Cardiac autonomic function in adolescents operated by arterial switch surgery

Falkenberg; short title: Autonomic function after arterial switch surgery

Cecilia Falkenberg, MD; Ingegerd Östman-Smith, MD PhD, Thomas Gilljam, MD PhD; Gavin Lambert, PhD; Peter Friberg, MD PhD

Division of Paediatric Cardiology, Institute of Clinical Sciences, Sahlgrenska University Hospital, Gothenburg University, Sweden (C.F., I.Ö-S, T.G); Human Neurotransmitters Laboratory, Baker IDI Heart and Diabetes Institute and Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia (G.L.): Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg University, Sweden (P.F.)

Prof Peter Friberg, Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg University, SE-413 45 Göteborg, Sweden.
E-mail: peter.friberg@mednet.gu.se, Telephone: +46 31 342 1596, Fax: +46 31 827614

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Abstract

Background

Children with transposition of the great arteries, in whom an arterial switch operation (ASO) is performed, have been shown to have an increased incidence of sudden death, which may be due to cardiac autonomic imbalance and repolarisation instability. We hypothesised that i) cardiac norepinephrine (NE) kinetics and ii) arterial baroreflex sensitivity (BRS), reflecting sympathetic activity and vagal function respectively, are altered in this group.

Methods and Results

17 children (15.8±1.5 years of age) with ASO-surgery in the neonatal period were studied. 17 had cardiac BRS assessed by spontaneous fluctuations of systolic blood pressure and RR-interval, and repolarisation was measured as QT variability index. Matched healthy subjects were controls. Cardiac vagal function and repolarisation pattern were unchanged following ASO-surgery. At cardiac catheterisation, we infused tritiated NE in 8 of these children to examine total body and cardiac sympathetic function at baseline and following 5 min of adenosine infusion to induce reflex sympathetic activation. Blood was sampled simultaneously from the aorta and coronary sinus. Cardiac fractional extraction of $^{[3H]}$NE was substantially lower in operated children, being 56±10 vs. 82±9% ($p=0.0001$). Following i.v. adenosine in the operated group, NE total body spillover doubled vs. baseline ($p<0.002$) and the coronary venous-arterial concentration gradient of $^{[3H]}$dihydroxyphenylglycol increased 4-fold ($p=0.04$).

Conclusions

Arterial switch operation performed neonatally appears to leave cardiac vagal function intact and, although cardiac sympathetic activation in response to adenosine occurs, cardiac neuronal NE reuptake is impaired. This may be pro-arrhythmic by reducing removal capacity of NE from the cardiac synaptic cleft.

Key Words

Congenital heart disease, autonomic nervous system, adolescents, heart, transposition of the great arteries

Background

Children born with transposition of the great arteries, in whom an arterial switch operation (ASO) is performed, have been shown to suffer from ischemic events and increased incidence of sudden death.[1-5] The ASO creates a suture line across the ascending aorta and pulmonary trunk as well as around the coronary ostia. The majority of the sympathetic nerves that enter the heart do so via the great arteries.[6] A large proportion of the sympathetic nervous inflow is therefore severed at the time of surgery, and denervation supersensitivity could perhaps account for the increased incidence of sudden death that occurs without evident coronary occlusion following the ASO.[1] While the mechanisms remain unknown they are likely to be associated with disturbances in the autonomic control of the heart, perhaps involving both the sympathetic and parasympathetic divisions. A recent report by us demonstrated elevated sympathetic activity and reduced cardiac baroreflex function in adults who had undergone Fontan surgery during childhood.[7]

It has been shown that one month after ASO there is virtually no [123]metaiodobenzyl-guanidine uptake in sympathetic nerve terminals in the heart,[8] thereby indicating that either the heart is denervated or that the norepinephrine (NE) transporter is not functional. After a year, in a fashion similar to what is observed following heart transplantation[9, 10] NE uptake is increased, suggesting partial reinnervation similar to that seen in adult patients following heart transplantation.[8, 10] In agreement with these data, physiological studies in

piglets subjected to ASO have demonstrated increased sensitivity to circulating NE 6-7 weeks after surgery, probably due to defective re-uptake.[11]
Normally, most of the released NE (>80 % in the heart) is taken up into the sympathetic nerve terminal via the uptake-1 mechanism.[12] Given the simultaneous process of release and uptake of NE, a measurement that adequately reflects NE release rate from an organ must take into account the active extraction of NE by that organ. By assessing total and cardiac NE kinetics[13, 14] it is feasible to examine the fractional extraction of NE across the heart as an index of NE reuptake. In addition, one may also measure the fall in NE specific activity, by means of radioisotope dilution, during a single passage through the heart, which also provides a valid index of released NE from cardiac sympathetic nerves.[15] Following reuptake into the sympathetic axoplasm, some NE is metabolised to dihydroxyphenylglycol (DHPG), which then diffuses into the plasma compartment.[15] Hence, [3H]DHPG produced from cardiac sympathetic nerves reflects infused [3H]NE that is taken up by neuronal uptake (uptake-1).[12, 15]

The arterial baroreflex sensitivity (BRS) is a marker of parasympathetic modulation of heart rate in response to blood pressure fluctuations that has been shown to convey prognostic information after a myocardial infarction.[16] Although the mechanisms underpinning the prognostic value of BRS are unclear, data indicate that a normal modulation of cardiac parasympathetic nervous activity could protect against ventricular arrhythmia.[17] Furthermore, increased lability of cardiac repolarisation, either spatial or temporal, is associated with ventricular

arrhythmias and sudden cardiac death.[18, 19] The QT variability index (QTVI) is a non-invasive measurement of subtle beat-to-beat fluctuations in the duration of the QT interval.[20]

Thus, it appears essential to determine the activity in both components of the autonomic nervous system and the value of QTVI in long term survivors, who neonatally underwent surgery for transposition of the great arteries and hence may present with damage or dysregulation of cardiac sympathetic and vagal innervation, to unravel any functional disturbance that could contribute to the reported heart problems in this group. Therefore, the present study explores: i) cardiac sympathetic nerve function in arterial switch operated adolescents using radiolabelled NE to determine cardiac fractional extraction of NE, specific activity of NE across the heart and the production of tritiated DHPG both at rest and during sympathetic activation induced by adenosine infusion, ii) cardiac BRS at rest to determine vagal activity, and iii) QTVI as an indicator of possible repolarisation abnormalities. Findings in these patients were compared with data from young healthy subjects.

