Doctoral Thesis for the degree of Doctor of Philosophy, Sahlgren´s academy

Consequences of Arterial Switch Operation in Children Born with Transposition of the Great Arteries

- A clinical and experimental study of the autonomous nervous system in the heart

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Figures 1, 2, 4, 5, 6 and 7 by Evelina Falkenberg

Consequences of Arterial Switch Operation in Children Born with Transposition of the Great Arteries

– A clinical and experimental study of the autonomous nervous system in the heart

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To my ”Winter star”, my ”Autumn leaf” and my ”Summer flower”

Julia, Evelina and Martha

With you everything makes sense

“It´s hard to make a “come-back” if you haven´t been anywhere!!”

-the Road House “The Centre of the Universe”, Western Australia

“The thing about growing up with Fred and George, ´said Ginny thoughtfully´, is that you sort of start thinking that anything´s possible if you´ve got enough nerve.”

-Ginny Weasley in “Harry Potter and the Order of the Phoenix”,

by J.K. Rowling (2003)

Abstract

Background*:* The introduction of the arterial switch operation (ASO) made it the procedure of choice for surgical correction of transposition of the great arteries. A majority of the sympathetic nerves innervate the heart alongside the great vessels; these are therefore likely to be damaged during the surgical procedure; imposing new challenges and questions that need to be addressed. The main aim for this thesis was to assess the long-term cardiac consequences on the autonomic nervous system after surgery (paper I and II) and to create an animal model allowing for cardiac physiological studies (paper III and IV).

Methods: Long-term follow-up in adolescents who had undergone ASO as neonates (n=17, 1 female, mean fractional shorting 32±5%) was performed. This included sympathetic nervous system function assessed through infusion of tritiated Norepinephrine ($^{[3H]}$NE) during heart catheterisation (n=8)(controls n=15) and blood samples analysed with high performance liquid chromatography. Samples were obtained both before and after adenosine stimulation as a response to sympathetic excitation. 24-hour heart rate variability (HRV)(n=15 in both groups) was measured both during the day and night using different algorithms. Baroreflex sensitivity and QT variability index (QTVI) (n=17 in both groups) were measured in awake patients. An animal model was developed using complex open heart surgery during cardiopulmonary bypass to mimick the arterial switch operation in piglets 8 weeks of age. The piglets surviving at least 5 to 6 weeks post-operation had follow-up of physiological response to catecholamines and were studied in vivo and in vitro using the Langendorff perfusion system.

Result*s:* In both groups the specific activity of $^{[3H]}$NE decreased from the artery to the coronary sinus, but to a lesser extent in the ASO group. The extraction fraction in the ASO group was 56±10% compared to 82±9% in the healthy subjects (p<0.001). The arterial to coronary sinus plasma concentration of $^{[3H]}$ dihydroxyphenylglycol (DHPG) was significantly increased in the healthy group (70%, p=<0.0001) but was not so in the ASO group (8%, p=0.5). The difference of endogenous DHPG increase from the arterial to the coronary sinus was significantly smaller in the ASO group (p=0.008). After adenosine infusion, the total body NE spillover increased in the ASO group (p=0.002), reflecting major sympathetic activation. $^{[3H]}$DHPG step-up from the artery to the coronary sinus increased 4-fold following adenosine. HRV frequency-domain at night-time, when cardio-parasympathetic drive is likely to be most pronounced, showed a significant decrease of normalized high frequency in the ASO group (52±20) compared to healthy subjects (68±15)(p=0.018). Time-domain showed no statistical difference between the two groups, neither during day-time nor night-time. Baroreflex sensitivity and QTVI did not show significant differences between groups. The animal model resulted in 14 out of 19 piglets surviving the mimicked ASO. Piglets operated with mimicked ASO had a significantly higher basal heart rate both in vivo (p=0.042) and in vitro (p=0.0056).

Conclusion*:* A disturbed but functioning sympathetic cardiac innervation was found in the ASO patients at long-term follow-up. The vagal tone seemed normal in terms of BRS, however, frequency-domain analysis showed a decreased parasympathetic tone at night time in the ASO group. The surgical challenges due to translocation of the coronary arteries and the consequences of an injured autonomic nervous system impose risks of decreased myocardial perfusion and arrhythmias. Thus, the present data suggest that these patients ought to have follow-up that includes autonomic nervous system assessment.

Key Words: Transposition of the great arteries, arterial switch operation, autonomous nerves system, norepinephrine, heart rate variability, cardiopulmonary bypass, piglets

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I **Falkenberg C**, Östman-Smith I, Gilljam T, Lambert G, Friberg P. **Cardiac autonomic function in adolescents operated by arterial switch surgery**. Accepted for publication in International Journal of Cardiology 25-Dec- **2012.** In press. DOI:10.1016/j.ijcard.2012.12.063

II **Falkenberg C**, Ekman M, Gilljam T, Friberg P. **Heart rate variability in adolescents who as neonates underwent neonatal arterial switch operation.** (Manuscript)

III **Falkenberg C,** Hallhagen S, Nilsson K, Östman-Smith I**. Anaesthetic, surgical and bypass techniques allowing long term survival after complex cardiac surgery in piglets.** (Manuscript)

IV **Falkenberg C,** Hallhagen S, Nilsson K, Nilsson B, Östman-Smith I. A **study of the physiological consequences of sympathetic denervation of the heart caused by the arterial switch procedure**. Cardiology in the Young (**2010**), 20, 150–158

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Abbrivations

$^{[3H]}$DHPG	Tritiated dihydroxyphenylglycol
$^{[3H]}$NE	Tritiated norepinephrine
ANS	Autonomic nervous system
ASD	Atrial septum defect
ASO	Arterial switch operation
AV-node	Atrio-ventricular node
BRS	Baroreceptor sensitivity
CHD	Congenital heart disease
CPB	Cardiopulmonary bypass
DHPG	Dihydroxyphenylglycol
ECG	Electrocardiogram
EPI	Epinephrine
EXcardiac	Cardiac extraction fraction
HF	High frequency
HRV	Heart rate variability
Hz	Hertz
LF	Low frequency
LV	Left ventricle
MAO	Monoamine oxidase
NE	Norephinephrine
NE_A	Arterial norepinephrine concentration
NE_V	Coronary sinus norepinephrine concentration
n.u	Normalized
PDA	Patent ductus arteriousus
PNMT	Phenyethanolamine-N-metyltransferas
QTVI	QT variability index

RMSSD	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SA	Specific activity
SBP	Systolic blood pressure
SDNN	Standard deviation of all RR intervals
SNS	Sympathetic nervous system
SVR	Systemic vascular resistance
TB	Total body
TGA	Transposition of the great arteries
U1	Uptake-1
U2	Uptake-2
VLF	Very low frequency
VSD	Ventricular septum defect

Introduction

Transposition of the great arteries (TGA)is a congenital heart defect that is found in approximately 5% of all newborns with congenital heart disease[1]. In this defect, the pulmonary artery arises from the left ventricle and the aorta from the right ventricle, a parallel instead of a sequential circulation exists. As a consequence, oxygenated blood from the lungs is prevented from reaching the systemic circulation unless there is mixing of venous and arterial circulation. Without points of mixing, such as an atrial septum defect (ASD) or patent ductus arteriousis (PDA), post-natal survival is not possible [2].

During the 20th century the quest for a feasible treatment for TGA was ongoing despite the many challenges the surgeons encountered. In the 50s, methods were developed for palliation procedures without extra-corporal circuits in addition to methods for corrections of heart defects in older children such as ventricular septum defect (VSD). Early pioneering attempts at correcting TGA by retransposing the arteries were quickly abandoned due to technical difficulties and the realisation that such surgery had to be performed in the neonatal period which at the time was not feasible[3]. The creation of an ASD in these patients proved to be a possible palliation as early as 1948. The idea of redirecting blood at the atrial level can be seen as a further development of that technique. Atrial redirection procedures were thus developed in children who either had a congenital ASD or a surgically created ASD in TGA patients. In 1958, Senning, in Sweden, published the first series of survivals[3] after the atrial redirection procedure. His method proved to be too surgical challenging and therefore Mustard designed a somewhat simpler technique in 1964[4] which became the preferred method throughout the “atrial redirection” era (figure 4).

Simultaneously, neonatal survival was improved by percutaneous balloon arterial septostomy[5] and by prostaglandin infusion for patency of the arterial duct in the late 1970s[6].

Nevertheless long-term complications after the Mustard procedure proved considerable. The technical difficulties with systemic and pulmonary venous obstruction could be overcome, whereas the post operative arrhythmias and systemic ventricular dysfunction proved fatal with a high degree of sudden cardiac death as well as terminal heart failure. Concurrently, infant cardiac surgery was developed in the early 1970s. The attention was re-focused on correction of TGA by re-transposing the great arteries, i.e. the arterial switch operation (ASO). Initial success was reported by Jatene, who operated in infants past the neonatal period[7]; a procedure which was further developed by Castaneda in neonates in 1982[8].

The early high mortality could be improved and ASO demonstrated fewer sequelae than previous methods[9 10]. Hence, new questions arose that needed to be addressed.

During an ASO, the arterial trunks are transected above the valvar complex, and two of the three sinuses are incised to accommodate the coronary arterial buttons. Transferral of the coronaries without obstructing flow is crucial when performing ASO.

The transection and translocation of the great arteries as well as translocation of the coronary arteries involve, as a consequence, transection of the cardiac sympathetic nerves. Jane et al (1986)[11] showed that a vast majority of the cardiac sympathetic nerves enter the heart alongside the great arteries and are therefore likely to be injured during the ASO. This may then cause denervation hypersensitivity of norepinephrine (NE) receptors and a disruption of the NE reuptake into the sympathetic nerve terminals (uptake-1)[12]. With time,

sympathetic nerves will to various degrees re-innervate the heart[13], although the sympathetic nerves re-innervating the coronary vascular bed will be imposed with the challange of passing not only the suture line crossing the wall of the great arteries, but also an additional suture line crossing the insertion of the coronary arteries at their new location in the root of the neo-aorta. The implication of an initial transection and a reinnervation of sympathetic nerves may impede the future function and possibly lead to deterioration of the heart after an ASO.

The autonomic nervous system (ANS) is a main regulator of the heart[14]. It has been shown that autonomic dysfunction, of either the sympathetic and parasympathetic division, is associated with clinical disorders such as ischemic heart disease and heart failure, and that it also predicts mortality[15-17]. Humans have low sympathetic activity (tone) and a dominant parasympathetic drive to the heart during resting conditions. Cardiac response to a parasympathetic burst allows for a quick dynamic vagal modulation of the cardiac rhythm. An impaired parasympathetic tone has been demonstrated to contribute to increased cardiovascular mortality and morbidity[18]. The anatomic innervation of postganglionic parasympathetic nerves after ASO is assumed to not be substantially injured, although considering the impact of the ANS in the regulation of heart rate, there might be a possibility also for disturbed cardiac vagal function.

The increased systemic vascular resistance (SVR) observed in patients with a Fontan circulation has been incriminated as a responsible factor for late failure of Fontan circulation. The mechanisms behind the increased SVR are, however, uncertain. Lambert et al. (2012)[19] investigated the muscle sympathetic nerve activity in Fontan patients and demonstrated that the increased sympathetic activity found was similar to that observed in patients with heart failure[20].

Patients with ventricular arrhythmia had a substantially increased NE spillover suggesting that cardiac sympathetic stimulation were associated with the arrhythmia[21]. It is pivotal to understand the adaptive physiological mechanisms imposed on the autonomic nervous system by congenital heart malformations and cardiac surgery to further enhance the treatment and long-term protection against heart failure.

