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# Assessment of microvascular function by use of transdermal iontophoresis

—  
methodological aspects

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**To my girls  
Judith and Yente**



# ABSTRACT

Assessment of microvascular function is of major importance in understanding the physiology of the vasculature and in investigating the vascular effects of pathological conditions such as diabetes, hypertension and sepsis. Transdermal iontophoresis can be used to non-invasively introduce vasoactive drugs into the skin. The response of the local cutaneous microvasculature to these drugs can be measured by laser Doppler flowmetry methods. Although the two techniques have been used together for over two decades, there are still important methodological issues to be resolved. This work is aimed at optimizing transdermal iontophoresis as a tool for microvascular assessment by focusing on methodological issues such as: non-specific vasodilatation, drug delivery protocols and analysis of blood flow data.

Non-specific vasodilatation, an increased blood flow during iontophoresis of non-vasoactive compounds, is an important problem as it interferes with the response to the administered drug. By investigating this effect in healthy volunteers, we found that the extent of the non-specific response differs between the positive and negative electrode and that it is dependent on the voltage over the skin and on the ionic strength of the vehicle in which the drug is dissolved. We also found that the extent of the non-specific response could be reduced by applying local anaesthetics and by pre-treatment with antihistamine drugs. These results suggest that non-specific effects are mediated by depolarization or hyperpolarisation of cells, triggering neural and histamine related mechanisms that result in vasodilatation of the local microvasculature.

To prevent non-specific effects from occurring during the experiments, our results show that the current strength and the total electric charge during iontophoresis should be limited to 0.02 mA and 12 mC, respectively. Furthermore, drug solutions at physiological ionic strengths should be used. Under these conditions, adequate responses to the most commonly used drugs, acetylcholine (ACh) and sodium nitroprusside (SNP), are obtained while no significant non-specific vasodilatation occurs.

The results of our investigations show that blood responses to ACh and SNP applied by a single iontophoretic pulse can well be described by dose-response models commonly used in pharmacodynamic studies *in vitro*. This enables a more powerful analysis and comparison between drugs or possibly patient groups compared with conventional analysis methods. Finally, we have incorporated the drug transport to the skin and the physiological response to the local drug concentration during iontophoresis of vasoactive drugs into a single pharmacological model. Validation of this model using measured responses to ACh and SNP shows that the commonly used assumption that the local drug concentration is linearly proportional to the electric charge may not be valid, due to a significant clearance of drugs from the microvascular bed during iontophoresis.

# LIST OF PUBLICATIONS

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\* *The author changed surname from Droog to Tesselaar in January 2006.*



# ABBREVIATIONS

ACh	Acetylcholine
ANOVA	Analysis Of Variance
ASA	Acetyl Salicylic Acid
Atr	Atropine
AU	Arbitrary Units
AUC	Area Under Curve
ED50	Dose eliciting half of maximum response
E <sub>max</sub>	Maximum effect
EMLA	Eutectic Mixture of Local Anaesthetics
FCD	Functional Capillary Density
ICU	Intensive Care Unit
LDF	Laser Doppler Flowmetry
LDPI	Laser Doppler Perfusion Imaging
LDPM	Laser Doppler Perfusion Monitoring
NaCl	Sodium Chloride
NO	Nitric Oxide
NOS	NO Synthase
OPS	Orthogonal Polarization Spectroscopy
PU	Perfusion Units
SEM	Standard Error of the Mean
SNP	Sodium Nitroprusside



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# 1 INTRODUCTION

This thesis is about measurement of microvascular blood flow responses elicited by pharmacological substances administered through the skin. One of the primary functions of microvascular blood flow in the human body is to ensure sufficient delivery of oxygen and nutrients to every cell within every organ. In order to achieve this, the healthy microvasculature must therefore respond to changes in metabolic demand of an organ. In many disease states, such as hypertension, diabetes, coronary artery disease, atherosclerosis and peripheral vascular disease, microvascular function is significantly impaired and this impairment may even precede disorders of the systemic circulation. Changes in the skin microvasculature, for instance, have been found to occur many years prior to the appearance of symptoms of microvascular disease of other organs in young patients with type 1 diabetes (Khan et al., 2000).

Transdermal iontophoresis is a technique for drug delivery to and through the skin, which together with laser Doppler flowmetry can be used to study the pharmacological effect of substances on the cutaneous microvascular blood flow. Because of its non-invasive character, iontophoresis and laser Doppler flowmetry provide an excellent combination of tools for studying microvascular function and it has been extensively used during the last two decades for physiologic evaluations. Nevertheless, the method has some important methodological drawbacks that have prevented its widespread use as a clinical test for microvascular function.

In this thesis, the anatomical and physiological principles of the microcirculation and the skin are outlined, as are some of the primary methods to investigate the physiology and pharmacology of the microcirculation. Moreover, the research is described that is aimed towards optimizing transdermal iontophoresis for microvascular assessment by resolving some of the main methodological issues of the technique, including the phenomenon of non-specific vasodilatation and the analysis of blood flow responses to vasoactive drugs applied by iontophoresis.



## 2 THE MICROCIRCULATION

The majority of cells in the human body are not in direct contact with the external environment, yet these cells must make exchanges with this environment, such as picking up oxygen and nutrients and eliminating wastes (Sherwood, 1997). Furthermore, chemical messengers must be transported between cells to accomplish integrated activity. To achieve these long-distance exchanges, the cells are linked with each other and with the external environment by vascular highways. Blood is transported to all parts of the body through a system of vessels that brings fresh supplies to the vicinity of the cells while removing their wastes.

These vessels, together referred to as the circulation, form a closed system. Arteries, which carry blood from the heart to the tissues, branch into a tree of progressively smaller vessels, with the various branches delivering blood to different regions of the body. When a small artery reaches an organ, it branches into numerous arterioles. The volume of blood flowing through an organ can be adjusted by regulating the internal diameter of the arterioles. Arterioles branch further within the organs into capillaries, the smallest vessels across which the majority of exchanges are made with surrounding cells. Capillaries rejoin to form venules, which further merge to form veins that leave the organs and eventually empty into the heart. The arterioles, capillaries and venules are collectively referred to as the microcirculation.

The walls of arterioles have a thick layer of smooth muscle that is richly innervated by sympathetic nerve fibres. The smooth muscle is also sensitive to many local chemical changes and to some circulating hormones. The smooth muscle layer runs circularly around the arteriole and upon contraction, the vessel's circumference (and diameter) becomes smaller, thus decreasing the flow through that vessel. This process is called vasoconstriction. Vasodilatation, on the other hand, refers to enlargement in the circumference and diameter of a vessel as a result of relaxation of its smooth muscle layer.

A variety of factors, both locally in an organ as well as extrinsic, can influence the level of contractile activity in arteriolar smooth muscle, thereby substantially changing the resistance to flow in such vessels. Local factors that

relax arteriolar smooth muscle include decreased concentrations of oxygen, increased concentrations of lactic acid, increased osmolarity, release of prostaglandins, and heat application. Histamine, although it does not normally participate in blood flow control, is released upon tissue injury or during allergic reactions and promotes smooth muscle relaxation. Extrinsic control mechanisms include neural and hormonal influences, with the effects of norepinephrine release from sympathetic nerve fibres being the most important. Hormones influencing arteriolar radius include epinephrine, norepinephrine, vasopressin and angiotensin II. In certain vessel beds, such as those in the skin, contractile activity is predominantly controlled by interactions with alpha-adrenergic receptors.

The mechanisms by which contractile activity of arteriolar smooth muscle is mediated are still under investigation. It has become clear that endothelial cells play a key role in the local regulation of arteriolar diameter. The vascular endothelium consists of a single layer of specialized epithelial cells that line the lumen of all blood vessels. Endothelial cells release locally acting messengers in response to chemical or physical changes in their environment. These chemical messengers act on the underlying smooth muscle to alter its state of contraction. A chemical mediator of major importance that causes relaxation of arterioles is nitric oxide (NO). NO is a small, short-lived molecule, which increases arteriolar diameter by inhibiting the entry of contraction-inducing calcium ions into the smooth muscle cells. Another important endothelial vasoactive substance is endothelin, which is one of the most potent vasoconstrictors yet identified.

Capillaries branch extensively to bring blood within the reach of every cell. It is interesting to note that the total surface area of the capillaries is approximately 1000 m<sup>2</sup> – the size of two tennis courts – and it is estimated that no cell in the human body is further away than 0.1 mm from a capillary (Pugsley & Tabrizchi, 2000). Exchange of materials across capillary walls is accomplished primarily by diffusion. Capillaries are ideally suited to enhance diffusion as their thin walls minimize diffusion distances and their extensive branching maximizes surface area and time available for exchange. Blood flows through capillaries at relatively slow velocity (~0.5 mm/sec). This slow velocity allows time for exchange of nutrients and wastes between blood and tissues. Capillary exchange is the entire purpose of the circulatory system; all other activities of the system are directed toward ensuring an adequate distribution of replenished blood to capillaries for exchange with all cells throughout the body.

## 3 THE SKIN

**B**eing the largest organ of the body, the skin not only serves as a mechanical barrier between the external environment and the underlying tissues but is also involved in defence mechanisms and other important functions (Sherwood, 1997). The skin consists of an outer epidermis and an inner dermis.

### 3.1 Epidermis

The epidermis is the outermost layer of the skin and varies in thickness between 40  $\mu\text{m}$  and 1.6 mm (Braverman, 2000). It is made up of numerous layers of epithelial cells. The inner layers are composed of living and rapidly dividing cells, whereas the cells in the outer layer are dead and flattened. The epidermis has no direct blood supply. Its cells are nourished only by diffusion of nutrients from a rich capillary network in the underlying dermis.

As the outer layer cells die, they form a keratinized layer that is airtight, fairly waterproof, and impermeable to most substances. It serves to resist passage in both directions between the body and the external environment. For example, it minimizes loss of water and other vital constituents from the body. The value of this protective layer in holding in body fluids becomes obvious in severe burns. Not only can bacterial infections occur in the underlying unprotected tissue, but even more serious are the systemic consequences of loss of body water and plasma proteins, which escape from the exposed, burned surface. The resultant circulatory disturbances can be life-threatening.

The skin impedes passage into the body of most materials that come into contact with the body surface, including bacteria and toxic chemicals. As a result, passage of drugs through the skin is very slow, and for substantial amounts of drugs to be absorbed by the skin, cutaneous patches have to be applied for long periods of time.

### 3.2 Dermis

Under the epidermis is the dermis, a layer of connective tissue that contains both elastin fibres (for stretch) and collagen fibres (for strength). The dermis contains an abundance of blood vessels which form the cutaneous microcirculation. The arterioles and venules of the cutaneous microcirculation form two separate plexuses: the upper and lower horizontal plexuses (Fig 3.1). The upper plexus, from which nutritive capillary loops arise, is situated in the papillary dermis. The lower plexus is formed by vessels that perforate into the dermis from underlying muscles and subcutaneous fat. The two plexuses are interconnected by arterioles and venules (Braverman, 2000).

