TPF/T×100 and TPE/T×100 (T: time interval for one heart beat), 1/3 filling fraction (1/3 FF%), 1/3 ejection fraction (1/3 EF%), 1/3 PER and 1/3 PFR values were calculated. The PFR (EDC/sec) was calculated as the maximum rate of increase in counts during the passive phase of filling, measured in enddiastolic counts. The TPF (msec) was defined as the time from the minimum volume to the peak filling rate. The first third filling fraction (1/3 FF%) was obtained by measuring the percent of the stroke volume in the left ventricle after one-third of the time between minimum volume and the subsequent R wave. The minimum value of the derivative curve is the PER (EDC/sec). The time interval from ED to PER represents TPE (msec). The time interval from enddiastole to endsystole on the volume curve is the time to endsystole (TES). 1/3 EF was the ejection fraction of the first third of the systolic interval. 1/3 PER and 1/3 PFR values are the value of the derivative curve at the point of 1/3 of the systolic and diastolic intervals, respectively.

Images were interpreted visually for myocardial wall motion and pump function and analyzed quantitatively using the functional parameters to compare the values before and after CABG, at rest and during hand-grip testing.

Radioimmunoassay (RIA)

Peripheral venous blood for the baseline determination of endothelin and ANP was taken before the operation in the morning. Blood samples before and after extra-corporeal circulation were taken from the right atrium. Samples were collected in tubes containing 7.5 mM/m*l* EDTA and 500 KIU/m*l* aprotinin, placed on ice and promptly centrifuged at 3000 rpm for 15 min at 4°C. Plasma was then frozen and stored at −70°C until the assay was performed. Determinations were done within one month after the sampling. The RIA was carried out using the Endothelin 1,2 {125 I} assay system (Amersham International plc, U.K.) and alpha-ANP {125I} assay system (Amersham International plc, U.K.). To extract ET from plasma, Amprep C2 columns (RPN 1913) were used and plasma was dried under nitrogen. Then it was reconstituted in 250 μl assay buffer and 100 μl of the sample taken for analysis. For the RIA procedure, standard synthetic ET dilutions ranging from 0.5 to 128 were prepared. 100 μl of the standard solutions and plasma extracts were pipetted into appropriate tubes and incubate for 24 hours at +4°C after addition of 100 μl of rabbit anti-ET1 serum to equal amounts of plasma. One hundred μl of 125I-ET-1 were then pipetted into all tubes and a second incubation for 24 hours at +4°C was carried out. After addition of 250 μl of donkey anti-rabbit serum and 10 minutes incubation at room temperature, the antibody bound fraction was separated in 15 minutes by magnetic separator (Amerlex-M). The radioactivity present in each tube was determined by counting for 60 seconds in a gamma scintillation counter. The sensitivity of the assay is about 1 fmol/tube and the intra-assay coefficient of variation is 4.1%. All samples were assayed in duplicate and processed in a single assay. The antibody included in this kit has a 100% cross-reactivity with synthetic endothelin-1, a 200% cross-reactivity with endothelin-2 and a 38% cross-reactivity with big endothelin-1, while it does not cross-react with endothelin-3. Endothelin and ANP levels are expressed as pmol/L. Plasma renin activity (PRA) was measured using RIA (Cis International, France). PRA activity results are expressed as ng/m*l*/hr. The normal PRA range is 0.2–2.8 ng/m*l*/hr in the supine position. Plasma aldosterone (Medgenix, Belgium) level was also measured using the RIA method. The normal plasma range is 7.5–150 pg/m*l* in the supine position. All values were corrected according to the hemodilution factor using changes in hematocrit. Maximal change in hematocrit was 30%.

Statistical analysis

All data are expressed as mean ± standard deviation. The Mann-Whitney U test was used to analyze the significance of the difference between the patients' and control group's parameters. The difference between the RV parameters at rest and during hand-grip and levels of the peptides during surgery were tested using Wilcoxon and Kruskall-Wallis analyses. The correlation between the function parameters was tested using linear regression analysis. A p value below 0.05 was accepted as significant.

RESULTS

The clinical characteristics of the patients are summarized in Table I.

Changes in the heart rate and systolic and diastolic blood pressure over 120 minutes are shown in Figures 1, 2 and 3, respectively.

