Spontaneous fluctuations of oxygen tension in tissue is similar to vasomotion of isolated pressurized arterioles

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ABSTRACT

The underlying mechanism of formation of spontaneous oscillations in tissue oxygen tension and their physiological significance remain unknown. The working hypothesis of this study were that the oxygen fluctuations are based on arteriolar vasomotion and that the frequency of oscillations in tissue oxygen tension is similar to spontaneous vasomotion of isolated pressurized arterioles. In addition we investigated whether oxygen fluctuations are altered under pathological conditions such as in malignant tissue.

In this study, pO_2 oscillations in the brain and skeletal muscles of rats were measured with an Eppendorf Oxygen electrode. Fluctuations in pO_2 and changes in local temperature were coherent. These fluctuations did not correlate with heart rate, respiration rate or electroencephalogram (EEG). Surgical sectioning of the sciatic nerve and intravenus (i.v.) injection of the ganglion blocker, mecamylamine, did not alter pO_2 oscillations in skeletal muscle. The histology of blood vessels in the measurement areas was assessed by light microscopy. Arterioles from muscle and brain surrounding the electrode track, were dissected, cannulated and pressurized in a myograph. Spontaneous oscillations were observed at normal physiological intravascular pressure. The frequency of spontaneous oscillations in vitro matched the frequency of pO_2 fluctuations in vivo. Pharmacological studies of these oscillations show that local administration of phenylephrine (PE), an α-adrenoreceptor agonist to skeletal muscle, in vivo, increased the amplitude and frequency of pO_2 oscillations but had only minor effects on oscillations in brain. Prostaglandin

(PGFα1) increased the amplitude of these oscillations in the brain but had only a moderate effect in skeletal muscles. Similar results were observed when phenylephrine and U46619, a thromboxane analogue, were administered to isolated pressurized arterioles. L-type calcium channel antagonist, nifedipine was shown to decrease the frequency and amplitude of these oscillations both in vivo and in vitro. In mice implanted with squamous cell carcinoma (SCCVII) tumours, pO_2 fluctuations (1-3 c/min) were observed in subcutaneous control and peripheral tumour tissue (2 c/min). These fluctuations were absent in most central zones close to necrosis areas. Injection of nicotinamide increased pO_2 significantly ($p < 0.01$) and also reinitiated pO_2 fluctuations in 5 out of 10 hypoxic tumour areas.

In conclusion, oscillations of oxygen tension recorded in tissue are similar to spontaneous vasomotion of isolated pressurized arterioles surrounding the electrode. These fluctuations are altered in hypoxic areas of malignant tissue.

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ABBREVIATIONS

ANOVA	analysis of variance
ATPase	adenosine tri phosphatase
HR	heart rate
IFP	interstitial fluid pressure
i.v.	intravascular
MABP	mean arterial blood pressure
NO	nitric oxide
PE	phenylephrine
PGFα1	prostaglandin Fα1
pO_2	tissue oxygen tension pressure
SR	sarcoplastic reticulum
SCCVII	squamous cell carcinoma
VOC	voltage gated calcium channels

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To my parents

1. INTRODUCTION

1.1. Historical Background

In 1954, L.C. Jr. Clark studied oxygen availability in cat brain and observed spontaneous oscillations in oxygen tension with a frequency of 6-12c/min and an amplitude of ±30% of the mean value. Vasodilating agents such as caffeine, papaverine and acetylcholine decreased the frequency and amplitude of these oscillations. He hypothesized that rhythmic changes in vascular tone adjacent to the electrode may be the underlying mechanism for formation of these fluctuations (Clark, 1958). Few years later, Misrahy et al. examined pO_2 oscillations in foetal cat brain. They noticed that administration of apresoline and adrenaline caused changes in the pattern of fetal brain pO_2 fluctuations (Misrahy et al., 1962). It was suggested that these agents may play a central role in maintaining extracellular oxygen balance but likely serve to alter it by increasing or decreasing cellular requirements for oxygen.

Following this study, Coopers et al. implanted large gold wire electrodes in brain of psychotic patients. A simultaneous measurement of EEG, blood pressure, heart rate and pO_2 showed that these spontaneous fluctuations were independent of systemic changes. They further examined the oxygen fluctuations and compared them with

temperature fluctuations recorded by a thermistor and established a correlation between fluctuations in blood flow and oxygen tension (Cooper et al., 1966).

The subsequent invention of oxygen microelectrodes has advanced our understanding of spontaneous pO_2 fluctuations. In a recent study, Hasidbeder et al. investigated mucosal and serosal oxygenation with a micro-electrode in an autoperfused, innervated jejunal preparation in pigs (Hasibeder et al., 1994). They concluded that jejunal mucosa demonstrates significant regular changes in oxygenation parameters that are locally mediated. They speculated further that the physiological basis for this phenomenon is the countercurrent arrangement of microvessels in conjunction with vasomotion.

The use of the laser Doppler velocimeter provided evidence for the presence of sustained time-dependent oscillations in blood flow, in the small tissue volumes of the human skin (Beinder et al., 1994; Bertuglia et al., 1996; Colantuoni et al., 1994), at frequencies similar to those found in previous oxygen measurement studies (Gniadecki et al., 1992; Morita-Tsuzuki et al., 1992; Schubert et al., 1995). These observations led to the conclusion that flowmotion originates from arteriolar vasomotion (Bouskela, 1989; Fagrell, 1985; Funk et al., 1983; Intaglietta, 1989). Others argued that rheological factors such as red blood cells and their locations at the arteriolar branching points may cause these fluctuations (Ellsworth et al., 1995; Kiani et al., 1994; Ragan et al., 1988).

Based on the above mentioned studies, arterioles close to the oxygen electrode are one of the major factors in formation of oxygen fluctuations. Thus the working hypothesis in this thesis was that arteriolar vasomotion causes fluctuations in tissue oxygen tension and that the frequency of spontaneous vasomotion of isolated pressurized arterioles is similar to spontaneous fluctuations in tissue oxygen tension.

In an attempt to find potential clinical implications of spontaneous oxygen fluctuations, few investigators examined their event under pathological conditions. Both hypoxia (Bertuglia et al., 1991; Hardwick, 1996) and hyperoxia proved to change the frequency of flowmotion and thus oxygen oscillations (Bertuglia et al., 1991; Schmidt et al., 1993). Similar alterations in flowmotion pattern appear under a wide range of pathological conditions. However, a direct link between fluctuations in flowmotion and disruption of tissue oxygen tension has not been demonstrated (for review see; (Duling and Klitzman, 1980; Leschke et al., 1992; Reiz, 1989; Selwyn et al., 1990; Tschoepe et al., 1993; Witte and Witte, 1995). The second hypothesis in this thesis was therefore to investigate whether spontaneous oxygen fluctuations alter under pathological conditions such as in malignant hypoxic tissue.

1.2. Physical factors affecting oxygen tension

Tissue oxygen tension can be modulated by several physical and chemical factors. Among the physical factors, vascular pressure and vascular network are of fundamental importance. Other factors such as tissue pressure and the extent of

tissue/blood exchange have been experimentally shown to affect oxygen tension but their physiological role in formation of pO_2 fluctuations, is uncertain.

1.2.1. Vascular pressure

The driving force of blood flow originates with the ejection of the blood into the aorta and pulmonary artery. Thus it has been shown that microvascular pressure in the individual branching orders is under a proportional relationship to the level of the systemic and pulmonary arterial pressures (Williams et al., 1988).

Frictional resistance encountered in the successive branching of the arterial tree dampens the pulse pressure but does not completely eliminate it. Recordings of capillary pressure show a pulse pressure of 1.0-2.0 cm H_2O (Intaglietta, 1968; Tooke and Williams, 1987; Williams et al., 1988). In mesenteric vessels, mean capillary pressures are consistently higher than plasma oncotic pressures by some 4-6 mm Hg. Pressures in individual capillaries fluctuate in both cyclic and irregular patterns (Intaglietta, 1968; Messmer and Kreimeier, 1989; Tooke and Williams, 1987). However, it has been considered unlikely that oscillations in capillary pressure by themselves would be sufficient to allow anything but marginal control of the oxygen exchange.

Microvascular pressure variability has both temporal and spatial characteristics. Temporally related factors include systolic-diastolic fluctuations as well as changes in pressure associated with variations in both the rate and amplitude of spontaneous

vasomotion (Gannon et al., 1983). The spatial characteristics of microvascular pressure variability are related to the architecture of the vascular network.

1.2.2. Vascular network

Recent microcirculatory research has increasingly focused on the architectural organization of the terminal vascular bed and its relationship to both the hemodynamic and exchange features (Dodd and Johnson, 1991; Koller et al., 1987; Pittman, 1995). The network concept of microcirculation was developed by taking into account not only the arrangement of vessels but also the mechanics of communication between the vascular segments constituting such vessel networks (Pittman, 1995).

As suggested by Ragan et al., an uneven distribution of red cells and plasma at the arteriolar branching point of each vessel will cause uneven distribution of red cells and blood flow in microcirculation (Ragan et al., 1988). Therefore, the architectural asymmetry of a network has a large influence on the heterogeneity of red cell flux distribution in the network. Furthermore, the uneven distribution of red blood cells at vascular branching points may cause uneven oxygen delivery to tissue (King et al., 1996).

Heterogeneity of velocity of a magnitude comparable to those observed by direct visualization of microcirculation can produce reductions in oxygen supply to small tissue regions of a degree that may limit oxygen delivery and thereby, tissue function.

Flow heterogeneity may also influence capillary hematocrit and/or red cell spacing by producing cell separation at bifurcations and a resultant reduction in mean capillary tube hematocrit. There is as yet no agreement on why and how hematocrit variations may influence tissue oxygenation (Duling, 1994; Duling and Damon, 1987).

1.2.3. Tissue pressure

Tissue pressure may be an important factor affecting microcirculation and oxygen transport. Increased pressure external to the blood vessels reduces transmural pressure and thus passively reduces vessel diameter (Hartsock et al., 1998). If external tissue pressure exceeds internal vascular pressure, the blood vessels collapse (Reneman et al., 1980).

Tissue pressure has direct effects on tissue oxygen tension. As shown by Matsen et.al, 1979 elevated tissue pressure decreases the tissue oxygen tension, but it does not reduce pO_2 to zero unless the tissue pressure exceeds the local arterial pressure (Matsen et al., 1979).

Sources of extravascular pressure under physiological conditions may be the distention of arteries as arterial pressure rises, capillary filtration, and lesion of veins as venous pressure rises (Kristiansen and Heyeraas, 1989). In pathological conditions such as edema (due to excessive filtration or lymphatic obstruction), hemorrhage, and cell swelling (due to hypoxia and injury) high tissue pressures have been observed (Mellander and Albert, 1994).

1.2.4. Blood-tissue exchange

In the early part of this century, August Krogh proposed a model of oxygen transport in capillaries. This model assumes that all oxygen is delivered to the capillaries by convection from small terminal arterioles and lost from these capillaries by diffusion. This model and its implications have been used extensively to interpret whole organ oxygen transport data in terms of diffusion from capillaries to tissues and to relate changes in microvascular hemodynamics to alterations in tissue oxygen transport.

Recently, Krogh's model has been reexamined because new exchange pathways between arterioles, venules and capillaries have been recognized. The proximity of capillaries, arterioles, and venules, along with the anastomotic connections and tortuosity of capillaries, provide the "complex spatial relationships" that lead to diffusive interactions between neighboring capillaries, between capillaries and nearby arterioles and venules, and between paired arterioles and venules (Duling and Berne, 1970) (Ellsworth and Pittman, 1990; Hoppeler and Weibel, 1998; Pittman, 1995; Ye et al., 1995).

In contrast to other substrates for tissue metabolism, oxygen can be exchanged between intra- and extravascular compartments without any important restriction by the vascular walls (Buerk and Goldstick, 1992; Hoppeler and Weibel, 1998; Yaegashi et al., 1996). Because the walls of all micro-vessels appear to be permeable, O_2

continuously diffuses between blood and interstitium. The direction of oxygen diffusion depends on the oxygen partial pressure difference (Popel and Gross, 1979).

In tissue, once oxygen is outside the blood vessel, it's movement through interstitial space occurs by diffusion and convection (Ellsworth and Pittman, 1990). Diffusion is based on the concentration gradient in the interstitium, and convection is proportional to the interstitial fluid velocity vi (cm/s). The latter, in turn, is proportional to the pressure gradient in the interstitium. Values of the transport coefficients are determined by the structure and the composition of the interstitial compartments as well as by the physiochemical properties of the solute molecule (Wiederhielm and Black, 1976).

1.3. Physiological factors affecting oxygen tension

Various physiological factors such as heart rate, respiration rate and the neuronal network may affect the basic oxygen tension in tissue. Three types of spontaneous pO_2 oscillations have been characterized by Kunze et al. (Kunze and Kunze, 1983). First, there are spontaneous changes that are not related to any visible event in the muscular scale recorded by the EMG-monitoring system but correlate with respiration cycles which change frequency after hyperventilation and disappear during apnea (Kunze, 1977). The second type of oscillations correlate with systemic heart rate. The third type of fluctuations is not related to any systemic factors and is hypothised to be due to vasomotion. Since all microvascular network are supplied

with vasomotor and sensory nerves, the neural network may play a substantial role in the control of oxygen exchange and tissue oxygen tension.

1.3.1. Neural control

Vasomotor innervation may include 1) sympathetic adrenergic postganglionic fibers 2) sympathetic postganglionic cholinergic fibers 3)sympathetic cholinergic fibers and 4) fibers with transmitters other than norepinephrine or acetylcholine. The activity of vasomotor nerves is subject to a large variety of cardiovascular regulatory reflexes that constitute the principal mechanisms of extrinsic microcirculatory control (Fleming, 1988).

Neurogenic control of the peripheral circulation is accomplished by alterations in nerve discharge to the pre- and postcapillary vascular network in various organs. Postganglionic sympathetic adrenergic nerves constitute the most important efferent pathway for neural control. The physiologic response of the microvasculature to neural influences depends on a number of factors, but the pattern of distribution of nerves to microvessels is one of the more important determinants (Bevan, 1987). In addition to their influence on the contractile state of vascular smooth muscle, adrenergic nerves also have a trophic influence on smooth muscle cells (Bevan, 1987; Bevan, 1984).

Sympathetic postganglionic adrenergic nerves supply the smooth muscle cells of arterioles in all organs. In some cases, innervation has been shown to extend to

terminal arterioles and precapillary sphincters (Baez et al., 1977). However, no evidence was found for central neural regulation of precapillary sphincters independent of arteriolar control (Baez et al., 1977). In most of the larger muscular vessels, nerve endings make contact only with the outermost layer of smooth muscle cells (Baez et al., 1977). Cell-to cell conjunction between endothelial cells and smooth muscle cells in small arterioles, terminal arterioles and precapillary sphincters transfers the neural electric signal.

Oscillations in neural network have been observed by Mayhew et al. They used imaging of scattered and reflected light from the surface of neural structures to reveal changes produced by local variation in blood volume and oxygen saturation related to neural activity. They found that a major source of variability in the captured light signal is a pervasive low-frequency (0.1-Hz) oscillation which apparently results from regional cerebral blood flow. This signal is present in the brain parenchyma as well as in the microvasculature and exhibits many characteristics of the low-frequency "vasomotion" signals observed in peripheral microcirculation. Concurrent measurements in the brain with a laser Doppler flowmeter revealed an almost identical low-frequency signal (Mayhew et al., 1996).

1.3.2. Spontaneous vasomotion

In 1946, the term, "vasomotion", was applied to spontaneous rhythmic contractility in the peripheral vasculature. Arteries from many vascular beds display vasomotion, i.e., rhythmic oscillations superimposed on a tonic contraction.

Experimental studies by Colantuoni et al. determined that the activity of contraction and relaxation is a characteristic of the arterioles and is specific to vessel size (Colantuoni et al., 1990; Colantuoni et al., 1984). The frequency of these oscillations is highest in the smallest blood vessels, the so-called terminal arterioles, which oscillate at the rate of 20±3 cycles per minute, the amplitude of these oscillations can be 100% of mean diameter. This phenomenon has also been observed as a periodic opening and closing of the lumen of these vessels. As we ascend the microvascular network towards the larger arterioles, both frequency and amplitude decrease to the extent that vessels of 100μm in diameter exhibit oscillation frequencies in the range of 1-3 cycles/min and amplitude of 10-20% of the mean diameter (Colantuoni et al., 1984).