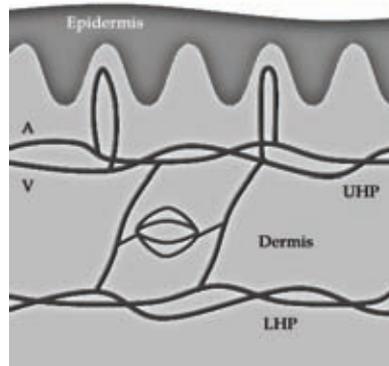


Figure 3.1. Schematic representation of the cutaneous microvasculature. A, arteriole; V, venule; UHP, upper horizontal plexus; LHP, lower horizontal plexus.

The blood vessels in the dermis do not only provide the skin with blood, but also play a major role in temperature regulation; at normal room temperature, twenty to thirty times more blood flows through the skin than is needed to meet the skin's nutritional need. The heat exchange between the skin surface vessels and the external environment is controlled by the calibre of these vessels and hence the volume of blood flowing through them. In the process of thermoregulation, skin blood flow can increase to 8 l/min compared to its baseline flow of from 250 ml/min (Charkoudian, 2003). This variation is mainly controlled by arteriovenous shunts located in the lower dermis. These allow direct passage of blood from the arterial to the venous side, bypassing the capillaries. The blood vessels in the skin diminish the effectiveness of the skin as an insulator by carrying heat to the surface, where it can be lost from the body by radiation and conduction-convection.

Special infoldings of the epidermis into the underlying dermis form the skin's exocrine glands – sweat glands and sebaceous glands – as well as the hair follicles. Evaporation of sweat released from sweat glands cools the skin and is important for thermoregulation. Sweat glands are innervated by cholinergic and adrenergic fibres from the sympathetic nervous system and respond to small increases in body temperature.



# 4 INVESTIGATING MICROVASCULAR FUNCTION

**O**ur current knowledge of microvascular function stems largely from in vitro preparations of isolated vessels or from intravital microscopy studies of whole microvascular beds in animal models (Clough and Church, 2006). These setups exhibit significant limitations associated with in vitro studies, which is why there is a strong need for an accurate, reproducible technique to assess microvascular function in vivo. In this chapter, the most important methods presently used for investigation of microvascular function will be outlined.

## 4.1 Isolated vessel preparations

Preparations of isolated blood vessels in organ baths are one of the most extensively used methods to study vascular function. Most commonly, arteries such as carotid, abdominal, femoral or buttock skin arteries with intact or denuded endothelium are used. Smaller arteries down to arterioles with an internal diameter of 100  $\mu\text{m}$  can be studied, albeit a delicate task that requires a microscope and a skilful experimenter. These methods enable investigation of mechanical, electrophysiological and biochemical properties of blood vessels in health and disease under highly controlled circumstances (Angus & Wright, 2000). Usually, the function of a blood vessel is assessed by mounting it on surgical wire hooks connected to a force or displacement transducer. The contraction or relaxation of the vessel during the application of varying stimuli can be measured accurately under the circumstances defined by the experimenter.

## 4.2 Intravital microscopy

A major technique to study the microvasculature in vivo is intravital microscopy, or microscopic observation and analysis of living tissues. Intravital microscopy can be applied in animals using various tissue preparations, and in humans, where it has been primarily used within the nailfold. Intravital microscopy, together with tracers and analysis algorithms,

is used to determine a wide range of morphological and functional vascular parameters such as vessel diameter, blood flow and vessel permeability.

#### *4.2.1 Animal preparations*

The tissue preparations used for intravital microscopy in animals are often exteriorized tissues such as the rat cremaster muscle and the hamster cheek pouch (Fukumura, 2006). These preparations have become popular as a result of the ease of preparation and good visibility of the vasculature and they have therefore often been used to study the effects of a broad range of pathological conditions on the microcirculation, such as the effects of hypertension. Also, chronic transparent window models such as the rabbit ear and the mouse dorsal skin chamber are used. The main advantage of these models is that they permit chronic assessment of vascular function, for up to a year.

#### *4.2.2 Nail fold capillaroscopy*

Intravital microscopy of human capillaries, or capillaroscopy, is most commonly applied to the nail fold. The skin fold surrounding the nail is used because it is easy to place a finger under the microscope and because the complete capillary loops in the nail fold can be visualized by optical coupling of the skin and the microscope objective with a thin oil layer. In this way, the capillary network in the nail fold can be studied in real time, sometimes combined with intravenous administration of fluorescent dyes. Capillaroscopy can be used to assess capillary morphology, capillary density and capillary blood velocity. The technique has been used to study the microcirculation in various disease states, such as Raynaud's phenomenon (Houtman, 1986), diabetes and hypertension (Shore, 2000). The reproducibility of the technique under strictly controlled conditions is fairly good. The limitation of capillaroscopy in practice is that an excellent image quality is required in order to collect representative and accurate data. The technique is rather time consuming as it is necessary to measure the velocity in several capillaries per subject in order to get a representative result. Capillaroscopy measurements also require sophisticated equipment and software setting, which further limits its clinical applicability.

#### *4.2.3 Optical polarization spectral imaging*

Orthogonal polarization spectral (OPS) imaging is a non-invasive intravital microscopy technique. It uses reflected light to produce real-time images of the microcirculation (Groner, 1999). Light from a source passes through a polarizing filter, and is directed towards the tissue by a set of lenses. As the light reaches the tissue, the depolarized light is reflected back through

the lenses to a second polarizing filter or analyzer and forms an image of the microcirculation on a CCD, which can be captured and further processed. Assessment of tissue perfusion is most often done by semi-quantitative scoring, using the functional capillary density (FCD), i.e., the number of perfused capillaries per observation area. OPS imaging produces excellent images of the sublingual microcirculation when the measurement probe is placed under the tongue. Movement artefacts, semi-quantitative measures of perfusion, the presence of various secretions such as saliva and blood and observer-related bias are some of the limitations of this technique.

### **4.3 Laser Doppler flowmetry**

Laser Doppler flowmetry (LDF) has been used to measure microvascular blood flow for over 30 years. Early work was on retinal blood flow (Riva et al 1972, Tanaka et al 1974) but was soon extended to other tissues (Stern et al 1975). Several thorough reviews on the theoretical background of LDF have been presented (Bonner and Nossal, 1990; Nilsson et al., 1991; Nilsson et al., 2003).

#### *4.3.1 Theory*

LDF is a method designed to provide continuous non-invasive measurement of microvascular perfusion. In LDF, low-power laser light impinges on the tissue surface and the individual photons migrate through the tissue in a random fashion, the exact statistical pattern of which is determined by the tissue optical properties (Leahy et al., 2004). Interaction with one or more moving objects such as blood cells shifts the scattered photons in frequency by an amount determined by the scattering angle, the wavelength of the laser light and the velocity of the scatterer. This frequency shift is generally named after the Austrian scientist Johan Christian Doppler who studied and explained the phenomenon in the beginning of the nineteenth century.

The backscattered and Doppler-broadened light carries information about both the speed of the blood cells traversing the scattering volume, which is reflected by the frequency broadening of the backscattered signal and their concentration, which can be derived from the fraction of total photons that is frequency-shifted. If a portion of the backscattered light is brought to impinge on the surface of a photodetector, the frequency-shifted and frequency-unshifted photons mix. The light intensity on the photodetector constitutes a fluctuating speckle pattern, the local intensity variations of which are mainly determined by the tissue perfusion. This perfusion is defined as the number of blood cells multiplied by the mean speed of the cells. The perfusion value is usually expressed in volts (V), in arbitrary units (AU) or in perfusion units

(PU). Currently available LDF systems cannot express the measurement in absolute units because of the enormous inter- and intra-individual variation in microvascular architecture, physiology, and optical properties (Leahy et al., 1999).

#### 4.3.2 Instrumentation

Two types of laser Doppler equipment can be distinguished: the laser Doppler perfusion monitor (LDPM) and the laser Doppler perfusion imager (LDPI). An LDPM consist of a probe containing an illuminating fibre, connected to an external laser, and one or several detecting fibres which transport the back-scattered laser light to a photodetector. The probe is placed on the tissue and the tissue blood flow within an area of about 2-3 mm in diameter is continuously measured. When an LDPI is employed, a scanner head is placed at typically 15 cm above the tissue, and a laser beam scans an area, resulting in a map of perfusion values.

An important advantage of LDPM is the high temporal resolution of the perfusion measurement. Blood flow response can be assessed in real time. However, the microvasculature is highly heterogeneous and blood flow varies considerably at distinct skin sites (Wårdell et al., 1994). In this respect, LDPI offers an advantage over LDPM, since the blood flow response is measured within a larger area and may be averaged over that area. The reproducibility of LDPI in measuring microvascular function in vivo has been found superior to LDPM (Morris & Shore, 1996). On the other hand, the scanning takes typically a few seconds to several minutes, depending on the spatial resolution and the properties of the scanning system. Whether this is a problem, depends on the dynamics of the blood flow response that is studied.

#### 4.4 Drug provocations

In isolated vessel preparations and often also in intravital microscopy models, pharmacological provocations are applied to study the interactions between drugs and the microvasculature. In fact, stationary parameters such as length or contractile force in blood vessels or baseline perfusion in skin microcirculation provide limited information about the underlying physiological or pharmacological mechanisms. A complicating factor in measuring cutaneous blood flow is that repetitive recordings generally do not result in identical perfusion values. This is due to inherent temporal and spatial variability of baseline microvascular blood flow and to other influences such as skin temperature (Salerud et al, 1983; Tenland et al., 1983). Therefore, measurement of microvascular blood flow responses to pharmacological provocations is preferable, as these responses are generally less variable than

baseline flow and can be related to specific physiological mechanisms or pharmacological actions.

#### 4.4.1 *Acetylcholine*

Acetylcholine (ACh) is a muscarinic receptor agonist that has been comprehensively studied in isolated vessel preparations. In 1980, Furchgott and Zawadzki demonstrated that the relaxation of vascular smooth muscle cells in response to ACh is dependent on the anatomical integrity of the endothelium. They therefore hypothesized that this relaxation is mediated by a substance released from the endothelium. In 1987, this substance was identified as nitric oxide (NO) produced enzymatically from the amino acid L-arginine by enzymes termed "NO synthases" (NOS). In the cutaneous microvasculature, vasodilatation in response to acetylcholine may be mediated by mechanisms other than NO. It has been suggested that prostaglandins are involved (Noon et al., 1998), although others have reported no appreciable impact from prostaglandins on cutaneous vascular response to acetylcholine (Morris and Shore, 1996; Berghoff et al., 2002). Furthermore, it has been suggested that acetylcholine induces vasodilatation through stimulation of sensory nerves (Berghoff et al., 2002). Despite conflicting evidence regarding the mechanisms by which the drug causes vasodilatation, ACh is nowadays commonly used to investigate endothelium-dependent vasodilatation in the cutaneous microvasculature.