The baseline mean endothelin level of patients was not significantly different from that of the control group (26.4±10 pmol/*l* and 24.1±7.2 pmol/*l*), but was lower than in the hypertensive group (34.6±6.4 pmol/*l*, Mann-Whitney

Table I. Clinical Data

Patient No.	Age	Sex	Coronary arteriogram	Baseline endothelin (pmol/l)	Baseline ANP (pmol/l)
1	58	m	LAD, Diag1, OM1, PDA	32.5	17.3
2	60	m	LAD, Lat Cx, Diag1, PDA	40.8	10.9
3	45	m	LAD, Lat Cx, Aneurisma	34.0	80
4	63	f	LAD, OM1, Lat Cx, PDA	35	11.1
5	47	m	LAD, OM1, Lat Cx	22.6	13
6	62	m	LAD, OM1, OM2, RCA, Diag1	15	9.4
7	58	m	LAD, RCA, Diag1	16	26.8
8	48	m	LAD, Diag1&2, RCA, OM1&2	15.5	13

LAD=left anterior descending; RCA=right coronary artery, Diag1=first diagonal branch; Diag2=second diagonal branch; OM1=obtuse marginal 1; OM2=obtuse marginal 2; PDA=posterior descending artery; Lat Cx=lateral circumflex artery.

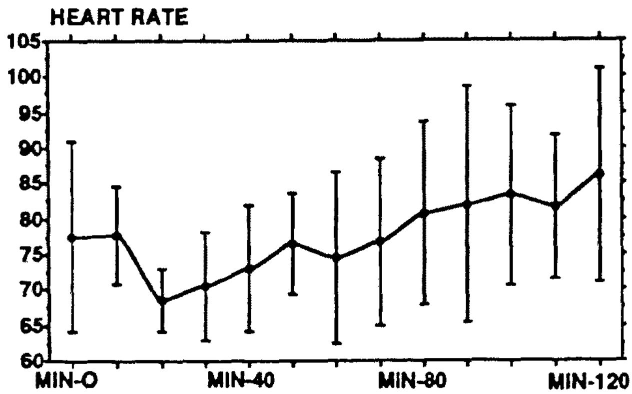

Fig. 1. Time course of the heart rate in 8 patients immediately after extra-corporeal circulation over 120 min. Each point is the mean value and bars indicate standard errors.

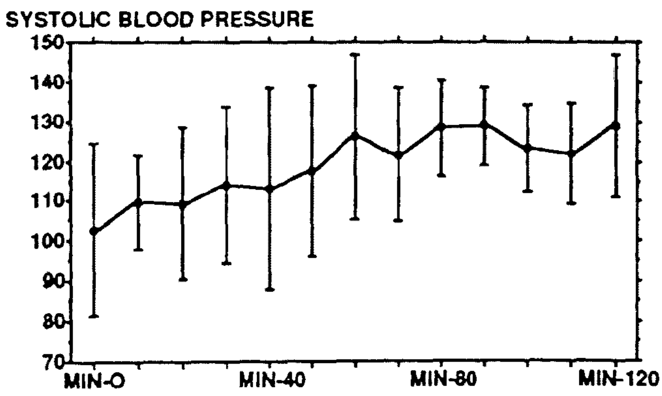

Fig. 2. Change in systolic blood pressure during the operation after extra-corporeal circulation.

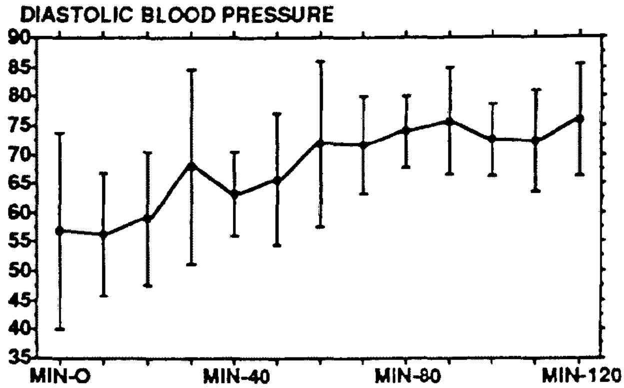

Fig. 3. Change in diastolic blood pressure during the operation after extra-corporeal circulation.

Table II. The Mean Values of Plasma Endothelin, ANP Aldosterone and Renin Activity.