Methods

Subjects

28 children born with transposition of the great arteries were operated with ASO in the neonatal period at Sahlgrenska University Hospital during the period from May 1983 to October 1991. 17 of these children underwent a long-term follow-up study of autonomic function. The patients were offered to participate in

consecutive order of birth. Four had died, two had moved to other regions, one had moved abroad, one declined the follow up; two participated although not in the investigations included in this article (one was excluded because of existing treatment with beta-blockers due to left ventricular dysfunction) and two did not participate due to unspecified reasons. One child born in 1994 was included because of request of the child's physician. Hence, 17 subjects (16 males/1 female, aged 15.8±1.5 years, weight 64.5±9.7 kg, height 174±10 cm) (see supplement which also includes clinical details) participated in this follow-up study, which included physical examination, electrocardiogram (ECG), pulmonary scintigraphy, BRS, cardiac ultrasound examination and exercise test with spirometry. After an overnight fast, all 17 patients underwent the cardiac catheterization procedure with full hemodynamic investigation. It was possible to achieve a stable catheter-position in the coronary sinus in 8 patients and they received tritiated NE ($^{[3H]}$NE) for studying cardiac sympathetic function. These subjects were given an antecubital intravenous (i.v.) line in the right arm for infusion of $^{[3H]}$NE and one i.v. line in the right hand for subsequent adenosine infusion. A catheter was also inserted via a femoral artery and placed in the descending aorta. The coronary sinus was catheterised via a femoral vein, jugular vein or left antecubital vein. During catheterization, anaesthesia was induced with pentobarbiturates 5.3±6.5 mg/kg, and light anaesthesia was maintained with isoflurane. The control group of healthy subjects who received $^{[3H]}$NE infusion (n=15) were sourced from the database of healthy subjects at the Baker IDI Heart & Diabetes Institute, Melbourne, Australia. Those subjects were

somewhat older than the aforementioned ASO group (19.7±1.0 years of age, p=0.0006, 1 female, weight 75.3±6.8kg, p=0.0006, height 178±11cm, p=0.29) and were given radiotracer infusion according to previously described protocols.[14]

The study protocols were approved by the local ethics reviewing committee at the Sahlgrenska University Hospital no Ö379-02, and at the Alfred Hospital, Melbourne, Australia, and all subjects, and if below 18 years, also their parents, gave informed consent.

Radiotracer Infusion

During the catheter studies participants received a tracer infusion of ^{3}H-labeled NE (specific activity of 11-25Ci/mmol; New England Nuclear, Boston, MA, USA) via a peripheral vein at 0.6 to 0.8 μCi/min, after a priming bolus of 12 μCi, for the measurement of NE kinetics by isotope dilution.

Once all the catheters and intravenous lines were in position the radiotracer infusion began. The first resting blood sample was collected after at least 15 minutes of infusion to ensure a steady state of the plasma concentration of $^{[3H]}$NE. Blood samples (10mL) were taken simultaneously from the coronary sinus and the aorta. Adenosine infusion was then started (140μg /kg/min), and after 5 minutes additional blood samples were taken simultaneously from the coronary sinus and aorta.

Assay of catecholamines

All samples were transferred immediately into pre-chilled tubes containing reduced glutathione and heparin. They were centrifuged at 4^{o}C and plasma separated for storage at -80 ^{o}C. NE was extracted from plasma (1mL) and samples of infusate (10µl) using alumina adsorption and separated by high performance liquid chromatography.[21, 22] Intra-assay coefficients of variation (CV) in our laboratory are 1.3% and 2.3% respectively; inter-assay CVs are 3.8% and 4.5%, respectively.

Calculations of norepinephrine kinetics

Total body plasma clearance of NE (CL_{TB}) and total body spillover of catecholamines (NE) into plasma (SP_{TB}) were calculated according to Esler et al.[13, 14] and cardiac fractional extraction of NE ($EX_{cardiac}$) was calculated as: $EX_{cardiac} = ({}^{[3H]}NE_A - {}^{[3H]}NE_V) / {}^{[3H]}NE_A$

NE_V is coronary sinus NE concentration (pmol/mL) and ${}^{[3H]}NE_A$ is arterial concentration of tritiated NE (dpm/mL). The specific activity (SA) was estimated according to the equation: $SA_{NE} = {}^{[3H]}NE_{AV}/NE_{AV}$

where ${}^{[3H]}NE_{AV}$ and NE_{AV} are the respective arterial-coronary venous increments in plasma concentration of ${}^{[3H]}NE$ (dmp/mL) and endogenous NE (pml/mL).[15] Further, the gradient of ${}^{[3H]}DHPG$, ${}^{[3H]}NE$ and DOPA between the descending aorta and coronary sinus was calculated.

Cardiac baroreflex sensitivity measurements and calculations

Prior to, and separated from the catheterization procedure, cardiac BRS assessment was performed in awake and non-sedated 17 ASO patients. 17 Swedish sex- and age-matched healthy adolescents were used as controls (1 female; age 15.7±0.3 years, weight 61.1±8.6 kg, height 173±7 cm). The BRS measurements were performed in a quiet environment with minimum disturbance. The same experienced research nurse performed all recordings. All individuals refrained from caffeine-containing beverages and exercise for the 12 h prior to the investigation and were fasting since at least 1 h. After 10 min of rest, ECG and beat-to-beat blood pressure were recorded over 20 min by Portapres® equipment (TNO Biomedical, Amsterdam, Netherlands), with the subject in supine position and with spontaneous, non-regulated breathing,[23-25].