#### 4.4.2 *Sodium nitroprusside*

In order to study the involvement of NO in a particular biological mechanism such as smooth muscle relaxation, it is desirable to administer exogenous NO. Because it is complicated to administer NO gas in solution or by inhalation there is increasing interest in compounds which generate NO in a controlled manner (the so-called "NO donors"). A frequently used NO-donor in vascular investigations is sodium nitroprusside (SNP), which is also applied clinically to reduce blood pressure, e.g. in hypertensive emergencies. NO is released from the nitroprusside molecule by a complex mechanism that is not completely understood, but the drug has a short half-life, indicative of its fast biotransformation (Feelisch, 1998). The vascular response elicited by SNP is considered to be independent of endothelial function.



## 5 TRANSDERMAL IONTOPHORESIS

**T**ransdermal drug delivery had its beginnings in the late 18th century, but it is only during the last twenty years that it has emerged as a preferred alternative to traditional oral drug delivery and injections (Singh & Maibach, 1994). The main reason for its growing importance is the increasing non-compliance to conventional medication systems such as oral and injectable drugs, due to their invasive nature and the potential side effects. Today a number of patches for drugs exist such as clonidine, fentanyl, lidocaine, nicotine, nitroglycerine, oestradiol, oxybutinin, scopolamine and testosterone. There are also combination patches for contraception as well as hormone replacement. However, for many other drugs transdermal delivery is not possible because the required transport rates through the skin barrier can not be obtained (Prausnitz et al., 2004). Therefore, different strategies have been used to increase the transport rates of drugs through the skin. In transdermal iontophoresis, this is accomplished by using an electric field to move both charged and uncharged molecules across the skin. The general principle of iontophoresis was introduced by Leduc in 1908 (Leduc, 1908), which introduced the term iontotherapy and described the principles of the process in detail. Since then iontophoresis has been used widely for transdermal drug delivery.

### 5.1 Theory

During iontophoresis, an electric field is applied across the skin by means of two electrodes (Fig. 5.1). A drug, usually consisting of charged molecules, is placed under one of the electrodes such that electrorepulsive forces drive the drug molecules away from the electrode, into the skin. This implies that a positively charged drug is placed under the positive electrode (anode), whereas a negatively charged drug is placed under the negative electrode (cathode). In this way, the rate of penetration of drugs through the relatively impermeable stratum corneum is greatly enhanced compared to passive diffusion (Singh & Maibach, 1996).

The transport of uncharged molecules can also be facilitated by iontophoresis (Pikal, 2001). At physiological pH, the skin acts as a negatively

charged membrane. When an electric field is applied across such a membrane, there is a net transport of water molecules across the skin, from the anode to the cathode. This electroosmotic flow results in an enhanced transport of uncharged molecules, especially if they are polar. Although the transport of molecules by electroosmosis is relatively small compared to electrorepulsion, iontophoresis of uncharged molecules has been successfully applied in glucose monitoring (Potts et al., 2002; Sieg et al., 2004).

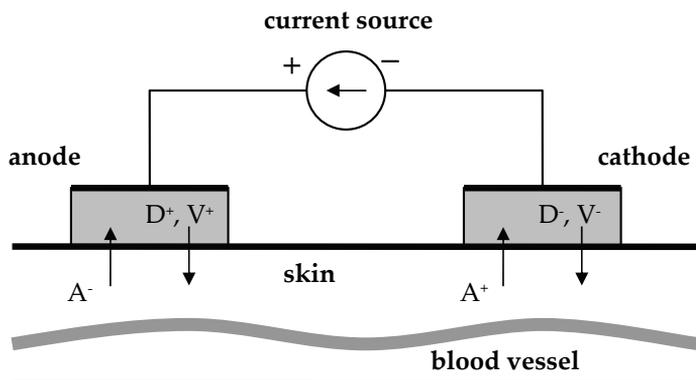


Figure 5.1. Schematic representation of transdermal iontophoresis.  $D^+$  and  $D^-$  are positively and negatively charged drug ions, respectively.  $V^+$  and  $V^-$  are positively and negatively charged co-ions in the drug solution.  $A^+$  and  $A^-$  are counter-ions travelling from the skin towards the electrode to maintain electroneutrality.

Considerable effort has been put into investigating the paths of transport of the drugs during iontophoresis. It is now generally believed that the molecules are transported mainly through the appendages in the skin such as sweat ducts and hair follicles. The use of pilocarpine in the diagnosis of cystic fibrosis suggests in itself that drugs travel through sweat ducts (Beauchamps & Lands, 2005). Also, a dot like pattern has been observed during iontophoresis of charged dyes (Cullander, 1992; Singh & Maibach, 1996).

## 5.2 Instrumentation

The electric field driving the drug ions into the skin is commonly created by means of a constant direct current, although high-frequency pulsed currents and alternating currents have sometimes been applied (Ishikawa et al., 2002; Shimizu et al., 2003). The current is generally delivered by a battery-operated source. Current densities of up to 0.5 mA/cm<sup>2</sup> are considered safe (Ledger,

1992) but in practice current densities vary between 0 – 0.2 mA/cm<sup>2</sup>. In this way, a certain iontophoretic charge is delivered, which is defined as current strength multiplied by time. The term dose is often used to indicate this charge, but should not be confused with the actual dose of the drug delivered to the skin, which depends on a number of factors, including the ionic mobility of the drug, ionic competition with background electrolytes in the chamber and degeneration of the drug in the chamber. In many studies however, the amount of drug delivered is considered linearly proportional to the iontophoretic charge applied. (Guy et al., 2000; Naik et al., 2000). Although platinum electrodes have been used quite often in the past, the electrodes used during iontophoresis are nowadays almost exclusively silver-silver chloride electrodes, because they do not alter the pH of the solution (Green et al., 1993).

### 5.3 Applications

The applications of iontophoresis can be divided into therapeutic and diagnostic applications. The major therapeutic value of iontophoresis lies in its capability of enhancing drug delivery through the skin, which makes it easier to apply higher dosages in shorter times. This has resulted in a wide range of therapeutic applications, such as tap water iontophoresis for the treatment of hyperhidrosis (Sloan & Soltani, 1986), local delivery of non-steroidal anti-inflammatory drugs in the treatment of rheumatic disorders (Garagiola et al, 1988), delivery of opioids against cancer pain (Mercadante & Fulfaro, 1999) and delivery of lidocaine for dermal anaesthesia (Greenbaum, 2001).

Iontophoresis has been used in several diagnostic applications, such as for diagnosing cystic fibrosis (Blythe & Farrell, 1984) and recently for monitoring blood glucose levels in diabetic patients (Potts et al, 2002; Sieg et al., 2004). The major advantage of iontophoresis in diagnostic applications is that there is no mechanical penetration or disruption of the skin. This lowers the risk for tissue trauma and infection and makes the method painless and easy to use. Another attractive aspect is that drugs are delivered locally, and at very low dosages. Possible systemic effects of the drugs are therefore avoided, and the timing of the drug delivery can be strictly controlled.

Apart from these therapeutic and diagnostic clinical applications, iontophoresis has been used widely in combination with laser Doppler flowmetry for the assessment of microvascular function. After its first application for the investigation of  $\alpha_1$ -receptors in finger skin (Lindblad & Ekenvall, 1986) the technique has been applied in patients with diabetes (Morris et al., 1995), Raynaud's phenomenon (Anderson et al., 2004) and cardiovascular disease (Andersson et al., 2005). Also, the effects of age (Rossi et

al., 2002) and altered physiological conditions like exercise (Lenasi & Strucl, 2004) and passive heating or cooling (Pergola et al., 1993) on microvascular function have been investigated.

In microvascular studies employing iontophoresis, the dissolved drugs are usually contained in a ring-shaped electrode chamber, which is mounted on the skin by double-adhesive tape. This type of electrode chamber allows for simultaneous drugs delivery and blood flow monitoring by laser Doppler flowmetry, either by insertion of an LDPM probe into the chamber or by scanning the chamber using LDPI.

# 6 METHODOLOGICAL CONSIDERATIONS

**A**lthough the combination of iontophoresis for transdermal drug delivery and laser Doppler flowmetry for blood flow measurement forms an excellent non-invasive tool for studying microvascular physiology, there are several methodological issues that pose some limitations for the technique if it is to be applied for clinical use. The most important methodological issues will be described here.

## 6.1 Non-specific vasodilatation

Currently, one of the main challenges in microvascular studies employing iontophoresis is the presence of the so-called non-specific vasodilatation. During iontophoresis of non-vasoactive compounds commonly used as vehicles for the active drug, such as deionized water, tap water, and sodium chloride, an increase in skin perfusion is often observed (Grossmann et al., 1995; Morris et al., 1995; Åsberg et al, 1999, Ferrell et al., 2002). Non-specific vasodilatation is an important confounding factor when iontophoresis is used to study the microvasculature, because it introduces a blood flow effect that is not a result of the drug under study. The mechanisms responsible for non-specific vasodilatation during iontophoresis are still not fully understood and the strategies applied so far to reduce or even prevent non-specific vasodilatation have important limitations. Hence, further investigations of the physiological origin of non-specific vasodilatation and possible strategies to avoid this phenomenon are needed.

## 6.2 Drug delivery protocols and data analysis

No general consensus exists about the protocol by which vasoactive drugs should be administered. In previous studies, iontophoretic charge densities varied from 2.1 mC/cm<sup>2</sup> to 27.8 mC/cm<sup>2</sup> for acetylcholine (Ferrell et al., 2002; Pellaton et al., 2002), and drug concentrations from 0.0015% up to 1% for sodium nitroprusside have been used (Maltz et al., 2004; Morris et al., 1995). Moreover, current strengths, number of current pulses, interval times

between pulses and electrode chamber sizes vary between the different studies. This makes comparisons of results between studies difficult.

