Correlations Between Redox-State Potential Changes in Different Tissues and the Heart Frequency in Vivo

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Abstract. Redosis evoked in different tissues by methylene-blue or menadione (oxidants), resulted in an increase in heart frequency, while oxidosis evoked by thiamine or cysteine (reductants) diminished the frequency. In isolated organ tissues where compensatory redox feed back overshoots are rarely to develop, owing to the low redox buffer capacity and lack of the influence of nervous and humoral factors, the heart frequency decreased in response to direct oxidosis induced by the application of oxidants, and increased following reductant application; this suggested an environmental type redox regulatory influence of the agents rather than specific action of the agents. This environmental type effect can result from direct action on isolated organs, or from direct and indirect actions in vivo. An increased redox-state potential resulted in decreased heart frequency and inversely. In a pathological situation provoked by complete strangulation of aortae, a significant oxidosis developed in parallel with a decrease in heart frequency. On increasing the redox buffer capacity by application of methylene-blue (oxidant), or thiamine (reductant) both the redox and the resulting heart frequency changes could readily be counteracted. When cigarette smoke was pumped through an intratracheal tube, a significant redosis developed in the heart ventricle in parallel with an increased heart frequency. These data show that regardless of the origin of redox-state potential changes in tissues, a shift to oxidosis decreases and a shift to redosis increases the heart frequency.

Key words: Rat heart frequency — Redox-state — Methylene-blue — Menadione — Thiamine — Cysteine

Introduction

It is generally believed that pacemaker mechanisms may not be a unique phenomenon found in only a few cells, but that these mechanisms can be operative

in most, or all excitable tissues; these mechanisms may have been lost in the development if it was more advantageous to the animal that the cell was driven by other activity. However, pacemaker mechanisms may reappear as soon as any of the factors change in favour of the oscillatory phenomenon. It is a peculiar fact that the heart and smooth muscle tissues of the alimentary tract, which have autorhythmicity capacities, show relatively low redox-state potential (Puppi et al. 1976). This primordially lower level may be due a.o. to the presence of the pentose-phosphate pathway in the metabolism of these tissues (Laborit 1965), while this pathway is missing in others, e.g. in skeletal muscles. These correlations and a number of literary data, showing that at a relatively low electron acceptor/donor quotient of the internal milieu is associated with an active pacemaking, while at a relatively high electron acceptor/donor quotient this mechanism is, impaired or fails to function (Laborit 1965; Uliyaninski et al. 1976; Puppi et al. 1979; Borchard et al. 1979; Otorii et al. 1977; Erdmann 1980; Takeo et al. 1981; Oliva and Savino 1981; Boschero et al. 1982), prompted us to investigate the effects of changes in the general tissue redox-state potential (E_o') in the organism on the heart frequency.

In contrast to the commonly held view that the quantity of redox agents and enzymes and the proportionality of oxidized and reduced forms of redox metabolites, or the activity of various redox enzymes, are kept in the organism within a narrow range, it could be shown that E_o' in tissues may rather substantially vary, owing to numerous reasons (for a review see Shapiro 1972; Puppi et al. 1980; Puppi and Dely 1983). Here, we shall give only some examples related direct to the heart. Stimulation or inhibition of isometric force development induced a decrease or increase in NADH fluorescence intensity corresponding to a more oxidized or reduced state of tissue pyridine nucleotides in heart muscles (Chapman 1972). Following acetylcholine application biphasic changes in E_o' were observed in frog heart (Puppi et al. 1976). Adrenaline and noradrenaline produced an initial improvement of E_o' followed by a sustained debasement, while isoproterenol produced only debasement (Otorii et al. 1977). An increase in contractile force of dog heart induced by noradrenaline and ouabain evoked an increase in NAD/ /NADH ratio (Bat Ami et al. 1983).

Materials and Methods

Our experiments were carried out on CFY rats weighing 250—300 g, anesthetized with Ketanest (15 mg/100 g body weight). Redox agents in solution were administered through a cannula inserted into the jugular vein. The animals were ventilated through a tracheal cannula at a frequency of 30/min and a volume of 2 ml per cycle. The chest was opened by median-sagittal incision. Platinum electrodes were inserted into the central part of the ventricle muscle, the medial vaste muscle or the liver, to a depth of 3 mm.

Redox agent solutions used were: 6.25 mmol/l methylene-blue (MB), or menadione (oxidants) and 96 mmol/l thiamine (THIA) or cysteine (CYST) (reductants).