	Baseline I	Before ECC II	After ECC III	2nd hour IV	5th day V
Endothelin (fmol/*l*)*	26.4±10.3	30.4±11	67.9±40.9	30.8±4.9	23.5±5.5
ANP (fmol/*l*)**	22.7±23.8	46.3±21.2	49.6±25.5	45.9±28.4	70.1±19.7
Aldosterone (pg/m*l*)***	99.4±44.4	226.7±146.1	155.9±60.7	309.8±178.5	71.3±31.9
PRA (ng/ml/hr)	1.4±1.4	2.9±2.4	1.0±0.9	1.4±1.4	2.2±2.7

ECC=Extra-corporeal circulation; p value is below 0.05; * I vs III, II vs III, IV vs III and V vs III; ** I vs III and I vs V; *** I vs II, I vs IV, II vs V, III vs IV, IV vs V.

U-test p=0.04). Although the mean ANP value was higher than in the control group (22.7±23.8 pmol/*l* vs 12.3±7 pmol/*l*, Mann-Whitney U-test, p=0.19), the difference was not found to be significant. Baseline ANP and endothelin levels did not correlate with each other.

The change in endothelin level during CABG was statistically significant (Kruskal-Wallis p=0.0005). Table II shows the mean endothelin values at baseline, during the operation and after the operation. ANP also showed a significant change (Kruskal-Wallis test, p=0.005), as did aldosterone (Kruskal-Wallis test, p=0.005). However, plasma renin activity did not show any significant change.

No significant correlation between the endothelin level and blood pressure was observed during or after the operation, however, diastolic blood pressure had a significant correlation with the level of plasma renin activity before extra-corporeal circulation (r=0.8 p=0.02) and a negative correlation with the plasma aldosterone level after extra-corporeal circulation (p=0.01, r=–0.9).

The mean values of the RV parameters before and after the operation are listed in Table III. Before the operation, the rest values of EF, PER, PFR, TPF and 1/3 FF were significantly decreased when compared to the control group

Table III. Mean Values of the Scintigraphic Parameters before and after Coronary Artery Bypass Surgery

	Preoperation			Postoperation			P Value*	
	I		II	III		IV		
	Rest	I vs II	Hand-grip	Rest	III vs IV	Hand-grip	I vs III	II vs IV
EF%	51.3±10**	NS	51.2±9	54.3±8.9	NS	57.8±11	NS	0.06
PER EDC/s	2.59±0.77**	NS	2.57±0.63	3.55±1.09	NS	3.69±0.98	0.01	0.02
TPE ms	144±39	NS	134.8±26	120±41	NS	100.8±29	NS	0.06
1/3 ER EDC/s	1.4±0.81**	NS	1.5±0.07	2.12±0.7	NS	2.3±0.8	0.06	0.03
1/3 EF%	18.1±10	NS	17.3±7	17.7±7	NS	19.7±8.3	NS	NS
TES ms	331±27	0.01	361±30	251±17	NS	264±36	0.01	0.01
PFR EDC/s	1.72±0.52**	NS	1.8±0.4	2.05±0.87	NS	2.4±0.89	NS	NS
TPF ms	167±39**	NS	155±21	105±46	NS	114±32	0.01	0.02
1/3 FR EDC/s	1.02±0.28	NS	1.12±31	1.33±0.6	NS	1.44±0.64	NS	NS
1/3 FF%	29.7±10**	NS	28±9.1	21.5±8.8	NS	25.1±13	NS	NS
TPE/T	14.7±3.7	NS	13.87±2.5	20.75±7	NS	17.5±5	NS	NS
TPF/T	17.6±4	NS	16.6±1.5	17.6±6.9	NS	19.7±5.4	NS	NS

* Wilcoxon analysis. ** Difference is significant when compared to control group; Mann-Whitney test, p<0.05.

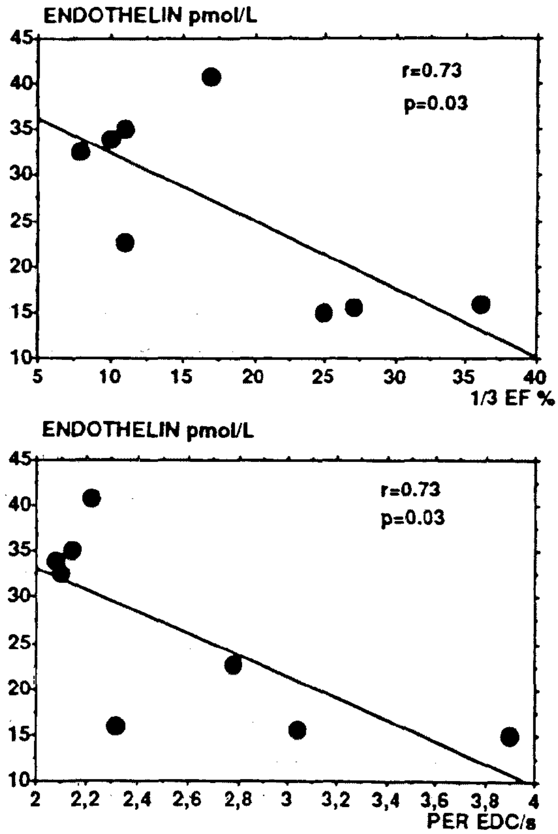

Fig. 4. The correlation between baseline circulating endothelin levels and left ventricular systolic function parameters (PER: peak ejection rate; 1/3 EF: one third ejection fraction)