The analysis of laser Doppler blood flow data during and after iontophoresis has been done in various ways. Some investigators have used the absolute increase in perfusion after one or multiple iontophoretic pulses (with or without subtraction of baseline perfusion values) as a measure of microvascular responsiveness. Others expressed the responses in terms of relative change with respect to baseline. Besides maximum responses (Abou-Elenin et al., 2001; Newton et al., 2001), the parameter of interest has been the area under the response curve (AUC) (Åsberg et al., 2000; Anderson et al., 2004) after a certain period of iontophoretic current application. However, both approaches have two major limitations. First, the maximum response seen during an experiment may not reflect the true physiological maximum. Second, the response over time to a drug that is applied by iontophoresis contains more information than the maximum response alone and by considering only the maximum response, important physiological characteristics may be overlooked. Therefore, many investigators have measured the perfusion response to increasing drug doses or iontophoretic charges (Morris et al., 1995; Khan et al., 2000; Åsberg et al., 2000; Christen et al., 2004).

When perfusion responses are measured after multiple iontophoretic charges, comparison of responses between drugs or between patient groups are almost exclusively analyzed using multiple-way analyses of variance (ANOVA). A limitation of ANOVA is that the method ignores the fact that doses or time points come in order and that it does not recognize trends. Therefore, analysis of variance may not really address the question that is asked in the experiments. In iontophoresis of vasoactive drugs, curve fitting based on dose-response models may be considered a superior technique compared to ANOVA, as it enables evaluation and comparison of pharmacologically or physiologically relevant parameters (Gabrielsson & Weiner, 2002).

### **6.3 Administered dose vs. iontophoretic charge**

The absolute dose administered during iontophoresis is inherently unknown. This dose is usually considered to be linearly proportional to the electrical charge, which is defined as the electrical current strength multiplied by the duration of the current pulse (Phipps et al., 1989). For example, a 60-second pulse with a current strength of 0.1 mA corresponds to an electrical charge of 6 mC. This proportionality between dose and charge is valid only

under restricted circumstances. In reality, part of the current is carried by counter-ions which travel from the body towards the electrode to maintain electroneutrality. Background ions present in the vehicle in which the drug is dissolved may also partially account for the electrical current (Mudry et al., 2006). These effects are likely to influence the rate of drug transport during iontophoresis.

Once the drug ions are transported into the skin, they may reside locally, diffuse towards underlying tissues or be transported away by the blood flow within the microvasculature. If their local effect is of importance, as is the case when vasoactive drugs are applied to study their local effect on the microvasculature, the clearance from the microvascular bed by means of passive or active transport routes forms an important consideration when estimating the locally administered drug dose. These considerations and their effect on the locally administered dose during iontophoresis have received little attention in studies performed up to date.

It can thus be concluded that there is a need for better understanding of the origin of non-specific vasodilatation. A protocol for iontophoresis of vasoactive drugs with which non-specific responses are avoided and which yield adequate drug-related responses is needed. Moreover, current methods for data analysis have limitations as they only take into consideration part of the information available in the blood flow data.



## 7 AIMS

The overall aim of this work is to develop and optimize iontophoresis as a clinical tool for assessment of microvascular function. This is mainly accomplished by investigating the phenomenon of non-specific vasodilatation and possible strategies to minimize it, and by developing new protocols and models to analyse blood flow responses to iontophoresis of vasoactive drugs. Specifically, the aims of the investigations were:

1. To investigate whether the extent of the non-specific blood flow response is related to voltage over the skin during iontophoresis and to investigate the involvement of sensory nerves and histamine-related reactions in the non-specific blood flow response.
2. To develop a protocol for iontophoresis of acetylcholine and sodium nitroprusside that minimises non-specific blood flow effects and maximises drug-related responses.
3. To improve the analysis of blood flow data by applying dose-response models, which take into account physiological response dynamics as well as drug kinetics during iontophoresis of acetylcholine and sodium nitroprusside.



# 8 MATERIAL AND METHODS

## 8.1 Subjects and environmental conditions

For all investigations, healthy volunteers with no previous history of vascular disease were included in the study after informed consent had been obtained (Table 8.1). All those involved refrained from taking drinks containing caffeine and from smoking tobacco or taking any kind of medication (with the exception of oral contraceptives) for at least 2 hours before the experiments. For all the experiments approval was obtained from the regional ethics committee at the Faculty of Health Sciences, Linköping University, Sweden, and procedures followed were in accordance with institutional guidelines.

*Table 8.1. Number of subjects included in the investigations, their mean age and gender.*

<b>investigation</b>	<b>experiment</b>	<b>N</b>	<b>age (years)</b>	<b>men</b>	<b>women</b>
I	anodal	8	33	4	4
	cathodal	8	33	4	4
II	anodal	8	30	5	3
	cathodal	8	31	6	2
III	NaCl	10	32	7	3
	ACh	10	32	7	3
	SNP	10	32	7	3
IV	ACh	10	24	3	7
	SNP	10	26	7	3
	ACh/Atr	5	26	4	1
	SNP/Atr	2	27	1	1
	ACh/ASA	10	28	6	4
V	ACh	10	25	5	5
	SNP	10	25	5	5

In all investigations, room temperature was kept at 23°C (+/- 1°C). Environmental light was dimmed to avoid interference with the laser Doppler measurements. Subjects acclimatized for 10 min before data were collected. The test areas were cleaned gently with 70% ethanol before each experiment.

During the experiment, subjects rested comfortably in a half-upright position with the test area of the forearm at the level of the heart.

## 8.2 Equipment

### 8.2.1 Blood flow measurements

A laser Doppler perfusion imager (PIM 2.0, Lisca Development AB, Linköping, Sweden) was used to measure skin perfusion during iontophoresis. The imager was either used in imaging mode, resulting in images consisting of  $16 \times 16$  measurement points over the area of iontophoresis, or in duplex scanning mode, which enables continuous measurement at  $4 \times 4$  adjacent measurement points at a scan rate of about 1 scan/s (Table 8.2).

Table 8.2. LDPI settings used in the investigations.

investigation	mode	Image size (# data points)	scan time (sec)	scan area (cm <sup>2</sup> )	scanner height (cm)
I	imaging	$16 \times 16$	15	1.8	15
II	imaging	$16 \times 16$	15	1.8	15
III	imaging	$16 \times 16$	15	1.8	15
IV	duplex	$4 \times 4$	1	1.5	15
V	duplex	$4 \times 4$	1	1.5	15

### 8.2.2 Drug delivery

Two different types of circular silver–silver chloride electrode chambers (Perimed AB, Stockholm, Sweden) were used, with an inner diameter (area) of 15 mm (1.8 cm<sup>2</sup>) and 14 mm (1.5 cm<sup>2</sup>), respectively. The chambers were attached to the skin with double-adhesive tape and filled with the appropriate drug solutions. To prevent leakage and evaporation, the chambers were covered with a glass cover slip. A battery-powered iontophoresis controller (PeriIont 382, Perimed AB, Stockholm, Sweden) was used to deliver a constant direct current to the skin. With this instrument, the current strength can be set between 0.02 mA and 1 mA, in steps of 0.02 mA. Depending on the experiment the positive or negative lead was connected to the iontophoresis chamber, resulting in anodal or cathodal iontophoresis, respectively.

## 8.3 Drug delivery protocols

Iontophoresis was used for drug delivery in all investigations, but current strength, pulse and interval duration, number of pulses and drug and vehicle concentrations differed between the investigations. The details of these parameters are presented in Tables 8.3 and 8.4. In the first three investigations, multiple pulses of current were applied during 60 or 120 seconds, separated by



Table 8.4. Overview of the drug solutions and the applied current strengths in the investigations. NaCl = sodium chloride, ACh = acetylcholine, SNP = sodium nitroprusside, Atr = atropine.

investigation	current strength (mA)	drug	drug concentration (%)	vehicle	final ionic strength (mM)
I	0.3-0.5	NaCl	0 – 1	dH <sub>2</sub> O	0 – 171
II	0.1	NaCl	0.9	dH <sub>2</sub> O	154
III	0.02-0.3	ACh/SNP	0.01 – 2	NaCl	154
IV	0.02	ACh/SNP/Atr	1	NaCl	154
V	0.02	ACh/SNP	1	NaCl	154

## 8.4 Data processing and analysis

When operated in imaging mode, LDPI generates a matrix of perfusion values. The LDPI software discards perfusion values below a threshold defined by the user, such that measurements on the electrode chamber are rejected. This yields approximately 200 measurement values per image in our experiments. For further analyses the geometrical mean of the values was calculated for each image. In duplex mode the measurement value generated by the software is the geometrical mean of the 4 × 4 measurement points. This value was used without further processing.

Perfusion data was analysed differently throughout the investigations. In the first investigation, three-way analyses of variance (ANOVA) for repeated measures were used to detect differences in increased perfusion between concentrations, current directions, and iontophoretic charge. In investigation II and III, two-way ANOVA for repeated measures were used with main effects of iontophoretic charge and treatment as independent variables. In all cases where ANOVA was used, Bonferroni post tests were done to test for differences between drugs, anode and cathode, treatments or iontophoretic charges, adjusting for multiple comparisons.

In investigation IV, the blood flow responses were analysed by fitting the  $E_{\max}$  model, based on classical receptor occupancy theory (Wagner, 1968), to the blood flow data. The model can be described by the following equation:

$$E(C) = E_{\min} + \frac{(E_{\max} - E_{\min})C(t)^n}{C(t)^n + ED50^n}$$

where  $E_{min}$  is the minimum response,  $E_{max}$  is the maximum response,  $ED50$  is the iontophoretic charge eliciting half of the maximum response and  $n$  is the Hill slope.  $C(t)$  is the iontophoretic charge as a function of time, defined as the current strength multiplied by the pulse duration:

$$C(t) = I \cdot t.$$

The perfusion responses as recorded by LDPI were normalized with respect to baseline and maximum responses, and the minimum and maximum responses were therefore kept fixed while the remaining two parameters ( $ED50$  and  $n$ ) were estimated from each response by fitting the model to the data. In investigation V, a more sophisticated model was introduced to explain the blood flow responses. This model will be described in chapter 9.

Whenever curve fitting was performed, the Levenberg-Marquardt algorithm was used to obtain best-fit parameters (Levenberg, 1944; Marquardt, 1963). When comparison between fit parameters was necessary, the significance of the difference of the mean values was tested using a paired two-tailed Student's t test. Differences between curves were evaluated using an F-test. For all analyses throughout the investigations, a p-level < 0.05 was considered significant.

Data were analyzed using LDPIwin software (version 2.3, Perimed AB, Sweden) and GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA). Data in the figures are presented as means  $\pm$  SEM.



# 9 REVIEW OF THE INVESTIGATIONS

## 9.1 Effect of voltage over the skin on non-specific vasodilatation

In investigation I, the aim was to study a possible role of voltage over the skin on non-specific vasodilatation. Therefore, NaCl was diluted in deionized water to different ionic strengths and administered to the skin of healthy volunteers by iontophoresis. During iontophoresis, the voltage between the electrodes and the blood flow response was measured.