Experimental animals were divided into five groups, 10 animals each. Group 1: After the onset of a steady-state value of E_o' has been reached, 1 ml Ringer solution was added to check the effect of volume changes on E_o' in m. vastus, liver and heart ventricle, and on the autorhythmic heart frequency. In these animals no significant changes in E_o' or heart frequency were observed.

The second group of animals received either oxidizing (1 ml methylene-blue or menadione) or reducing (1 ml thiamine or cysteine) agent solution through the cannula, and E_o' in m. vastus medialis, in the liver or in the heart ventricle, and the heart frequency were measured.

The aortae of the third group animals were completely strangulated with catgut just below the left subclavicle artery and E_o' in the heart ventricle and the heart frequency were measured.

The fourth group of animals received during aortic strangulation as above 1 ml oxidizing (MB) or 1 ml reducing (THIA) agent solution and E_o' in heart ventricle and the heart frequency were measured.

In the fifth group, cigarettes were fixed at the air input of the respirator and the animals were inhaling smoke for 7 min.

To measure redox-state potential (E_o'), the potentiometric method of Cater et al. (1957) was used. Electronically sharpened platinum wires (tip diameter 150 μm) were inserted into the heart ventricle, m. vastus medialis or the liver. The electrodes were enamel insulated to 0.5 mm of their tips. The reference electrode (Ag/AgCl) was placed into the intraperitoneal cavity of the animal. Following compensation of the electrode potentials, E_o' was read as the potential difference between the platinum and Ag/AgCl electrodes.

The redox potential is a quantity which characterizes the oxidizing or reducing capacity of a chemical or biological system. Redox potential exists only in a system at equilibrium. Living systems are open or non-equilibrium systems in which any measurement of E_h is a steady-state approximation. Hence, with biological objects we prefer to speak of "redox-state" or "redox-state potential". Since pH actually influence this parameter, we use symbol E_o' which means redox-state potential at physiological pH.

Although the platinum electrode was fixed in the beating ventricle very loosely so that it could follow the muscle movements readily, some injury to the cell membranes must have occurred. Nevertheless, this was of no importance for our measurements, since 1, the platinum — versus Ag/AgCl system measures only metabolic (redox) and no membrane potentials (Shvetz—Teneta—Gurii 1980). This fact seems to rule out the possibility that local electric fields initiated by membrane voltage would significantly interfere with our E_o' measurements. 2. There are observations suggesting that no significant redox-barriers exist between extra- and intracellular compartments (Cater et al. 1957; Bartoli and Siess 1978). This has also been strengthened by the observation (Puppi, unpublished results) that E_o' recorded in different tissues showed no significant alterations following osmotic disruption of the cells in hypotonic solution; similar values of E_o' were also measured in homogenates of the same tissues. The above seemingly surprising statements are easily explained by the findings of Clark et al. (1981) and Sun et al. (1983) that there are trans-plasmamembrane redox enzyme systems which mediate and equilibrate the redox-state between the extra- and intracellular compartments.

A high input resistance digital potentiometer (Radelkisz OP-208) was used, with a thermostat-controlled temperature correcting amplifier, placed in the intraperitoneal cavity of the animal. Slow changes in E_o' (1—60 s or 1—10 min) measured by the potentiometer were recorded continuously using a potentiometric recorder. These slow potential changes seemed to have purely redox origin, since they could not be detected by saline bridges.

To record heart frequency, two saline bridges were placed on the surface of the left heart ventricle and the right atrium, and potential changes were recorded through Ag/AgCl electrodes in saline reservoirs using a potentiometric recorder. Three hours following the administration of redox agents, the effects weakened. The absolute values of heart frequencies varied between 470—502 beats/min.

Table 1. Correlations between changes in redox-state potential ($\Delta E_o'$) of heart ventricle, *musculus vastus medialis* and liver induced by oxidants (methylene-blue; MB, and menadione) and the heart frequency (Δf) in vivo. Note an early oxidizing shift (insignificant) and then a significant shift to relative redosis, which is in good negative correlation to the heart frequency. The animals received 1 ml MB or menadione solution (6.25 mmol/l).