According to Gao et al [26] the time series of SBP and RR interval from the entire period of recording (20 min) were scanned to identify baroreflex sequences, which were defined as three or more consecutive beats in which successive SBP and RR intervals concordantly increased or decreased, with the threshold set at 1.0 mmHg and 5.0 ms, respectively, and a shift of +1 between the blood pressure pulse and the RR interval, as suggested by Bertinieri *et al*. [27]. Linear regression was applied to each sequence and only those for which the square of the correlation coefficient (r^2) was greater than 0.85 were accepted for further analysis. The spontaneous BRS was calculated, reflecting the average regression slope for all the linear regressions, thus reflecting cardiac vagal activity.

QT interval variability index

A period of 5 min with less than 5% atrial/ventricular ectopic beats was chosen for the temporal QT interval variability analysis using a computer algorithm.[26, 28] RR interval mean (RRm) and variance (RRv) and QT interval mean (QTm) and variance (QTv) were derived from the respective time series. QTVI, which represents the log ratio between normalized QT and RR interval variability, was calculated according to the equation.[20, 29]

$$\mathrm{QTVI} = \log_{10}\left[\frac{\mathrm{QTv}/\mathrm{QTm}^2}{\mathrm{RRv}/\mathrm{RRm}^2}\right]$$

Statistical methods

Numerical distributions are presented by their mean±SD. Mann-Whitney U-test was used for inter-group comparisons, and Wilcoxon rank sum test was performed for paired comparisons given that the distributions of the data from switch-patients were not normally distributed. Statistical significance was defined as $p<0.05$.

Results

The ASO group presented with a rather normal cardiac function, reflected by a fractional shortening of 32%, hence no evidence of heart failure (c.f. supplement).

Noradrenaline kinetics

Total body NE spillover was lower in ASO patients compared with healthy controls, being 579±154 and 2875±1246 pmol/min for the former and latter groups, respectively ($p<0.001$). The lower total body NE spillover observed in ASO patients was based on both a lower total body NE clearance vs. healthy subjects (1287±329 and 3036±986 mL/min, $p<0.001$) and a reduced arterial plasma NE concentration (see table 1).

In healthy subjects 82±9% of the $^{[3H]}$NE entering the coronary vascular bed was extracted by the heart. Arterial switch patients also had lower coronary venous than arterial plasma concentrations of $^{[3H]}$NE ($p<0.001$) (fig 1a), but the magnitude of cardiac fractional $^{[3H]}$NE extraction was substantially decreased (56±10, $p=0.0001$) compared with healthy subjects. The specific activity of plasma $^{[3H]}$NE, decreased from arterial to coronary sinus sampling sites in both healthy subjects (79±11%) and in ASO patients (69±14%), demonstrating release of endogenous NE also in the heart of ASO-subjects, although with a trend towards a smaller fall in the latter group ($p=0.09$) (fig 1b). In healthy subjects at rest, coronary sinus plasma concentrations of tritium labelled DHPG were 70% higher than arterial concentrations ($p<0.0001$), whereas in ASO patients arterial and coronary sinus plasma concentrations of $^{[3H]}$DHPG did not differ significantly (+8%, $p=0.5$) (fig 1c). Concentrations of endogenous DHPG were 81% higher in coronary sinus than arterial plasma in healthy subjects ($p<0.0001$) while in ASO patients the positive gradient between the arterial and

coronary sinus sampling sites was 29%, (p=0.018) (fig 1d); with the size of the increase being significantly smaller than that of the control group (p=0.008).

In healthy subjects there was consistent arterial-to- coronary sinus-step-up in plasma concentration of DOPA across the heart, whereas in ASO patient's plasma DOPA concentrations did not show a consistent step-up between the two sampling sites (fig 1e). Coronary sinus-arterial DOPA concentration gradients usually parallel NE spillover rates.[30]

Adenosine infusion in the ASO group resulted in a doubling of total body NE spillover, from 579±154 to 1194±343 (pmol/min, p=0.002). Likewise, total body NE clearance increased from 1287±329 at baseline to 1673±299 (pmol/min, p<0.001) following adenosine. Cardiac fractional extraction of $^{[3H]}$NE remained low being 25±17% following in the operated group i.v. adenosine. The within-patient step-up from artery to coronary sinus in $^{[3H]}$DHPG concentration was 3.9±16.9 dpm/ml at baseline, and increased 4-fold after adenosine infusion to 16.1±9.9 dpm/ml (p=0.049).

Baroreflex sensitivity and QT interval variability index

Cardiac BRS showed similar values for ASO patients vs. healthy subjects, being 13.5±5.6 (n=17) and 18.0±12.3 (n=17) ms/mmHg respectively, (p=0.1). Resting heart rate was similar in both groups (66 beats/min). Furthermore, QTVI between groups was similar, being -1.47±0.35 in the ASO patients and -1.43±0.27 in the healthy subjects.

Discussion

This study establishes a reduced function of cardiac sympathetic nerves, and no overt signs of vagal dysfunction in 15 year-old subjects operated neonatally for transposition of the great arteries. It is important to study the autonomic nervous system after previous ASO surgery given that both the sympathetic and parasympathetic divisions are involved in prognostic determination of long-term cardiovascular health.[31-33]

The present study utilised the gold standard radiotracer dilution approach to study whole body and cardiac NE kinetics.[13, 14] The technique involves measurements of specific activity of the infused [3H]NE in plasma, i.e. the degree to which the infused [3H]NE dilutes with the endogenous plasma NE. The greater the dilution is, the lower the specific activity and the larger the NE spillover into the plasma compartment. The [3H]NE specific activity was higher to start with in the ASO group as a result of background plasma endogenous NE levels being lower than in controls. However, the majority of circulating NE derives from NE released from organs other than the heart, which makes it necessary to study NE kinetics in the heart specifically. In the ASO patients, the spillover of NE into plasma from cardiac sympathetic nerves was shown to be present by the decrease in the specific activity of tritiated NE from arterial to coronary sinus venous plasma, but the decrease was 35% smaller than that observed in the healthy subjects. These findings clearly show that functioning cardiac sympathetic nerve terminals releasing NE are present even in ASO subjects.