It was found that the voltage over the skin was dependent on the ionic strength of the NaCl solution both with anodal and cathodal iontophoresis, although the effects were different between the polarities (Fig 9.1). With both anodal and cathodal iontophoresis, the use of deionized water resulted in a significantly higher voltage over the skin ( $p < 0.001$ ).

An increase in blood flow was found at both electrodes, but the extent of this increase depended on the ionic strength of the vehicle and on the electrode polarity. With anodal iontophoresis, the extent of the vasodilatation was similar for different ionic strengths of NaCl, while with cathodal iontophoresis, after 4 pulses (72 mC) the vasodilatation response increased with decreasing ionic strengths. The strongest blood flow response during cathodal iontophoresis was seen when deionized water was used ( $p < 0.001$ , Fig 9.2).

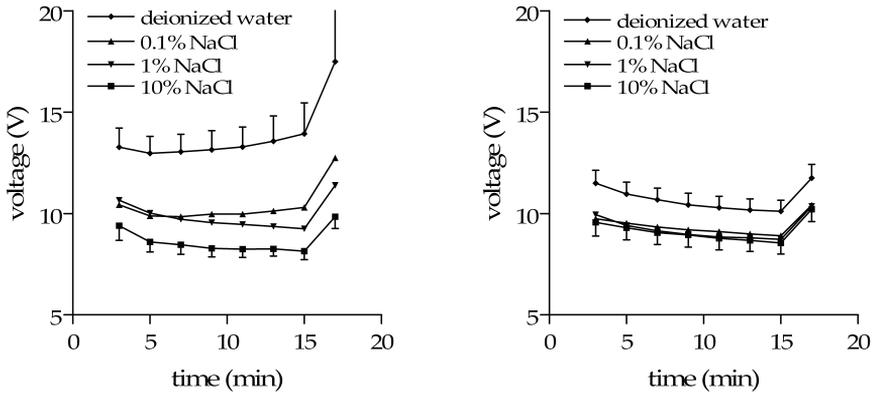


Figure 9.1. Voltage over the skin during anodal iontophoresis (left) and cathodal iontophoresis (right) of NaCl at different ionic strengths ( $n=8$ ).

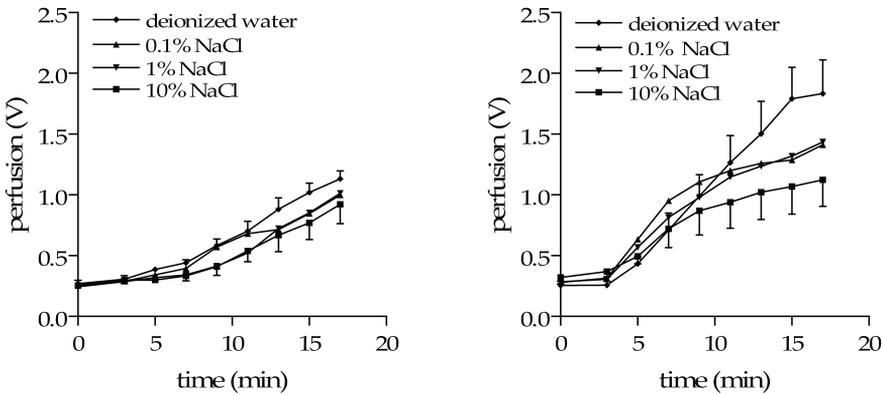


Figure 9.2. Blood flow response during anodal iontophoresis (left) and cathodal iontophoresis (right) of NaCl at different ionic strengths ( $n=8$ ).

The spatial distribution of the blood flow response was characterised by inspection of the perfusion images. The response was usually highly heterogeneous within the chamber. Vasodilatation usually started at distinct spots, which expanded during current application (Fig. 9.3).

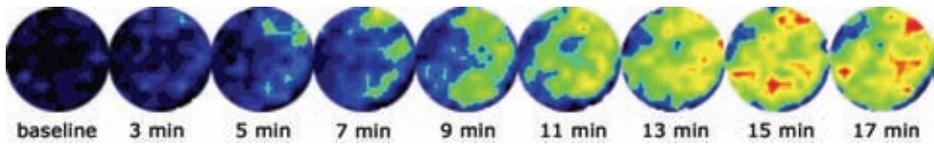


Figure 9.3. Typical sequence of perfusion images obtained during cathodal iontophoresis of sodium chloride in investigation I, showing a highly heterogeneous blood flow response within the electrode chamber.

## 9.2 The role of neural and histamine-related mechanisms in non-specific vasodilatation

In investigation II, we studied the involvement of sensory nerves and histamine-related mechanisms in non-specific vasodilatation. 0.9% NaCl was administered by iontophoresis in healthy volunteers who had been treated with a lidocaine-prilocaine mixture (EMLA), an oral H<sub>1</sub>-receptor antagonist (cetirizine), or both.

The main finding of this investigation was that non-specific responses were significantly suppressed both by pre-treatment with EMLA and oral cetirizine compared with untreated controls (Fig 9.3). The effect was different for anodal and cathodal iontophoresis. With anodal iontophoresis, suppressed non-specific responses were observed after cetirizine treatment. The suppressive effect was significant in EMLA-treated sites and approached significance in untreated sites ( $p=0.04$  and  $p=0.06$ , respectively). The effect was most pronounced after five current pulses, i.e. at iontophoretic charges of more than 60 mC. With cathodal iontophoresis, oral cetirizine only suppressed non-specific responses in untreated sites and only during the first three pulses, i.e. at iontophoretic charges up to 36 mC. In EMLA-treated sites, no effect of oral cetirizine during cathodal iontophoresis was found.

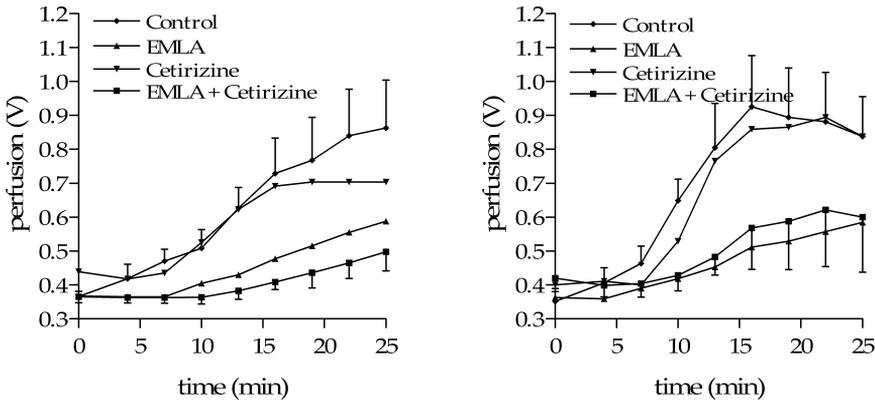


Figure 9.3. Effect of cetirizine on non-specific vasodilatation in EMLA-treated and untreated skin sites during anodal (left) and cathodal (right) iontophoresis of 0.9% NaCl ( $n=8$ ).

### 9.3 Preventing non-specific vasodilatation

The aim of investigation III was to find the best combination of protocol parameters in iontophoresis of ACh and SNP in order to obtain adequate microvascular responses to the drugs, while non-specific responses are avoided. Therefore we administered NaCl, ACh and SNP by iontophoresis using different drug concentrations, current strengths and at increasing cumulative iontophoretic charges.

Non-specific responses were stronger with cathodal iontophoresis compared with anodal iontophoresis and increased with increasing current strength and number of pulses (iontophoretic charge). No significant non-specific responses were seen with 0.02 mA at iontophoretic charges below 12 mC ( $7.8 \text{ mC/cm}^2$ ), except in two subjects who reacted strongly even at low iontophoretic charges and current strengths. Blood flow responses to the drugs increased with current strength, iontophoretic charge and drug concentration up to a level where a plateau in the responses was reached. The maximum blood flow response was independent of current strength at drug concentrations of 1%, and was reached at an iontophoresis charge of 4.8 mC ( $2.7 \text{ mC/cm}^2$ ) with ACh and 9.6 mC ( $5.4 \text{ mC/cm}^2$ ) with SNP.

The most important result of the investigation therefore is that it is possible to obtain adequate responses to the drugs, while significant non-specific effects can be avoided. This was realised by limiting the current strength during iontophoresis to 0.02 mA ( $0.013 \text{ mA/cm}^2$ ) and the total

iontophoretic charge to 12 mC (7.8 mC/cm<sup>2</sup>) and by applying 1% solutions of drugs, dissolved in saline to physiologic ionic strength.

The non-specific blood flow response and the blood flow response to ACh and SNP applied by 0.02 mA and at 1% concentrations is shown in Figure 9.4.

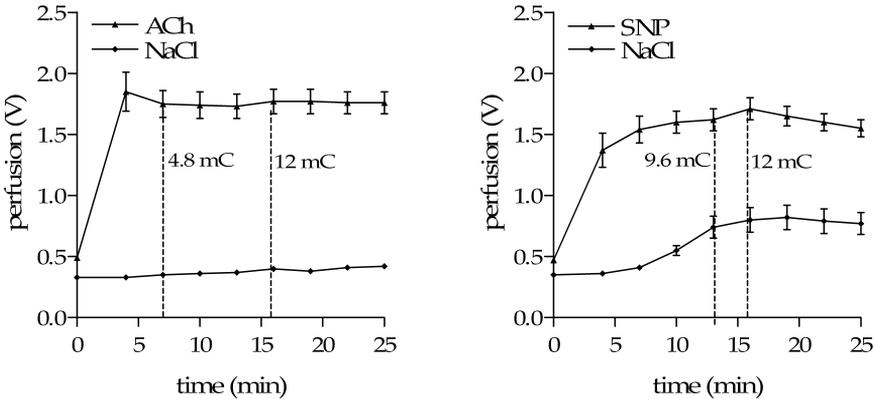


Figure 9.4. Non-specific blood flow response to NaCl and drug-related blood flow response to ACh (left) and SNP (right) applied by 0.02 mA and at 1% drug concentrations ( $n = 10$ ).

### 9.4 Dose-response analysis of blood flow responses

In investigation IV, the hypothesis was tested that the blood flow response as measured by LDPI during a single iontophoretic current pulse can be modelled by the sigmoidal  $E_{max}$  model, which is a dose-response model commonly applied in pharmacodynamic studies in vitro. In pilot experiments we noted that the responses resembled dose-response curves known from in vitro experiments. We administered ACh and SNP by iontophoresis in healthy volunteers using a single 10-minute current pulse, measured the blood flow responses with LDPI and compared these responses with organ bath experiments with isolated bovine mesenteric arteries. Also, we studied the effect of atropine as a muscarinic receptor antagonist and the effect of acetylsalicylic acid (ASA) as a prostaglandin antagonist on the dose-responses.