The overall aim of this thesis is to examine the pathophysiological aspects of ASO on the heart's autonomic nervous system to further unravel possible mechanisms underlying cardiac arrhythmias, sometimes present in adolescents who underwent ASO as neonates. The present study combines assessment of the functional sympathetic and vagal nervous innervation and function of the heart, using NE radio-tracer infusion, baroreceptor sensitivity and heart rate variability, in clinical long term follow-up to identify possible abnormalities of cardiac innervation.

In order to further study the mechanistic pathophysiology and receptor sensitivity after denervation a "mimicked" ASO long-term survival model in piglets was developed to explore sympathetic nervous changes by means of physiological and pharmacological studies both in vivo and in vitro.

Background

Transposition of the great arteries

Ventriculoarterial discordance is defined as the pulmonary artery arising from the morphological left ventricle (LV) and the aorta originating from the morphological right ventricle. Transposition of the great arteries, also known as d-transposition (referring to the D-loop which is a loop of the embryonic heart tubes), has the inflow portion of the right ventricle located to the right of the

morphological left ventricle. The great arteries become parallel rather than crossing as in a normal heart, as the aorta tends to be on the right and anterior (figure 2)[22].

In a d-transposition, the systemic and pulmonary circulations run in parallel, preventing oxygenation of systemic blood through the pulmonary circuit. It is not compatible with extra-uterine life unless there is a mixing between the pulmonary artery and the systemic circulation (figure 3). An ASD is generally required since a patent duct alone has been proven to be insufficient to alleviate cyanosis. Consequently, in cases without an ASD a Rashkind balloon arterial septostomy is required if surgery is not performed within the first days of life[5].

TGA is not to be confused with congenital corrected transposition of the great arteries, where there is atrial-ventricular discordance and ventricular-arterial discordance, which, unless there are additional defects, is a non-cyanotic condition, (figure 6).

Arterial Switch Operation

The ASO starts by transecting the pulmonary arteries and aorta above the sinuses, followed by detaching the coronary arteries with a “button” from the aortic wall. The great arteries are switched and sewn into their new positions and the coronaries are sutured into the “neo-aorta”[7], (figure 5). The “French manoeuvre”, in which the pulmonary artery bifurcation is transferred in front of the distal ascending aorta[23], improved the technique and therefore also the outcome.

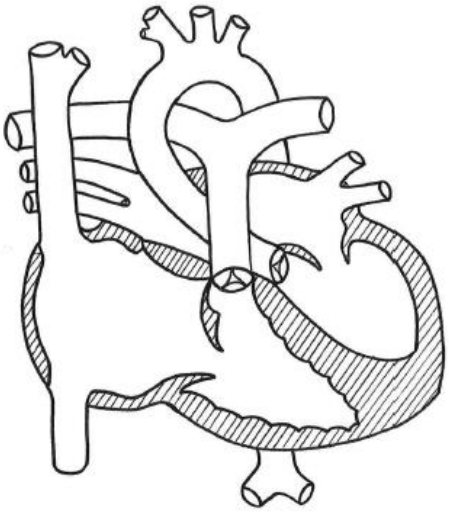

Figure 1. Anatomy of a normal heart

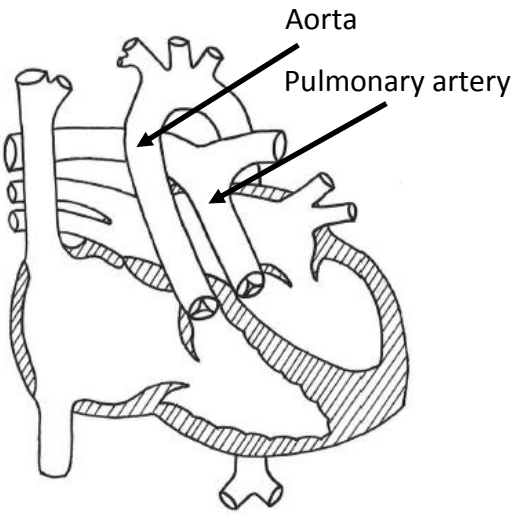

Figure 2. Anatomy of transposition of the great arteries

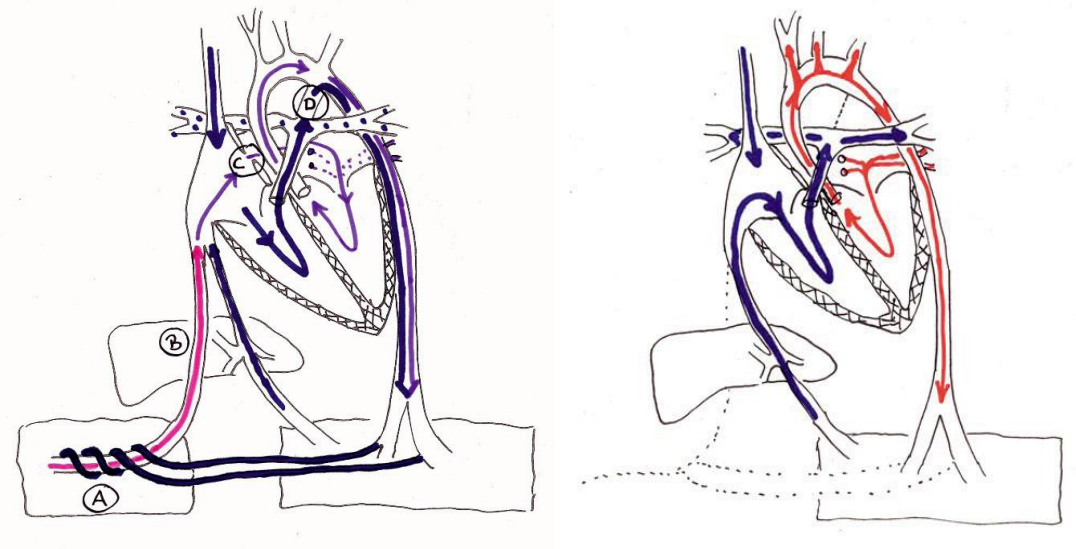

Figure 3. Schematic pictures showing the normal fetal circulation to the left and the normal postnatal circulation, with closed fetal connections, to the right. There are four shunts enabling the dirculation during fetal live: the placenta (A), Ductus venosus (B), the Patent Foramen Ovale (C) and the Patent Ductus Arteriousus (D). (with permission from Anne de-Whal Granelli)

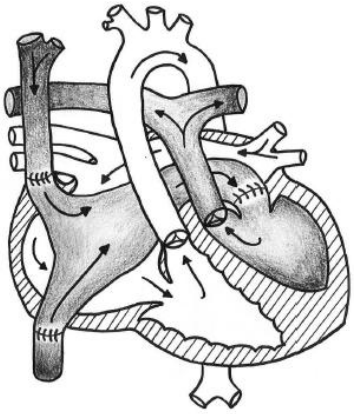

Figure 4. Anatomy post-Mustard-operation.

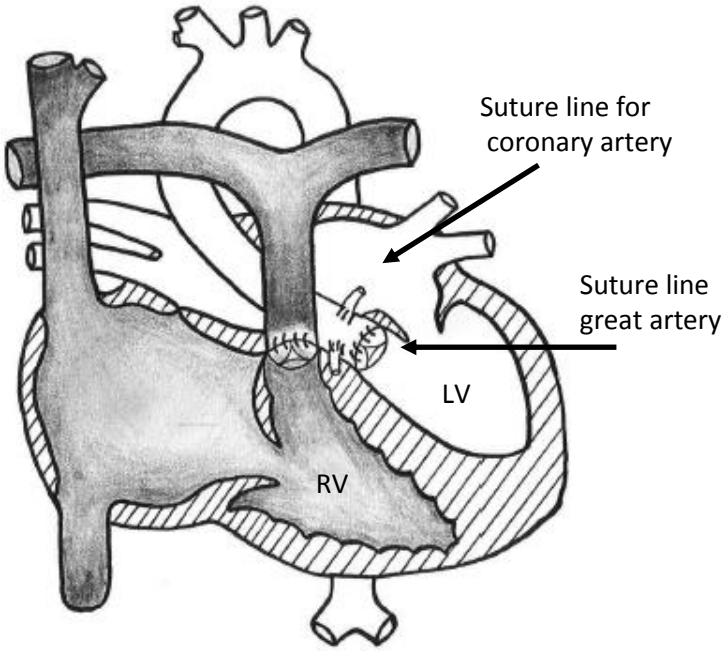

Figure 5. Anatomy post Arterial Switch , Operation. RV= right ventricle, LV= left ventricle.

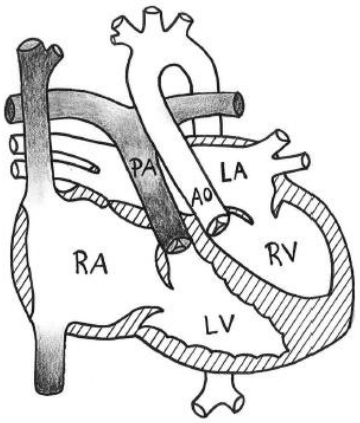

Figure 6. Congenital corrected transposition of the great arteries. RA= right atrium, LV= left ventricle, PA= pulmonary artery, LA= left atrium, RV= right ventricle, AO= aorta.

A potential complication is kinking, distortion compression and complicated coronary artery anatomy such as intramural coronaries which all might cause obstruction of the coronary flow causing ischemia. It has been shown that many ASO patients with obstructions in the coronary arteries and ischemia are asymtomatic or have diffuse symptoms therefore routinely investigations needs to be performed to detect potential ischemia even if there are no traditionally symptoms[24, 25]

Autonomic nervous system

The autonomic nervous system, the internal regulatory nervous system, regulates a large part of our organs and their functions. It includes the insular cortex, amygdala, hypothalamus, periaqueductal gray matter, parabrachial complex, nucleus of the tractus solitarius, and ventrolateral medulla[26], (figure 7). The ANS consists of three entities: the sympathetic nervous system (SNS), the parasympathetic nervous system and the enteric nervous system with plexus myentericus and plexus submucosus[27]. The latter system will not be discussed in this thesis.