However, a clear-cut reduction in cardiac fractional extraction of [3H]NE was obvious in ASO patients compared with the healthy subjects, suggesting loss of re-uptake sites in functioning sympathetic neurons. One may surmise that the transposing of the great arteries neonatally has lead to some functional deficit in the regenerated sympathetic nerve fibres that have reinnervated the heart growing in along the great vessels, thus explaining the reduced fractional extraction of NE across the heart. This deficit might be due to reduced density of sympathetic nerve terminals, but changes in cardiac ultrastructure and microvascular flow might also contribute. Although not to the same magnitude, the situation in the ASO patients mimics the pattern observed in patients who underwent cardiac transplantation, where cardiac fractional extraction of NE was severely reduced.[9, 10]

Norepinephrine disposition in the heart is heavily dependent upon the process of neuronal re-uptake, due largely to the fact that the sympathetic innervation of the heart is particularly dense and that the synaptic cleft width is narrow. Hence, normally only a minimal amount of NE released from the heart reaches the circulation. This means that defective re-uptake may exert functional consequences. Indeed, dysfunctional neuronal re-uptake of NE is a major component of denervation supersensitivity since the released transmitter remains longer at the myocyte receptor, a concept that is corroborated by findings in ASO-piglets.[11] In the present study, precursors and metabolites of NE, such as DOPA and DHPG, were also measured. Most plasma DHPG emanates from

intraneuronal metabolism of NE, and cardiac DHPG is a reflection of leakage of NE from storage vesicles. A normal cardiac DHPG spillover thus reflects adequate NE stores. A fully sympathetically innervated heart usually shows a large positive gradient (step-up) between arterial plasma and coronary venous DHPG concentrations. Likewise, this is also true for $^{[3H]}$DHPG plasma concentrations, an even better estimate than endogenous DHPG concentrations, in determining cardiac NE neuronal function. Both ASO patients and healthy subjects demonstrated a step-up of DHPG plasma concentration across the heart, although to a lesser extent in the ASO-group. However, there was no statistically significant difference in terms of step-up regarding $^{[3H]}$DHPG in the ASO group. One may speculate that the reserve capacity in terms of cardiac sympathetic responsiveness may be hampered in some ASO patients. However, these patients, when exposed to substantial sympathetic activation by means of adenosine infusion, demonstrated an increase in both cardiac NE- and $^{[3H]}$DHPG arterio-venous plasma concentration gradients, indicating still functioning cardiac sympathetic nerves. Since the ASO group did not exhibit the same step-up across the heart in plasma DOPA concentrations, this might be indicative of a reduction in NE synthesis.[30] Taken together, cardiac sympathetic function is disturbed in ASO patients, reflected as a clearly reduced fractional extraction of NE across the heart.

Interestingly, cardiac vagal function, reflected as an estimate of baroreflex sensitivity, obtained by means of spontaneous fluctuations of blood pressure/R-R

interval sequences,[23-25] revealed only a slight non-significant reduction between ASO patients and healthy subjects.[34] This finding is important given that reduced baroreflex sensitivity is associated with both total and cardiovascular mortality in adult populations.[16] Afferents and efferents controlling the baroreceptor reflex run along with the XI and X cranial nerves and are, thus, completely different from cardiac sympathetic nerves.[35, 36] They might be affected due to snaring of caval veins at institution of bypass but they are not directly severed by the surgery as is the case for sympathetic nerves in the ASO patients. This probably explains why the baroreceptor reflex is still intact in these patients compared to age-matched healthy subjects. Furthermore, *given that resting cardiac function and blood pressures were in the normal range in the ASO group, it provides a rational for this group to show a preserved BRS. However, BRS measurements represent but one mode of investigating cardiac vagal function. Several other techniques prevail, such as heart rate variability assessing both time- and frequency domain variables, in order to unravel any possible dysfunction in the ASO group. We have only analysed 20 min of blood pressure and heart rate fluctuations in our subjects, which might not be enough to detect any cardiac vagal dysfunction in the ASO group.*

Another feature, besides cardiac autonomic activity, that can influence cardiac rhythm is the QT pattern. In healthy subjects QTVI is negative due to logarithmic transformation. *We used the QT variability index given, which considers the pattern of the QT-interval and thus makes it even more accurate than for*

example only assessing QT interval and QTc, and in addition, it takes into account the variability of the R-R interval. The present study explored the possibility that QT variability index, which reflects repolarisation stability, is altered in ASO patients. In the presently examined patients this value was similar in both groups thus indicating no difference in repolarisation pattern in ASO patients.[20, 37]

Limitations of the study

All subjects refrained from coffee and tea from 10 pm the evening before the examination. The healthy subjects were allowed a very light breakfast without coffee and tea in the morning of the catheterization. Total body NE spillover was clearly higher in the healthy subjects compared with the patient group. One major difference in protocol design was that the patients were analysed under anaesthesia, also including barbiturate. Previous preclinical work has demonstrated that barbiturates are without effect on norepinephrine uptake or release in rat cerebral cortex slices.[38] While such treatment may lower the total body spillover importantly we obtained simultaneously blood samples from the arterial and coronary sinus sites in both groups. In addition, augmentation of sympathetic activity due to adenosine was produced while the patients were under anaesthesia, allowing us to draw valid conclusions about sympathetic responsiveness. Notably, total body NE spillover increased substantially and cardiac fractional extraction decreased further with adenosine in spite of the anaesthetic state. The healthy subject group, which was studied for cardiac

neurochemical analyses, was slightly older than the ASO group. Using the techniques described in this report it was not ethical to invasively study healthy subjects of as young as 15 years old, hence our healthy cohort were 19 and able to give their own consent.

Naturally, a larger group of patients examined for sympathetic function would have made our case stronger; however this is a difficult and elaborate invasive experimental performance in adolescents. We feel confident that our finding of a decreased cardiac fractional extraction of NE in the patient group is valid, in particularly as they are in agreement with experimental findings in ASO-operated piglets.[11]

In conclusion, in ASO patients the present study has provided evidence for disturbed, albeit still functioning, cardiac sympathetic innervation in ASO patients. There was a clear-cut reduction of fractional extraction of NE compared with sex- and age matched adolescents. The other division of cardiac autonomic function, namely cardiac vagal activity, appeared normal. Thus, ASO surgery performed neonatally mainly affected cardiac sympathetic function, leaving vagal innervation intact. The reduced neuronal re-uptake of NE into sympathetic nerve terminals may result in a higher synaptic cleft NE concentration and hence, more postsynaptic beta-receptor stimulation, perhaps indicating a higher risk for arrhythmia.