Several investigators have examined vasomotion in different organs. When intravital microscopy and laser Doppler flowmeters have been employed simultaneously, an equivalence between oscillations in blood flow and vasomotion has been observed (Colantuoni et al., 1994; Lossius and Eriksen, 1995; Ren et al., 1993).

Variant coherence analysis indicated that spontaneous fluctuations in flow are caused by vasomotion of arterioles with a frequency of around 0.1 Hz. (Bertuglia et al., 1996).

Two main hypotheses have been advanced to explain the mechanism of vasomotion. Some investigators suggested that vasomotion may be regulated by oxygen availability in tissue (Feigl, 1975; Gorczynski and Duling, 1978; Sullivan and Johnson, 1981) as well as by various metabolic factors (Carroll et al., 1996; Johnson and Henrich, 1975). Others suggest that fluctuations in tissue oxygen tension are caused by spontaneous vasomotion which is consequently based on myogenic tone and intravascular pressure (Bouskela and Wiederhielm, 1979; Johnson and Henrich, 1975).

The first hypothesis suggests that vasomotion is formed by changes in the chemical makeup of the downstream tissue milieu accompanying either an excess or diminished flow (Ceriello et al., 1990). In general, some substrates or by-products of metabolism act to relax vascular smooth muscle; an exception is oxygen which at tensions above normal produces arteriolar and precapillary narrowing by acting on smooth muscle or endothelial cells (Bouskela, 1989; Mombouli and Vanhoutte, 1995).

Both metabolic factors in low concentrations and tissue oxygen concentration may cause dilation of small arterioles and vasomotion (Segal and Duling, 1986) (Mombouli and Vanhoutte, 1995; Montorsi, 1991). In organs and tissues in which the principal function of blood supply is to support local metabolism, metabolic flow

autoregulation provides a complete feedback circuit, with blood flow and metabolism interacting to determine local concentrations of substrates and products (Mombouli and Vanhoutte, 1995). The existence of metabolic or functional chemical control of blood flow is accepted, but the interactions of such a control with the myogenic response remains to be determined.

Several of these vasoactive metabolites have been identified. The possible involvement of adenosine, ATP, lactate, pH, hyperosmolarity, potassium, and inorganic phosphate has been discussed. (Cameron et al., 1994) (Hudlicka and el Khelly, 1985). It has also been suggested that cyclic generation and destruction of specific vasoactive agents of both polypeptide and prostaglandin classes may be involved (Bertuglia et al., 1994; Gerstberger et al., 1988).

Oxygen is another potent modulator of vasomotion. In this relation, Hoper and Kessler have found a mitochondrial oxidase enzyme that responds to a decrease in oxygen tension by reducing oxidative requirements of the cell. They postulate that such a sensor enzyme in endothelial and smooth muscle cells provides a feedback mechanism such as vasomotion for adjusting pressure and blood flow in accordance with extracellular oxygen levels (Kessler et al., 1978).

In addition, two distinct oxygen-sensitive mechanisms of arteriolar dilation in porcine coronary arteries have been identified (Rubanyi and Paul, 1985), both of which regulate vascular tone. One is activated at relatively high pO_2 values (10-40%),

and the vasoconstriction induced by this mechanism is mediated by vascular prostaglandin synthesis. The other is expressed at low pO_2 values (near zero), and the depression of mechanical activity by this mechanism may be related to the limitation of oxidative energy metabolism (Dora and Kovach, 1981; Furchgott, 1966; Hudlicka and el Khelly, 1985; Johnson and Henrich, 1975; Proctor et al., 1981; Vern et al., 1988; Ye et al., 1995). Perhaps both metabolic factors and oxygen provide a feedback system by regulating vascular tone which consequently affects oxygen delivery to tissue. Therefore, oxygen is a regulator and may also be regulated by vasomotion (Tsai et al., 1993).

Vasomotion has also been demonstrated in isolated pressurized arterioles and it has been proposed to be based on the myogenic tone (Osol et al., 1988). In this relation, the role of L-type voltage-operated Ca^{2+} channels (VOCs) in myogenic responsiveness was studied in a number of preparations such as in cannulated rat mesenteric small arteries (Wesselman et al., 1996). This study show that any intervention that blocked the VOCs also blocked myogenic responses (Wesselman et al., 1996). Therefore, calcium is considered the main factor in formation of spontaneous vasomotion.

One of the important factors in formation of vasomotion in isolated arterioles is intraluminal pressure. The tension oscillations are closely associated with

corresponding oscillations in membrane potential and are known to be associated with oscillations in intracellular Ca^{2+} concentration.

The influence of transmural pressure on vasomotion is assumed to be located at the smooth muscle level. Upon reduction of extracellular Ca^{2+}, vasomotion continues as long as a tonic contraction is obtained (Gustafsson et al., 1994). In contrast, pharmacological blockade of voltage-operated Ca^{2+} channels (VOCs) totally abolishes vasomotion, even though a sustained contraction may still be still obtained. Inhibition of the handling of Ca^{2+} in the sarcoplasmic reticulum (SR) abolishes the oscillations while facilitating Ca^{2+} release with caffeine increases the frequency of vasomotion. Thus, both VOCs and the SR are essential in generation of vasomotion (Gustafsson et al., 1994). These findings support either K^+ channels or Cl^- channels to be directly involved in the feedback system of oscillation since vasomotion has been reported to be immediately abolished when the electrogenic effect of the Na^+,$K(^+)$-ATPase is inhibited by Quabain (Gustafsson, 1993).

Further investigations into the mechanism of vasomotion shows that vasomotion may be generated via an endothelium-dependent feedback system in the vascular smooth muscle (Park et al., 1996). The tension oscillations might dependent on the endothelium as a feedback mechanism, apparently by increasing smooth muscle cyclic GMP via the release of nitric oxide, the contraction phase of vasomotion ends and dilation phase starts. However, recent in vivo studies by Buerk et al. showed that

endothelium release of nitric oxide (NO) is not required for vasomotion and that spontaneous, low-frequency NO fluctuations observed in the cat optic nerve head are a passive phenomenon caused by natural variations in shear stresses (Buerk and Riva, 1998). Other findings by Bertuglia et.al also suggest that vasomotion is not directly related to nitric oxide in conscious animals while NO blockade stimulates vasomotion in smaller arterioles of anesthetized hamsters which did not display vasomotion prior to the blockage (Bertuglia et al., 1994).

1.3.2.1 Smooth-muscle cell

Smooth muscle is responsible for major microvascular adjustments of pressure and flow. Relaxation or contraction of specially arranged fibers control the circumferences and compliance of the vessel wall. In some instances single smooth muscle cells extend to the capillary entrance (precapillary sphincters) and control the supply of the capillaries. This control may have a direct effect on capillary contractility and therefore tissue oxygen tension (Duling et al., 1987; Hayashida et al., 1987).

Concerning the origin of the oscillatory behavior of arterioles, we should note that smooth muscle cells exhibit spontaneous membrane polarization. This spontaneous membrane oscillation slow waves are regular and resistant to tetrodotoxin and sympathomimetic or parasympathomimetic antagonists, findings indicative of myogenic activity. It has been suggested that spontaneous membrane

depolarization may be because of continuous SR Ca^{2+} uptake and release (Byron and Taylor, 1993). In addition, it has been shown that groups of cells that are in contact tend to oscillate in synchrony (Iwashima et al., 1997). The process by which this takes place is not established, but is presumed to be a combination of mechanical signal transmission related to the stretch sensitivity of these cells and the transmission of signals via gap junctions (Gustafsson, 1993). These two mechanisms constitute a pathway for the coupling of signals necessary to produce the phenomenon of "frequent entrapment" which is likely responsible for the coordinated oscillations of the cells present at the arteriolar bifurcations (Colantuoni et al., 1985).

1.3.2.2. Pacemaker theory

The concept that active pacemakers control vasomotion was developed by Colantuoni et al., following the observation that the onset of contraction first appeared in the immediate vicinity of bifurcations and then propagated up- and downstream from this location (Colantuoni et al., 1984). Given the evidence that the activity propagates along the vessel, it also became apparent that at given locations the pattern of vessel diameter changes could be quite complex, as the activity of different pacemaker cells becomes superimposed on each other (Kannan et al., 1997).

The presence of pacemaker cells is also suggested by studies of the smooth muscle of larger vessels where pacemaker-like regions are observed to exist both in terms of contractile activity and electrical events (Goligorsky et al., 1995). However, in the

microcirculation, the only distinctive anatomical feature seen at the bifurcations is a thickening of the layer of smooth muscle cells, without any other specific characteristics (Colantuoni et al., 1985).

It is important to note that this phenomenon has independent frequencies and phases in different branches of a given arteriolar network, which leads to the hypothesis that vasomotion is a locally controlled phenomenon, further supporting the concept that it is regulated by signals from pacemaker cells (Gorczynski and Duling, 1978).

1.3.2.3 Gap Junctions

The gap junction coupling is important for the organization of the tissue as an electrical syncytium. Propagation of vasomotor responses along arterioles is initiated via a local change in membrane potential that spreads electronically through gap junctions coupling smooth muscle cells, endothelial cells, or both (Segal and Duling, 1989).

It has been suggested that conduction of vasodilator response along the arterioles is mediated via direct communication between cells composing the arteriole. The cell-cell coupling enabled by gap junctions and reported for both endothelial and smooth muscle cells indicates that either one cell type or both cell types could conduct a vasomotory stimulus along the vessel (Little et al., 1995; Segal and Duling, 1987).

1.3.2.4. Capillaries and endothelial contractility

The phenomenon of "capillary contractility" was first described more than 100 years ago. Recent reports on this phenomenon in the frog mesentery have been based on the observation of dramatic reductions of luminal diameter and blood flow during electrical stimulation of the capillary wall (Biswal and Hudetz, 1996).

In 1991 Ragan et.al video recorded spontaneous cyclic contractions of endothelial cells in capillary walls that often stopped the flow of blood cells in different preparations such as spleens of rats and mice. They concluded that these spontaneous capillary contractions are primarily of endothelial origin (Ragan et al., 1988). Since then, an old concept has been revitalized and the existence of independent capillary contractility has been reinvestigated by a few investigators.

Indeed, experiments mainly in cold-blooded animals have shown that individual endothelial cells can contract in response to specific stimuli (Stepp and Tulenko, 1994) or spontaneously (Ragan et al., 1988). However, such physiological phenomena have not been demonstrated in warm-blooded animals. Some investigators argue that capillary contractility cannot be regarded as a general regulatory principle controlling the available surface area for oxygen exchange. It seems unlikely, therefore, that capillary contractility represents a major mechanism of flow because vasomotor

adjustment within the mammalian tissue occurs only in those vessels with recognizable smooth muscles (Tyml et al., 1984).

1.4. Functional consequences of vasomotion on tissue oxygen tension

Vasomotion might affect oxygen tension via several mechanisms. One mechanism is indirect via changes in blood flow. Principally, vasomotion alternates the hydraulic conductivity of the active vessels. This effect results from the substantial non-linearity introduced by the dependence of this parameter on the fourth power of the radius according to Poisueuilles Law. An additional effect is due to the substantial variations in hematocrit density induced by vasomotion which affect the local viscosity of blood (Papenfuss and Gross, 1985) and thus oxygen delivery.

The changes in vascular hydraulic resistance induced by vasomotion ultimately affect capillary pressures and therefore fluid balance (Intaglietta et al., 1996). This relation has not been verified objectively. Since vasomotion in arterioles is a factor in determining overall vascular resistance, we could presume that it has an effect on systemic blood pressure.

Another aspect of flow regulation by vasomotion relates to the inability of red cells to pass through very small capillaries (for humans: 2.8 μm). In such conditions, if the terminal arterioles are subject to vasoconstrictor stimuli that cause the arteriolar diameter to fall below a critical value, blood flow will stop and the tissue becomes ischemic. Under these circumstances, vasomotion would provide the necessary escape

for red blood cells to pass through arterioles and flow is maintained due to oscillatory behavior by vasomotion through the capillaries supplied by the arterioles. This in turn, will allow blood flow to be maintained by vasomotion (Biswal and Hudetz, 1996).

1.5. **Spontaneous fluctuations of oxygen under pathological conditions**

Adequate oxygen tension is essential to maintain normal physiological conditions. In many pathological conditions such as diabetes and ischemic heart, oxygen tension is lower than normal levels which consequently leads to tissue hypoxia and necrosis. Often disruption of oxygen supply is associated with abnormal vascular structure. Conditions such as diabetic retinopathy is directly connected to vascular abnormality, extensive angiogenesis and consequently, inadequate oxygen supply. Although a direct link between abnormal vascular function and oxygen tension has not been demonstrated, a few investigators have shown that arteriolar vasomotion is altered under diabetic and hypertensive conditions (Cameron et al., 1994; Grunwald et al., 1996; Osol et al., 1988; Stansberry et al., 1996; Tooke et al., 1987).

In other conditions such as hypoxia in tumours, insufficient oxygen supply is a major therapeutic obstacle (Chaplin and Trotter, 1990; Gulledge and Dewhirst, 1996). Low tumour oxygenation can lead to distant metastasis (Brizel et al., 1996). Extensive studies have been conducted to understand and improve tumour oxygen supply.

1.5.1. Fluctuations of oxygen in malignant tissue

Hypoxia in tumours has been linked to several factors. Tumours grown in mice typically exhibit regions of hypoxia believed to result from two different processes: chronic oxygen deprivation due to consumption/diffusion limitations, and periodic deprivation resulting from transient reductions in tumour blood flow (Chaplin and Trotter, 1990). The relative contribution of each is, however, not generally known. Abnormal vascular architecture or extensive angiogenesis have been also regarded as important factors in formation of hypoxia. Recent studies in this field show that most blood vessels in tumours do not undergo vasomotion (Shan et al., 1997).

Secondary effects of the absence of vasomotion in tumours have not been investigated. It is not known whether tumour blood flow and oxygenation are affected by abnormal vasomotion. Recent theoretical studies by Dewhirst et.al suggest that knowledge of tumour arteriolar pO_2 is important in understanding O_2 transport in tumours and that the increased arteriolar pO_2 could reduce hypoxia in some circumstances (Dewhirst et al., 1996).

Most recent studies by laser Doppler flowmeters show lower frequency (1c/25-60 minutes) fluctuations in murine breast cancer and R3230Ac tumour oxygen tension (Dewhirst et al., 1997; Kimura et al., 1996) which do not exist in normal tissue. These fluctuations appear to be connected to progression of hypoxia. However,

the higher frequency (1-3 c/min) of vasomotion-induced oxygen fluctuations have not been identified in tumours.

In this study SCCVII tumours were used for oxygen measurement studies. As previous studies (Steinberg et al., 1991) showed and the histologic features of squamous cell carcinomas in these mice are identical to the human type of squamous carcinoma grown any where else in the body.

1.5.2. Treatment of hypoxic tumour tissue

Tumour hypoxia has been a major concern both in radiation and chemotherapy. The identification of a compound that can modify the dynamic fluctuations in microregional oxygen delivery in tumors could have important implications for radiation therapy.

Nicotinamide, an agent previously reported to reduce hypoxia and increase the irradiation response of experimental tumors, has been evaluated for its effect on the occurrence of acute hypoxia in the murine squamous cell tumor SCCVII (Ono et al., 1993). Several mechanisms of action have been proposed for this compound. It has been suggested that nicotinamide reduces tumour interstitial fluid pressure (IFP), thereby reducing transient vessel non-perfusion and acute hypoxia, and radiosensitizing tumours (Peters et al., 1997).

2. MATERIALS AND METHODS

2.1. Animals & Surgical procedure

Rats were used for oxygen measurements and measurements of local blood flow. Oxygen measurements were performed in skeletal muscle and brain since these organs did not require extensive surgery and anesthesia.

2.1.1. Rats

Wistar rats were used for this study and the animals weighed between 250-350 gr. All animal experiments were conducted according to University of British Columbia animal care unit experimental regulations. Animals were anesthetized with pentobarbitone (60 mg/kg). The heads of 8 rats were placed in a stereotaxic apparatus (model 900, David Kopf, Tujunga, CA). The scalp and connective tissue were removed and holes were made in the frontal skull with a dental drill (Pronto Dental Equipment, OH, USA). The skull was periodically cooled with sterile saline during drilling to access the cerebral cortex. After the surgery, oxygen electrode was inserted into the drilled hole and the insertion area was covered with a cloth moist with physiological PSS (see 2.7.).