The aim of the ANS and its regulation of cardiovascular functions is to make appropriate adjustments for the diverse physiological demands of the body and maintain homeostasis. The moment-to-moment regulation is achieved by integration of the sympathetic and parasympathetic components of the ANS. Main neurotransmitters in the ANS are NE and epinephrine (EPI) and acetylcholine. Dopamine (also an endogenous catecholamine) and other transmitters (for example neuro peptide Y) and their roles will be discussed briefly in this context

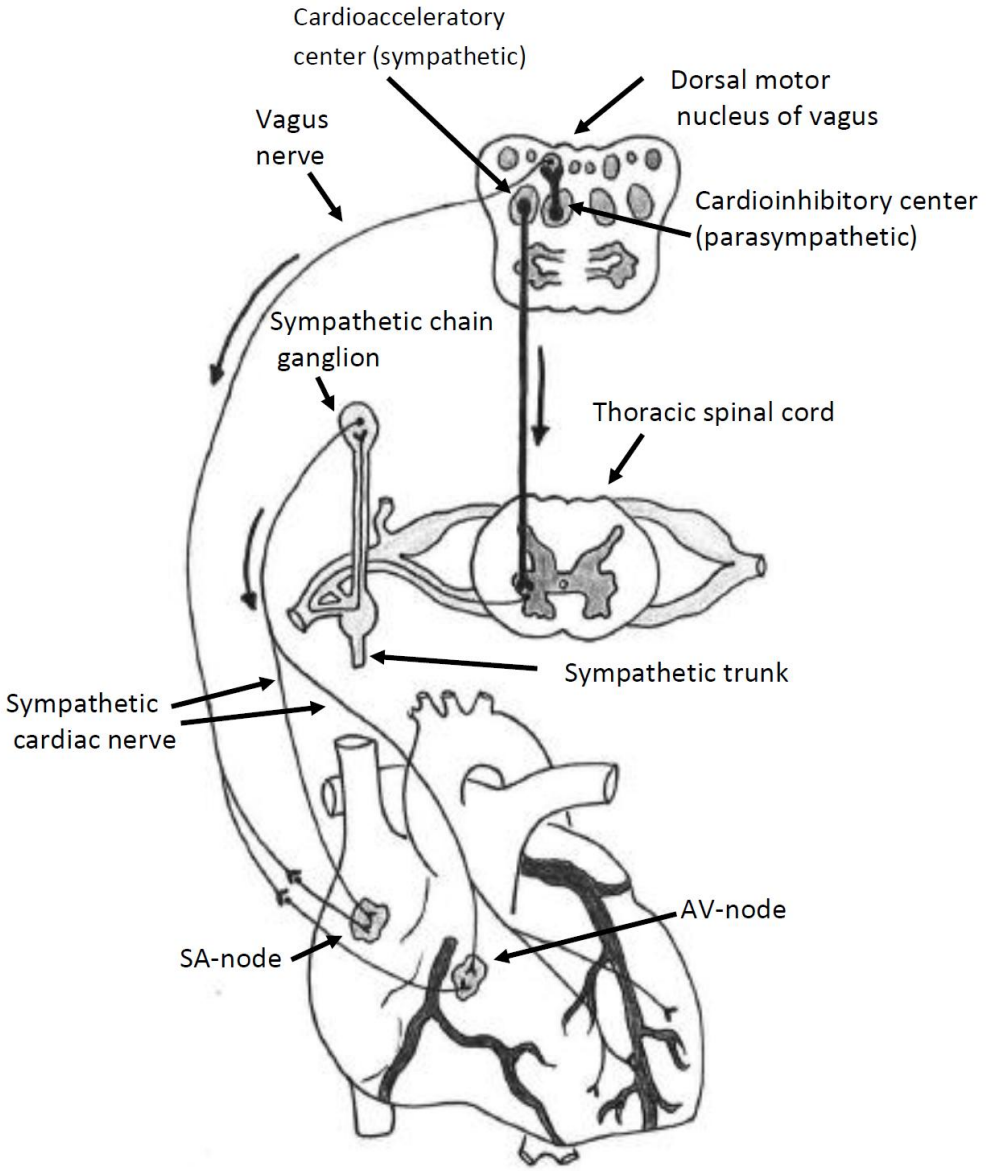

Figure 7. A schematic picture of the sympathetic and parasympathetic innervation of the heart.

Sympathetic nervous system

According to Goldstein (1990, 1995) the regional innervation in the heart is heterogenic and it is especially dense in the atria and the base of the ventricles[28-30]

Fibres from the SNS also innervate the adrenal medulla and trigger release of EPI and, to a substantially lesser extent, release of NE.

Neurochemistry of the sympathetic neuron

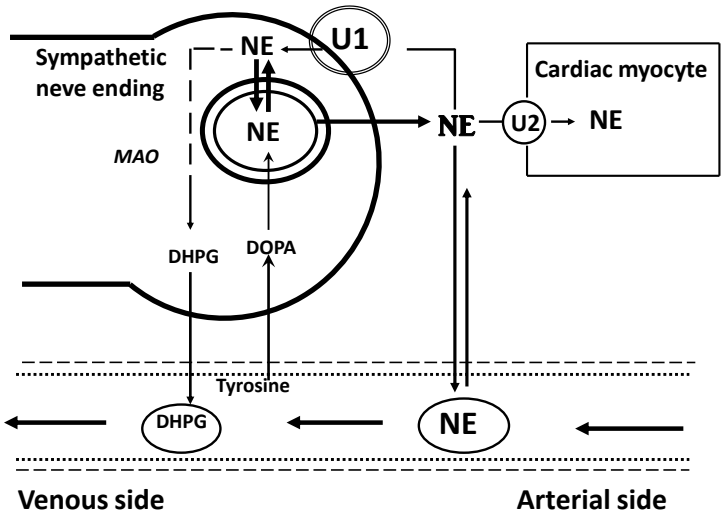

Figure 8. A schematic picture of the sympathetic nerve terminal and the release of norepinephrine (NE). DHPG=dihydroxyphenylglycol, U1=re-uptake-1, U2=re-uptake-2, MAO=monoamine oxidase.

The precursor to NE is dopamine[31]. The enzyme β-hydoxylase hydroxylates dopamine into NE. The final stage of synthesis is in the synaptic vesicles. An action potential triggers the nerve-terminal to release NE into the synaptic cleft after which NE binds to NE-receptors postsynaptically, (figure 8). The vast majority of released NE will be taken up into the sympathetic nerve-terminals and its vesicles -- the so called neuronal re-uptake-1. A leakage from the vesicles of NE into the terminals will expose NE to monoamine oxidase (MAO) that degrades NE to dihydroxyphenylglycol (DHPG) -- the predominant intra-neuronal metabolite. This metabolite reflects the turnover of NE and will be released into the plasma and can, as well as NE, be measured in the blood[32-34]. The majority of NE entering the sympathetic axoplasm by reuptake or by leakage from vesicles will be transferred back into storage vesicles. A small amount will escape and be exposed to the above described metabolism[35].

The re-uptake of NE into the nerve terminal can either be as uptake-1 (which in the heart is almost 92 % of released NE)[36, 37] or as uptake-2 (inactivated by the post-synaptic cell), and a few % spills over into the blood stream.
Sympathetic preganglionic nerves release acetylcholine in the adrenal medulla, which will trigger the phenyethanolamine-N-methyltransferas (PNMT) to catalyse the transformation of NE into EPI in the adrenomedullary cells; EPI is then released directly into the blood and is an agonist for both α-and β-receptors[38]
NE and EPI have various actions at the different receptors; in general α-receptors promote vasoconstriction while β-receptors affect the inotropic and chronotropic capacity of the myocardium and produce vascular relaxation[38].
Denervation owing to transection of sympathetic nerves by ASO is apt to cause a disruption of the NE neuronal turnover, which may lead to a denervation hypersensitivity of the NE- receptors[12]. To explore the consequences of nerve denervation, an animal model was developed in the present study. The advantages of such a model are that the SNS can be studied through experimental approaches including dose-response patterns to catecholamine in vivo. In an isolated heart preparation, in vitro, function and sensitivity of sympathetic nerves and receptors can be studied, excluding any other regulatory and counter-regulatory mechanisms such as vagal or humoral responses.

The Parasympathetic nervous system

The parasympathetic ganglia are usually located close to or inside the innervated organ, for that reason, vagal innervation consists of preganglionic fibres; it innervates the heart and lowers the heart rate. The right vagal nerve for the most part innervates the sinus node, whereas the left vagal nerve

mainly innervates the AV-node. The vagal nerve also innervates the atrial muscle[39]. The vagal nervous system could be described as “rest and digest”. Otto Loewin won (together with Sir Henry Dale) the Nobel Prize in physiology or medicine in 1936 for the identification of a endogenous neurotransmitter, acetylcholine[40]. The receptors that mediate the effects of acetylcholine are nicotinic and muscarinic receptors. Muscarinic agonists inhibit NE release from sympathetic nerve terminals and are selectively blocked by atropine. Cholinergic transmission in the parasympathetic ganglia and cholinergic stimulation of the EPI secretion from the adreno-medullary cells are mediated by nicotinic receptors[38].

Most of the inactivation of acetylcholine occurs extracellularly through acetylcholinesterase, immediately after the release from the nerve endings. This instantaneous inactivation makes it almost impossible to measure acetylcholine in blood. Other methods have been developed to determine parasympathetic nervous system function.

The pressure-sensitive nerve endings in the walls of the atria of the heart, the carotid sinuses and the aortic arch, are called baroreceptors. They are stimulated by central reflex mechanisms allowing physiological adjustment through close interaction between the sympathetic and parasympathetic nervous systems, with the aim of regulating changes in blood pressure via altering heart rate, vasoconstriction and vasodilatation. Baroreceptors are crucial for maintaining homeostasis[41]. The adjustment to alterations in blood pressure causes beat-to-beat oscillations in RR-intervals. The oscillations can be measured as heart rate variability[42] (HRV) (figure 9).

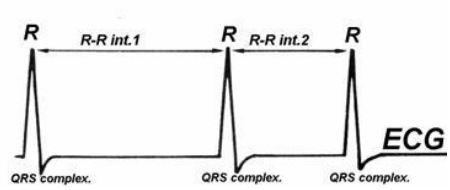

Figure 9. Heart rate variability.

Heart rate variability can be assessed by various means. Time domain analysis measures the changes in heart rate over time or the intervals between successive normal cardiac cycles. Frequency domain (power spectral density) analysis describes the periodic oscillations of the heart rate signal as different frequencies and amplitudes; and provides information regarding the amount of their relative intensity (termed variance or power) in the heart's sinus rhythm. Different frequencies have been identified to represent different parts of the autonomous nervous system. High frequency (HF) (range 0.15-0.4Hz) is considered to be driven by respiration and originates mainly from the parasympathetic nervous system. Parasympathetic blockage, by for example atropine, will reduce HF but it also reduces a significant part of the low frequency (LF, range 0.04-0.15 Hz). Ganglionic blockade eliminates the residual LF fluctuation, which implies that both sympathetic and parasympathetic modulations contribute to LF[43, 44] (figure 10). There is also very low frequency (VLF), considered to reflect thermoregulatory cycles[45] or plasma rennin activity[46].

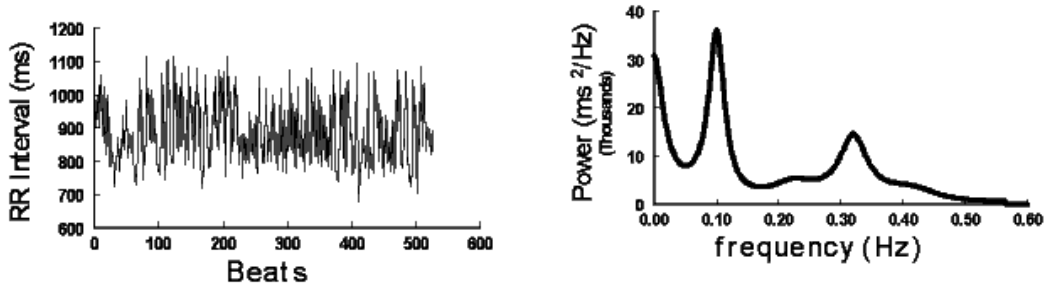

Figure 10. A schematic picture of heart rate variability with RR-interval in ms per beats and a diagram showing the peaks of low frequency (LF) and high frequency (HF) variability.

By measuring baroreceptor sensitivity (BRS), the entire baroreflex loop, from spontaneous blood pressure oscillation to heart rate adjustment, is evaluated (figure 11). BRS is a non-invasive method to assess the tone of the vagal nervous system. A normal modulation of cardiac parasympathetic nervous activity is considered to protect against ventricular arrhythmia[47].

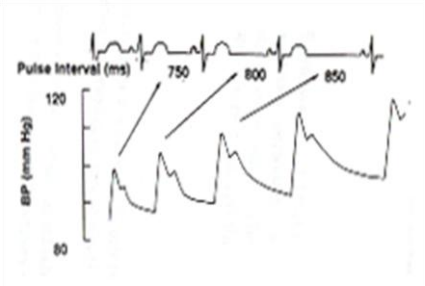

Figure 11. Showing how systolic blood pressure (SBP) modulates the pulse (RR) interval.