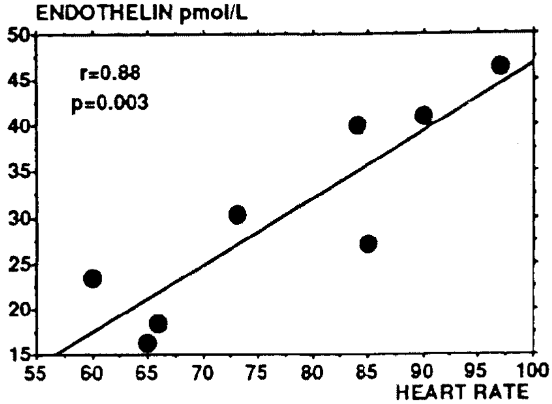

Fig. 5. The correlation between baseline circulating endothelin levels and heart rate before extra-corporeal circulation.

(Mann-Whitney U-test, $p<0.05$). During the hand-grip test, the TES value was prolonged significantly, demonstrating impairment of systolic function. The other parameters did not show any significant improvement. After the operation, the mean PFR value was significantly higher than that of the pre-CABG. The mean TES and TPF values were shortened significantly when compared with those of pre-CABG. During the hand-grip test, significant improvements in PER and 1/3 ER were observed. Although there was no change in EF before the operation as a response to hand-grip, a slight increase in EF was observed postoperatively (from 54.3%±8.9 to 57.8±11). The mean TES and TPF values were prolonged in response to hand-grip. However, their values normalized according to the heart beat TES/T and TPF/T did not change significantly.

Baseline endothelin levels were negatively correlated with rest PER and 1/3 EF values (Fig, 4) ($r=-0.73$, $p=0.03$ and $r=-0.73$, $p=0.03$, respectively). Heart rate and endothelin values before extra-corporeal circulation were also correlated significantly ($r=0.88$ $p=0.003$) (Fig. 5). The other scintigraphic parameters did not correlate with either endothelin or ANP values.

Discussion

Numerous biological reports have attempted to describe the effects of the exogenous administration of endothelin, and its mechanism. However, the physiological and pathophysiological roles of endogenous endothelin remain to be established. Recent studies have demonstrated that it is present in plasma in low concentrations and its plasma concentrations are increased in various pathological states.[18)-30)] However, according to our knowledge there are no reports on the changes in endothelin levels during CABG-surgery and its relationship to atrial natriuretic peptide. Therefore, we investigated the changes in circulating endothelin levels during CABG-surgery and their relation to the changes in atrial natriuretic peptide, aldosterone and renin levels in patients who had an improvement of left ventricular function after surgery as evidence of successful revascularization. Left ventricular systolic and diastolic function was evaluated using RV parameters because this method and quantitative parameters have proven to be reliable and reproducible in the routine assessment of left ventricular function, size and wall motion.

Our results showed that the baseline endothelin levels of the patients were similar to those of healthy volunteers. Normal concentrations were reported to be 1–11 pmol/*l* in 15 healthy subjects by Schuller et al[32] and 5.1±0.5 pmol/*l* in 25 healthy subjects by Davenport et al.[21] Our healthy group had a significantly higher level of endothelin when compared with these data. However, the difference may depend on the specificity of the assay system used in each laboratory. The plasma level of endothelin after declamping increased significantly. However, within two hours it returned to the initial values. Pittet et al[30] found no significant change in endothelin levels in patients studied 24 hours after open heart surgery compared to controls. The increase we observed might have been due to either mechanical injury of the endothelial cells during the procedure or to myocardial ischemia. It is known that ischemic conditions may enhance the production of endothelin. The decline in endothelin levels in our group may indicate the absence of ischemia. Watanabe et al[33] suggested that coronary occlusion damages endocardial cells of the artery and part of the cardiomyocytes, triggering the release of endogenous big endothelin. Big endothelin is then converted to endothelin-1 on the cell membrane and the increase in plasma endothelin-1 causes strong vasoconstriction leading to extension of the myocardial infarct size. Intracoronary administration of endothelin has been shown to produce a profound and long lasting reduction of coronary blood flow with electrocardiographic evidence of myocardial ischemia and depression of myocardial contractility.[34] The effect of endothelin on hemodynamic parameters is known to be long-acting.[35] Maximum response was seen after one hour of infusion and return of flow to basal values took 2 hours. However, it is unknown whether sufficiently high levels would be reached *in vivo*.[6] Meanwhile, the results of Lerman and colleagues[36] support the concept that endothelin has biological action at pathophysiological concentrations, although several studies have established its vasoconstrictor action at pharmacological concentrations.