In a second group of 8 rats, small incision were made in the skin of left legs and a small catheter was placed in the skeletal muscle of the hind limb to facilitate the incursion of the needle electrode in skeletal muscle. Animals were placed in a

temperature-regulated (37 °C) tubular restrainer (Harvard Instruments, MASS) 30 minutes before the oxygen measurements.

To find whether the spontaneous fluctuations in pO2 were local and independent from systemic changes, in 3 rats, systemic measurements were conducted. EEG electrodes (Harvard Instruments, MASS) were placed on the head of rats and a catheter (Baxter Healthcare, IL, CA) was placed in the carotid artery. This catheter was connected to a blood pressure transducer. In addition, this catheter was used to sample blood for oxygen and hematocrit measurements.

In another group of rats which were treated with drugs, an intravenous line was established, and fluid administration of saline and drugs were started.

After surgical procedure, anesthesia was maintained at a low dose of 0.2 mg/kg once during the oxygen measurement experiments after animals had recovered from surgery and no ventilation was necessary.

2.1.2. Mice

Mice were used for oxygen measurements in tumour tissue. A group of 15 mice 10-14 week-old C3H/Km mice. SCCVII sarcoma cells were prepared as described (Twentyman et al., 1980). Briefly, $5x10^5$ cells were injected subcutaneously in the dorsal midline of each mouse approximately 2 cm from the base of the tail. Studies began 4 weeks later when mean tumor volume was between 500-1000 mm^3 and necrosis area had developed. The shape of the tumour was half spherical and the

necrosis area was identified by the change in the color to a darker shade at the central areas of tumours. Often minor bleeding was observe in necrosis areas. Tumour volume did not exceed the size recommended by animal care unit. The animals were restrained in perspex jigs with heated operating pads to maintain body temperature. Animals were anaesthetized by inhaled Metofan (Halotane) (2ml / kg) (Janssen, ON). A polyethylene catheter (id 0.5 mm, OD 1.0mm) was surgically placed in the tail vein and secured, remaining in situ for 20 minutes before the start of pO_2 measurements.

2.2. pO_2 measurements

2.2.1. Oxygen measurements in rats

Oxygen tension was measured in 2 groups of 8 rats while they stayed in the restrainer. In the first group the oxygen tension was measured in the hind limb skeletal muscle and in the second group the oxygen tension was measured in the frontal brain. The values of oxygen tension were determined using a polarographic O_2 -sensitive needle electrode (Eppendorf, Hamburg, Germany) used for clinical oxygen measurements. Probe diameter was 350 μm, its response time 2 seconds, and the sensitivity: 3.0 PA. Calibrations were performed in 0.9% saline solution equilibrated with calibration gases immediately before and after the measurements. Calibration was conducted in sterile, physiological common-salt solution (pH:7.8). Sterile filtered air and pure nitrogen alternately bubble the solution and thus set both 100% and 0%

O_2 partial pressure in the liquid within 2 to 3 minutes. Temperature effects were incorporated within these calibrations.

The electrode was connected to a stepper motor which automatically advances in the tissue. The diverse motion decompresses the tissue. For pO_2 measurements in the brain, electrodes were inserted in the frontal brain and recordings were made at depth of 3-5 mm. pO_2 values were recorded for 10 minutes. A 2 minute recording was used for most studies.

A second type of oxygen electrode was used. Two double barreled oxygen sensitive microelectrodes (Linsenmeier and Braun, 1992) were introduced into the hind limb and tumour tissue. These two electrodes were used for simultaneous oxygen measurements. The electrode was 10-12 μm wide and was calibrated in saline with 4, 8, and 21% O2 with a sensitivity of 0.45±0.01 pA/Torr (mean and standard deviation ; n=35). Current from the microelectrodes was measured with model 614 electrometer (Keithley Instruments, Cleveland, OH), whose output was fed through a buffer amplifier with 30-HZ low-pass filter and 60-HZ notch filter to an FM tape recorder (Model store 4DS, Racal Recorders, Southhampton, England). Later the signals were digitized for 30 seconds at 40 HZ by an IBM compatible computer and converted back to pO_2 using the calibration that had been obtained during the experiment. A fast Fourier transform analysis of the signal (Labtech notebook, laboratory technologies Corp., Wilmington, MA) was done in order to obtain the power spectrum of pO_2.

Before the FFT analysis, slow drifts in the signal were removed by subtracting the best fitting quadratic function from the pO_2 Vs time records. The oxygen electrode responds very rapidly and did not distort the measurements.

2.2.2 Control measurements

For the next series of experiments, 24 rats were divided into 3 groups and the following control measurements were made; the oxygen electrode was inserted in the cornea (1-2 mm depth) of anaesthetized rats and recordings of oscillation were made. These measurements were conducted at the cornea of rats since this is an avascular organ of body and thus no fluctuations should be registered at this area. Size of cornea was not larger than the oxygen electrode. Animals in this part of the study were anaesthetized with Halotane before the recordings were made. This procedure was chosen since recordings in cornea required extensive anesthesia and baritone compounds are known to decrease the amplitude of oxygen fluctuations in tissue (Braun et al., 1992).

Measurements were also made in hind limb of animals that had died 2-3 minutes prior to the recordings in order to observe whether the oxygen electrode made accurate oxygen measurements.

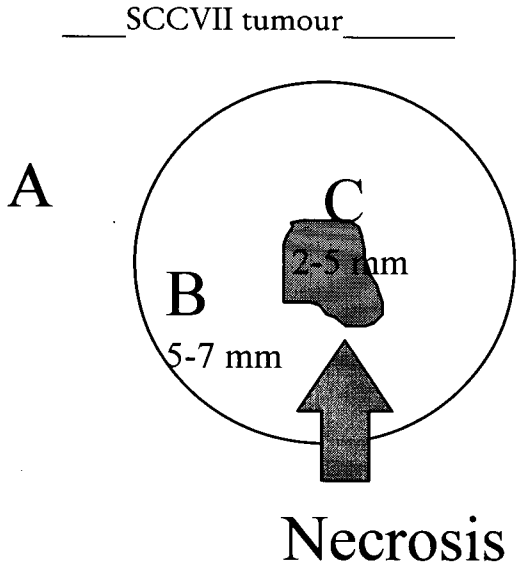
In order to investigate the effect of muscle motor control (EMG), sciatic nerves were sectioned, and the wounds were closed by surgical suture. pO_2 in hind limb muscle was measured after animals had recovered from anesthesia. In addition the

same group of rats received 3 mg/kg /min mecamylamine to block the effect of sympathetic nerves. Oxygen tension was measured in the skeletal muscle (hind limb), before and 3 minutes after the injection of the drug.

2.2.3. Oxygen measurements in mice

Tumour oxygen tension was determined by two types of oxygen electrodes. To characterize oxygen fluctuations, oxygen measurements were conducted using gold oxygen electrode (KIMOC-6650, Eppendorf, Hamburg, Germany).

A small incision was made in the skin overlying the tissue to be assessed and the O_2 electrode was inserted. Position of the electrode remained constant throughout the experiment. The oxygen electrode was allowed a few minutes of adaptation to the tissue environment then the pO_2 was measured as follows: The electrode was placed in an area close to necrosis in the center of tumours. Areas of necrosis were identified by its darker color and the width of this area was 2-4 mm^2. The size and the areas of necrosis was identified as previously described by (Steinberg et al., 1991). Previous reports which showed that oxygen values in areas close to necrosis were 5 mm and 18 mm in peripheral areas of SCCVII tumors and contained 0.86 and 8.4%, of the tumour areas respectively (Trotter et al., 1990). The electrode was then moved 1 cm away from the initial recording spot and a new incursion was made at the peripheral tumour areas and the oxygen tension was recorded. A control measurement was conducted in the subcutaneous area outside the tumours.



Schematic figure showing different zones of pO_2 measurement and spontaneous fluctuations in oxygen tension. A) outside tumour area (control measurements), B) peripheral tumour zones, C) central tumour zones close to necrosis.

In addition, a second type of electrode as described above was used for simultaneous pO_2 measurements (see above) in 5 of the animals. Two electrodes were placed in the tumours within 2, 5 mm and 8 mm from each other. The initial simultaneous recordings were conducted within 2 mm and the incursion points were marked. One of the electrodes was then moved to 5 and 8 mm apart from the other electrode. After 3 minutes stabilizing period, recordings were made. This procedure was repeated in the subcutaneous tissue.

In another set of experiments, 10 mice were injected with nicotinamide (1 mg/g intraperitonealy) and 5 mice were injected with saline (0. 5 ml of 0. 9% NaCl). pO_2 was recorded 3 minutes before the injection, at the time of the injection and 1 hour after the injections. Data were analyzed by fast Fourier transform analysis.

2.3. Flow measurement by thermocouples

The objective of this part of study was to investigate whether the local changes in blood flow correlate with spontaneous changes in oxygen tension. For this purpose a thermocouple was used to measure changes in local temperature which is an indirect measurement of local blood flow. As previously described by Schichino et.al (Shichino et al., 1991) changes in local blood flow result in changes in local temperature. For this purpose, a blunt thermocouple (DA 330μm) (National Instruments, TX, USA) with 0. 025 sec response time was placed adjacent to the Eppendorf pO_2 electrode. Animals were kept in a restrainer with a constant temperature of 37^0 C. Sensitivity of measurements was adjusted to 0.0015 ^{0}C. A data acquisition system was used to record temperature signals (National Instruments, TX, USA). Fast transform analysis of the data obtained by the thermocouple was performed as described for the microoxygen electrode described in section 3.3.2.

2.4. Histology of the measurement site

Histology of the area of oxygen measurement was studied to observe whether any severe bleeding or trauma had been formed after the incursion of the oxygen

electrode. In addition, number of blood vessels and their arrangement around the oxygen electrode was studied. India ink was used to mark the insertion site of the pO_2 electrode in skeletal muscle and brain. Oxygen electrode was dipped in India ink and then inserted into the tissue. Tissue was cut and fixed in formalin, and blocks were sectioned. Serial sections of brain were stained with Van Glieson stain. Skeletal muscle was stained with Masson trichrome stain. Sections obtained from tumours in mice were stained by H&E method.

2.5. Isolated pressurized arterioles

The objective of this part of study was to investigate whether vasomotion can be observed in isolated arterioles under pressurized conditions without the influence of blood flow. Furthermore, frequency of vasomotion in these arterioles was compared to spontaneous fluctuations in tissue oxygen tension. Middle cerebral and small branches of the femoral artery were obtained from adult male Sprague-Dawley rats (250-350 g). The animals were anaesthetized with intraperitoneal injection of sodium pentobarbital (Somnotol, 30 mg/kg) and heparin (Hepalean, 100 IU/kg), and killed by decapitation. For cerebral arteries, the brain was removed and immersed in cold oxygenated physiologic saline solution (PSS). A segment of artery (0.6-1.0 mm long) was dissected from surrounding connective tissue and transferred to the experimental chamber of a pressure myograph filled with oxygenated PSS at 20°C. For skeletal muscle small arteries, a muscular branch of the femoral artery was isolated. The artery

(1-2 mm length and 100-200μm diameter) was roughly cut out from the animal and carefully dissected free from surrounding tissues in cold oxygenated PSS and transferred to the myograph. One vessel (cerebral or skeletal) was taken from each experimental animal. The proximal aspect of the artery was fed onto a glass microcannula (tip diameter ~60 μm), and tied with a single strand (20 μm) of braided 4-O nylon suture. After flushing the artery with PSS to remove intraluminal blood, the distal aspect of the vessel was similarly cannulated and tied. The intraluminal pressure was gradually increased to 60 mm Hg with an electronic pressure servo system (Living Systems, Burlington, VT) different intraluminal pressures 40-180 mm Hg were examined to observe the effect of pressure on formation of vasomotion. The bath temperature was slowly increased to 37°C, and the vessel was then equilibrated for 60 minutes. During this equilibration period, vessels spontaneously and reliably developed myogenic tone, with significantly reduced luminal diameters. Once attained, myogenic tone and vessel diameter remained stable unless perturbed by changes in transmural pressure or the addition of vasoactive compounds. Intravascular pressure was kept at levels previously suggested as the physiological range pressure. (Davis and Sikes, 1990). The PSS (see 2.7.) entered the experimental chamber at a flow rate of 20 ml/min, passing through an external reservoir that was bubbled with 95% O_2 - 5% CO_2. A heating pump connected to a heat exchanger maintained the PSS at 37°C. Buffer temperature and pH were monitored by microprobes situated in the

tissue bath: pH was maintained at 7.40 ± 0.04 U by adjustment of the gassing rate. The arteriograph containing a pressurized artery was placed on the stage of an inverted microscope with a monochrome video camera attached to a viewing tube. Arterial dimensions were measured using a video system that provides automatic continuous digital measurements of luminal diameter and wall thickness. Information is updated every 17 ms, and the precision of the diameter measurements is within 1% (Nilsson and Sjoblom, 1984).

Cerebral and skeletal muscle arteries were equilibrated and allowed to develop myogenic tone. Vessels which manifest spontaneous vasomotion (5 of 8 cerebral, 4 of 4 skeletal) were used to study the pharmacological properties of vasomotion for comparison with cerebral and skeletal pO_2 oscillation.

2.6. Drug treatment

The purpose of this part of the study was to further investigate the similarities and pharmacological properties of spontaneous oxygen fluctuations and spontaneous vasomotion of isolated pressurized arterioles in vitro. A number of vasoactive agents were used both in vivo and in vitro to further investigate similarities between vasomotion of isolated arterioles and fluctuations in tissue oxygen tension. For this purpose 3 groups of 5 rats were used for in vivo experiments. Vasoconstrictors and calcium antagonists were injected to these animals and the doses were chosen based on previous recommendations; phenylephrine (0.2mg/kg/min, intraarterial infusion)

(Bouskela and Cyrino, 1997), prostaglandin (1mg/kg/min)i.v. (Bouskela and Cyrino, 1997), and nifedipine (0. 1mg/kg) were injected i.v (Bouskela and Cyrino, 1997). Nifedipine was used to investigate the importance of calcium in formation of these fluctuations. Effect of these agents on fluctuations of oxygen tension was recorded 2 minutes before the injection and 5 minutes after the injection of each drug. Effects of these drugs were compared between oxygen tension in hindlimb and brain.

Parallel to these experiments, similar vasoactive drugs were added to the buffer bathing the isolated pressurized brain and femoral arterioles. In all in vitro experiments, phenylephrine (0.01-0.3 μM) was added to the buffer and since prostaglandin did not show maximum effect in the bath a tromboxane analogue U46619 was used. Initial dose of 0.01 μM and following doses of 0.03, 0.1 and 0.3 μM was added to the bathing solution. Each drug was examined on 4 different femoral and 5 different brain arterioles. Results from administration of these drugs were compared between isolated arterioles from both organs. These results were further compared to the effects on oxygen tension in vivo.

2.7. Materials

The ionic composition of the PSS was (in mM) 118 NaCl, 24. 9 $NaHCO_3$, 4. 7 KCl, 1. 18 KH_2PO4, 1. 17 $MgSO_4$, 1. 6 $CaCl_2$, 11. 1 glucose, and 0. 026 EDTA. Phenylephrine and nifedipine were purchased from Sigma Chemical (St. Louis, MO),

and U46619 was purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA).

2.8. Statistical analysis

All data obtained through the pO_2 electrode (Eppendorf) were processed by Fourier statistical analysis software (Coulbourn Instruments, PA). Data obtained by the microelectrode and the thermocouple were analyzed by the Labtech FFT soft ware. Linear regression was used to measure correlation between oxygen and temperature measurements. In the case of drug administration, effects of changes in blood pressure were compensated by measuring the dependence of these oscillations on blood pressure. Analysis of co-variance (ANCOVA) was used for this purpose with mean arterial pressure (MAP) as a co-variant and the effect of vasoactive compounds as a factor. A value of $p < 0.05$ was considered statistically significant. In tumour study, variability between and within tumours was estimated using two different oxygenation parameters, the median pO_2 and the proportion of pO_2 measurements less than or equal to 5 mm Hg. Student t-test was used to compare differences between different tumour areas.