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Disclosures

None.

Figure Legend

Fig 1a-e

Plasma concentrations and specific activity of $^{[3H]}$Norepinephrine (NE) and plasma concentrations of dihydroxyphenylglycol (DHPG) and $^{[3H]}$DHPG and dihydroxyphenylalanine (DOPA) in healthy subjects and arterial switch operated patients. Asterisks indicate significant changes in comparison between artery and coronary sinus. ***= $p<0.001$, *=$p>0.05$.

Table 1.

Arterial and coronary sinus concentrations of endogenous norepinephrine (NE), tritiated NE ($^{[3H]}$NE), endogenous dihydroxyphenylglycol (DHPG), tritiated DHPG ($^{[3H]}$DHPG), dihydroxyphenylalanine (DOPA), and specific activity (SA) of NE in healthy young subjects and in arterial switch operated adolescents (ASO) pre and post adenosine infusion. The healthy subjects were awake during the procedure.

	Healthy subjects (n=15)	**ASO patients (n=8)**	**P=between healthy and ASO patients**	**ASO patients after adenosine**
NE (pmol/mL)				
arterial	1.0±0.4	0.46±0.1, n=8	0.0001	0.73±0.3†, n=8
coronary sinus	0.9±0.3	0.76±0.4*, n=8	0.55	1.14±0.6, n=7
DHPG (pmol/mL)				
arterial	5.3±1.3	7.3±1.0, n=8	0.0006	7.9±2.2, n=8
coronary sinus	9.6±2.4***	9.4±2.5*, n=7	0.87	8.5±2.3, n=7
DOPA (pmol/mL)				
arterial	6.6±1.3	10.9±4.0, n=8	0.019	11.6±4.8, n=8
coronary sinus	7.9±1.7*	12.1±2.6, n=7	0.0047	12.6±2.5, n=7
$^{[3H]}$NE (dpm/mL)				
arterial	485±155	468±152, n=8	0.8	348±85†, n=8
coronary sinus	86±52***	214±72***, n=8	0.0023	281±101, n=7
SA $^{[3H]}$NE (dpm/pmol)				
arterial	548±256	1027±271, n=8	0.001	518±208†, n=8
coronary sinus	101±47***	328±154***, n=8	0.0005	309±206, n=7
$^{[3H]}$DHPG (dpm/mL)				
arterial	27±12	51±17, n=8	0.005	63±23, n=8
coronary sinus	47±16***	54±20, n=7	0.4	78±26, n=7

Mean±SD, *p<0.05, ***p<0.001 indicate statistically significant difference between the arterial and coronary sinus sites within the same group. In the ASO group only the 7 patients with samples from both aorta and coronary sinus are included in the comparison. †indicates statistically significant difference between ASO group before and after adenosine infusion.

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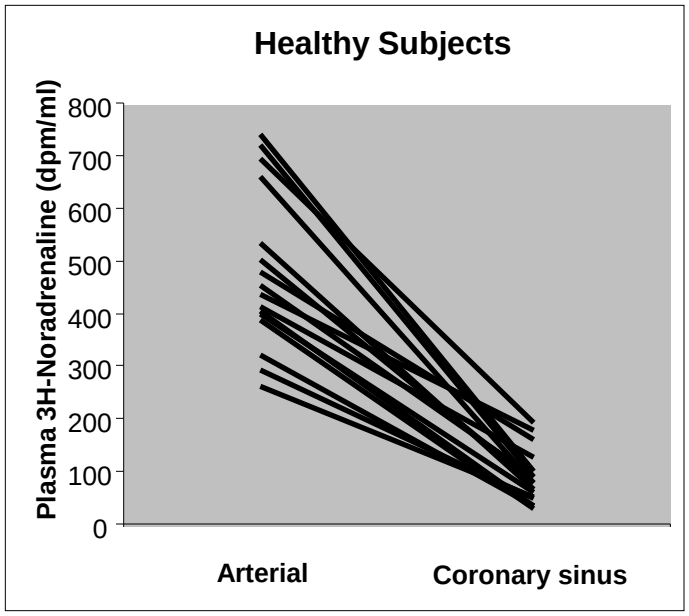
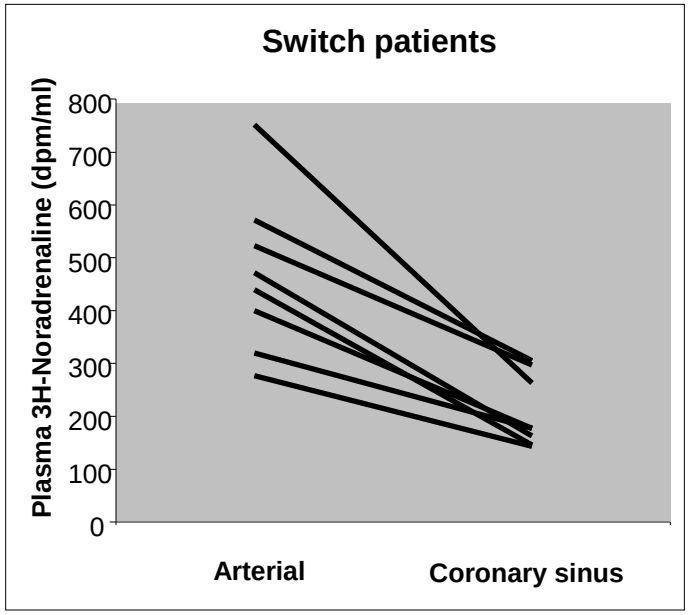
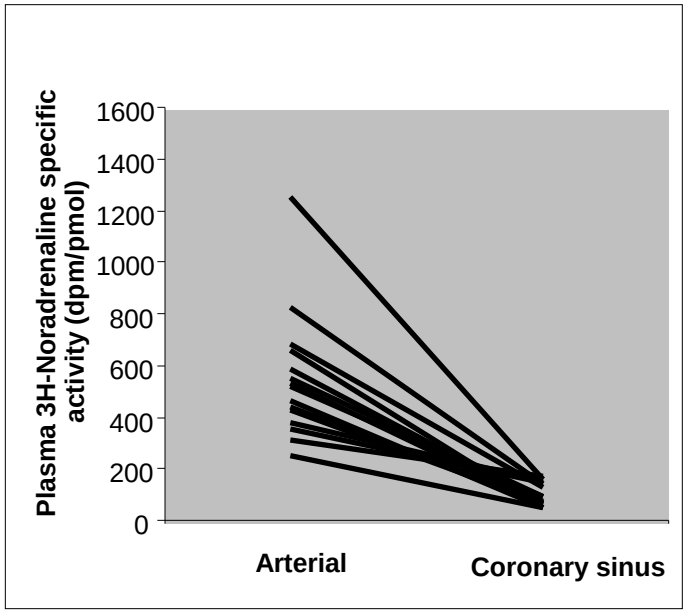
Fig 1