The electrocardiogram (ECG) can also be used to assess the QT variability index (QTVI) that evaluates the repolarization of the heart and also considers also the RR-interval[48, 49]**.** A high value of QTVI indicates a repolarization disturbance that is a factor contributing to arrhythmogenesis.

Current perspectives and unresolved issues

For patients who underwent ASO as correction for TGA in their neonatal lives, usually within the first 2 weeks of life, cardiac problems such as arrhythmias, heart failure and ischemic events have been reported. This may be related to disturbances of the autonomic nervous system.

- How can the ANS function in ASO patients be assessed in a feasible clinical context, and what do the results predict in regard to future potential heart failure?

- To what extent can potential denervation hypersensitivity promote arrhythmia in the recently ASO-operated neonate?

- Is re-innervation of the heart heterogeneous and potentially pro-arrhythmic?

- Is an animal model creating the same transection of nerves that innervates the heart possible in order to gain more knowledge of ANS post-ASO.

Hypothesis

Arterial switch operation performed in neonates impairs cardiac autonomic nervous function.

Aims of the study

- To determine cardiac sympathetic neuronal function, cardiac baroreceptor sensitivity and QT variability index in long-term follow-up after arterial switch operation in adolescents.

- To assess heart rate variability during night and day, respectively, expressed both in time and frequency domains in long-term follow-up after arterial switch surgery in adolescents.

- Create a long-term survival piglet model, mirroring the ASO operation in neonatal humans, to enable physiological and pharmacological studies of sympathetic function.

- To explore sympathetic responsiveness, determined as heart rate, to catecholamines after “mimicked ASO” in piglets in vivo and *ad modum* Langendorff perfusion.

Methodological Considerations

Study design

This thesis is based on two projects. The first one is a clinical long-term follow-up of teenagers born with d-TGA. The second project is to develop a new experimental piglet model, mimicking arterial switch surgery in humans to enable us to do physiological and pharmacological studies.

Study groups

Teenagers:

Twenty-eight children born between May 1983 and October 1991 who underwent surgery in the neonatal period using the ASO at the Sahlgrenska University Hospital were in consecutive order of birth offered to participate in a clinical long-term follow-up study. Seventeen of these participated in the follow-up. Two additional children, born 1994 and 1995 were added upon request from their paediatricians.

Healthy subjects:

Controls for cardiac neurochemical studies with tritiated NE were obtained from a database at the Baker IDI Heart & Diabetes Institute, Melbourne, Australia. BRS and QTVI were assessed in controls derived from a database at the Department of Clinical Physiology at the Sahlgrenska University Hospital. HRV controls were recruited from a database of healthy adolescents at the Department of Clinical Physiology at the Sahlgrenska University Hospital/Östra Sjukhuset.

Ethics: The protocols were approved by the ethics board of the University of Gothenburg (no Ö379-02, and at the Alfred Hospital, Melbourne, Australia) and conducted in accordance with the declaration of Helsinki (initial statement and amendments found on the homepage of the World Medical Association,

www.wma.net). All subjects, and if below 18 years, also their parents, gave informed consent.

Catheterisation Procedures

After an overnight fast, catheterisation was performed during anaesthesia. Full haemodynamic measurements were obtained. In 8 patients, a stable catheter-position was obtained in the coronary sinus; these patients received an intravenous (i.v.) infusion of tritiated NE ($^{[3H]}$NE) (specific activity of 11-25Ci/mmol; New England Nuclear, Boston, MA, USA) via a line in the right arm. When steady state was achieved, blood was sampled simultaneously from the catheter in the coronary sinus and from a catheter in the descending aorta, (figure 12 and 13). A subsequent adenosine infusion was given in the right hand, and under continuous i.v. infusion of $^{[3H]}$NE, blood samples were obtained from the same positions as during resting state.

Figure 12. Cecilia Falkenberg and Thomas Gilljam at the heart catetherization laboratory taking samples of tritiated norepinephrine.

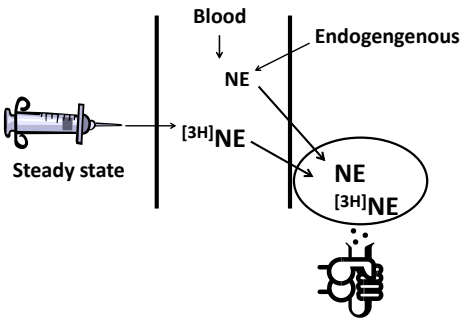

Figure 13. A schematic picture of tritiated norepinephrine ($^{[3H]}$NE) infusion in the circulation.

Plasma Norepinephrine Kinetics

Depolarisation of the nerve membrane releases NE from the sympathetic nerve terminals. When released into the plasma NE is efficiently removed by neuronal and extra-neuronal uptake[36, 37]. Only a small amount will spill over into the circulation(figure 8)[37]. When estimating the release of NE from an organ into plasma, the NE extraction has to be considered. Esler et al. (1979, 1984)[32, 50] showed that the concentration of arterial NE is determined by the ratio of the fraction of endogenously released NE that appears in plasma (the spill over rate) to the removal of NE from plasma per unit time (clearance) from the arterial plasma.

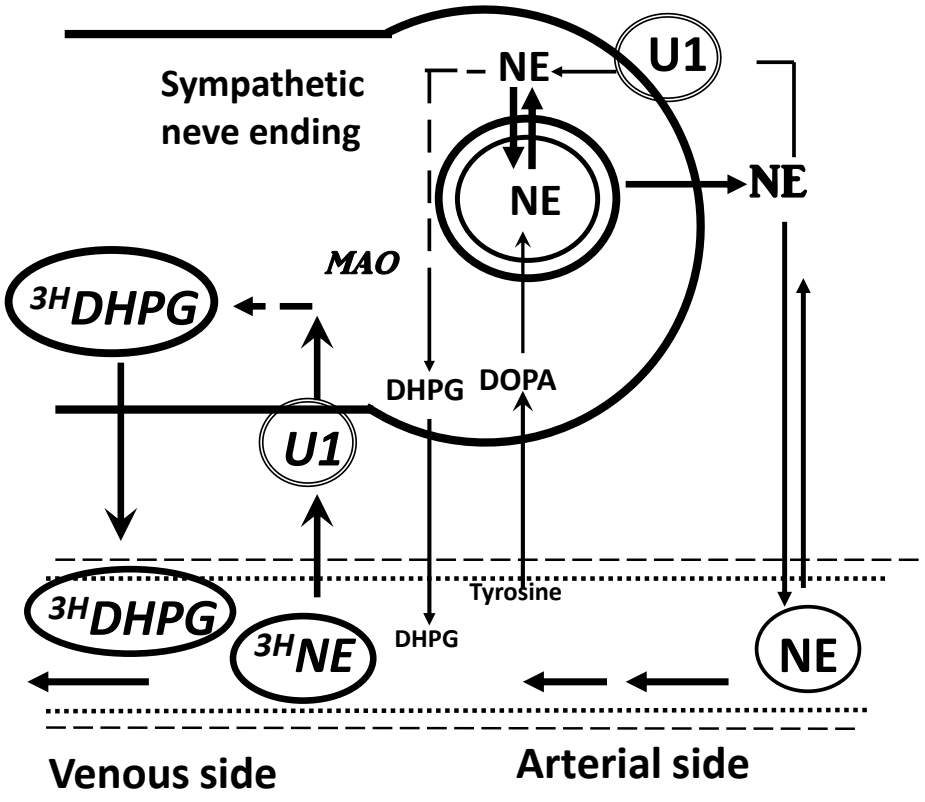

Figure 14. Schematic picture of sympathetic nerve ending with norepinephrine turn over, including uptake of tritiated norepinephrine ($^{[3H]}$NE) and its metabolising by monoamine oxidase (MAO) to tritiated dihydroxyphenylglycol ($^{[3H]}$DHPG). U1= re-uptake-1, U2= re-uptake-2, DHPG= dihydroxyphenylglycol.

Plasma clearance can be calculated as:

NE clearance= $^{[3H]}$NE infusion rate/arterial plasma $^{[3H]}$NE concentration

Whole body NE spillover can be calculated as:

Whole-body NE spillover= arterial plasma NE concentration x NA plasma clearance

Cardiac fractional extraction of NE (EXcardiac) is calculated as:

$$EXcardiac = (^{[3H]}NE_A - ^{[3H]}NE_V) / ^{[3H]}NE_A$$

NE_V is coronary sinus NE concentration (pmol/mL) and $^{[3H]}NE_A$ is arterial concentration of $^{[3H]}$NE (dpm/mL).

Specific activity (SA)(isotope dilution) across an organ can be used to estimate the endogenous release of NE[37].

$$SA_{NE} = {}^{[3H]}NE_{AV}/NE_{AV}$$

$^{[3H]}NE_{AV}$ and NE_{AV} are the respective arterial-coronary venous difference in plasma concentration of $^{[3H]}$NE (dmp/mL) and endogenous NE (pml/mL).

The gradient of $^{[3H]}$DHPG, $^{[3H]}$NE and DOPA between the descending aorta and coronary sinus was also calculated (paper I).

Heart Rate Variability

Heart rate variability and blood pressure variability are believed to be the result of a dynamic interplay of uncountable variations of the cardiovascular situation and the response of the cardiovascular regulatory systems to these variations[41]. HRV is usually evaluated from the RR-interval changes on beat-to-beat basis (a Tachogram) and may be expressed in time-domain or frequency-domain units. The latter requires transformation. Time-domain description is based on RR

changes over a period of time or number of beats. The frequency-domain description is based on the strength (amplitude) of a set of harmonics, i.e. sine-wave like oscillations, reflecting corresponding variability of the tachogram. Different algorithms are used to translate heart rate and blood pressure variability into a range of frequencies with different amplitues[46, 51]. In humans, recorded frequencies usually range between 0 to 0.4 Hz and are divided into high frequency (HF)(range 0.15-0.4Hz) and low frequency (LF)(range 0.04 to 0.15), while very low frequency (VLF)(range ≤0.04Hz) has also been identified[52]. Heart rate variability evaluates the degree of autonomic modulations rather than the level of autonomic tone[53].

All 24-hour ECG analyses were performed using the ASPECT Holter System 3.80/3.81 (Danica Biomedical AB, Borlänge, Sweden). An automatic algorithm detecting the QRS-complex was applied to the data-set[54], (paper II). ECG data were recorded using 100Hz sampling frequency. An automatic algorithm detecting the QRS-complex onset was applied to the data set[54]. Prior to data analysis, the data-set was corrected automatically for missing or ectopic beats. Periods of more than 4% corrected beats were rejected from further analysis. On a beat-to-beat basis, the QRS onset was defined to a nominal resolution of 1 ms using an envelope interpolation delineation technique described by Nygård et al (1983)[54].

The above procedure was carried out for the entire recording, resulting in consecutive QRS onset intervals with 1 ms nominal resolution. From the QRS-complex positions, an RR-tachogram was generated with a 5 Hz resolution from which the frequency analysis was performed.

Each individual continued with his/her everyday life with no restrictions during the recording. We aimed at analysing periods when cardiac vagal drive was assumed to be maximal, i.e. during the night-time. Hence, HRV results from

such recordings would reveal the maximal cardiac vagal drive that can be obtained at resting conditions. Day-time was also obtained for comparison. The highest (day-time) and the lowest (night-time) heart rates, during 5 min episodes, were identified for further analysis.

Cardiac baroreflex sensitivity

Increases or decreases in arterial blood pressure generate a response by mutual changes in the sympathetic and parasympathetic activity[30]. The baroreflex control of heart rate evaluates the sensitivity of the response in the entire baroreflex loop to spontaneous blood pressure oscillations[55, 56], and is a sensitive method to detect cardiac vagal dysfunction that convey risk of cardiovascular morbidity and mortality[18].