3. RESULTS

3.1. Systemic measurements

All of the rats adapted to the restraining devise rapidly, showing no signs of distress. Under control conditions, MABP was 179±1 mm Hg and in most animals heart rate remained at 290-295/min. Respiration rate was maintained at 84-89 /min. Blood gas and hematocrit remained constant during all experiments. Fluctuations of EEG recordings during pO_2 measurements did not correlate with those obtained by oxygen electrode and remained constant under control conditions. Overall, no correlation among rhythmic changes in systemic measurements and oxygen fluctuations was observed (in all 3 rats examined over a period of 3 minutes). The extent of neural control over skeletal muscle oxygen tension was examined after section of the sciatic nerve. No changes in the pO_2 oscillations in the skeletal muscle were observed after the section. Administration of i.v. ganglion blocker mecamylamine did not affect these oscillations. Thus, the neural control of pO_2 fluctuations was considered minimal (Table 1 shows a summary of systemic measurements).

Table 1: Systemic measurement values in a rat anaesthetized with 20mg/kg pentobarbitone after 10 and 20 seconds of recording. (n=3) ±SD

Measurement	Heart rate	Respiration rate	Blood pressure	Hematocrit	Blood paO_2
10 seconds	298±3	89±7	179±1 mm Hg	22.4±5%	83±4
20 seconds	330±2	89±4	180±6 mm Hg	22.4±2%	83±3

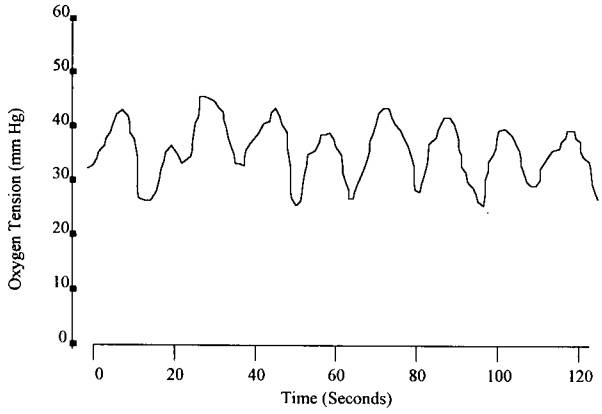
3.2. Oxygen measurement

3.2.1. Oxygen measurement in rats

In 8 animals used for this part of the study, the oxygen tension was 43±2 mm Hg in skeletal muscle and 25±3 mm Hg in the brain (±SD, $p<0.05$). The oxygen tension was similar in all animals if anesthesia were not too deep and if 30 minutes were allowed for recovery from surgery. Figure 1 shows an example of the fluctuations in the skeletal muscle. Fast Fourier transform analysis of the pO_2 fluctuations is shown in Figure 1b. A main frequency of 4 c/min with an amplitude of 20±2.9% of mean value was observed in skeletal muscle. In addition, a secondary frequency of 12 c/min with an amplitude of 15±2.7% of the mean pO_2 was present in these recordings (n=8) ($p<0.05$). In brain (n=8), oscillations of oxygen were faster but occurred at a lower average level of pO_2 (Figure 2a). The oscillations had a main frequency of 10 c/min and amplitude of 15±5.7% of mean value ($p<0.05$). A secondary frequency of 24c/min was observed. Figure 2b shows a representative spectrum of Fast Fourier analysis of pO_2 values recorded in the cerebral cortex. No oscillations were recorded in control measurements in dead animals (fig 3) which indicates that the electrode was functioning well and was not recording inaccurate oxygen tension. No oscillations were observed when the recordings were conducted in the cornea of rats since this organ is avascular (fig 4) and fast Fourier transform analysis did not show any frequency in these control recordings (fig not shown).

Fig 1)

a



b

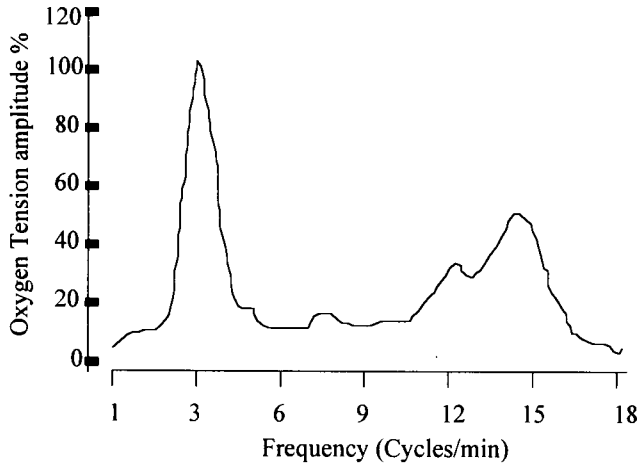
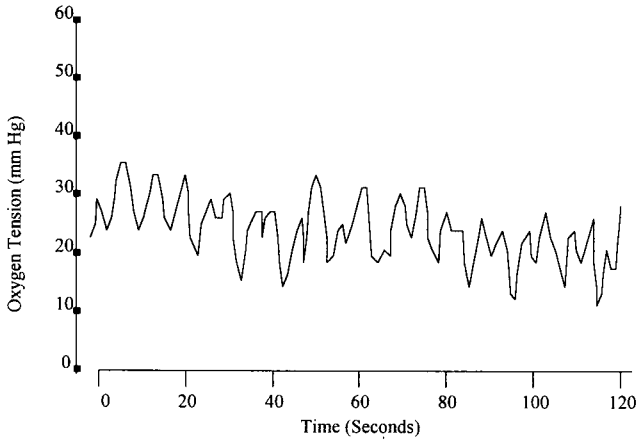


Fig 1: A representative recording of oxygen tension in rat skeletal muscle. Oxygen measurements by gold oxygen electrode in hind limb; a) Spontaneous oscillations of oxygen recorded during 2 minutes. b) Fast Fourier transform spectral analysis are shown for the frequency range <15 cycles/min. A main frequency of 4c/min was observed.

Fig 2)

a



b

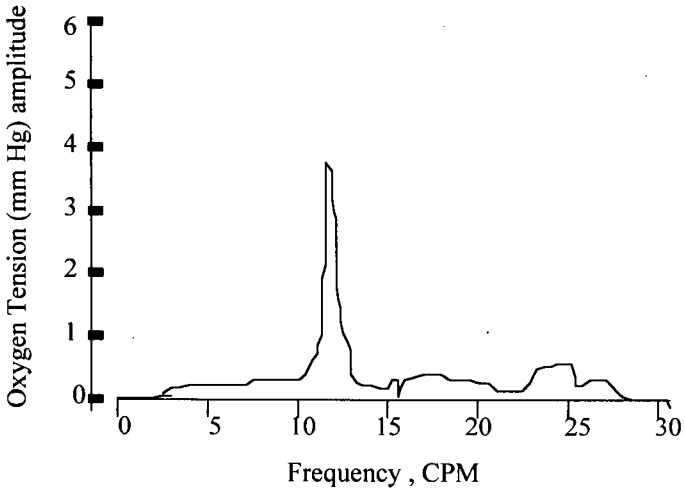


Fig 2: A representative recording of oxygen tension by Eppendorf oxygen electrode at 10 mm depth in rat brain cortex. a) Spontaneous oscillations of oxygen recorded during 2 minutes. b) Fast Fourier transform spectral shows a main frequency of 12c/min.

Fig 3)

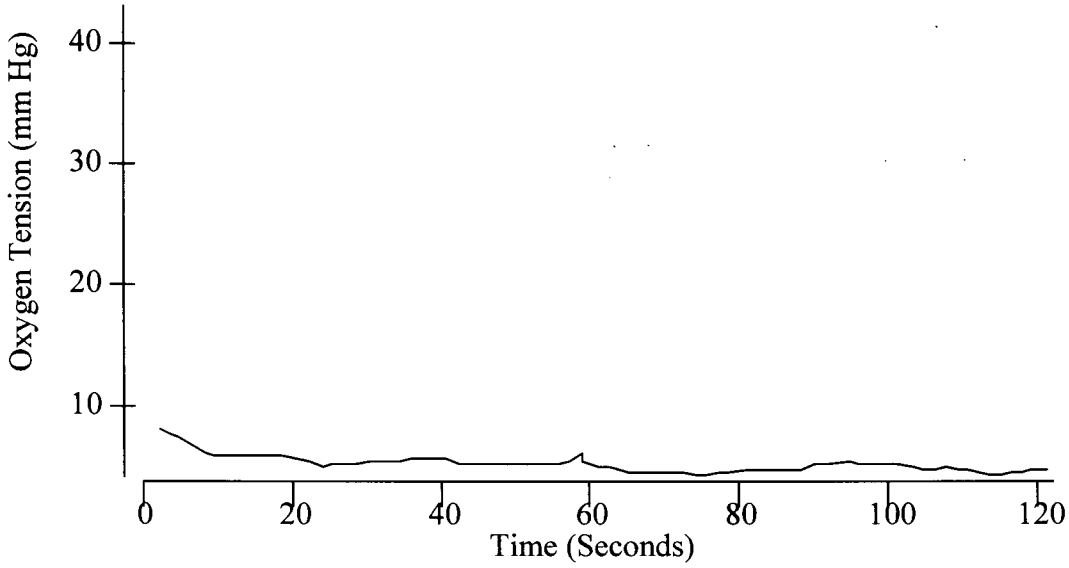


Fig 3: A representative recording of oxygen tension in dead animal in rat brain cortex.

Rats had been killed 3 minutes prior to recordings.

Fig 4)

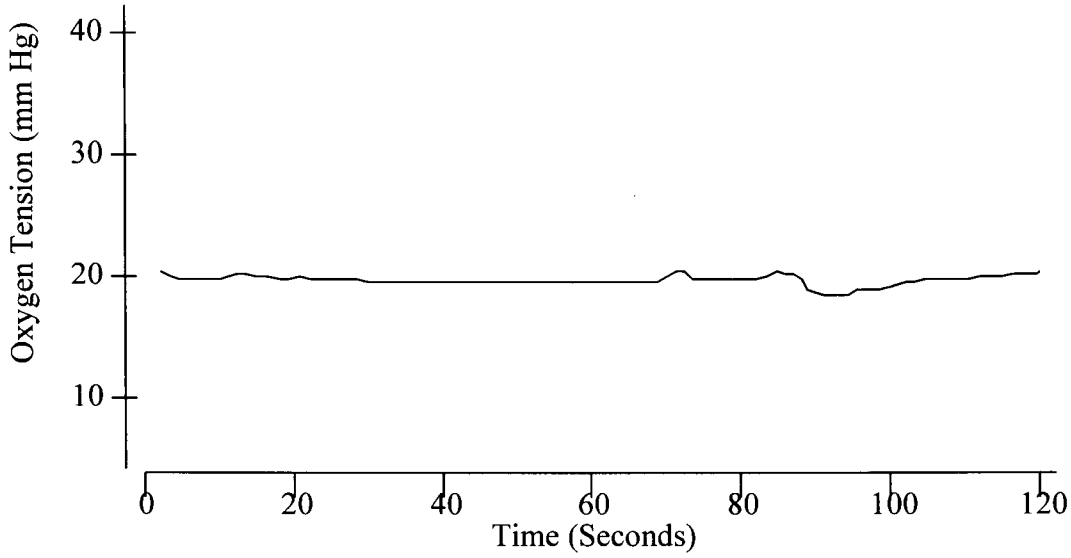


Fig 4: A representative recording of oxygen tension by Eppendorf oxygen electrode in cornea of anaesthetized rats.

3.2.2. **Spatial relationship and location of pO_2 fluctuations**

The spatial characteristics of pO_2 fluctuations were examined by simultaneous oxygen measurements with two platinum electrodes inserted at various distances from each other. In all 5 rats studied, the coherence between the pO_2 curves obtained from electrodes were placed 2 mm apart from each other but the coherence was very low (>0.8) when the electrodes were located at a distance of 5 mm from each other in skeletal muscle. Figure 5 shows that oscillations recorded in skeletal muscle within a 2 mm area were synchronous but when recordings were conducted 5 mm apart, the oscillations were out of phase. This observation is an indication of the local character of these oscillations. We did not determined with the FFT method whether there was a shift in phase in all different recordings.

Fig 5)

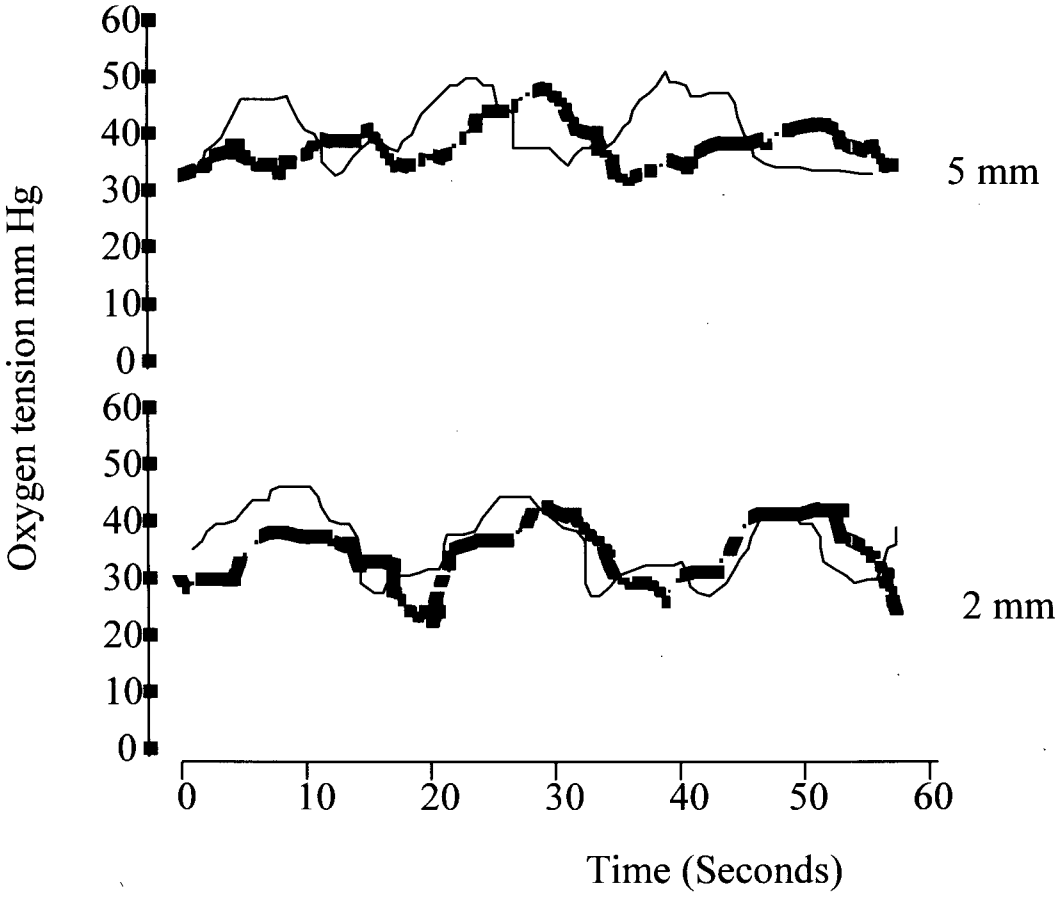


Fig 5: Simultaneous recording of fluctuations in oxygen tension with two electrodes. Electrodes were positioned within a) 2 mm, b) 5 mm distance from each other.

3.3. **Histology of oxygen measurement site**

After locating the oxygen electrode track by India ink, the incursion area was examined. Although erythrocytes were present in most sections, no obvious signs of bleeding were observed. Severe signs of tissue compression was observed closer to the surface of the incursion point in tissue. Figure 6 shows a section of skeletal muscle stained with Mason's trichrome stain. Blood vessels are the darker circles indicated by arrows. Few blood vessels were identified close to the tip of the electrode.

Fig 6: Cross section of skeletal muscle stained with Mason's trichrome x160. This method was used to stain the elastic of arteriolar wall darker and muscle fibers were stained lighter. Arrows show blood vessels near the tip of the needle electrode. Oxygen electrode incursion track was identified by India ink (E).

3.4. **Correlation with changes in local temperature**

In order to examine a possible correlation between the oxygen oscillations and the changes in local blood flow, temperature clearance and pO_2 were simultaneously recorded. Figure 12 depicts the original tracings of pO_2 and temperature clearance values measured at the same time and plotted on identical time scales. As shown in the Figure 7, a strong coherence of the two types of oscillations was observed when the oxygen electrode and the thermocouple were placed adjacent to each other. Linear regression of values obtained by the thermocouple correlates with that obtained by the oxygen electrode ($r=0.87$, $p<0.001$), indicating a close correlation between changes in temperature (blood flow) and pO_2 oscillations. This experiment was performed in 5 animals and a close correlation between oxygen and temperature was observed in all animals studied. Oxygen fluctuated at 4.2 ± 1.2 c/min and the temperature fluctuated at 3.9 ± 2.1 c/min ($p<0.05$).