time after injections (min)	$\Delta E_o'$ (mV) and ± S.E.			Δf (%) and ± S.E.		
	measured in and (evoked by)			Correlated by the $\Delta E_o'$ in		
	heart ventricle (MB)	m. vastus med. (menadione)	liver (menadione)	ventricle	m. vastus medialis	liver
0.5	+ 1.5 (±1.9)	+ 2.5 (±1.8)	+4 (±2.1)	− 0.2 (±0.5)	−0.5 (±0.5)	−0.7 (±0.7)
1	+ 3 (±4)	+ 5.5 (±3)	+10 (±4)	− 1 (±0.6)	−0.8 (±0.5)	−0.9 (±1.1)
5	−10 (±4)	−13 (±3)	−26 (±5)	+15 (±2)	+5 (±0.8)	+6 (±0.9)
10	−13 (±3)	−18 (±3.5)	−35 (±5)	+18 (±1)	+6 (±2)	+6 (±1.2)

Table 2. Correlations between changes in redox-state potential ($\Delta E_o'$) of heart ventricle, musculus vastus medialis and liver induced by reductants (thiamine, THIA; and cysteine, CYST) and the heart frequency (Δf) in vivo. Note an early shift (insignificant) to redosis followed by a significant shift to relative oxidosis, which is in good negative correlation to the heart frequency. The animals received 1 ml THIA, or CYST solution (96 mmol/l).

time after injections (min)	$\Delta E_o'$ (mV) and ± S.E.			Δf (%) and ± S.E.		
	measured in and (evoked by)			Correlated by the $\Delta E_o'$ in		
	heart ventricle (THIA)	m. vastus med. (CYST)	liver (CYST)	ventricle	m. vastus medialis	liver
0.5	− 2 (±1.3)	−0.3 (±0.3)	− 0.8 (±0.5)	+ 1.2 (±1.5)	+0.9 (±1.1)	+1.8 (±0.9)
1	− 1.2 (±0.9)	−1.1 (±1.1)	− 0.2 (±0.5)	+ 1.1 (±0.7)	+1.1 (±1.5)	+2 (±1.1)
5	+14 (±2)	+2.5 (±1.1)	+ 7 (±3)	− 9 (±3)	−6 (±2)	−7 (±1.8)
10	+17 (±4)	+6 (±1)	+12 (±1)	−11 (±3)	−6 (±2.1)	−7 (±1.5)

Results

Correlations between changes in E_o' in different tissues and the autorhythmic heart frequency in normal hearts

Tables 1 and 2 show that there is a standard and, starting from the 5th min, following the administration, significant negative correlation between redox agents

induced changes in E'_o in different tissues and heart frequency. The administration of oxidants (MB or menadione) resulted in the development of redosis followed by an increase in the heart frequency. Following the administration of reductants (THIA or CYST) oxidosis developed which was parallelled by a simultaneous decrement in the frequency of autorhythmic heart contractures. Following the administration of both oxidant and reductant drugs only short-lasting shifts in E'_o (insignificant changes) could be observed, followed by a significant compensatory overshoot of the endogenous redox feed back mechanisms.

Correlations between ventricular E'_o and the frequency of autorhythmic contractures in pathological states

A pathological state was induced by total strangulation of the aortae. This was followed by a gradual increase in E'_o (Fig. 1 and 2). During the first minutes following strangulation when changes in E'_o have not reached significant levels, the heart frequency pronouncedly increased ; subsequently the frequency was decreasing in parallel with the increasing E'_o.
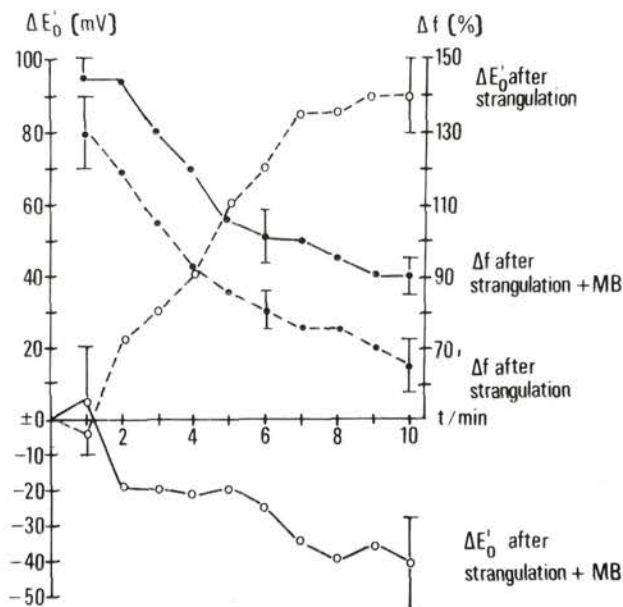

Fig. 1. Correlation between redox-state potential changes ($\Delta E'_o$) in heart ventricle and the heart frequency (Δf) induced by complete strangulation of the aorta with subsequent administration of 1 ml (6.25 mmol/l) methylene-blue solution (oxidant) in rat heart in vivo. Note the gradually developing oxidosis after strangulation and the simultaneous decreases in heart frequency. MB decreased E'_o and counteracted the frequency diminution. Vertical bars indicate S.E.M.