ECG (RR-intervals) and beat-to-beat systolic blood pressure (SBP) recordings were registered during 20 min using Portapres equipment in awake patients and healthy subjects[57-59], (paper I). The time series of SBP and RR-intervals were scanned by the computer to identify baroreflex sequences, which are defined as three or more consecutive beats in which successive SBP and RR-intervals concordantly increase or decrease by the classical criteria suggested by Bertinieri et al[56]. A change in SBP of 1mmHg and in RR-interval of 5 msec is recorded.

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[48, 49]. A template QT interval was defined, for one beat which was used for finding the QT intervals of all other beats. The QT variability is measured and

calculated as the QT variability index adjusting to the RR-interval and logarithmic transformation[60], (paper I).

Complex cardiac surgery during cardiopulmonary bypass in piglets

Animal study groups:

Piglets of the Swedish Landrace -Yorkshire x Hampshire breed from a commercial breeder were used. They were purchased in groups of four littermates. Male piglets were abandoned early in the series because they had to be castrated, in order to be kept for long term survival. Unless castrated, they tend to become too aggressive to be kept at the research facility. In accordance with Swedish Animal Welfare guidelines the piglets were 8.5 weeks of age at the time of surgery.

Ethics: The study protocol was approved by the ethics committee for animal studies at Gothenburg University (no 150/2002 and 150/2005). It conformed to the principles of the American Physiological Society.

At the start of this study there was, to the best of our knowledge, limited information regarding complex heart surgery during cardiopulmonary bypass (CPB) in piglets of only 8 weeks of age[61, 62].

The experimental protocol combined the paediatric cardiac surgery procedures at the Queen Silvia Children's Hospital with available information from a literature search on this topic and veterinary experience at from Experimental BioMedicine at Gothenburg University. If, for a particular drug, no veterinary use or pharmaceutical dosing for pigs was available in the literature, paediatric doses in mg/kg were used (picture 15-17). As this study progressed, the equipment used for CPB changed to improve the procedure (figure 18)(paper III).

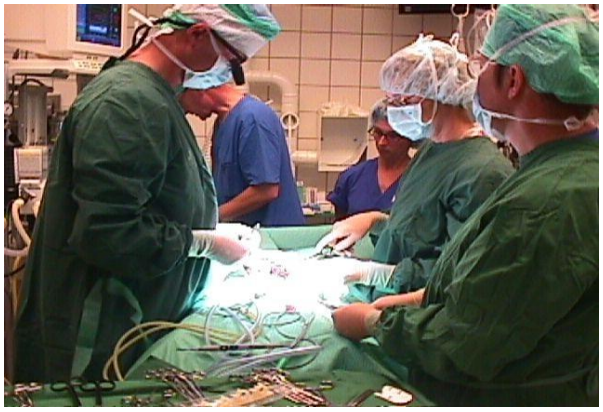

Figure 15. From the left, Stefan Hallhagen, Krister Nilsson, Nadia Karlsson, Cecilia Falkenberg, and Ingegerd Östman-Smith.

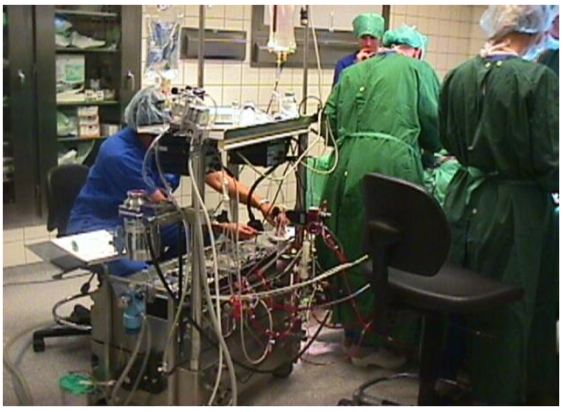

Figure 16. The cardiopulmonary bypass set-up.

Figure 17. Piglets post-arterial switch surgery, picture taken the same day as the surgery was performed.

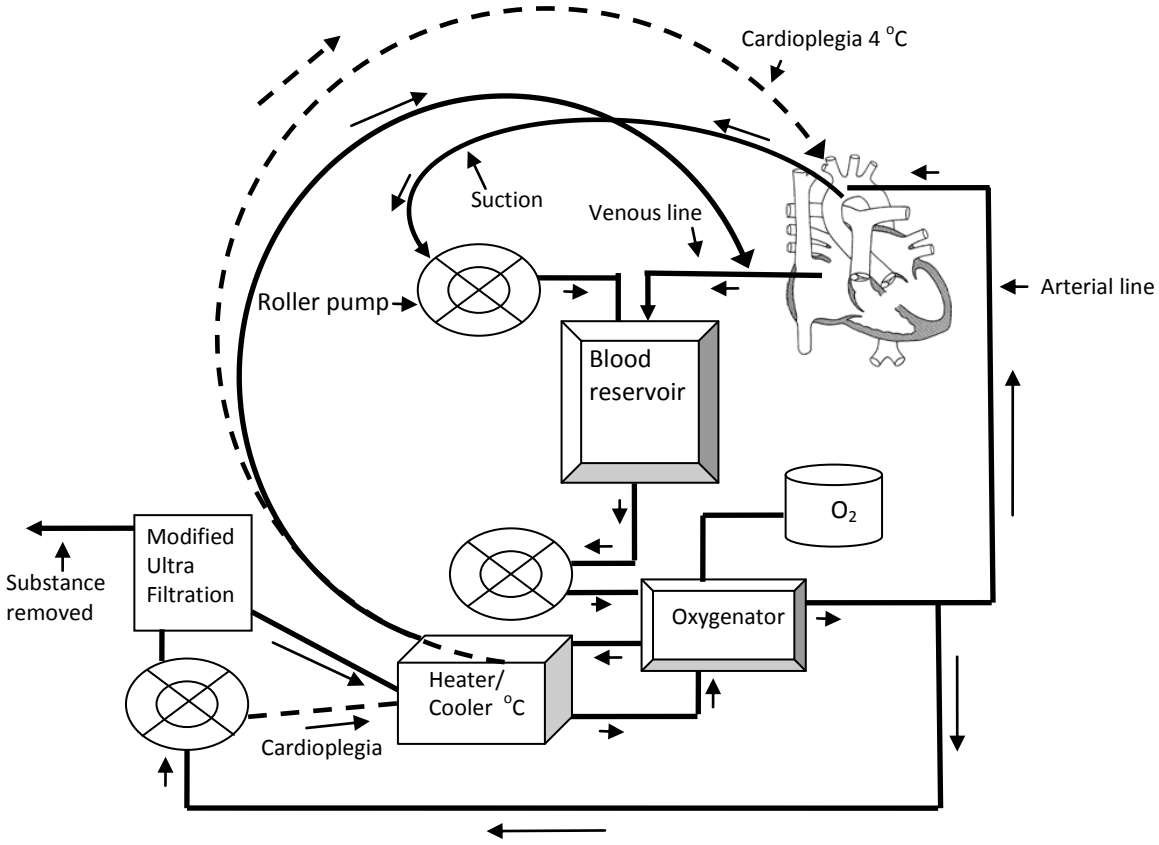

Figure 18. Schematic set-up of the cardiopulmonary bypass.

In Vivo Study

A dose-response study was performed at an average age of 6 weeks post-surgery[12, 13] in the anaesthetised animal (figure 19) there was a 60 to 90 min recovery period to prevent possible confounding factors by residual NE response when starting the second dose-response curve with EPI between the dose-response studies of NE and EPI. Doses of NE and EPI corresponding to 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 μg/kg were given. In order to be able to measure the response of every dose in relation to basal heart rate and blood pressure, heart rate and blood pressure responses were recorded until baseline levels were regained. Subsequent dosing schedules were given once more in the same fashion. Heart rate and blood pressure were monitored continuously

using a Datex-Ohmeda S/5 (Datex-Ohmeda Division Instrumentarium Corp., Madison, Wisconsin, USA (paper IV).

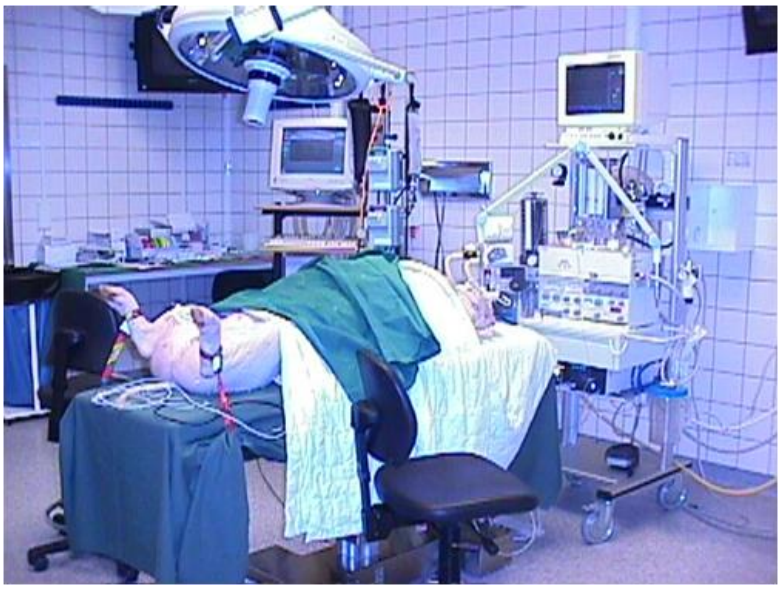

Figure 19. The set-up for in vivo physiological study

In Vitro study

In the Intact body both nerves and humoral influences naturally affect regulation of heart rate. By extracting the heart and performing a perfusion with Krebs solution[63] *ad modum* Langendorff[64, 65] on the beating isolated heart, (figure 20), a dose-response study of NE allowed assessment of the sensitivity of alpha and beta receptors, as well as coronary flow without interaction by nerves and humoral effects (paper IV).

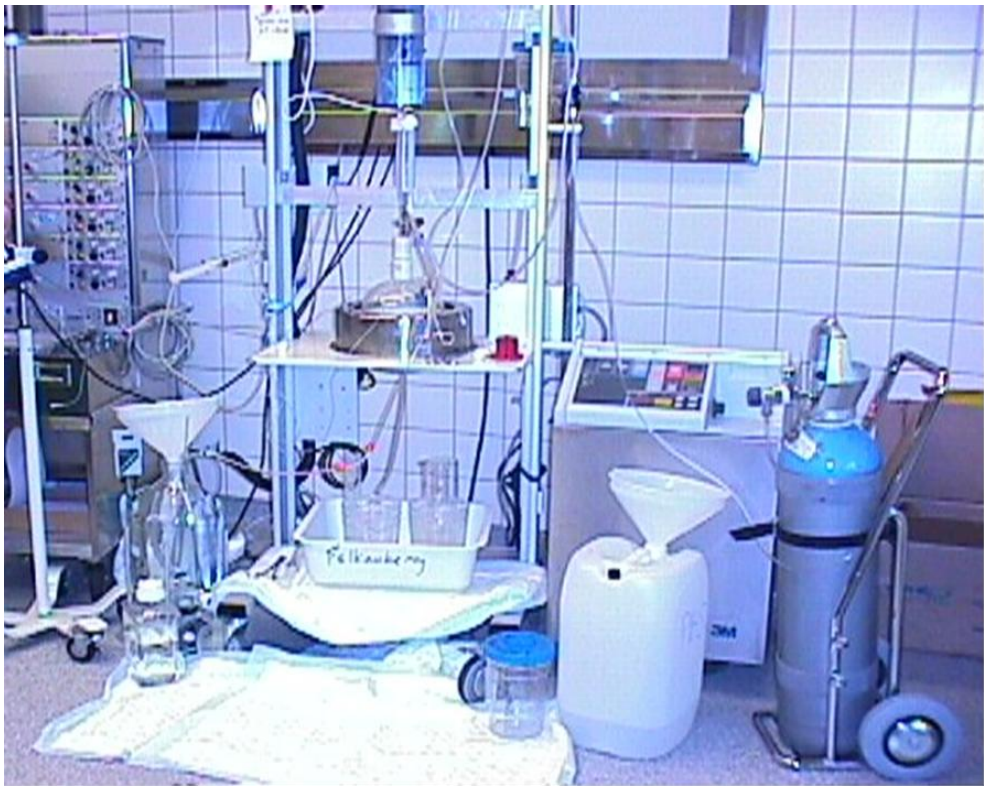

Figure 20. Langendorff perfusion. To the left the polygrass, in the middle the Langendorff perfusion device, and to the right the heat exchanger from a cardiopulmonary bypass machine.