Fig 7)

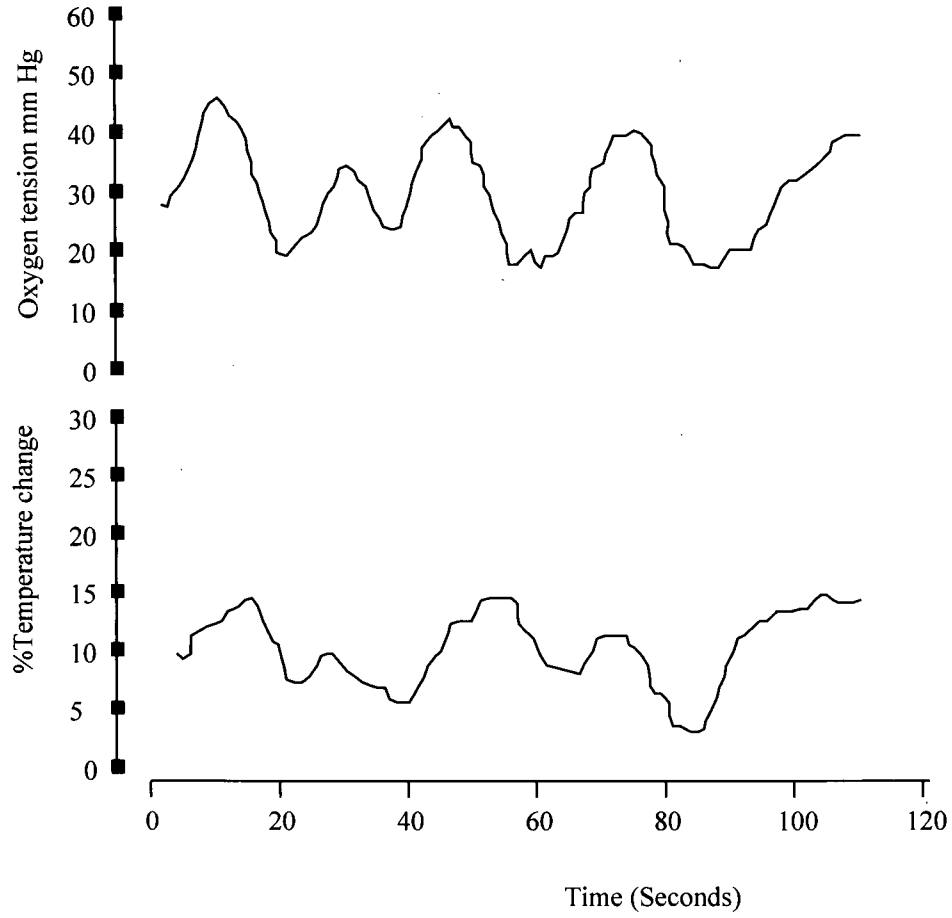


Fig 7: Representative recording of simultaneous measurements of pO_2 and temperature fluctuations in rat skeletal muscle using gold oxygen electrode and micro-thermocouple. The oxygen electrode and the thermocouple were positioned adjacent to each other. Spontaneous fluctuations recorded by two methods were compared with each other. A close correlation of temperature and pO_2 values was obtained.

3.5. **Isolated pressurized arteriole**

The average diameter of isolated pressurized arterioles obtained from both organs was measured at 20 mm Hg. Cerebral arteries had an average diameter of 126 ± 6 μm (n=5) and the skeletal arteries had an average diameter of 130±23 μm (n=4). During the initial 1/2 hour stabilization period, at the pressure range of 60-120 mm Hg, arterioles exhibited spontaneous vasomotion. The frequency of these oscillations was similar to spontaneous fluctuations in oxygen tension showed in figure 1 and 2. Figure 8 shows a representative tracing of spontaneous oscillations in diameter of pressurized brain. The femoral artery vasomotion was estimated 4±1 c/min and the brain arteriole vasomotion was at 10±2 c/min ($p < 0.05$).

Fig 8)

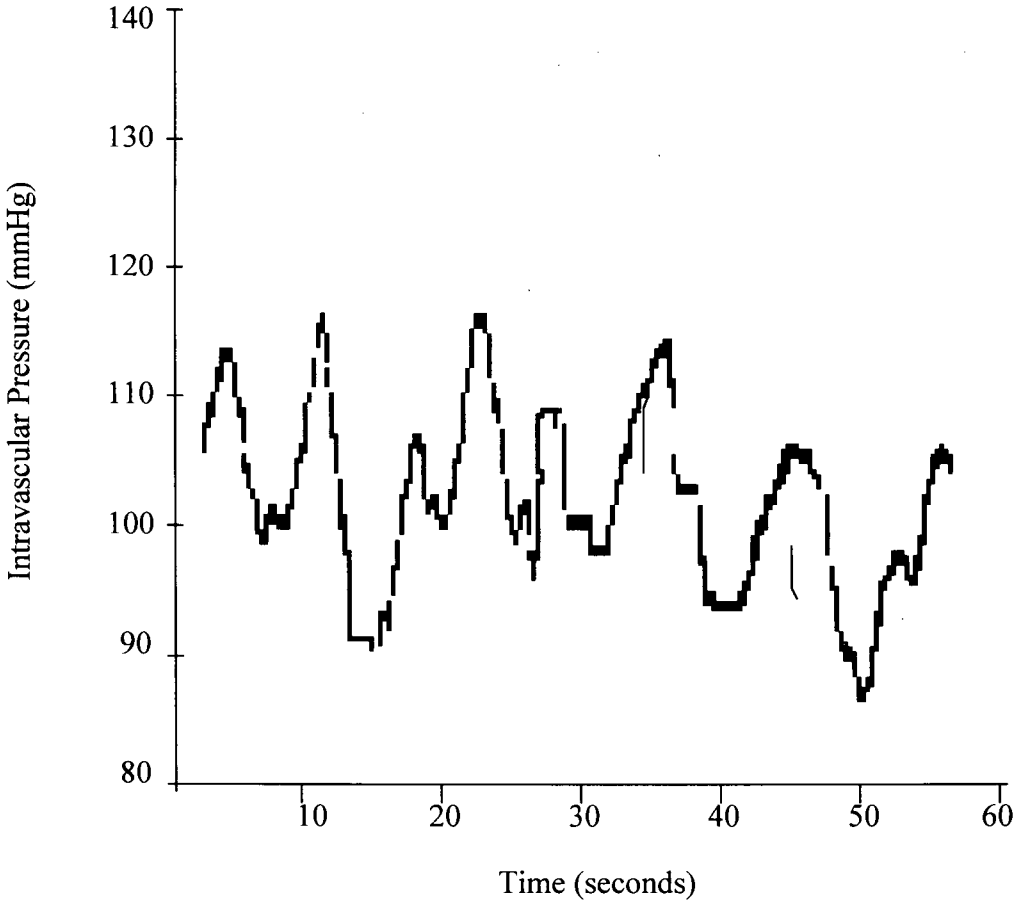


Fig 8: A representative recording of spontaneous vasomotion of middle cerebral arteries of adult male rats. The two ends of the artery was fed onto a glass microcannula (tip diameter ~60 μm), and tied. The intraluminal pressure was gradually increased to 60 mm Hg, the bath temperature was 37°C, and the vessel was then equilibrated for 60 minutes.

3.6. **Drug effect**

3.6.1. Vasoconstrictors in vivo

Following the in vivo administration of phenylephrine the blood pressure increased by 30±15 mm Hg ($p<0.05$) compared to control conditions, stabilizing at a lower mean level of 120 to 130mm Hg. Frequency of oscillations increased from 4.±0.9 to 6.5±1.3 c/min ($p<0.05$). Administration of phenylephrine also increased the amplitude of the oscillation significantly by 72±3.4 % ($p< 0.05$) (Fig 9a). Administration of phenylephrine did not cause significant changes in brain pO_2 (Fig 9a). This observation can be explained by presence of fewer α-adrenoreceptors in the brain arterioles than the skeletal muscle.

To examine whether the observed effects were receptor specific, prostaglandin was administered i.v. while pO_2 was monitored in brain and skeletal muscle. This increased blood pressure from 110 to 135 mm Hg. The amplitude of pO_2 fluctuations increased in the brain significantly by 62±2% ($p<0.05$) (Fig 9b) but remained stable in the skeletal muscle since the action of prostaglandin and tromboxane is decreased in this tissue (Chatziantoniou and Arendshorst, 1993; Coupar, 1980; Suematsu et al., 1991).

In order to find out whether the changes in oscillations were due to a consequent change in arterial pressure, ANCOVA was performed with the normalized blood pressure as a covariant variable and with PE and PGFα1 as factors. These results

indicated that PE and PGFα1 exerted an effect independent of its effect on arterial pressure ($p < 0.05$). These results were reproducible in all 5 animals.

Table 2: Effect of vasoactive agents on pO_2 fluctuations vs. effect of the same agents on spontaneous vasomotion of isolated pressurized arterioles.

Experimental group	phenylephrine	PGF α1/ U46619	Nifedipine
Brain			
In vivo	Frequency	Frequency + +	Frequency-
	Amplitude	Amplitude + +	Amplitude-
In vitro	Frequency	Frequency +	Frequency-
	Amplitude	Amplitude +	Amplitude-
Skeletal muscle			
In vivo	Frequency +	Frequency	Frequency-
	Amplitude +	Amplitude	Amplitude-
In vitro	Frequency +	Frequency	Frequency-
	Amplitude +	Amplitude	Amplitude-

Fig 9a

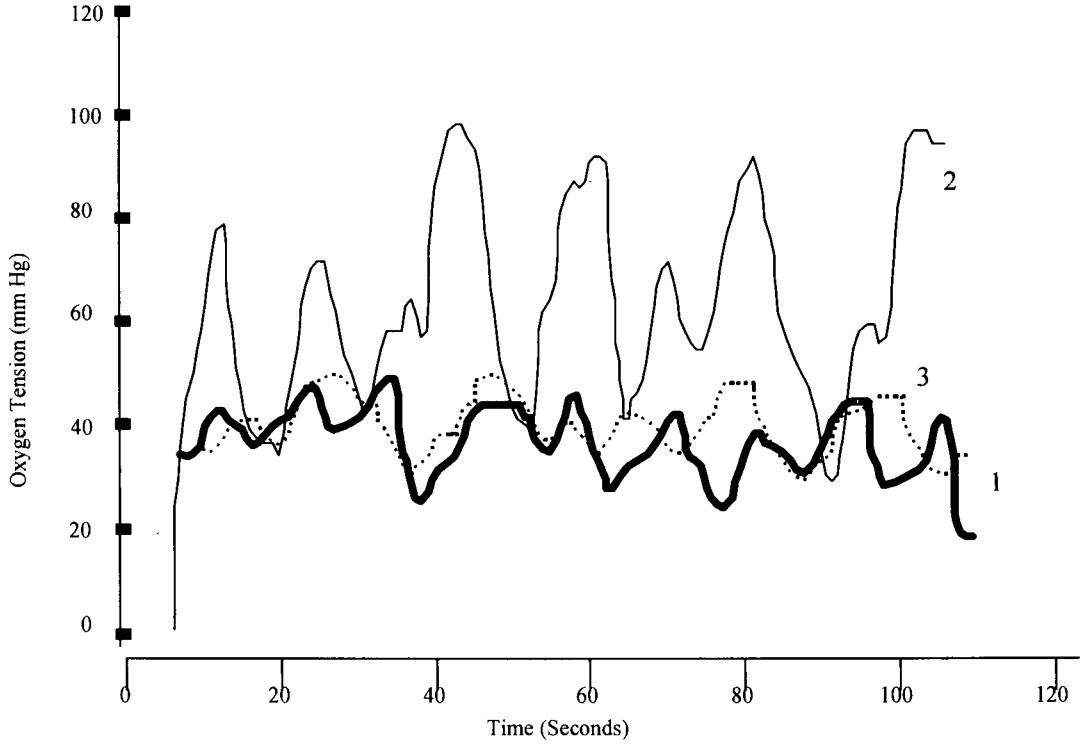


Fig 9a) Effects of vasoactive agents on pO_2 oscillations in skeletal muscle. 1) pO_2 oscillations under normal conditions. 2) following the administration of phenylephrine to skeletal muscle the amplitude of pO_2 oscillations increased. 3) prostaglandin (PGFα1) moderately increased the frequency and amplitude of pO_2 fluctuations.

Fig 9b)

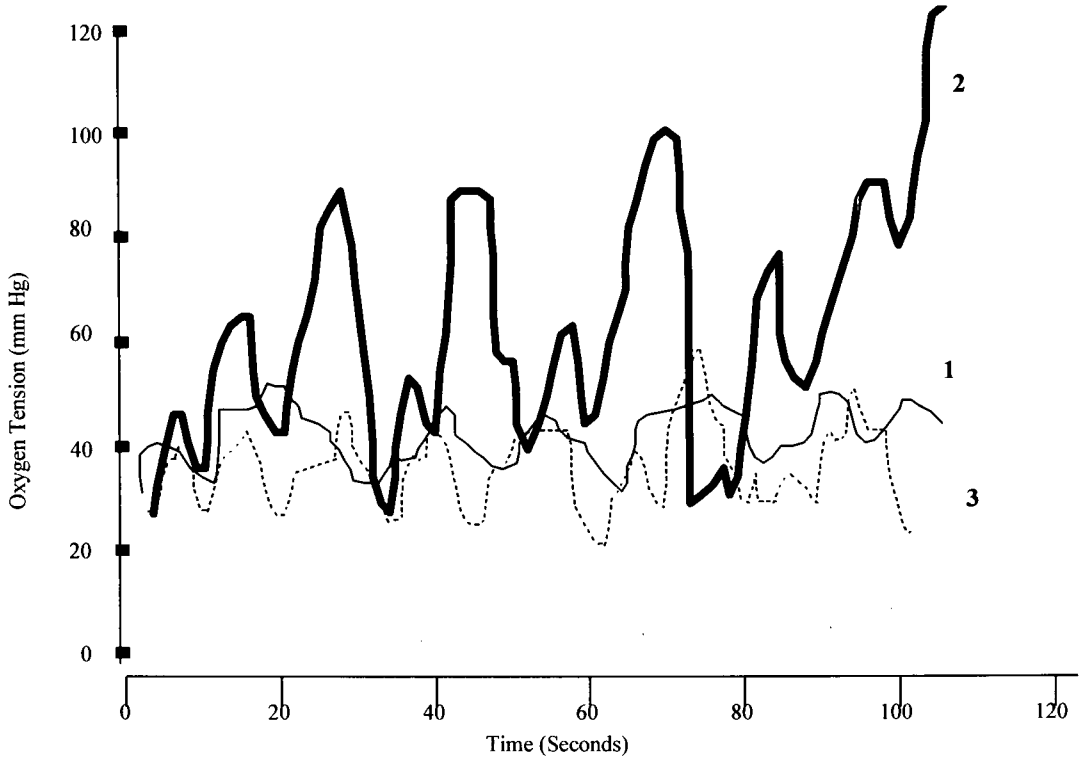


Fig 9b) Representative recording of fluctuations in pO_2 in rat brain 1) under normal conditions 2) administration of prostaglandin in vivo, (1mg/kg/min) i.v. increased the amplitude and frequency of these oscillations. 3) phenylephrine had minor effects on the oscillations.

3.6.2. Effect of vasoconstrictors of isolated arterioles

When the same vasoactive drugs were administrated to isolated pressurized arterioles, a close correlation with above mentioned in vivo results was obtained. As shown in previous recording, phenylephrine had a receptor specific effect. It caused strong potentiation of vasomotion in skeletal arteries. The amplitude of vasomotion cycles increased by 72±3% ($p < 0.05$) and the frequency was not changed from 4.1 c/min(Fig 10a). Phenylephrine was without vasoactive effect in cerebral arterioles ($n = 6$)(Fig 10b). In contrast, U46619 caused a graded potentiation of vasomotion and constriction in cerebral arteries ($n = 4$). The amplitude of vasomotion cycles increased by 81±2% and the frequency remained at 10.2 c/min. U46619 caused only constriction (without vasomotion) in skeletal arteries ($p < 0.05$). The effects observed were similar in all animals.