MB added simultaneously with aortic strangulation could completely abolish the strangulation induced endogenous increment in E'_o and a decrease in E'_o occurred. The decreasing tendency of the heart frequency was accordingly significantly inhibited ($p < 0.05$ 10 min following the administration).
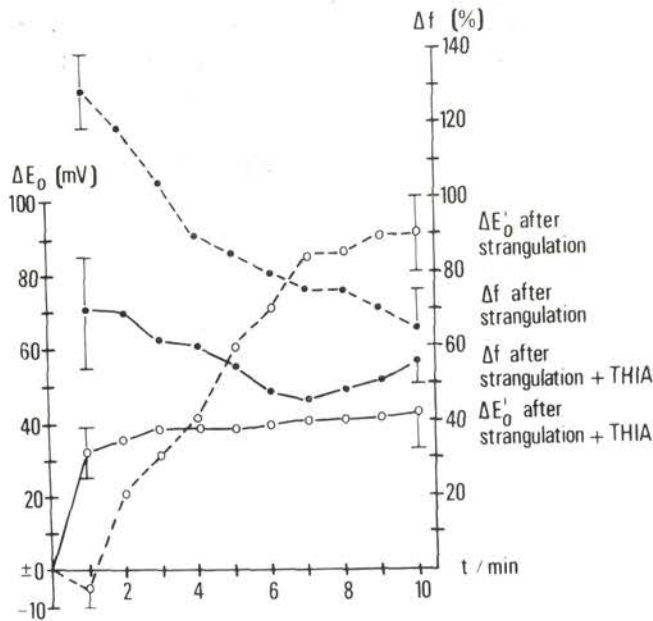

Fig. 2. Correlation between redox-state potential changes ($\Delta E'_o$) in heart ventricle and the heart frequency (Δf) induced by complete strangulation of aorta, with subsequent administration of 1 ml of thiamine (reductant) solution (96 mmol/l) in rat heart in vivo. Note the gradually developing oxidosis after strangulation and the simultaneous decrease in heart frequency. THIA had a biphasic effect on E'_o in parallel with the resulting changes in f. Vertical bars indicate S.E.M.

During the first few minutes following simultaneous aortic strangulation and thiamine administration, THIA induced compensatory type oxidosis additively enhanced the increase in E'_o of endogenous origin. After four min however, endogenous oxidosis reached a rather high level, and thiamine begun acting as a reductant, counteracting significantly the oxidosis of endogenous origin. The decreasing tendency of the heart frequency, typically occurring in the absence of THIA was completely abolished. Hence, a reducing agent capable of counteracting the increment in E'_o can also abolish the strangulation induced increase in heart frequency.

It should be mentioned that during the first minutes, characterized by additive oxidosis, no tachycardia occurred in contrast to strangulation without THIA; this is

in good accordance with the assumption of the significant role of tissular E_o' in modulating heart frequency.

In an other series of experiments, the animals were exposed to intense cigarette smoke for 7 min. A decrease in E_o' by 20 ± 6 mV was observed in the heart ventricle and the heart frequency simultaneously increased by 15 ± 5 %. Although effects of the cigarette smoke are multifactorial, the high correlation between redox changes and the heart frequency is an interesting phenomenon even in this case.

Discussion

Our results have shown that oxidosis induced by direct action of oxidants ($+ \Delta E_o'$ in vitro) or elicited by endogenous biochemical mechanisms (in vivo) impairs the frequency of autorhythmic cycles; on the other hand, redosis ($- \Delta E_o'$) induced by direct effect of reductants (in vitro), or elicited by endogenous biochemical mechanisms (in vivo) promotes the frequency of autorhythmic cycles.

Since no significant changes in E_o' or in heart frequency were observed in the first experimental group of animals (receiving Ringer solution only), and since the agents used have no special site of action and are chemically very different, the effects obtained can be considered as being due to redox changes. On the other hand, the question necessarily arises, whether changes in the redox-state potential were due to changes in the frequency, or whether changes in the frequency were due to changes in E_o'.