Statistical analysis

Statistical analysis was performed on commercial software "Statgraphics Plus v5.2 and Graph Pad Prism 4). Numerical distributions are presented by their mean±SD. Mann-Whitney U-test was used for inter-group comparisons, and Wilcoxon rank sum test was used for paired comparisons given that the distribution of the data from switch-patients were not normally distributed. The dose-response curve from the Langendorff perfusions was assessed by Graph Pad Prism comparing four-criteria analysis (bottom, top, Hill slope, effective concentration for 50 percent of maximal response). Statistical significance was defined as $p<0.05$.

Results and Discussion

Congenital heart diseases (CHD) occur in approximately 0.8% of live births. They are a heterogeneous group of patients who have due to surgical and medical advances experienced incremental increases in survival beyond childhood[66]. Estimates based on the current surgical mortality rate predict that almost 1 in 150 young adults will have some form of CHD during the next decade[67, 68]. Despite excellent surgical results long-term survival may lead to patophysiological challenges and hence morbidity and mortality[66].

Studies convey results of good early outcomes for TGA patients[69]. However, it is important to study long-term consequences regarding plausible impairment of both the sympathetic and parasympathetic division of the ANS[66], development of the coronary arteries[70] and also myocardial ischaemia[69]. These can all potentially be detrimental to the health of the TGA patient.

Long-term follow-up protocols with feasible methods for investigating and assessing the post-operative situation and prognosis are clearly of great value. Non-invasive methods are preferred due to the risk imposed with invasive methods; however, they do not always have the desired sensitivity and specificity and therefore need to be complemented with clinical invasive methods, such as heart catheterization. In spite of all the scientific and methodological developments it is not possible to study all potential consequences of ASO, and the detrimental effects of imbalances in the ANS on morbidity and mortality in humans[18]. The need for an animal model to enable post-surgical investigations of injury and repair of the ANS to unravel some of the research questions is imperative.

Sympathetic neurochemistry in humans

Sympathetic nervous system activation has been associated with the development and complications of cardiovascular diseases[19, 71, 72].

In heart failure, increased sympathetic activity is seen as a compensatory mechanism[73]. With the use of "state of the art NE kinetics technique"[50 32] used in the present study, the ASO group demonstrated clear-cut evidence of functioning cardiac sympathetic nerve terminals releasing NE at resting conditions.

The substantial fall in specific activity across the heart supports the existence of functioning cardiac sympathetic nerve terminals; however a trend was noted towards a smaller fall in the ASO group, (figure 21). The greater the dilution of endogenous NE across the coronary vascular bed, the lower the specific activity. This indicates specifically endogenous NE release from cardiac sympathetic neurons.

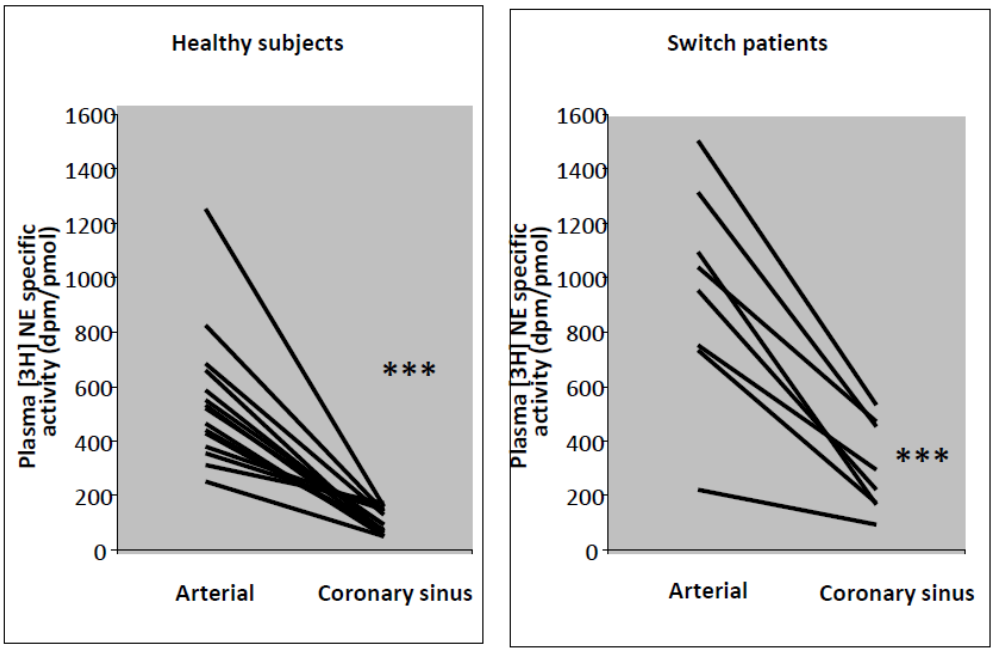

Figure 21. A statistically significant decrease of specific activity for tritiated norepinephrine (NE) from the arterial to the coronary sinus in healthy subjects (left panel) and switch operated patients (right panel).

The extraction fraction of [3H]NE across the heart was markedly decreased compared to healthy subjects suggesting loss of re-uptake sites (neuronal uptake-1) in the sympathetic neurons[37, 74, 75], (figure 22).

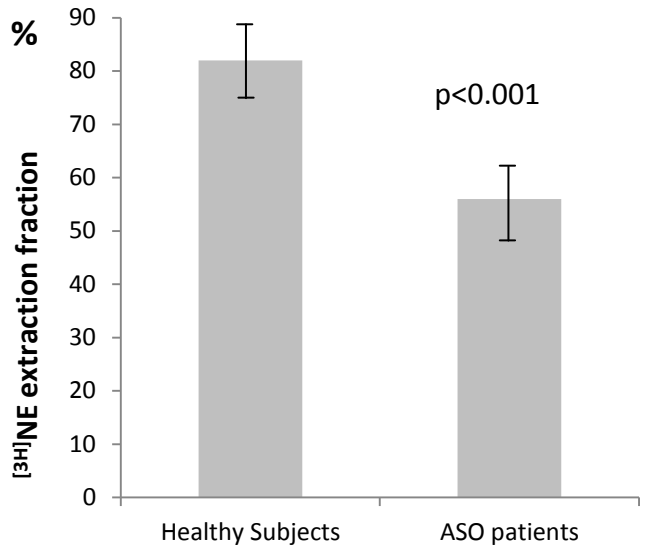

Figure 22. The tritiated norepinephrine (NE) extraction fraction across the heart was reduced in the arterial switch operated (ASO) group vs. healthy subjects.

With the dense sympathetic nerve innervation of the heart and narrow synaptic clefts, the neuronal re-uptake mechanism is of major importance for sympathetic nerve function in the heart (paper I).

Cardiac DHPG is a measure of the NE turnover in myocardial tissue. A normal cardiac DHPG spillover reflects adequate NE stores and is an excellent method to determine cardiac NE neuronal function, based on the fact that a positive veno-arterial step-up usually prevails in healthy hearts.

[3H]DHPG can only be measured if [3H]NE has been infused and taken up and being metabolised in the sympathetic nerve terminals (figure 14). Therefore, a

measurable concentration of [3H]DHPG reflects functioning sympathetic nerve terminals.

Both the endogenous DHPG and the [3H]DHPG had a lower gradient between the arterial and coronary sinus in the ASO group (figure 23). These findings are consistent with a lower NE extraction fraction, suggesting that cardiac sympathetic neuronal function is disturbed in ASO patients at least to a limited degree, given also the clear-cut fall in NE specific activity across the heart.

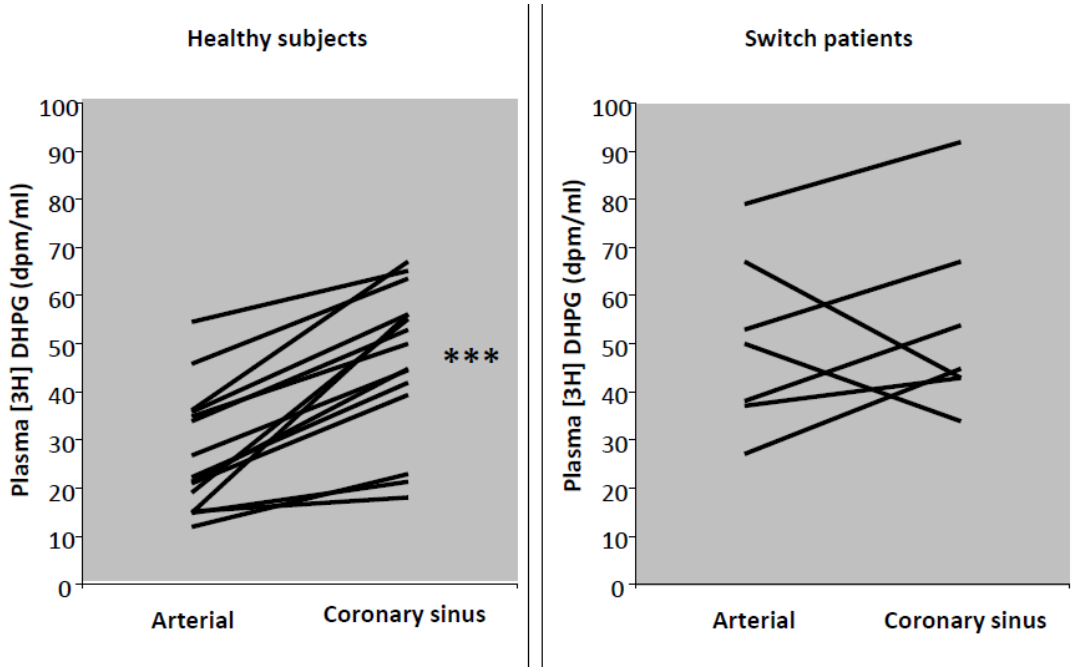

Figure 23. The healthy subjects demonstrated a positive step-up between the arterial and coronary sinus for tritiated DHPG (left panel), a finding which could not be demonstrated in the arterial switch operated group (right panel).

To further activate the sympathetic nerve system in general an adenosine infusion was given to these ASO patients, which resulted in massive increase of total body NE spillover supporting the contention of a substantial generalised sympathetic activation, (figure 24).