Fig 10a)

1)

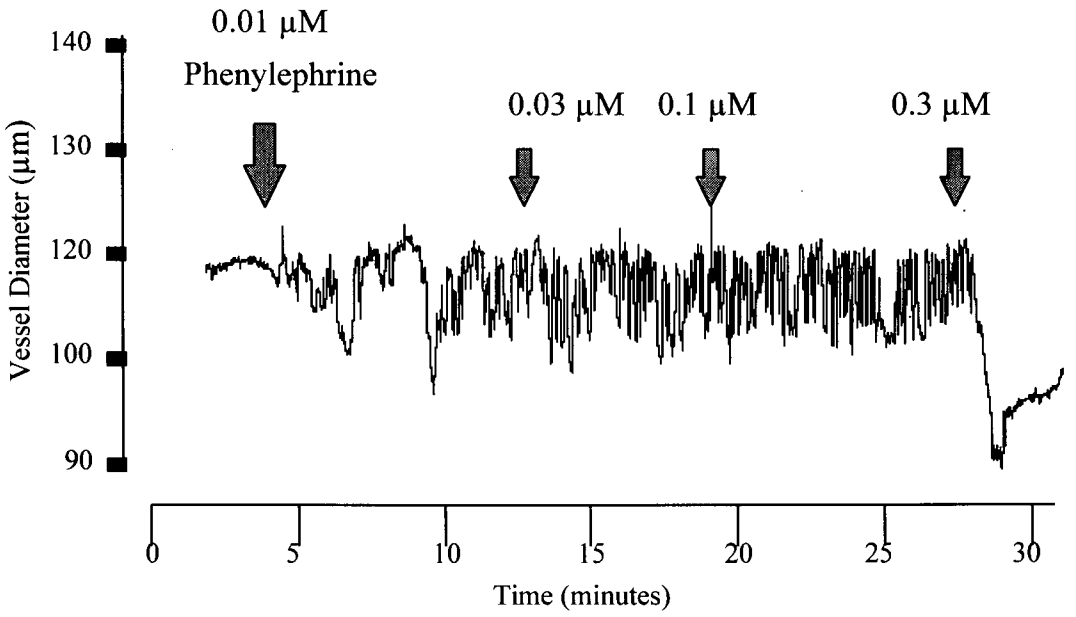


Fig 10a) Effect of vasoactive agents on isolated pressurized skeletal arterioles. 1) phenylephrine increased amplitude and frequency of spontaneous oscillations of skeletal muscle arterioles. 2) vasomotion did not change by administration of prostaglandin.

2)

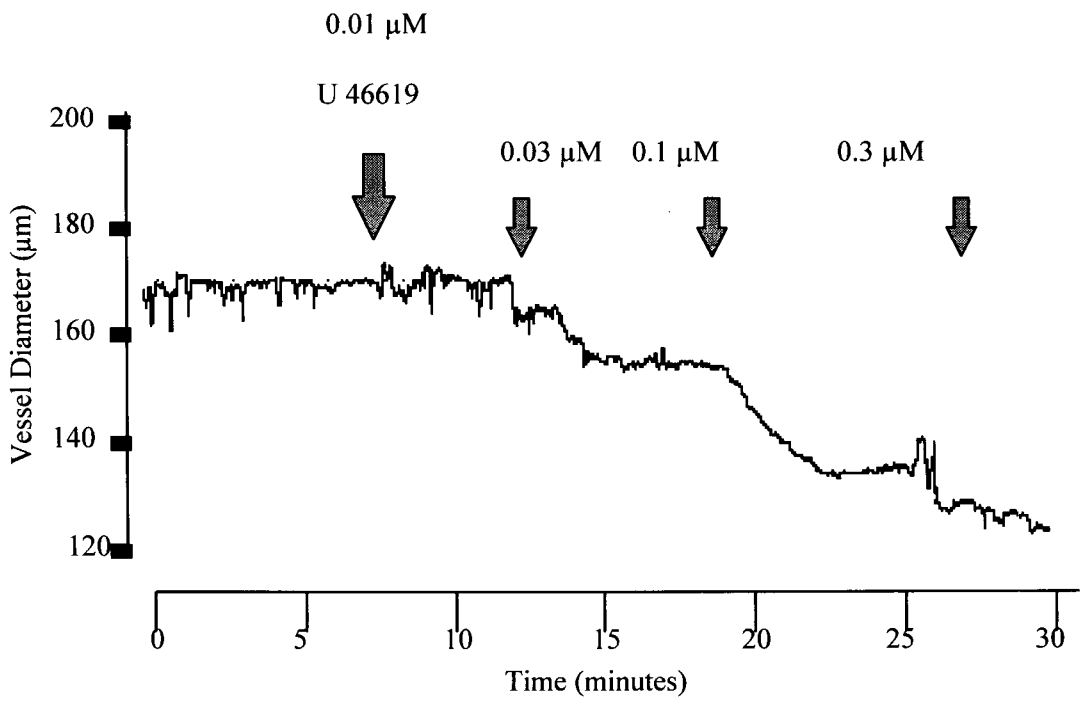
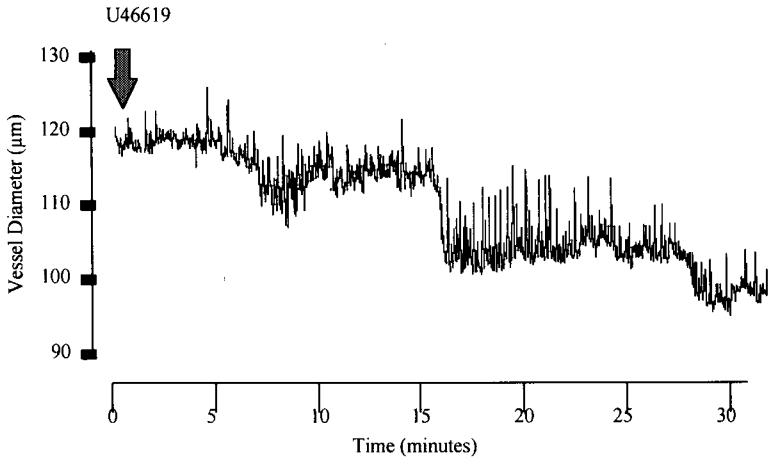


Fig 10a) Effect of vasoactive agents on isolated pressurized skeletal arterioles. 1) phenylephrine increased amplitude and frequency of spontaneous oscillations of skeletal muscle arterioles. 2) vasomotion did not change by administration of prostaglandin.

Fig 10b)

1



2

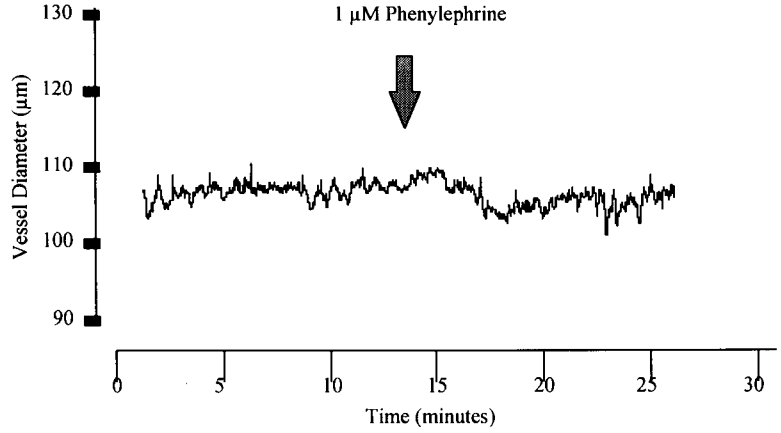


Fig 10b) Effect of vasoactive agents on isolated pressurized brain arterioles. 1) U46619 increased amplitude and frequency of spontaneous oscillations of brain arterioles. 2) vasomotion did not change by administration of phenylephrine.

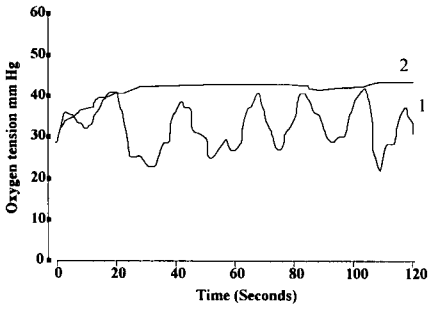
3.6.3. Effect of nifedipine

Administration of nifedipine, increased oxygen tension in tissue by 25±4 %. It decreased the frequency of pO_2 fluctuations in 2 minutes after the injection by 99±4% in skeletal muscle ($n=4$) and 90.3±4 % in the brain ($n=6$) ($p<0.05$). In addition, oscillation amplitude was decreased by 82±2% in the brain and by 84±1% in skeletal muscle ($p<0.05$). Fig 11a shows a representative recording of oxygen tension in the brain of rats after treatment with nifedipine.

Isolated pressurized cerebral arteries treated with nifedipine were dilated and a dose dependent attenuation of vasomotion frequency similar to the in vivo results was observed (Fig 11b). Nifedipine decreased the frequency of fluctuations in skeletal muscle by 87±1% and in brain by 89.±3.% ($p<0.05$). The amplitude of vasomotion was decreased by 77±1 in skeletal muscle and 81±2% in the brain ($p<0.05$).

Fig 11)

a



b

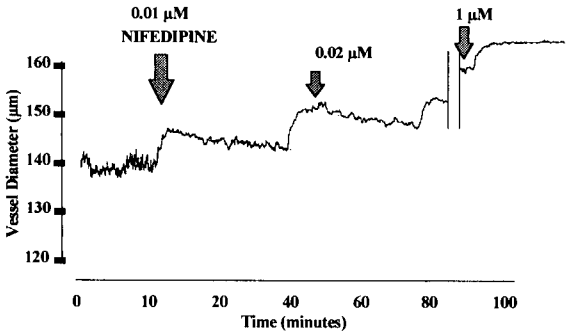


Fig 11: Effect of calcium antagonist on spontaneous oscillations in brain. a) pO_2 oscillations in brain (1) were abolished (2) after the injection of (0.1mg/kg) nifidipne. Recordings were taken 2 minutes after the injection. b) Spontaneous vasomotion in isolated pressurized arterioles was abolished after addition of 0.01 μM - 1 μM nifedipine.

3.7. Oxygen tension in SCCVII tumour

Mean oxygen tension in tumours was more variable than in normal tissue. The variations in oxygenation between tumours was greater than the variations within tumours. Experiments where pO_2 values were lower than 1 mm Hg were discarded as recommended by electrode manufacturer's protocol.

Average oxygen tension in subcutaneous sites was 43±5.1 mm Hg (range 38-48, n=10). Fourier Fast transform of data showed that in subcutaneous pO_2 oscillates at 3c/min (Fig 12a) with an amplitude of 22±5.3% of the mean value. Oxygen tension in peripheral zones of tumours was 12±4.3 mm Hg (range 12-24) and oscillations occurred at 2c /min rate with an amplitude of 15±6.42% of the mean value (±SD, $p<0.05$)(Fig 12b). These fluctuations occurred at pO_2 values as low as 8 mm Hg.

Hypoxia was observed in the central zones of tumours near the necrotic tissue (3±4.4 mm Hg n=10). No oscillations in recordings were observed in hypoxic areas value (±SD, $p<0.05$)(Fig 12c). However, one of 10 mice showed fluctuations with low amplitude (5% of the mean). Table 3 summarizes oxygen tension values and characteristics of these fluctuations in subcutaneous and different tumour zones. Fourier fast transform analysis of these oscillations are shown in figure 13.

Table 3: Oxygen tension and pO_2 fluctuation frequency at different zones of SCCVII tumors (All data are expressed as means SE in mm Hg)

Tissue	Mean pO_2 mm Hg	Oscillation frequency	Oscillation amplitude
peripheral tumor	12±2.3	2±0.6	15±6.4%
hypoxic tumor	3±4.4	_	_
subcutaneous	43±5.1	3±0.7	22±5.3%

n = 10, mean ±SE.

Figure 12)

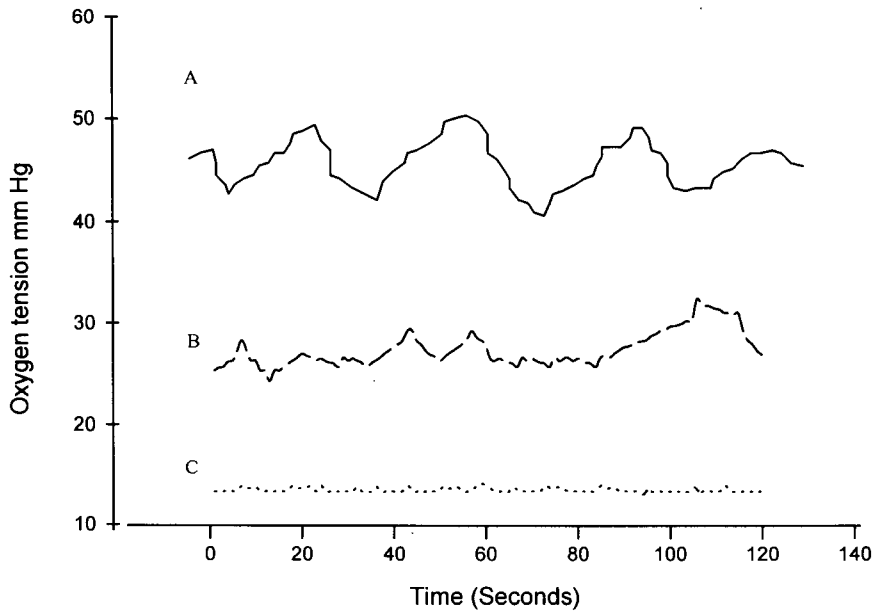


Fig 12. Representative recording of oxygen tension (pO_2) in SCCVII tumours. A) subcutaneous, B) peripheral, C) central tumour tissue in mice with implanted SCCVII tumor.

Fig 13)

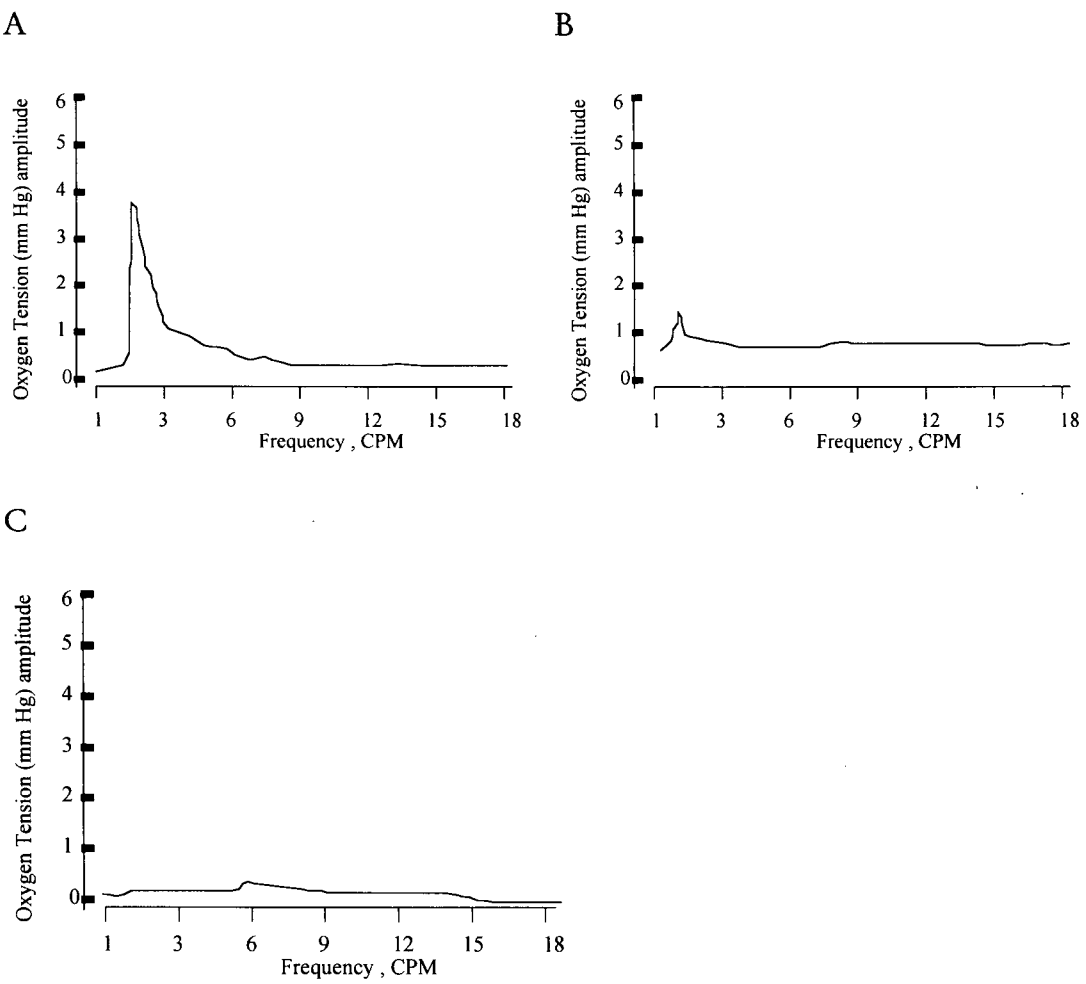


Fig 13. Representative figure of statistical analysis of pO_2 oscillations by fast Fourier transform analysis. Power spectrum of frequency analysis is shown for A) subcutaneous control tissue, B) peripheral and C) central tumor zone.