Besides the vasoconstrictor effect of endothelin, positive inotropic and chronotropic effects probably mediated by the endothelin receptors on the myocytes have also been documented.[7)-10] The increase in sinoatrial nodal rate in a dose dependent manner seen in the isolated right atria of guinea pigs was reported first by Ishikawa et al.[10] The positive chronotropic effect of endothelin was reported to be very potent. In our study, the observed correlation between heart rate and plasma endothelin levels before extra-corporeal circulation might be evidence of a positive chronotropic effect. However, in the other points we could not find any significant correlation. In the literature there are also results indicating that endothelin has a potent negative chronotropic effect due to activation of the inwardly rectifying, muscarinic K^+ channel.[37] Vierhapper et al[38] did not observe a change in heart rate at the plasma concentrations (4–20 pmol/l) obtained with a low dose infusion in humans.

Masaki and colleagues reported that endothelin-1 had potent inotropic activity in guinea pig atrial strips.[7] Subsequently, high-affinity receptors were identified in mammalian atria and ventricles.[7] Endothelin induced a positive inotropic effect on cardiac myocytes either by increasing cytosolic calcium or increasing contractile amplitude.[39] It does not begin to have an effect on the cardiac myocyte until a concentration of 0.3 nM is reached, 10 fold higher than its EC50 on vascular muscle.[6] However, a dose dependent decrease in cardiac function and in the indices of cardiac contractility has been reported by Prasad et al.[40] The decrease in the index of cardiac contractility may be primarily due to ischemia of the myocardium from constriction of the coronary blood vessels that supply the myocardium. Interestingly we observed a negative correlation between the baseline endothelin levels and systolic function parameters, i.e., peak ejection rate (PER) and one-third ejection fraction (1/3 EF) values. This finding seems to be in agreement with the results of Prasad et al.[40]

Endothelin is a potent direct stimulus for the release of ANP, independent of its effects on mean arterial blood pressure.[14] Its infusion is reported to increase plasma ANP concentrations with a close correlation between the increase in plasma ANP and endothelin concentrations. However, some reports showed that endothelin infusion did not change the level of ANP, although its plasma concentrations rose 50-fold.[38] We could not observe any correlation between endothelin and ANP levels either at baseline, or during and after the operation. In addition, there was no relation between the systolic or diastolic blood pressure and ANP levels, although within the normotensive subjects, a positive correlation was reported between the endothelin-like immunoreactivity and blood pressure.[21] Curello et al[41] reported an increase in ANP levels after declamping in six patients undergoing CABG-surgery. Significantly elevated postoperative ANP concentrations in coronary artery bypass patients were reported by Aschcrof et al,[42] which is in agreement with our results. We observed an increase in the mean ANP level after extra-corporeal circulation and it remained higher than the baseline values. It has been suggested that release of endothelin not only by the vascular endothelial cells but also by the endocardial endothelium may be of physiological or pathophysiological importance in the regulation of myocardial function.[43),44)] However, the role of endogenous endothelin under pathophysiological conditions is not yet known. According to the results of the present study, the circulating endothelin levels of patients with coronary artery disease whose ventricular function was improved after surgery, were normal and the increase in levels observed during CABG-surgery returned to baseline values in two hours. The observed increase in endothelin might be due to either endothelial injury during surgery or to a transient myocardial ischemia and/or hypothermia or to a combination of both. ANP values increased during the operation and remained higher than initial values. Baseline endothelin values were negatively correlated with systolic function parameters and this finding might be evidence of the negative affect of endothelin on cardiac contraction due to induced vasoconstriction. However, this must be considered with caution due to the relatively small sample size and because confirmation is necessary before any definitive conclusion can be drawn.

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