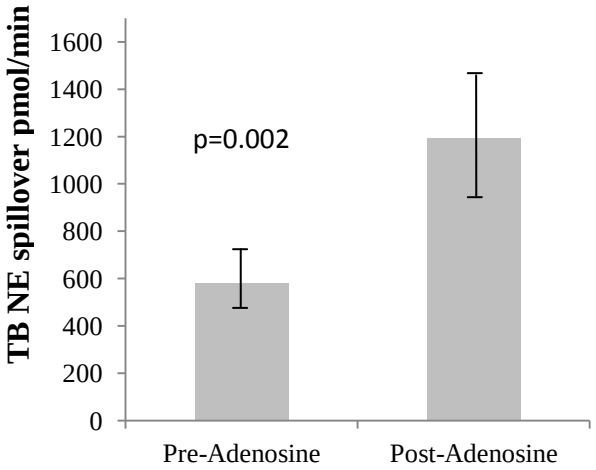

Figure 24. Total body (TB) Norepinephrine (NE) spillover increased in the arterial switch operated group after sympathetic stimulation following adenosine-infusion.

After the adenosine infusion, the $^{[3H]}$DHPG step up from the arterial to the coronary sinus was increased 4-fold in the ASO group, pre-adenosin $^{[3H]}$DHPG, 3.86±16.9 dpm/mL vs. post-adenosin 16.1±10 dpm/mL.

We speculate that the loss of cardiac sympathetic neurons, due to a heterogenous innervation, is compensated for by increased NE release per neuron of the remaining cardiac neurons.

Patients with heart failure demonstrate increased cardiac release of NE and a decreased uptake-1. They also have a decreased leakage of NE from the vesicles to the axoplasma of the sympathetic nerve terminals, aiding in preventing depletion of NE in the vesicles. The increased cardiac release of NE in such patients leads to an amplified stimulation of NE in the adrengenic post-synaptic receptor resulting in a down regulation of these receptors[35].

Consistent with such reasoning, the impairment of the sympathetic nerve terminals caused by the ASO in the present study could impose an augmented risk for these patients to subsequently develop heart failure and or arrythmia.

Heart rate variability, baroreceptor sensitivity and the QT interval variability index

The regulation of changes in sequential beat-to-beat intervals reflects the interaction between the parasympathetic and sympathetic nervous system; i.e. the physiological regulation of the heart[76 77]. A high level of variability is considered to reflect a healthy, responsive system able to regulate physiological processes; i.e. the ability to respond to internal or external stressors by diminishing parasympathetic activity and allowing sympathetic activity to dominate followed by a recommencement of parasympathetic dominance after the stress is relieved[78, 79] On the other hand, a low level of variation reflects an ANS that is less responsive to changing conditions[80] A way to evaluate LF and HF is the ratio between them which is considered a marker of change in the relative balance between sympathetic and parasympathetic activity[81, 82].

Heart rate variability is easily assessed in a patient using ECGs and computer algorithms. However, interpretation of the HRV variables and the implications of the results are intriguing. A survey of the topic resulted in a so called "Task Force" which had to develop an appropriate standard and also of give an exposé of both the usefulness of HRV and addressing the difficulties in interpretation[52]. HRV can be influenced by several factors other than innervation and function of the ANS of the heart, such as the release of adrenomedullary catecholamines and variations in the renin-angiotensin system[46, 83]. Patients who have received heart transplants and therefore have had a complete denervation of the heart show variation of HRV attributed to humoral effects and to respiration[84], clearly showing that analysis of HRV has potential pit-falls.

In the present study, the ASO group showed a statistically significant difference for normalized (n.u) LF and HF, but not for LF/HF ratio, in the 24-hour frequency analysis. In the 5 min recording during night-time, which represented the lowest recorded values of mean heart rate for 5 min (healthy subjects heart rate 56±8 and ASO group 52±10) there was a statistically significant difference between the healthy group and the ASO group LF n.u, HF n.u and LFn.u/HFn.u ration (table 2, paper II). The incremental increase of the HF in the night-time compared to daytime for both groups and a corresponding decrease in LF for both groups, (figure 25) suggests that ASO patients in long-term follow-up may have a moderately reduced cardiac vagal influence that is only detectable during sleep when the cardiac parasympathetic drive is considered higher than during day-time.

In adults with acquired cardiac disease, sympathetic activation and parasympathetic withdrawal have been studied[85]. Adults undergoing coronary bypass surgery have reduced HF power, reflecting a reduced ability to regulate cardiac ANS physiological function. The HF power subsequently returns to pre-surgical levels within 30-60 days[86].

Feeding during infancy is a challenge to homeostasis and a reduction in parasympathetic activity (measured by HF power) during feeding in healthy infants has been studied[87]. Adaptive reduction in HF power during feeding was reduced in infants early after surgical correction for TGA compared to matched healthy infants[88]. This study may suggest that vagal tone is affected by surgery which would be consistent with findings in our study demonstrating a lower

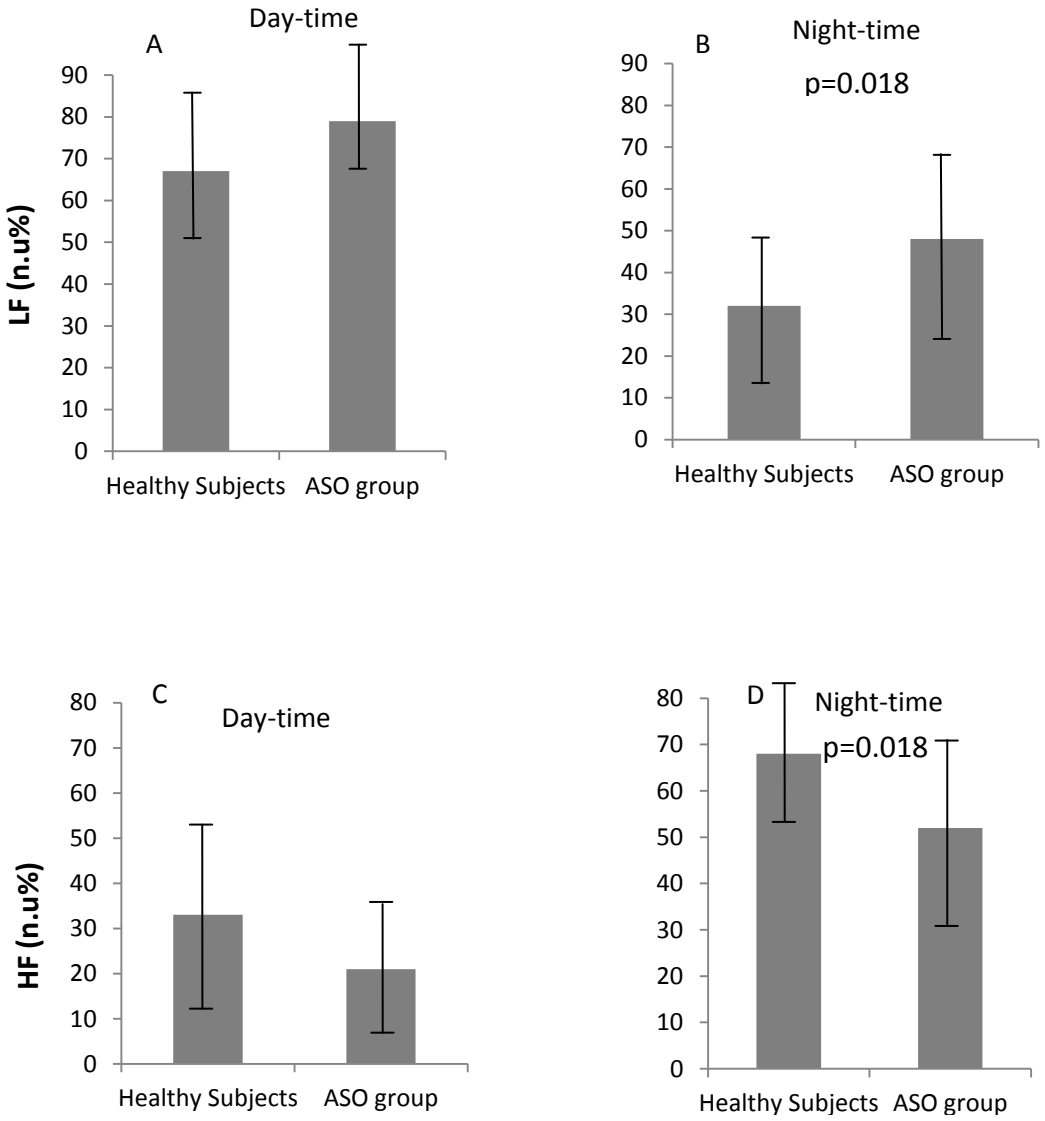

Figure 25. A: Low frequency (LF) normalised (n.u) % day-time in healthy subjects and ASO group. B: LF n.u % night-time in healthy subjects (32±15)(mean±SD) and ASO group (48±20). C: High frequency (HF) n.u % day-time in healthy subjects and ASO group. D: HF n.u % night-time in healthy subjects (68±15) and ASO group (52±20).

parasympathetic tone during night-time in the ASO group compared to healthy subjects. In neonatal studies, however, one must consider that results may be affected by examination in the hospital setting (such as the TGA infants prior to surgery) versus the home environment.

In alignment with Kaltman et al (2006)[88], our study could be interpreted as showing that the passage of time after surgery improves the parasympathetic function of cardiac rhythm regulation. Therefore detecting any impairment is challenging and is mainly shown during circumstances when the parasympathetic system tone is more influential than the sympathetic, i.e. during night-time and sleep, (paper II).

HRV can has also been measured and analysed in time-domain. SDNN is considered to respond to total power of the frequency domain and RMSSD to HF. However, the present study showed no difference in regard to time-domain between the two groups.

Baroreceptor reflexes impose a strong inhibitory effect on sympathetic outflow and stimulate the vagal neurons. It is relevant to study these reflexes because they operate within very short time frames and also with high frequencies of heart rate. Baroreceptor afferents can transmit blood pressure fluctuations accurately within high ranges of HRV. Cardiac vagal function, estimated by BRS, revealed only a trend toward a reduction in the ASO group vs. controls. The anatomical differences in innervation by the vagal nerve vs. the sympathetic nerves of the heart impose less risk for vagal innervation to be damaged by the surgical technique. This could be an explanation for preserved cardiac vagal function, (paper I). The BRS was recorded at rest and analysed during day-time with a more prominent sympathetic and less vagal tone prevailing, while parasympathetic efferent activity is known to be the most influential for governing heart rate during sleep. Since significant differences existed between

the groups at night-time in regard to high frequency HRV, the BRS results might have been different had the examination been performed at night.
The QT interval variability index represents myocardial repolarisation and is changed in patients with heart failure[89]. In our study, there was no statistical difference between the two groups, although ultrasound examination revealed signs of heart failure based on measurements of fractional shorting of the LV in some of the patients, (see supplement, paper I). Regardless of the cause of heart failure, it is of the outmost importance for long-term survival to detect and treat it. The QT variability index could therefore be useful in the clinical long-term follow-up programme.