The dose-response model, details of which are described in chapter 8, could be accurately fitted to both the in vivo perfusion data as well as the in vitro data, with  $r^2$  values typically  $> 0.9$ . Dose-response parameters differed significantly for ACh and SNP.  $ED_{50}$  was larger for SNP compared with ACh

in both the in vitro and the in vivo experiments. Hill slopes were different between ACh and SNP in the in vivo experiments but not in the in vitro experiments, where the slopes were close to unity (Table 9.1). Pooled data from the in vitro and in vivo measurements are presented in Figure 9.5.

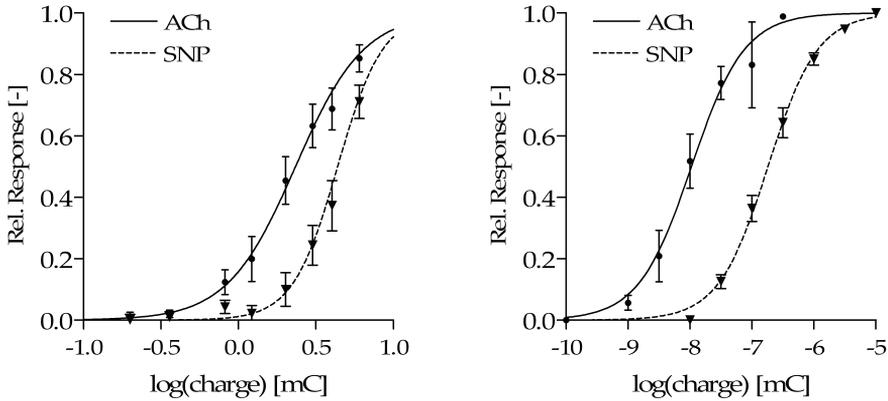


Figure 9.5. Average responses of the cutaneous microcirculation in vivo during iontophoresis (left,  $n=10$ ) and in isolated bovine mesenteric arteries in vitro (right,  $n=7$ ) to increasing doses of ACh and SNP.

Blood flow responses to iontophoresis of ACh were suppressed after iontophoretic pre-treatment with atropine. This suppressive effect was stronger with increasing concentrations of atropine. Pre-treatment with atropine did not alter the blood flow responses to SNP. No significant effect of pre-treatment with ASA on the blood flow response to ACh was found.

Table 9.1. Best-fit parameters of the blood flow responses to iontophoresis of ACh and SNP in vivo and the responses of isolated vascular strips to the same drugs in vitro. Significance levels of differences between drugs are also shown.

	In vivo			In vitro		
	ACh	SNP	$p$	ACh	SNP	$p$
$\log(ED50)$	0.37	0.64	< 0.0001	-8.0	-6.7	< 0.0001
Hill Slope	2.0	3.0	< 0.0001	1.0	1.1	0.61

## 9.5 Modelling iontophoretic transport and blood flow responses

Analysis of blood flow responses during iontophoresis of vasoactive drugs by an empirical model as presented in investigation IV enables efficient use of the measurement data and may therefore increase the sensitivity of the

method. However, the limitation of fitting the response with an empirical model is that it is not based on the physical processes underlying the response to the drug in question. The main objective of investigation V was to develop a mechanistic time-response model, in which both the physical transport of drugs into the skin and the physiological response to these drugs are taken into account and to apply this model to the blood flow response elicited by iontophoretically applied ACh and SNP.

The proposed model can be divided in two parts. The first part describes the transport kinetics of drugs from the iontophoresis chamber into the microvascular bed, based on a one-compartment approach (Gabrielsson & Weiner, 2002). The second part describes the response dynamics of the microvasculature elicited by the local concentration of the drugs, according to classical receptor occupancy theory (Wagner, 1968). The derivation of the model is presented in detail in investigation V. Here we will only present the resulting equations. The model is schematically depicted in Figure 9.6.

The assumptions of the model are:

1. A constant input of drugs from the electrode into the microvascular bed;
2. A first order clearance from the microvascular bed due to diffusion and blood flow;
3. A lag time between the drug dose in the electrode and the microvascular bed;
4. Binding of drugs to receptors according to the law of mass action.

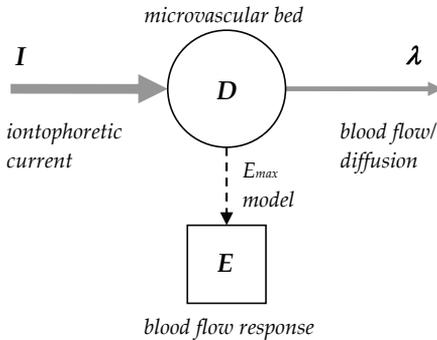


Figure 9.6. Schematic representation of the model used in investigation V, incorporating the drug transport into and out of the microvascular bed and the local blood flow response.

The above assumptions yield the following expression for the drug dose  $D$  in the microvascular bed as a function of time:

$$D(t) = \frac{nI}{ZF\lambda}(1 - e^{-\lambda(t-t_0)})$$

where  $n$  is the transport number of the drug ions,  $I$  is the current strength,  $Z$  is the valence of the drug ions,  $F$  is Faraday's constant,  $\lambda$  represents the clearance from the microvascular bed and  $t_0$  is the lag time. In the microvascular bed, the drug causes a dose-dependent response, which is written as:

$$E(D) = \frac{E_{\max}D(t)}{D(t) + K}$$

where  $E_{\max}$  is the maximum possible response to the drug and  $K$  is the drug dose that elicits half the maximum response (similar to  $ED_{50}$  in investigation IV).

Combining the two equations yields the blood flow response as a function of time:

$$E(t) = \frac{E_{\max}(1 - e^{-\lambda(t-t_0)})}{(1 - e^{-\lambda(t-t_0)}) + \frac{\tau\lambda}{I}}$$

where  $\tau = KZF/n$  is a parameter that combines the efficacy of the drug and the transport number and  $E_{\max}$  is the physiological maximum of the blood flow response. Hence, the model contains four unknown parameters:  $E_{\max}$ ,  $\lambda$ ,  $t_0$ , and  $\tau$ . The proposed model is applied to blood flow responses in healthy subjects elicited by ACh and SNP, iontophoretically applied by a single 10-minute pulse using three different current strengths.

We found that for both ACh and SNP, not only the speed of the response but also the maximum response depended on the current strength, with the highest current strength resulting in the fastest response and the highest maximum response. The model could be fitted accurately to the perfusion data obtained with LDPI (Fig. 9.6).

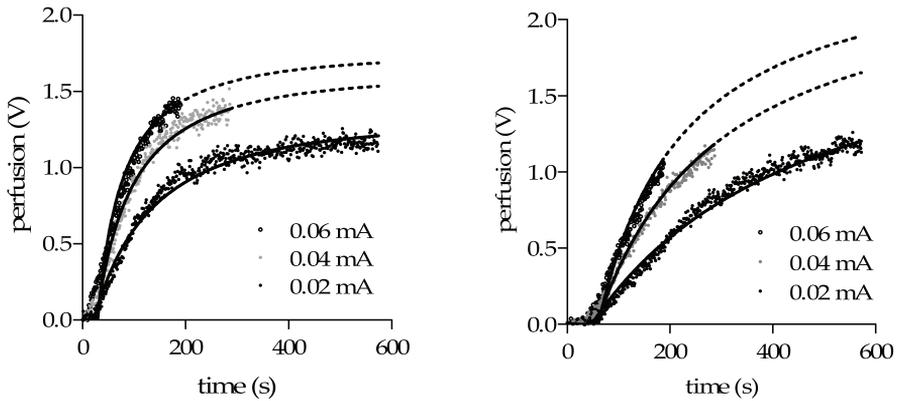


Figure 9.6. Average blood flow responses to iontophoresis of ACh (left) and SNP (right) at three different current strengths ( $n=10$ ). Solid lines are the responses predicted by the proposed time-response model. Dashed lines are the extrapolated responses predicted by the model.

The clearance rate constant  $\lambda$  for SNP was significantly smaller than for ACh. The lag time  $t_0$  increased with increasing current strength and was significantly larger for SNP than for ACh. The parameter  $\tau$ , reflecting the effects of drug efficacy and transport number, was significantly larger for SNP compared with ACh. The maximum response parameter,  $E_{max}$ , was significantly larger for SNP. Parameters could be estimated with narrow confidence intervals and are summarized in Table 9.2.

Table 9.2. Best-fit parameters and 95% confidence intervals of blood flow response to iontophoresis of ACh and SNP, obtained with the proposed time-response model. \* indicates significant differences between drugs.

	Acetylcholine			Sodium Nitroprusside			
$I$ (mA)	0.02	0.04	0.06	0.02	0.04	0.06	
$E_{max}$		2.1 (2.0 – 2.2)			2.7 (2.4 – 3.0)		*
$\lambda$ ( $h^{-1}$ )		13.7 (12.6 – 14.8)			2.7 (1.8 – 3.6)		*
$\tau$ (mC)		3.4 (3.1 – 3.7)			10.7 (9.1 – 12.3)		*
$t_0$ (s)	21.8 (18.7-25.0)	27.0 (25.1-28.9)	29.2 (27.8-30.6)	47.3 (43-51.6)	57.4 (55-59.8)	60.5 (58.7-62.3)	*



# 10 DISCUSSION

**T**ransdermal iontophoresis of vasoactive drugs provides a non-invasive tool for assessment of microvascular responses elicited by pharmacological provocations when it is combined with laser Doppler flowmetry for blood flow measurements. Although iontophoresis combined with LDF has been frequently used in experimental settings for more than two decades, several methodological issues prevent it from being used commonly as a clinical tool. In this thesis, a number of investigations are presented that are aimed at resolving some of these methodological issues and that hopefully will lead to a larger applicability of the technique.

## 10.1 Non-specific vasodilatation: possible mechanisms

Vasodilatation during iontophoresis of non-vasoactive compounds such as water or NaCl has been observed by many investigators. The non-specific effect was first investigated by Grossmann et al. (1995), who found an increase in blood flow upon iontophoresis of sodium, lithium, chloride, nitride, acetate, and bicarbonate ions. This was attributed to membrane hyperpolarisation and pH changes induced by the ions in the vehicle. The non-specific response at the cathode was found stronger than the response at the anode.

This difference between electrode polarities was also observed by Morris et al. (1995), who further suggested an effect of vasoactive substances present in the sweat ducts. Later studies have suggested effects of local sensory nerve activation (Morris & Shore, 1996; Berghoff et al., 2002), since the non-specific response could be largely reduced by using a topical anaesthetic agent (EMLA). Berliner (1997) investigated non-specific vasodilatation during tap water iontophoresis, and found that the response at the cathode could be suppressed by pre-treatment with aspirin, suggesting an involvement of prostaglandins.