Healthy Subjects
Plasma 3H-Noradrenaline (dpm/ml)
800
700
600
500
400
300
200
100
0
Arterial
Coronary sinus


Switch patients
Plasma 3H-Noradrenaline (dpm/ml)
800
700
600
500
400
300
200
100
0
Arterial
Coronary sinus


Plasma 3H-Noradrenaline specific activity (dpm/pmol)
1600
1400
1200
1000
800
600
400
200
0
Arterial
Coronary sinus

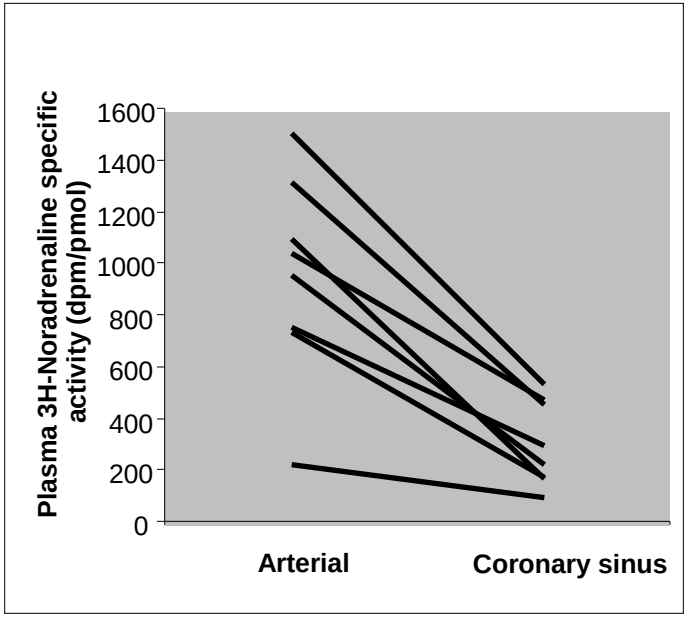
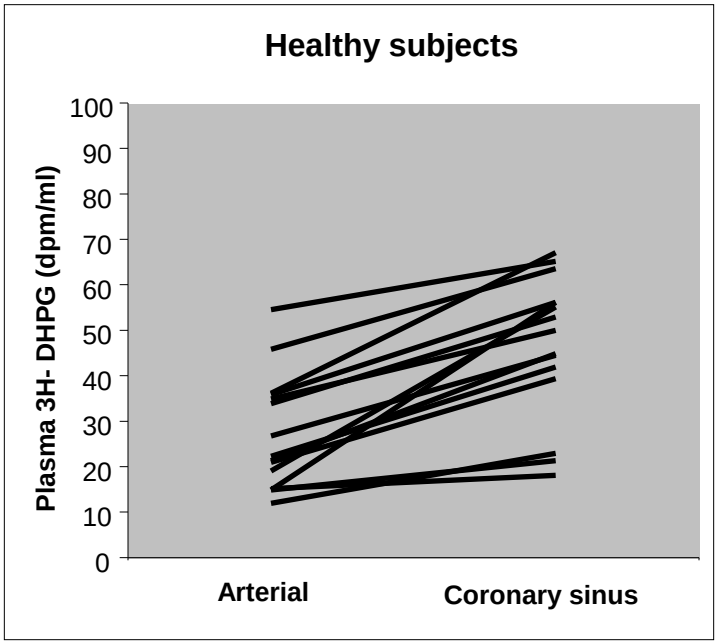
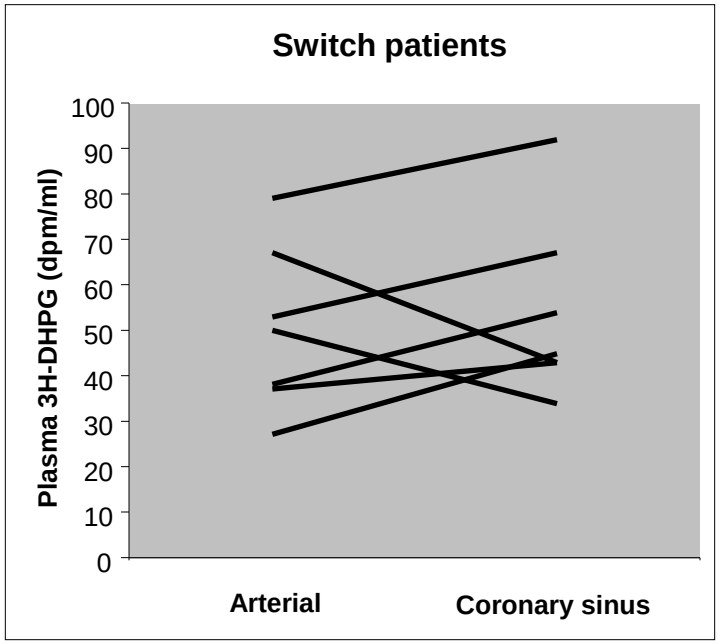
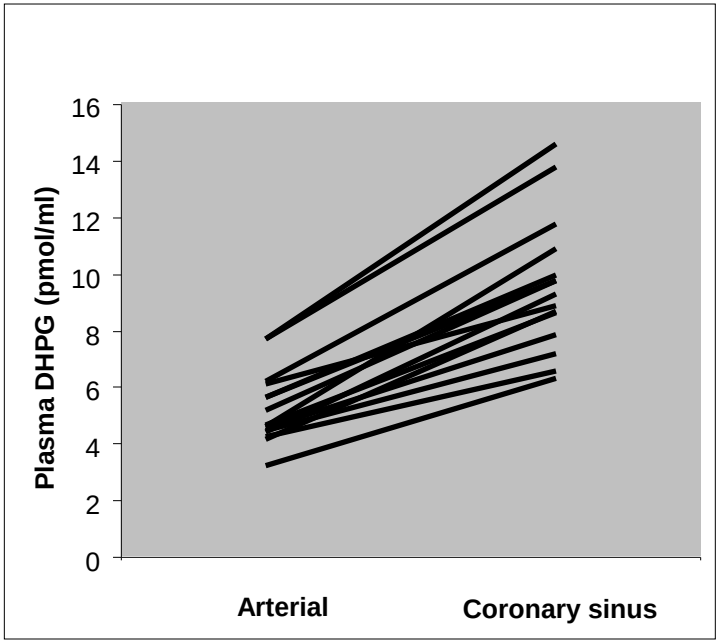