Physiological consequences of sympathetic denervation in piglets

To our knowledge, a mimicked arterial switch operation had not been performed in 8 week old piglets before (paper III). The logistic challenges were many. It was essential to have highly experienced paediatric cardiac surgeons, paediatric anaesthetists, perfusionists and the experience of animal models by the staff at the research centre present. With, to our knowledge, limited available information about this type of cardiac surgery in piglets we had to resolve several problems. Piglets' hearts are very sensitive and cardiac surgery can easily cause arrhythmias. Hence, every detail from pre-operative medications to post-operative care had to be scrutinised and reflected upon in order to succeed. We usually performed surgery two days in a row and I stayed at the research centre to observe and care for the animals, including administration of drugs such as antibiotics and morphine, for at least the first 24 hour postoperatively. If their status then was satisfactory the staff at the research centre would continue with the observation at that point. The lack of a post-operative intensive care unit was solved in such a manner that the piglet

was extubated on the table and was observed and cared for until midnight on the day of operation, and thereafter, at least every two hours or more if needed. There was a protocol for post-operative pain relief and, if needed, other medical treatments. After the introduction of blood transfusion of the "ASO" piglets at the time of surgery the general condition of the piglets improved after surgery. They were able to be mobilised and I could feed them within the first few hours after they had been extubated and moved to the post-operative pre-heated cubicles used for observation. All piglets had pleural drains; this had to be removed at the first sign of the piglet wanting to move. Initially, we also performed transection of either the pulmonary or the aortic artery. The aim was to study the consequences of denervation and re-innervation if only one of the great arteries had been transected; resulting in a lesser degree of injury to the autonomic nervous system of the heart than through an ASO. This had to be abandoned due to the vast amount of resources needed; therefore we prioritized the mimicked ASO. As we developed the ASO model in piglets we aimed to accomplish the same denervation that the children experience after an ASO (paper IV). It became obvious that, if successful, the model as such could be used not only for mimicking ASO, but for a range of complex cardiac surgery procedures unravelling potential deficiencies.

We performed open heart surgery during CPB on 23 piglets, out of which 2 underwent pulmonary transection and 2 underwent aortic resection. Of these 4, all survived. The ASO imposed a critical challenge compared to these 4 previously mentioned piglets due to translocation of the coronary arteries. With every additional task, the risk for complications and decreased survival is enhanced.

Combining the paediatric protocol used at the Queen Silvia Children's with the experience of the veterinarians and what we could obtain from the literature proved successful with survival of 14 out of 19 mimicked ASO in piglets (paper III).

Physiological studies of heart rate and blood pressure during a dose-response curve of NE and EPI were performed in all surviving piglets, including all those with a sham operation.

To execute the physiological study in piglets, we commenced with a pilot of 6 pigs to gain experience with the device built for the Langendorff perfusion. The great amount of Krebs' solution needed, 50 L, was warmed to 37 degrees °C through a CPB heat exchanger connected to the device and was oxygenated. The Krebs' solution was pumped up to a container 60 cm above the heat chamber containing the beating isolated heart. Hence, the perfusion pressure was 60 cmH_2O. This perfusion pressure was chosen because we wanted a good perfusion at the same time as the tissues would be preserved for perfusion fixation with formaldehyde, and not damaged, for future histology. Between the container and the heat chamber, turbulence was created of the flowing Krebs' solution to ensure that the infused drug was properly mixed in the Krebs' solution before it reached the heart in order to be evenly distributed into the coronary arteries. The polygrass was connected to the pipe between the container and heat chamber and every heart beat created a pulsatile wave in the pipe that could be recorded by the polygrass. In this manner, we could measure the effect on heart rate by NE infused into the isolated heart.

Both the in vivo and in vitro experiments were performed in pigs up to 6 months of age. However, the size of the heart at that age made the challenges of a successful Langendorff perfusion not feasible despite the very large device built by us.

A total of 56 pigs were involved in the study of which 5 of the ASO operated piglets succumbed; hence, the in vivo and in vitro studies were performed in 51 animals.

Ten ASO piglets and 13 shams were studied at 5-7 weeks post operatively and, of these, the ASO piglets had a statistically significantly higher baseline heart rate both in vivo and in vitro (paper IV, figure 26A and B).

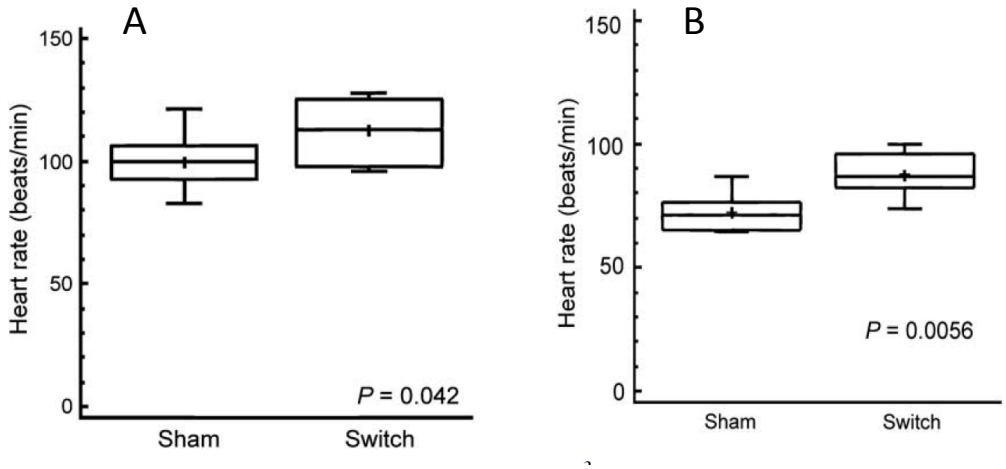

Figure 26. Basal heart rate in vivo (A), and in vitro (B).

It has been reported that chronic spinal cord interruption reduces sinus node cycle length[90], while a chronic spinal cord stimulation increases sinus node cycle length[91]. If increased basal heart rate is a result of impaired sympathetic innervation, this finding is consistent with the decreased extraction fraction found in ASO adolescents compared to healthy subjects (paper I), hence, more available NE in the synaptic cleft, concluding that the ASO operated adolescents have a hampered function of the sympathetic nerve terminals.

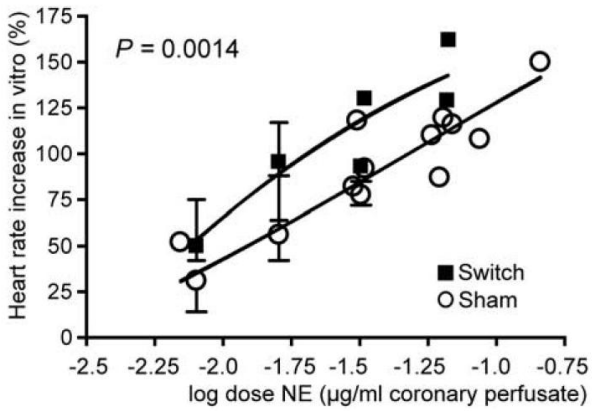

Figure 27. Dose-response curve to norepinephrine (NE) in vitro. NE dose range used corresponded to 0.047-0.38 µmoles per litre perfusate. Statistical method used is four-criteria analysis (bottom, top, Hill slope and 50 % of maximal response).

The dose-response curve with NE in the Langendorff perfusion would have been possible to optimize if more physiological doses could have been infused, especially in the lower dose ranges. However, the challenges imposed by a Langendorff perfusion are many, as mentioned above. If the osmotic pressure of the solution cannot be maintained within physiological values, the heart will quickly develop oedema, which further increases the risk of failure of the experiment. Hence, we had a very limited timeframe of approximately 20 to 30 minutes in which to perform the experiments.

The results of the NE dose-response pattern in the isolated heart, using a four-criteria statistical analysis (bottom, top, Hill slope and 50 percent of maximal response) showed an increased sensitivity to catecholamine stimulation that is consistent with other studies following denervation of sympathetic nerves[12]. The amount of NE needed to increase the heart rate by 80% from baseline was statistically significantly less in the "ASO" hearts compared to the controls. It may be assumed that this was due to denervation hypersensitivity post ASO operation. However, if adjusting baseline heart rate (given that the basal heart rate was higher in the operated group) before every given dose of NE with the

relative increase of heart rate in percent after every dose, no statistical difference between the two groups could be found. Limitations with an experiment such as this, with the difficulties imposed by the procedure as well as giving proper dosing, could affect the results in a manner not foreseen by us. Possible denervation hypersensitivity is challenging from a clinical perspective; increased sensitivity for catecholamines, which are used to increase inotropic and chronotropic response in the heart, could be a potential hazard.

Coronary perfusion

The coronary flow at baseline, in vitro, was not different between the two groups (Fig 6a, paper IV), although the increase in coronary flow per gram heart weight after maximal NE stimulation was lower in stimulated ASO hearts. Reports regarding myocardial perfusion have been controversial with contradictory results regarding perfusion defects and coronary flow reserve[92, 93]. In addition, the methods to assess myocardial perfusion including control groups have been debated[94-96]. Study of cardiac NE function, (paper I), concluded that the ASO patients had functioning but likely loss of NE nerve terminals. As angiogenesis can be prevented by chemical sympathectomy[97] and is stimulated by for example neuropeptide Y, a nerve growth factor and transmitter released from sympathetic nerve terminals[98, 99], it is essential to have functioning sympathetic nerves for normal tissue growth. Reports of silent ischemia in some ASO patients stress the importance of a thorough follow-up of the coronary status in these patients.

General Conclusions

Survival and long-term good health in a child born with heart malformation consist of a long sequence of challenges starting with the diagnosis and continuing through treatments such as surgical and/or medical treatment and follow-ups all through life.

The sympathetic nervous innervation of the heart, not only showed as endogenous release of NE, but also showed as a functioning system while exposed to sympathetic stimulation with adenosine in the ASO group. In addition, the decreased extraction fraction could possibly result in a net increase of NE concentration in the synaptic cleft leading to increased stimulation of the post-synaptic receptors. This could potentially create the same situation as for adults with heart failure where there is an increased sympathetic stimulation to the heart. Tritiated DHPG can only be found if tritiated NE has been extracted and metabolised in the sympathetic nerve terminals. The endogenous release of NE and the decrease of tritiated DHPG in the ASO group support the conclusion that the sympathetic nervous system in the heart of the ASO patients is functioning, although moderately impaired.

The cardiac parasympathetic division of ANS, the effects of which are known to be most pronounced during night-time, was found to functioning as well as in the controls. However, when analysed using normalized HF at the lowest measured heart rate at night, there was a difference between the two groups. An imbalance of the parasympathetic nervous system is known to have potential detrimental effect on cardiovascular prognosis. The impairment found in the sympathetic system combined with a potentially decreased tone in the parasympathetic system, makes the long-term prognosis challenging to evaluate in order to avoid possible cardiac arrhythmias.

Animal studies are essential in exploring potential hazards of complex heart surgery, which cannot be performed in humans. The increased basal heart rate, the potentially increased sensitivity in the isolated heart to NE, and the difference in coronary perfusion after adrenergic stimulation, support the clinical findings of an altered heart innervation after ASO and moves the frontier of knowledge forward.

Increased sympathetic activity in the heart is a compensatory mechanism in the failing heart. The ASO patients' prognosis is closely linked as to whether or not they develop heart failure. If the children post-operatively have an increased sensitivity to catecholamines, as shown in the animal model, there might be a risk for arrhythmias. The neurochemical analysis reporting a functioning, but impaired sympathetic nervous innervation and a potentially impaired cardiac parasympathetic tone, also impose a future risk for arrhythmias and heart failure.

Clinical Implications

Bonnet et al. concluded that a delay of post-birth diagnosis in TGA children resulted in an increased incidence of metabolic acidosis and multi-organ failure[100]. The introduction of pulse oximetry screening before discharge improved total detection rate of duct dependent circulation to 92% [101] . This approach could be an important tool for early detection of TGA.

The clinical challenges in long-term survival for these patients are many. Sustained coronary perfusion is an important issue and silent ischemia makes it important to follow myocardial perfusion continuously in these patients, whether or not they are presented with ischemic symptoms. The debate, over which methods are the best or the most feasible when it comes to autonomic assessment, must also be taken into account the risk posed by invasive

methods. It is important to assess the ASN in ASO patients who, if they have an imbalance of the ASN system, have a potential danger of developing arrhythmias and heart failure. Early detection will enable them to receive treatment to protect their hearts, and treat potential negative consequences post-ASO during their lifetimes.

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