The difference in response between anodal and cathodal iontophoresis has led to the idea that the mechanisms of non-specific vasodilatation should be interpreted in terms of voltage across the skin, rather than ionic current through the skin. A number of studies have focused on the effect of the

voltage, and it was first shown by Åsberg et al (1999) that addition of a high concentration of sodium chloride to the vehicle resulted in a lower voltage across the skin and in parallel a substantial reduction of the non-specific vasodilatation. This effect was later found to be present even at lower concentrations of sodium chloride (Ferrell et al., 2002).

In our investigations we observed a relationship between the ionic strength of the vehicle during iontophoresis, the voltage across the skin and the magnitude of the non-specific response. The use of deionized water as a vehicle for the drug is associated with a higher voltage over the skin and a pronounced non-specific effect, especially in cathodal iontophoresis. Adding NaCl ions to the vehicle lowers the voltage over the skin and reduces the non-specific vasodilatation. These findings, together with previous studies, suggest that vasodilatation is a result of depolarisation or hyperpolarisation of nerve cells, smooth muscle cells or other cells as a result of the applied voltage during iontophoresis. Depolarization of nerve cells has been shown to induce vasodilatation by release of various neuropeptides, such as calcitonin gene-related peptide, substance P and neurokinin A (Westerman et al., 1987; Wårdell et al., 1993). Hyperpolarization of smooth muscle cells causes vasodilatation through closure of voltage-dependent calcium channels (Feletou & Vanhoutte, 1996).

Neuropeptides released from nerve endings also promote the release of histamine from dermal mast cells, which dilates post-capillary venules, activates the endothelium, and increases blood vessel permeability (Lewis, 1927, Huttunen et al., 1996). Furthermore, when histamine is applied experimentally by iontophoresis, it induces a distinct wheal, flare, and itching (Furue et al., 2001; Kirchner et al., 2002). We therefore hypothesized that electrical current during iontophoresis causes release of histamine from dermal mast cells, possibly through the release of neuropeptides by afferent nerve endings or through other, unknown pathways.

The results of our investigations on the role of histamine indicate that in addition to involvement of nerve cells, non-specific vasodilatation is partly mediated by histamine-related mechanisms. The role of histamine appeared to be different with anodal and cathodal iontophoresis. During anodal iontophoresis, oral antihistamine treatment with cetirizine only suppressed non-specific responses at iontophoretic charges higher than 60 mC. This suggests that non-specific vasodilatation during anodal iontophoresis is partly mediated by the release of histamine, but only at high iontophoretic charges. At EMLA-treated sites, where non-specific responses were suppressed compared with untreated sites, cetirizine further suppressed the responses at

charges higher than 36 mC, which suggests that histamine-mediated vasodilatation occurs independent of the neural pathway.

The variability between subjects in non-specific blood flow responses during anodal iontophoresis was decreased after antihistamine treatment. This can be explained by the fact that the histamine antagonist particularly suppressed the response in subjects who responded strongly. This finding suggests that strong non-specific responses during anodal iontophoresis may be mediated in certain individuals by a local inflammatory reaction.

During cathodal iontophoresis, a right-shift of the blood flow response curve was observed when cetirizine was given as a pre-treatment. When local sensory nerves were blocked with EMLA, cetirizine had no effect. This suggests that the effect of histamine during cathodal iontophoresis is mediated by the release of neuropeptides from local sensory nerves.

## **10.2 Preventing non-specific vasodilatation**

In addition to the studies that have sought for possible mechanisms of non-specific vasodilatation, others have focused on strategies to prevent non-specific vasodilatation in the experimental setting. The first approach to deal with the non-specific response was to subtract the blood flow response to the vehicle in which the drug is dissolved, from the response to the drug (Morris et al, 1995; Morris & Shore, 1996). After iontophoresis of acetylcholine or sodium nitroprusside, control measurements were made with the respective vehicles, such as deionized water or saline. The problem with this approach is that it may introduce erroneous results because of the variability in perfusion response between skin sites, which may be as high as 20% (Kubli et al., 2000). Even if drug and vehicle responses are measured at the same site, it is difficult to assess the two responses independently, since the tissue may have been affected after the first measurement. Another complication is that the ionic strength of the vehicle alone is likely to differ from that of the vehicle and the drug together. The ionic strength is an important factor that influences the extent of the non-specific vasodilatation as found in our investigations and in other studies (Åsberg et al., 1999; Ferrell et al., 2002). These points make it doubtful that the response to the vehicle and the response to the drug are independent and that subtraction is justified.

Another strategy that has been used to suppress non-specific effects is the use of high molar concentrations of sodium chloride as the vehicle, in order to reduce the voltage across the skin. We have seen in this work that this indeed lowers the non-specific responses. However, other investigators found that the variability in drug response between subjects increased in the presence

of high concentration sodium chloride vehicles (Åsberg et al., 1999; Ferrell et al., 2002). The effect of ionic competition in the electrode chamber is likely to be the cause of this increased variability. Ionic competition occurs when drug ions and vehicle ions are both transported into the skin during iontophoresis. This effect becomes increasingly important when the number of vehicle ions in the drug solution increases (Mudry et al., 2006). Thus, although vehicles with a high ionic strength may reduce non-specific vasodilatation in the absence of drug ions, they may also reduce the response to the drugs during iontophoresis as a result of ionic competition. In fact, Khan et al. (2004) showed that adding high molar concentrations of sodium chloride did not lower the resistance of acetylcholine and sodium nitroprusside solutions, and concluded that deionized water is therefore a better vehicle for iontophoresis.

Topical anaesthetic agents have been found to suppress the non-specific response in iontophoresis almost completely. Mostly, lidocaine-prilocaine cream (EMLA) has been used. Unfortunately, EMLA is also known to affect vascular tone as the local anaesthetic has vasoactive properties in itself (Bjerring et al., 1989; Arildsson et al., 2000). This is obviously a confounding factor in the measurement of the microvascular response to vasoactive drugs. Nevertheless, this strategy of suppressing non-specific vasodilatation has been used quite often (Morris & Shore, 1996; Kubli et al., 2000; Pellaton et al., 2002; Hanneman et al., 2002; Christen et al., 2004).

Ferrell et al. (2002) observed that the use of larger electrode chambers resulted in less non-specific vasodilatation. In fact, using larger chambers is equivalent to using lower current strengths, since the current density per unit area decreases in both cases. In both cases, the drawback is that the effective amount of drug delivered per surface area decreases, resulting in weaker microvascular responses.

An obvious strategy to reduce non-specific effects is to limit the current density and total iontophoretic charge so that the mechanisms of non-specific vasodilatation are not triggered. This is a strategy that has been applied successfully by a number of investigators and their protocols were usually based on pilot experiments (Newton et al., 2001; Hamdy et al., 2001; Khan et al., 2000). The disadvantage of limiting current densities and total charges is that the response to the drug may not be maximized. In those studies, blood flow measurements were made only at a few iontophoretic charges or even only at a single charge. It is therefore difficult to interpret to what extent the responses had reached plateaus. Nevertheless, limitation of the current density and total charge seems to have the least drawbacks compared with suppression of non-specific effects by topical anaesthetics or by calculation of

drug-minus-vehicle responses. The main risk of this approach is that adequate plateaus in drug responses are not obtained as a result of the limited drug dose applied. In this work it is shown that, by limiting current strength and iontophoretic charge and by using drug solutions at physiologic ionic strength, it is indeed possible to minimize non-specific vasodilatation while plateaus in blood flow responses to ACh and SNP may still be obtained.

It must be noted however that we observed a strong non-specific reaction in 2 out of 10 subjects when NaCl was administered with cathodal iontophoresis. The fact that this strong reaction occurred already at low iontophoretic charges, independent of current strength, indicates an inflammatory reaction or hypersensitivity to the electric current, in agreement with the findings in investigation II. This observation stresses the significance of a control measurement to test for non-specific responses.

### **10.3 Analysis of blood flow responses**

There is no consensus about the best protocol for iontophoretic drug delivery. The protocols that have been used so far for assessment of microvascular function may be divided into single pulse and multiple pulse protocols. The advantage of applying multiple current pulses is the possibility to measure the maximum response after each separate iontophoretic charge. In this way, cumulative dose-response curves can be constructed. In fact, these protocols are very similar to the protocols used in studies on isolated vessel preparations.

In the first investigations, drugs were delivered to the skin with 8 successive current pulses. However, it was soon realised that a practical disadvantage of this approach is that the protocols tend to be lengthy, as a substantial time is needed for the response to reach its peak with slow-acting drugs. Also, it can be questioned if successive iontophoretic charges can really be considered as cumulative doses, considering the possibility of clearance of the drug out of the skin during the interval between pulses, a factor that is not significant *in vitro*.

It was therefore decided that in later investigations, a single pulse protocol would be applied. Single pulse protocols are faster, and they may either be applied with a single blood flow measurement at the end of the current application, or with blood flow measurements made at regular intervals during current application. An essential difference of using this protocol instead of a multiple pulse protocol, however, is that the blood flow responses at a certain time point are related to the iontophoretic charge applied at the very same time point. These responses may not be the maximal

responses to that iontophoretic charge, since no time delay is allowed for the response to maximize as is done in multiple pulse protocols. It may therefore be expected that dose-response curves obtained with a single iontophoretic current pulse will be right-shifted compared with dose-response curves obtained with multiple pulse protocols.

Despite this essential difference, the blood flow responses in our experiments could be very well characterised by a general pharmacodynamic dose-response model, at least for acetylcholine and sodium nitroprusside. This enables powerful assessment of the blood flow responses, since they can be interpreted in terms of conventional pharmacodynamic parameters such as *ED50*, *Hill slope* and maximum response. Also, it enables not just a qualitative comparison of responses between drugs or between patient groups, but rather a quantitative, statistically supported comparison of well-defined parameters.

In studies using iontophoresis for microvascular assessment, the iontophoretic charge is generally used as an estimate for the dose administered to the local microvasculature. The rationale for this assumption is Coulomb's law, stating that a charge (carried by drug ions) is equal to strength of the applied electric current (ions/second) multiplied by the time during which these ions are transported. Also this relation has been verified in vitro for model inorganic ions and drug ions (Phipps et al., 1989). The situation during iontophoresis is however essentially different from in vitro circumstances. The influx of drugs may be considered a zero-order infusion process during an extended period of time, typically several minutes. During this time, a clearance of drug ions out of the microvascular bed as a result of diffusion and active transport by blood flow is likely to occur. Furthermore, counter-ions and background ions in the vehicle partially account for the current transport, to an extent that may be dependent on the drug formulation and other factors.