Plasma 3H-Noradrenaline specific activity (dpm/pmol)
1600
1400
1200
1000
800
600
400
200
0
Arterial
Coronary sinus

Fig 2


Healthy subjects
Plasma 3H- DHPG (dpm/ml)
100
90
80
70
60
50
40
30
20
10
0
Arterial
Coronary sinus


Switch patients
Plasma 3H-DHPG (dpm/ml)
100
90
80
70
60
50
40
30
20
10
0
Arterial
Coronary sinus


Plasma DHPG (pmol/ml)
16
14
12
10
8
6
4
2
0
Arterial
Coronary sinus

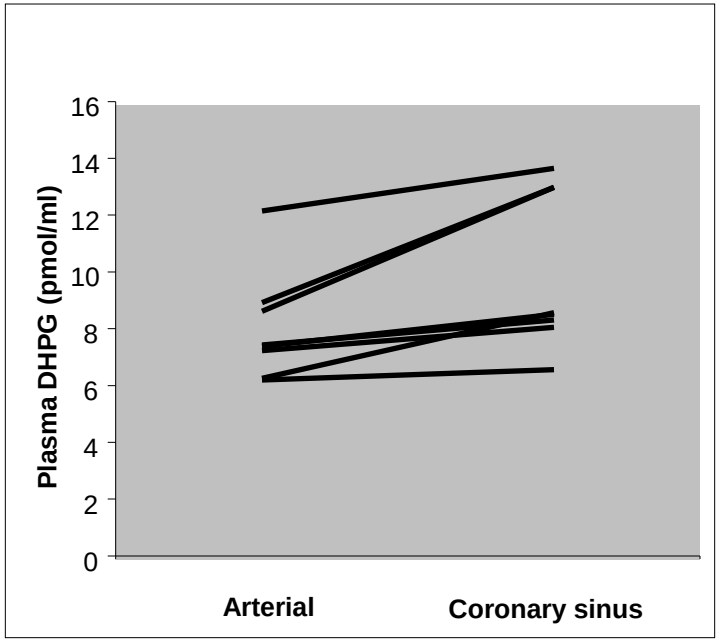
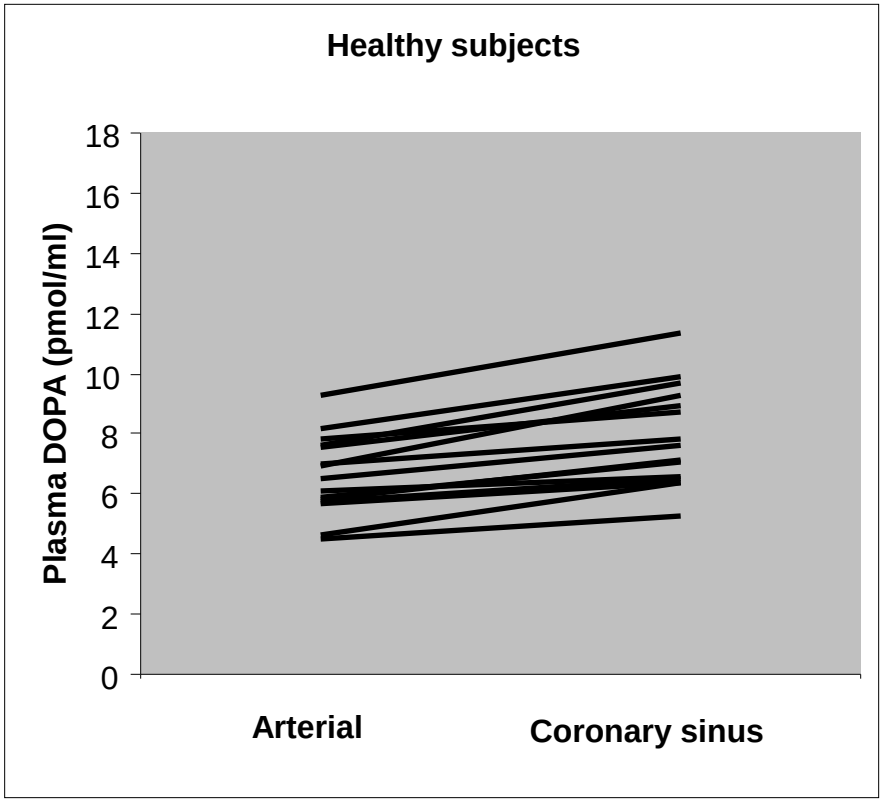
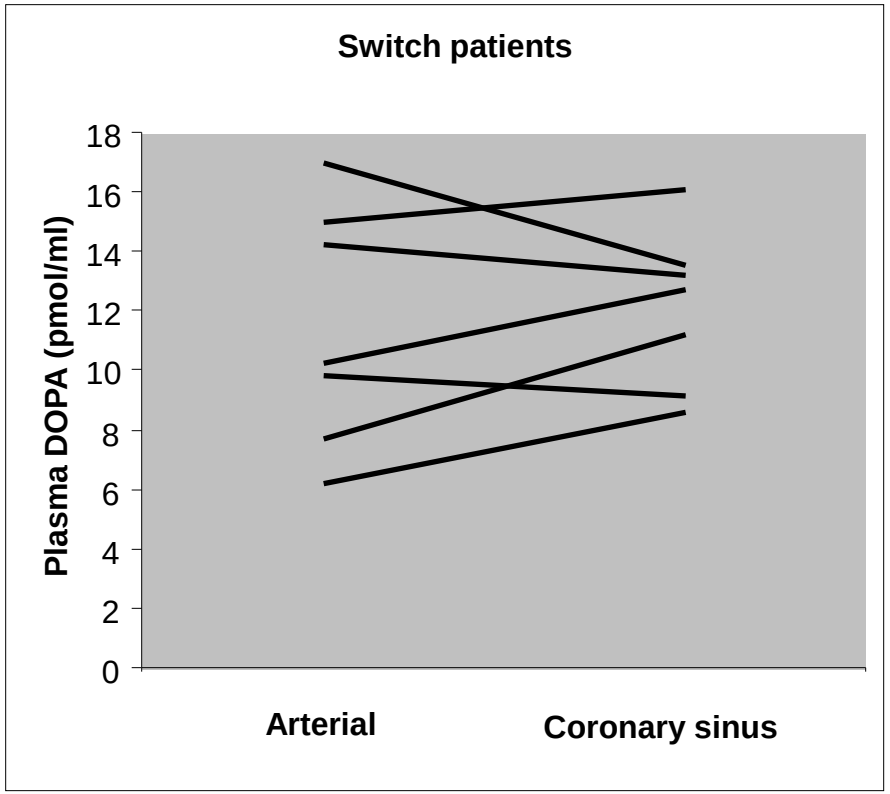