3.7.1. **Spatial relation of oxygen fluctuations in tumours**

When micro-electrodes were used for simultaneous oxygen measurements and we observed that pO_2 fluctuations in subcutaneous tissue were synchronous within 5 mm distance. These experiments were then repeated in peripheral tumour zones and observed that pO_2 fluctuations were asynchronous when the two microelectrodes were positioned within 8 mm distance (Fig 14). Results from all 5 mice recordings were similar.

Fig 14

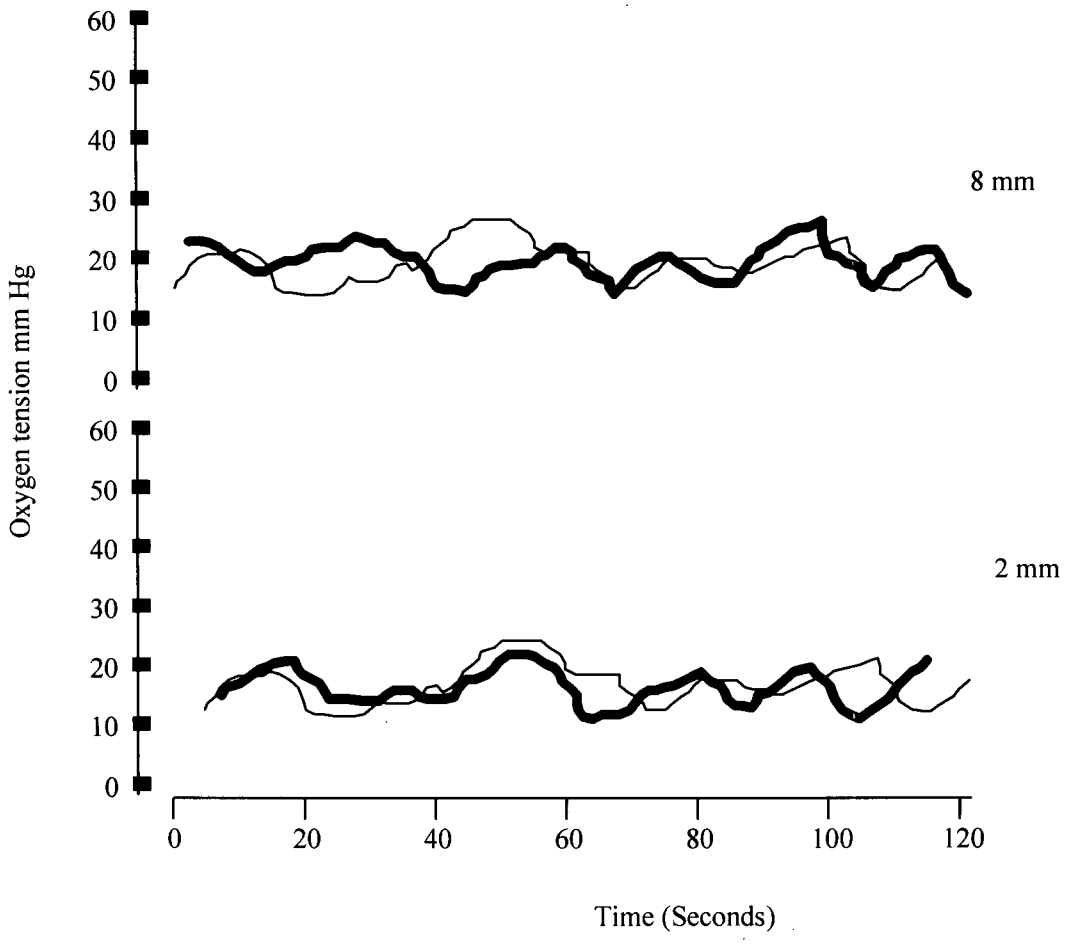


Figure 14. Representative examples of pO_2 fluctuations in oxygen tension recorded when two platinum oxygen micro-electrodes, positioned within 2 mm and then 8 mm distance in peripheral zone of the SCCVII tumors.

3.8. **Histology of the measurement site in tumours**

After each pO_2 measurement with the Eppendorf electrode, the number and arrangement of blood vessels surrounding the electrode were examined by light microscopy. No obvious difference in the number of blood vessels was observed between central and peripheral zones. Fig 15a shows the electrode track in tumours. Typical arrangement of blood vessels surrounding the electrode is shown in figure 15b.

Fig 15. Histological sections of oxygen electrode track in SCCVII tumours. E shows an oxygen needle electrode track in histological section of a SCCVII tumour. Arrows show blood vessels located close to the oxygen electrode in peripheral tumour zone magnification x 140. b) shows the histology of areas close to necrosis and arrows show blood vessels.

3.9. **Effects of nicotinamide on tissue oxygen tension**

Treatment of C3H mice bearing 500-750-mg subcutaneous tumors with nicotinamide (1.0 mg/g intraperitoneally) 1 hour prior to oxygen measurement resulted in reinitiation of pO_2 fluctuations in 4 out of 10 rats. It also caused seizures in 5 out of 10 rats and these symptoms were sufficiently serious in 2 of the mice that also had dyspnea. Fig 16 shows the changes in pO_2 observed at hypoxic areas after the administration of nicotinamide. A summary of these observations in all 10 mice is presented in Table 4.

Table 4: Measurement of pO_2 in hypoxic zones close to necrosis before and after injection of nicotinamide (1000 mg/kg, i.v.). n=10

Experiment	Mean pO_2	% change in pO_2	Oscillations c/min before nicotinamide	Oscillations c/min after nicotinamide
1	2±1.2	-	0	0
2	2±6.7	-	0	0
3	5±7.6	52.3%	2	3
4	2±3.1	-	0	0
5	2±1.4	-	0	0
6	8±2.1	10.2%	1	5
7	2±7.4	45.2%	0	0
8	3±1.9	21.5%	0	2
9	3±5.3	-	0	0
10	8±4.4	63.7%	0	3

Fig 16

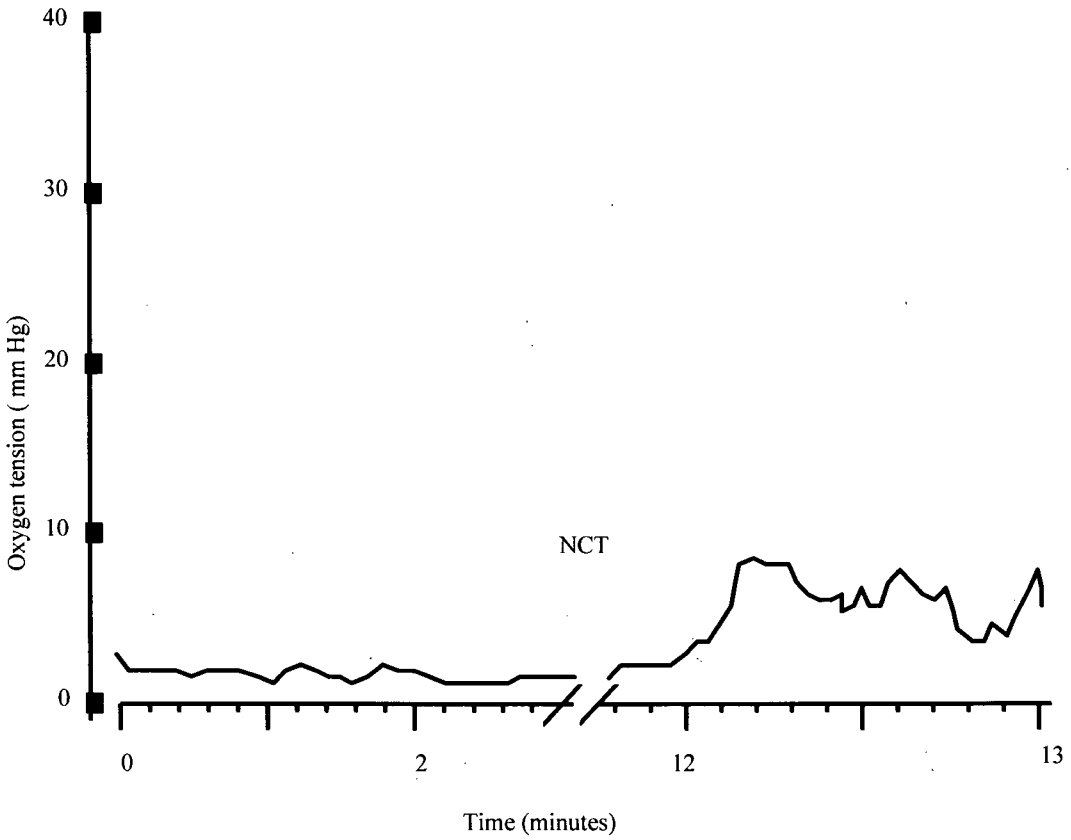


Fig 16. Effect of injection of nicotinamide (1.0 mg/g intraperitoneally) on oxygen tension in hypoxic zone of SCCVII tumour. Representative recording shows measurements taken at 2 minutes before the injections, at the injections and 1 hour after the injection of nicotinamide.

4. DISCUSSION

Fluctuations in tissue oxygen tension have been recorded as early as 1958. To date the exact mechanism of formation of these fluctuations remains unknown. The work in this thesis has shown that pO_2 oscillations are similar to spontaneous arteriolar vasomotion. The important finding in this study is the frequent occurrence of large amplitude oxygen fluctuations that are independent of the cardiac and respiratory cycles. The findings in this study support the hypothesis that spontaneous vasomotion of small arterioles may cause changes in extracellular concentration of oxygen as well as facilitate exchange of nutrients and metabolic products between the blood and cells.

4.1. **Methodological considerations**

The frequency and amplitude of oscillations recorded with the Eppendorf oxygen electrode correlate with previous observations of spontaneous changes in oxygen tension in brain and skeletal muscle (Misrahy et al., 1962).

The zone of measurement with the Eppendorf electrode, produces a mix of data from the extracellular space and immediately adjacent extravascular O_2 (Vaupel, 1995). This conclusion is based on several technical specifics of the electrode. In addition, the recordings were not intravascular as histological studies did not show consistent signs of bleeding at the measurement sites or incursion channels. The electrode used in this study was mainly the Eppendorf gold electrode. However, another type of microelectrode was used for spatial studies. This electrode was not very different from

the Eppendorf electrode since the actual size of the gold electrode was 12 μm. However, the overall size of the Eppendorf electrode was larger (350 μm) and that the large size of this electrode may cause more severe tissue compression and damage which would consequently affect tissue oxygen tension.

Oscillations in tissue pO_2 recorded by the oxygen electrode are a physiological phenomena distinct from other systemic fluctuations such as respiration, heart rate and EEG. Since sectioning of the sciatic nerve did not influence oxygen fluctuations, we conclude that pO_2 oscillations observed in skeletal muscle do not originate from skeletal muscle contractions. This observation is consistent with previous reports where myogenic activity of skeletal muscle (EMG) was recorded and no fluctuations in EMG were observed (Kunze, 1976). In addition, injection of ganglion blocker did not inhibit oxygen oscillation in brain or skeletal muscle suggesting that neural control of pO_2 oscillations is minimal.

4.2. **Localization of oxygen oscillations**

It is important to note that arterioles rather than the down stream capillary bed may be the main source of oxygen supply and pO_2 fluctuations. It is increasingly evident that small arterioles are of equal importance as capillaries in systemic tissue oxygen supply (Buerk et al., 1993; Hsu and Secomb, 1992; Intaglietta et al., 1996; Secomb and Hsu, 1994). Thus diffusive exchange between arterioles, venules and capillaries plays an important role in distributing oxygen throughout the tissue (Hsu

and Secomb, 1992; Intaglietta et al., 1996; Pittman, 1995; Secomb and Hsu, 1994; Ye et al., 1995). It has been shown that a substantial portion of oxygen delivery to hamster skin is provided by the arteriolar network, suggesting that the contribution of the capillary network oxygenation in this tissue is relatively small (Duling and Berne, 1970) (Torres Filho et al., 1996). It is therefore reasonable to assume that even in the skeletal muscle and the brain, a major part of oscillations shown in the recordings presented in this study may have been the result of oxygen release from arterioles.

The frequency of pO_2 oscillations do not completely match those obtained by other methods such as laser Doppler flowmetry (LDF) (Bertuglia et al., 1996; Biswal and Hudetz, 1996; Linden et al., 1995), (Morita-Tsuzuki et al., 1992). The sampling volume of laser Doppler flowmeter contains a broad spectrum of vessels. In other words, the laser Doppler beam hits a larger tissue volume than the oxygen electrode and it penetrates deeper into the tissue. The LDF signal should primarily reflect blood flow in capillaries, which could have very complex behavior due to influences from upstream oscillations.

The spatial location of arterioles is of importance in formation of oxygen fluctuations. Recordings with the duplicate oxygen electrode show that when the distance between electrodes increases, fluctuations became asynchronous. It is possible that arterioles located within a limited area originate from a single larger arteriole. In support of this theory, Manil et.al show that as the distance between electrodes

increases, several large arterioles may be present within the measurement range. Manil et.al have also shown that when these fluctuations are recorded simultaneously in the frontal, parietal and occipital region of rat's brain, the value of coherence function of pO_2 fluctuations is very low, suggesting a local control mechanism of these fluctuations (Manil et al., 1984).

Size of the arterioles used in the pressurized setup was often at the level of first order arterioles. The frequency 3-12 c/min observed via intravital microscopy has been attributed to third order arterioles. It is possible that under pressurized conditions, isolated arterioles increase in size and therefore are considered as first order arterioles. We observed that these blood vessels undergo similar frequency of vasomotion as the third order arterioles observed in vivo, via intravital microscopy (Colantuoni et al., 1984).

4.3. **Correlation of these fluctuations with local temperature**

In this study, the oxygen electrode and the thermocouple were positioned side by side and simultaneous recordings were made. Measurements with the thermocouple represent local blood flow as shown previously by Shichino et al. (Shichino et al., 1991). Fluctuations recorded by the thermocouple may reflect periodic changes in flow velocity, hematocrit or vascular volume; the latter could be associated with changes in vessel diameter or with capillary recruitment which

consequently leads to changes in local blood flow and registration of temperature change by the thermocouple.

Similar fluctuations in blood flow have been recorded with laser Doppler flowmeters (Bertuglia et al., 1996; Bollinger et al., 1991; Colantuoni et al., 1994). Fluctuations recorded by the thermocouple are also in accordance with fluctuations in capillary hematocrit and with capillary pressure in testicles as previously reported by Damber et al. (Damber et al., 1992).

In our experiments, the thermocouple and the duplicate oxygen electrodes were placed adjacent to each other. It is possible that they were close to the same feeding arteriole or were placed in the same capillary bed. Coherent diameter oscillations have occasionally been seen in the pial artery tree without predominant localization or restriction of arteries of a given size (Auer and Gallhofer, 1981). Simultaneous vasomotion of arteries and veins has also been observed (Hundley et al., 1988). Coherence in vasomotion is suggested by the presence of vascular volume oscillations in the cerebral cortex (Dora and Kovach, 1981).

As observed with duplicate oxygen electrodes used by Clark et.al, the correlation distance of the cerebral cortex is about 1.5 mm (Clark, 1958; Cooper et al., 1966) which may indicate the size of the vascular area participating in the blood flow oscillations.

4.4. Mechanism of vasomotion

Results in this study show that pressurized arterioles undergo spontaneous vasomotion at the same frequency as pO_2 oscillations in vivo. A correlation between frequency of pO_2 oscillations and individual arteriolar vasomotion rate has not been reported previously. However, reports on frequency of vasomotion of arterioles observed via intravital microscopy are similar to recordings presented in this study (Colantuoni et al., 1984). This resemblance provides further support for direct vascular control of pO_2 oscillations.