In pilot experiments we noticed that, when different combinations of current strengths and pulse durations were used while iontophoretic charges were kept equal (i.e. constant charge of 12 mC using  $0.02 \text{ mA} \times 600 \text{ s}$ ,  $0.04 \text{ mA} \times 300 \text{ s}$  and  $0.06 \text{ mA} \times 200 \text{ s}$ ), the response curves were shaped differently. Maximum responses as well as *ED50* values differed for the different current strengths used. This suggested that the dose was not proportional to current strength multiplied by pulse duration.

In experiments where applied drug concentrations are unknown, time-response data often contain useful information about drugs kinetics as well as the pharmacodynamic characteristics (Gabrielsson, 2000). The kinetics of the transport of drugs into and the clearance of drugs out of the microvascular bed

were therefore considered in a time-response model for iontophoresis of vasoactive drugs. This one-compartment model also included the physiological response of the microvasculature to the local concentration of drugs based on the classical receptor occupancy theory (Wagner, 1968).

The results of fitting this model to blood flow responses elicited by iontophoretically applied ACh and SNP indicate that there is indeed a significant clearance out of the skin, which is dependent on the drug. This implies that the drug dose in the microvascular bed is not linearly proportional to current strength multiplied by pulse duration in a typical protocol for iontophoresis of vasoactive drugs. Also, the model indicates that the true physiological maximum response is never reached during the experiments because the maximum amount of the drug in the skin is limited by the clearance rate.

Applying the time-response model to blood flow responses elicited by vasoactive drugs applied by iontophoresis enables assessment of pharmacological mechanisms. For instance, it is interesting to note that the clearance rates of ACh and SNP were different. The relatively slow clearance of SNP could be explained by its fast biotransformation. The NO molecule is released very easily from the nitroprusside and has a high affinity for its enzyme receptors, which are located on the smooth muscle cells at the perimeter of the arterioles. The receptors for ACh reside on the intravascular endothelium and ACh must therefore cross the vessel wall before it can act on its receptors. This may cause ACh to be more susceptible to washout by intravascular blood flow.



# 11 CONCLUSION

The investigations presented in this thesis have been aimed towards resolving some of the main methodological issues in iontophoresis of vasoactive drugs for microvascular assessment, by investigating possible mechanisms of non-specific vasodilatation, by optimizing drug delivery protocols to prevent non-specific vasodilatation, and by applying new approaches for analysis of blood flow data.

From the results of the investigations it is clear that ionic strength of the vehicle in which the drug is dissolved during iontophoresis influences the extent to which non-specific vasodilatation occurs. This can be attributed to differences in voltage over the skin, resulting in depolarization or hyperpolarisation of cells, triggering vasodilatation through a variety of mechanisms. These mechanisms include neural and histamine pathways, as non-specific responses were largely inhibited by applying local anaesthetics and pre-treatment with an oral anti-histamine drug.

It is possible to construct protocols that yield maximized blood flow responses to ACh and SNP while non-specific vasodilatation is minimized. This can be accomplished by limiting current strength and total iontophoretic charge and by using drug solutions at physiological ionic strengths.

Blood flow responses measured by LDPI during a single pulse of iontophoresis of ACh and SNP can be accurately described by pharmacological dose-response models that are commonly applied in microvascular studies on isolated vessel preparations. By applying a time-response model that incorporates drug transport kinetics as well as blood flow response dynamics, it is possible to assess pharmacological mechanisms of microvascular responses. Validation of this model indicates that the assumption that the local drug dose in the microvascular bed is linearly proportional to the iontophoretic charge may not be valid. This assumption should therefore be used with care.



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## SVENSK SAMMANFATTNING

**M**ikrocirkulationen, som inbegriper kroppens minsta blodkärl, transporterar syre och näringsämnen till våra celler. Vissa sjukdomar, som diabetes, hjärt-kärlsjukdom och akut blodförgiftning leder till förändringar hos mikrocirkulationen. Mekanismerna bakom dessa förändringar är delvis okända. Det finns därför ett stort behov av kliniska mättekniker som kan bedöma mikrocirkulationens funktion. Vid jontofores placeras en elektrod tillsammans med ett läkemedel på huden. När en svag elektrisk ström anbringas transporteras läkemedlet ner genom hudlagren. Effekterna av ett kärlaktivt läkemedel som appliceras på detta sätt kan sedan avläsas non-invasivt med laser Doppler-teknik. En stor fördel med jontoforesmetoden, förutom att den är non-invasiv, är att läkemedelsdoserna som tillförs kroppen är mycket små och därmed ger de inte upphov till några systemiska bi-effekter. I avhandlingen presenteras forskning, vilkas målsättning är att lösa några av de viktiga frågorna kring transdermal jontofores så att tekniken optimeras för att den skall kunna brukas som ett verktyg vid kliniska undersökningar av mikrocirkulationen.

Den första delen ägnas ett fenomen som kallas ospecifik vasodilatation. Det uppstår vid jontofores av substanser som är inte kärlaktiv, som vatten och koksaltlösning. Resultaten från dessa försök indikerar att den ospecifika vasodilatationen beror på framför allt spänningen över huden, vilken i sin tur är relaterad till jon-koncentrationen hos läkemedelslösningen. Vidare registreras att mekanismen bakom den ospecifika vasodilatationen delvis är neuralt medierad genom att de till stor del går att förhindra med hjälp av lokal bedövning. Dessutom leder förbehandling med anti-histamina läkemedel till minskade ospecifika reaktioner, vilket också indikerar att lokala inflammatoriska processer är inblandande.

Den andra delen av avhandlingen ägnas att optimera försöksprotokollen för jontofores. Till att börja med utvecklas ett protokoll som ger ett adekvat läkemedelssvar samtidigt som ospecifika effekter minimeras. Det visar sig är möjligt genom att begränsa strömstyrkan och den elektriska laddningen under jontoforesen och genom att använda läkemedelslösningar som har en fysiologisk jonstyrka. Resultaten visar också att blodflödesförändringen som

registreras under jontofores av acetylkolin och natriumnitroprussid kan beskrivas med hjälp av konventionella dos-responsmodeller, vilket möjliggör en mer exakt analys av det mikrocirkulatoriska svaret samt underlättar jämförelse mellan olika läkemedel eller patientgrupper.

Slutligen presenteras en mekanistisk model för det mikrocirkulatoriska svaret vid jontofores. Modellen beskriver läkemedlets transport från elektroden ner genom huden, clearance i huden vilken beror på diffusion och det lokala blodflödet, samt förändringen i blodflöde som sker på grund av läkemedlet. Modellen valideras genom försök på försökspersoner och resultaten visar att förändringarna i blodflödet åstadkommet av acetylkolin och natriumnitroprussid med denna modell kan beskrivas på ett exakt sätt. Vidare visar resultaten att det sker en betydande clearance av läkemedel i huden under jontofores. Detta har väsentlig betydelse när man ska uppskatta den lokala jontoforesdosen.

## NEDERLANDSE SAMENVATTING

**D**e microcirculatie, een verzamelnaam voor de kleinste bloedvaten in het lichaam, voorziet onze cellen van zuurstof en voedingsstoffen. In ziektebeelden zoals diabetes of hart- en vaatziekten en in patiënten met ernstige sepsis is de functie van de microcirculatie verstoord, deels door onbekende oorzaak. Er is dan ook behoefte aan een klinisch toepasbare techniek om het functioneren van de microcirculatie te kunnen beoordelen. Transdermale iontoforese is een methode om geneesmiddelen door middel van een zwakke elektrische stroom de huid in te transporteren. Bij iontoforese van stoffen die de bloedvaten verwijden of vernauwen, kan het effect van deze stoffen op de microcirculatie in de huid worden gemeten met behulp van laser Doppler technieken. De combinatie van iontoforese met laser Doppler technieken maakt bestudering van het functioneren van de microcirculatie mogelijk op een niet-invasieve manier en met zeer lage doseringen van vasoactieve stoffen.

In dit proefschrift wordt een onderzoek beschreven dat gericht is op het wegnemen van enkele belangrijke problemen bij transdermale iontoforese en op verbetering van de methodiek, zodat deze optimaal kan worden ingezet als klinisch hulpmiddel voor het bestuderen van de microcirculatie in combinatie met de laser Doppler techniek. In het eerste deel is het zogenaamde niet-specifieke effect van iontoforese bestudeerd. Tijdens iontoforese van stoffen zoals water of fysiologisch zout, die geen farmacologisch effect hebben op de bloedvaten, treedt toch vaak vaatverwijding op onder de elektrodes. Uit de resultaten van dit onderzoek blijkt dat deze niet-specifieke reactie afhankelijk is van de elektrische potentiaal over de huid, welke samenhangt met de ionconcentratie in de toegediende vloeistof. Het is aannemelijk dat de niet-specifieke vaatverwijding deels ontstaat via neurale mechanismen, aangezien deze nauwelijks optreedt bij lokale verdoving van de huid. Tevens blijkt toediening van antihistamine voorafgaand aan iontoforese de niet-specifieke vaatverwijding deels te onderdrukken, hetgeen wijst op de rol van een lokale ontstekingsreactie.

Het tweede deel van het onderzoek is gericht op het optimaliseren van protocollen voor iontoforese ten behoeve van metingen aan de microcirculatie.

Ten eerste is een protocol opgesteld dat leidt tot een adequate reactie van de microcirculatie op de toegediende stoffen, terwijl niet-specifieke effecten worden voorkomen. Dit blijkt mogelijk te zijn door de stroomsterkte en de totale lading tijdens iontoforese te beperken en door gebruik te maken van vloeistoffen in fysiologische concentraties. Het onderzoek laat tevens zien dat de bloedstroom in de microcirculatie tijdens iontoforese van enkele belangrijke vaatverwijdende stoffen, toegediend volgens bovengenoemd protocol, kan worden beschreven met gangbare dosis-effect modellen. Dit verbetert de analyse van de resultaten en vereenvoudigt de vergelijking tussen reacties op verschillende typen medicaties of tussen patiëntgroepen.

Ten slotte is een mechanistisch model voorgesteld voor de reactie van de microcirculatie op vasoactieve stoffen toegediend met iontoforese. Dit model beschrijft enerzijds het transport van deze stoffen van de elektrode naar de huid en de eliminatie uit de huid ten gevolge van diffusie en bloedstroom. Anderzijds beschrijft het model de fysiologische reactie van de bloedvaten op de aanwezigheid van deze stoffen in de huid. Validatie van dit model door metingen bij gezonde vrijwilligers laat zien dat de microvasculaire reactie tijdens iontoforese van vaatverwijdende stoffen goed door het model kan worden beschreven. Tevens blijkt uit de resultaten dat er tijdens iontoforese een substantiële eliminatie van de stoffen uit de huid plaatsvindt. Dit effect is belangrijk omdat het de lokale concentratie beïnvloedt van stoffen die zijn toegediend via iontoforese.