Plasma DHPG (pmol/ml)
16
14
12
10
8
6
4
2
0
Arterial
Coronary sinus

Fig 3


Healthy subjects
Plasma DOPA (pmol/ml)
18
16
14
12
10
8
6
4
2
0
Arterial
Coronary sinus


Switch patients
Plasma DOPA (pmol/ml)
18
16
14
12
10
8
6
4
2
0
Arterial
Coronary sinus

Nr	Sex	Age at FU (year)	Height (cm)	Weight (Kg)	Diagnosis pre-operation	Operation	Complication post-op (and later follow-up)	Drugs at Follow-up	FS (%)	Examination
1	M	16.1	168	58.5	TGA,	ASO, FO closed with suture	phrenic nerve palsy, spontaneously resolved	None	40	[3H]NE+BRS
2	M	15.6	174	61.0	TGA, ASD secundum	ASO, ASD closed with suture	heart failure, subendocardial infarction, dialysis, 1.5 month post.op colonic ileus which needed surgery	None	24	[3H]NE+BRS
3	M	16.0	192	72.0	TGA, FO	ASO, FO closed with suture	peri-op cardiac tamponade	None	31	[3H]NE+BRS
4*	M	14.9	172	56.8	TGA	ASO	heart failure, dialysis and respiratory support for several weeks	Folic acid 5mg 3times/w, trimetroprim 80mg and sulfametoxazole 400mg b.i.d., metotrexate 15mg 1/w i.m., etanercept 20mg s.c. 2times/w, naproxene 250mg when needed	28	[3H]NE+BRS
5	M	17.3	172	61.0	TGA, ASD	ASO, ASD closed with suture		None	27	[3H]NE+BRS
6	M	15.4	165	60.0	TGA, ASD	ASO, ASD closed with suture		None	38	[3H]NE+BRS
7	M	11.9	151	57.5	TGA, VSD, ASD, TAPR	ASO, VSD and ASD patch, TAP		None	33	[3H]NE+BRS
8	M	16.4?	180	66.0	TGA	ASO		None	37	[3H]NE+BRS
9	M	16,3	186	67.0	TGA, VSD	ASO, closure of VSD		None	29	BRS
10	M	15.3	183	67.0	TGA, VSD	ASO, closure of VSD	Hypertension at follow-up	None	35	BRS
11	F	15.8	176	60.0	TGA	ASO	Later stent in LPA	None	29	BRS
12	M	16.2	182	92.0	TGA	ASO		None	41	BRS
13	M	15.6	173	53.0	TGA	ASO		None	24	BRS
14	M	19.7	163	77.0	TGA	ASO	hepatitis C, laterTAP PI, homograft in PA, systemic hypertension	peginterferon alfa-2a 135μg 1/w s.c., enalapril 10mg b.i.d.	30	BRS
15	M	15.9	183	62.7	TGA	BT shunt initially, at 3 month of age ASO		None	34	BRS
16	M	15.7	172	56.0	TGA, VSD, CoA	ASO, VSD closing, carotid flap	Re-op patch PA due to supravalvular stenosis	None	28	BRS
17	M	14.9	171	56.0	TGA	ASO		None	38	BRS

FU= follow-up, TGA=transposition of the great arteries, ASD=atrial septal defect, ASO=arterial switch operation, FS=fractional shortening, FO=foramen ovale, [3H]NE=tritiated norepinephrine, BRS=baroreceptor sensitivity, post-op=post operatively, peri-op=peri operatively, w=week, b.i.d.=twice a day, i.m.=intramuscular, s.c.=subcutaneous, VSD=ventricular septal defect, TAPR=total anomalous pulmonary venous return, LPA= left pulmonary artery, TAP=trans-annular patch, PA= pulmonary artery, PI=pulmonary insufficiency, BT=Blalock-Taussig shunt, CoA= coarctation of the aorta, re-op=re-operation, *=patient developed rheumatoid arthritis later in follow-up