The exact mechanism of oscillatory vasomotion is not well understood. Previous investigations show that oscillations of oxygen as well as fluctuations in blood flow are pressure dependent (Hudetz et al., 1992; Manil et al., 1984). Results presented in the in vitro study of this thesis, also show that pressure is of importance in the generation of these fluctuations. As isolated pressurized arterioles undergo vasomotion only under a certain range of intravascular pressure.

The pressure dependence is also compatible with the hypothesis of pacemaker cells in arterioles suggested by Goligorski et al. (Goligorsky et al., 1995). These pacemakers are mainly present at the branching points of arterioles, where most prominent changes in blood pressure takes place (Colantuoni et al., 1985; Goligorsky et al., 1995). Moreover in support of the pacemaker theory, we observed that

recordings of both oxygen tension fluctuations and vasomotion of isolated arterioles sustain a recurring cycle of pO_2 fluctuations over longer periods of observation.

It is possible that the initial vasomotory signal originates from the blood pressure acting at the branching points causing oscillatory intra and extracellular calcium fluxes in smooth muscle cells located at the pacemaker regions. Such changes in calcium can further cause excitation-contraction in vascular smooth muscle cells by opening and closing voltage or receptor operated calcium channels (Casteels et al., 1985). The signal is initiated at the pacemaker sites, then conducted along the vessel via gap junctions. Repetitive excitation of the pacemaker cells by pressure may be the origin of the regular cycles of oscillations observed in this study.

Our results also demonstrate that other factors previously described as important in formation of vasomotion such as shear stress and blood flow and their effect on endothelium may not be determinant factors. Our in vitro experiments show that spontaneous vasomotion is generated and maintained without the presence of shear stress or blood flow and that the intravascular pressure is the most important factor in formation of these fluctuations.

If vasomotion is divided to a relaxation and a contraction period, during relaxation, membrane distention activates calcium influx through stretch operated channels. The elevation in cytoplasmic calcium concentration stimulates actin and myosin interaction and initiates a constriction. Once constriction has started, the

stretch operated calcium channels are deactivated by physical deformation of the membrane and the cytoplasmic calcium is decreased by normal extrusion mechanism and potassium efflux is initiated by the inward calcium current and serves to hyperpolarize the membrane, facilitating relaxation (Osol et al., 1988).

Following the stimulation of the α-adrenoreceptors, pO_2 oscillations in skeletal muscle display large pseudosinusoidal oscillations. Similar effects were observed after administration of prostaglandin into the brain. The increase in frequency of these oscillations may have been the effect of vasoconstrictors on the receptor operated Ca^{2+} channels of small arterioles (Wesselman et al., 1996). Our results show that increased vasomotory response of arterioles has a secondary effect on oxygen fluctuations. Overall, these observations plead for an organ specific origin of oxygen fluctuations and further links them to vasomotion of isolated arterioles.

4.5. **Physiological consequences of vasomotion**

Several metabolic and circulatory parameters show spontaneous oscillatory behavior. It has been suggested that oscillations reflect the operation of metabolic autoregulation to provide optimal blood flow (Cooper et al., 1966). This effect may be formed by wave forms of diameter changes in vasoactive arterioles. In addition, arteriolar vasomotion may have a secondary affect on downstream capillaries.

In this relation, previous studies show that pressure in individual capillaries fluctuate in both cyclic and irregular pattern so that they are above plasma oncotic pressure at one time (25-30 mm Hg) and below it at another time (10-15 mm Hg), providing a time-dependent sequence alternatively favoring filtration and then absorption of oxygen (Intaglietta, 1968; Messmer and Kreimeier, 1989). These fluctuations may have been induced by vasomotion of precapillary arterioles which are transferred downstream to the capillary bed. Thereby maintaining blood flow and oxygen tension in the microcirculation at times capillary perfusion is compromised.

Furthermore, a recent intravital study of vasomotion in cremaster muscle has demonstrated that tissue oxygen tension in a capillary bed drops several mm Hg when arterioles are in the contraction phase of vasomotion and it increases when blood vessels dilate (Donias et al., 1998). Such vasomotion-induced fluctuations may cause changes in the extracellular concentration gradient of oxygen. These changes may be gradual or sharp depending on the frequency and amplitude of vasomotion.

Consistent with this theory, Tsai and Intaglietta have calculated the effect of vasomotion on tissue oxygenation. Using a mathematical model they show that variability in hematocrit and blood velocity caused by vasomotion produces fluctuations in tissue oxygenation. They argue that, depending on the frequency

and amplitude of vasomotion, dynamic changes of several mm Hg of magnitude in tissue oxygen tension are possible (Tsai et al., 1993).

Changes in tissue pO_2 may also be the result of alteration in arteriolar volume during different phases of vasomotion. It is possible that the arteriolar surface in the dilation phase is expanded and therefore higher oxygen level is registered by the oxygen electrode. However, simultaneous measurements of vascular volume changes and fluctuations in pO_2 are required to investigate this hypothesis. Moreover, synchronous vasomotion and volume change of several arterioles may serve as a mechanical mixer. Simultaneous volume changes in these microvessels may cause alterations in the concentration gradients through extracellular stirring of nutrients.

It is possible that mechanical mixing of plasma by arterioles may enhance oxygen transport, although it is impossible to assess the physiological importance of such phenomena. To be able to investigate that, a model has to be developed that includes oxygen transport in both the red blood cell and tissue, the oxygen release by the red blood cell, and the oxygen consumption in the tissue.

4.6. Oxygen tension and pO_2 fluctuations in SCCVII tumours

Part of the purpose of this study was to characterize spontaneous oscillations in tissue pO_2 in different parts of SCCVII tumours. The main finding in the tumour oxygenation study is that spontaneous fluctuations of oxygen are

absent in centrally located hypoxic zones of SCCVII tumours (Hardwick, 1996). These results also demonstrate that the Eppendorf oxygen electrode can be used in tumours to provide real time mapping of microregional fluctuations. The observation of low oxygen tension and hypoxia recorded by this electrode are consistent with those of previous investigations using other types of micro electrodes (Brizel et al., 1996; Horsman et al., 1994; Olive, 1967; Olive, 1994).

There are several possible explanations for the absence of pO_2 fluctuations in hypoxic zones. One reason may be that because the oxygen electrode used in this study is not as accurate as previously used micro-electrodes, it may not register fluctuations at lower oxygen levels. On the other hand, pO_2 fluctuations were observed in a few peripheral zones with low oxygen tensions. It is therefore concluded that the absence of oscillations is not due to technical insufficiency of the oxygen electrode.

The absence of oxygen fluctuations in hypoxic areas might have been due to lower vascular density in these regions. In certain types of tumours, vascular density decreases and intercapillary distances increase in the advancing stage of the disease. However, based on the morphological data available by the electrode manufacturer and from our histological studies, the number of blood vessels are similar in a nominal volume of tissue measured by the Eppendorf electrode in both hypoxic and non-hypoxic measurement zones.

It is known that arterioles in central hypoxic tumour zones are structurally and functionally different from normal vessels (Oda et al., 1984). In this connection, lack of arteriolar vasomotion has been reported in a variety of tumours (Shan et al., 1997; Vaupel and Gabbert, 1986). Preexisting normal vessels in tumours exhibit vasomotion (Vaupel et al., 1989). These blood vessels may be present in the peripheral tumour zones and thus fluctuations in oxygen tension might be more prominent in these areas.

In addition to arteriolar vasomotion, vascular arrangement surrounding the oxygen electrode may affect formation of pO_2 fluctuations. Our findings with duplicate micro-electrode recordings in peripheral and central tumour zones supports this hypothesis. A comparison between fluctuations in subcutaneous tissue and tumour tissue in this study showed that in subcutaneous tissue pO_2 oscillations were synchronous if the two oxygen electrodes were placed within 2 mm distance. In tumour areas this distance was increased to 8 mm. The close correlation between each of the data sets obtained from different peripheral tumour sites suggests that these oscillations are in fact a local phenomenon originating from the same preexisting vessel. The role of these small arterioles in regional blood flow fluctuations are thought to require synchronized vasomotion of many vessels possibly achieved by myogenic mechanism described previously (Cavalcanti and Ursino, 1996; Griffith, 1996; Ursino and Fabbri, 1992).

In this study, we have not measured the effect of low or high oxygen availability either in vivo or in vitro conditions. Such studies are of immense importance since it is increasingly known that oxygen affects vasomotion. It would be specially important to study effect of low oxygen tension or hypoxia on pressurized arterioles and its effect on vasomotion in these arterioles.

4.7. Modification of pO_2 fluctuations in SCCVII tumours by pharmacological agents

A final factor that may contribute to the absence of pO_2 oscillations in central tumour zones is elevated extravascular pressure. The precise level of pressure in the fluid phase of the interstitium remains a controversial issue primarily because of the questionable nature of the methods available for measuring this force. However, recent measurements by Jain et.al show that elevated interstitial fluid pressure (IFP) is a pathophysiological characteristic of most human and experimental tumors and may be responsible, in part, for the poor distribution of blood-borne therapeutic agents and low blood flow rate in tumors (Znati et al., 1996). In addition it has been proposed that the changes in IFP and pO_2 may occur before any macroscopic changes in tumor volume after radiation therapy of tumours. Radiation-induced decrease in IFP could be, in part, responsible for the increased uptake of monoclonal antibodies and increased tissue pO_2 (Znati et al., 1996).

In our study, nicotinamide reinitiated pO_2 fluctuations in some central tumour zones. It has been suggested that nicotinamide reduces tumour IFP and thereby decreases transient vessel non-perfusion and acute hypoxia (Peters et al., 1997). The exact mechanism by which these changes occur is not well understood.

In addition, recently, Chaplin et.al showed that nicotinamide can modify dynamic fluctuations in microregional oxygen delivery in tumours (Chaplin et al., 1990). It is possible that nicotinamide reduces IFP, thus reducing external pressure on some of the arterioles and permitting reinitiation of vasomotion and consequent fluctuations of tissue oxygen tension. These direct effects on vascular resistance together with the reduction of interstitial fluid pressure could be combined to improve the homogeneity of tumour perfusion (Hirst et al., 1995; Horsman et al., 1989).

The impact of nicotinamide and similar agents on tumour vasomotion and tumour IFP requires further study. Perhaps, investigating fluctuations of pO_2 in slow growing AT-17 tumours that contain more differentiated and normal structured blood vessels may facilitate a better understanding of tumour oxygenation and ultimately improved tumour therapy.

5. Summary and Conclusions

The delivery of essential substrates for cellular oxidative processes requires activation of some control system. The structural entity around which such controls

operate is the most peripheral portion of the arterial tree, the arterioles, which present a bridge between large systemic arteries and microscopic capillaries.

It is possible that arteriolar vasomotion provides a means of autoregulating blood flow during alterations in arterial pressure to maintain a constant capillary pressure. Arteriolar vasomotion also regulate nutrient supply to maintain sufficient oxygen and nutrient levels in the extracellular space.

Findings in this thesis show that spontaneous vasomotion of isolated pressurized arterioles is similar to fluctuations of oxygen tension in brain and skeletal muscle. These findings also indicate that the oscillations were dependent on intravascular pressure and Ca^{2+} concentration, and the role of metabolic products in the generation of these fluctuations is minimal.

This study therefore supports the hypothesis that fluctuations of oxygen tension are formed by arteriolar vasomotion based on myogenic tone. It also supports the hypothesis that pO_2 fluctuations are altered under pathological conditions such as hypoxia in malignant tissue. Further experimental work is required to determine whether and under what circumstances consequences of vasomotion on oxygen transport become essential to other tissue dynamics and cellular homeostasis.

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6. APPENDIX

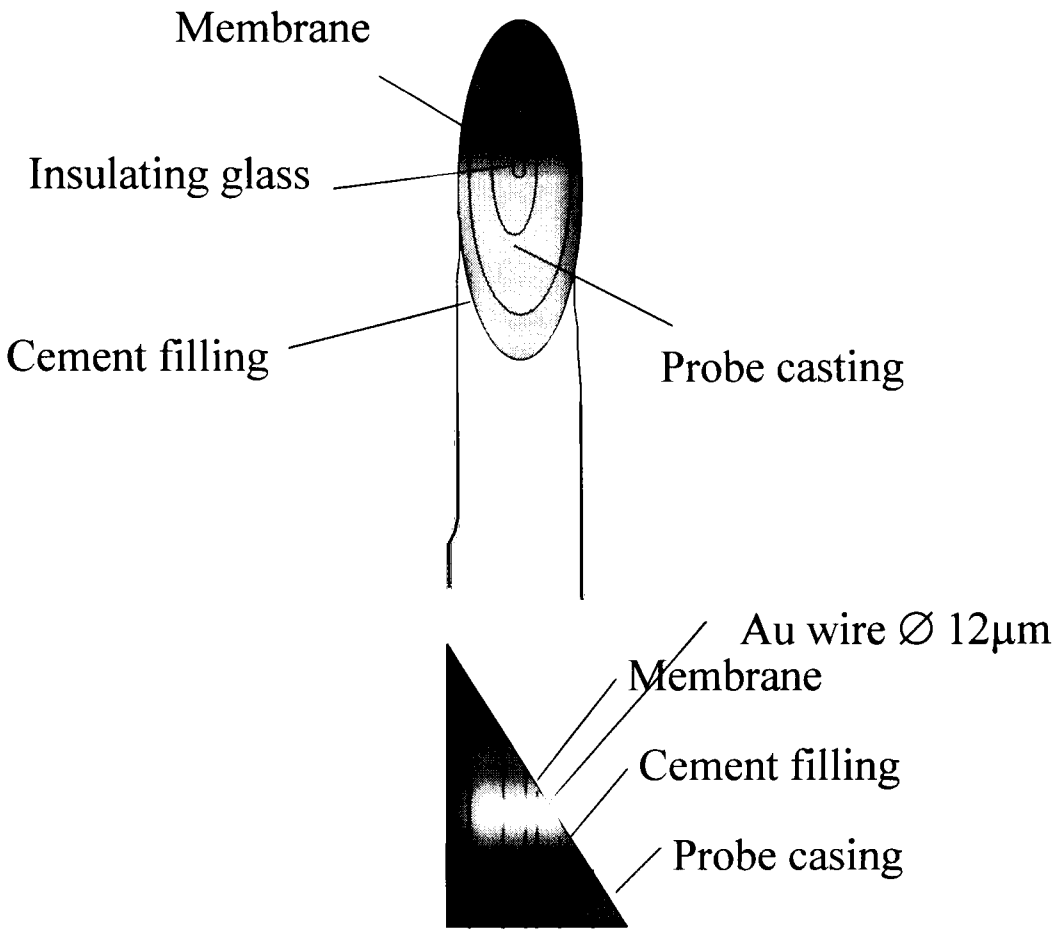
Technical data (Eppendorf oxygen electrode)

The electrode casing tube is made of steel (D: 0,5 mm) and a sloping cut of the probe tip (D: 0,33 mm), with a glass-insulated gold micro-cathode (D: 12µm), recessed and covered with teflon membrane.

Technical data of the probe in 0.9% NaCl solution at 37°C and 700 mV polarization compared with an Ag/AgCl reference electrode:

sensitivity:	6±3.0 pA/mm Hg pO_2
Current ratio of O_2 free to saturated O_2 solution	< 10% of air value
Convection dependency of Current	< 5%
Reaction time for pO_2 changes	< 500 ms (T_{90})
Current drift at constant pO_2	< 10%/h
pH cross-sensitivity	±1% (pH 6,5-9,5)
Temperature cross sensitivity	2,44%/ °C

This electrode has no cross-sensitivity to N_2O and enthrane. This electrode can not be used with halothane anesthetics.



Physical principle of measurement

The oxygen partial tension (pO_2) of the tissue is measured polarographically with a fine needle O_2 electrode. The membrane-sheathed gold electrode is pretensioned with a typical negative voltage of 700 mV (=polarization voltage) against the AG/AgCl counter electrode. The subsequent current in the measurement circuit is proportional in amperage (0.01 to 2,00 nA) to the oxygen partial tension in the electrolyte or tissue.

Saurer Electrolyte: $O^{2+} 4e + 4H \rightarrow 2H_2$

Neutraler: $O^{2+} 4e + 2H_2O \rightarrow 4OH^-$