For redox agents, the reply is easy: Since significant changes only occurred after their administration, it should hardly be supposed that it were frequency changes which evoked the alterations. The problem is more difficult to solve with strangulation. Following aortic strangulation, E_o' began gradually increasing, the increase being, however not significant in the first few minutes. In this period a very intensive tachycardia was observed. The tachycardia may be explained as resulting from complex cooperative influences of baro- and chemoreceptor reflexes, pH, nervous and humoral mechanisms. It is known, for example that when the resistance of the aorta is increased, the heart works with higher strength, and tachycardia develops (Neely et al. 1967). Parallelly with the increase in E_o', this factor also begins to work and a gradual decrease in frequency develops. The significance of the redox factor is well seen in Fig. 2. When E_o' was higher (induced by thiamine), even during the first minutes, the tachycardia evoked by strangulation was significantly lower than without thiamine. In summary, one of the consequences of aortic strangulation is a gradual increase in E_o'. If this factor is actually operative, it should be modified by redox agents. Since effects of this factor could readily be intensified or inhibited by oxidants or reductants

a redox-modulatory site of heart frequency seems to exist and be of importance in vivo as well.

In in vivo experiments it certainly is highly plausible to assume that the redox action not only occurred through a direct action on the heart, but also via nervous or humoral pathways. Indeed, it is known that redox agents can modulate almost any physiological process (for review see Puppi et al. 1980; Puppi and Dely 1983). However, even if these indirect complex mechanisms are operative, the inverse relationships between tissue E'_o and heart frequency is valid.

In analysing differences between in vitro and in vivo data, we should bear in mind that in vitro, oxidants induce oxidosis, and reductants redosis within a short time, since in an isolated organ the metabolic redox homeostatic mechanisms get quickly exhausted owing to a low redox capacity. On the other hand, in in vivo experiments the heart can utilize all the redox capacity of the whole organism, and it can answer to effects of reducing agent with a compensatory oxidosis and vice versa. This latter view seem to be supported by the observation that redox metabolites circulate in the blood in rather great quantities. The concentration e.g. of the most important redox metabolite, glutathione is 43 μmol/l (Igarashi et al. 1983). According to numerous workers (e.g. Hommes 1964; Zhabotinsky 1974) rhytmical cyclic changes in E'_o of tissues observed especially after increased work are attributed to the rebound effects of biochemical feed back systems. It is known that methylene-blue injected into rabbits increases (not decreases!) reduced glutathione in erythrocytes (Smith et al. 1977), etc.

On the basis of our recent knowledge, the supposed biochemical mechanisms of the increase in E'_o following strangulation, can be outlined as follows: 1. Hypoxia increases lipid peroxidation (Sjöström and Crapo 1983); 2. The stimulation of heart activity shifts the NAD/NADH quotient to higher values (Chapman 1972; Bat Ami et al. 1983).

Following strangulation, the injection of MB induced a greater decrease in E'_o than did thiamine; the decrease in heart frequency was however counteracted more intensely in the latter case. Presumably, the redox feed back mechanisms are differently sensitive to an increase in redox buffer capacity induced by oxidants or reductants, and this difference is the cause of different reactions of the coupled non-redox mechanisms.

Excitatory events in cells, including autorhythmicity, have specific underlying ion flux programmes. Unfortunately, the question concerning the ionic mechanism of pacemaker activity has not yet been elucidated. In the sixtieth, it was assumed that the pacemaking ability of the heart results from a high resting sodium conductance as compared with a voltage and time dependent decrease in potassium conductance (Dudel and Trautwein 1968; Sperelakis and Lehmkuhl 1966). In the seventieth, I_k decay was believed to be the controlling factor in induced pacemaking in frog atrium (Brown et al. 1976; Noma and Irisawa 1976; Noma et al.

1978). Later, substantial claims for the importance of I_{si} in SA node pacemaking was made by the same authors (Irisawa and Noma 1982; Noma et al. 1980; Yanagihara and Irisawa 1980). Recently, in an up-to date review by Brown (1982) the author emphasized that neither i_f, which is highly dependent on both Na^+ (Di Francesco and Ojeda 1980) and K^+ (Brown et al. 1980), nor any other single current is the sole "pacemaker current" in the Sa node; rather, multisited cooperative, time dependent or independent relationships between the specific ion fluxes are responsible for the functioning of pacemaking. Actually, any influence which promotes these ion flux programmes will increase, and any impair to the primordial mechanisms will decrease the autorhythmic process.

The exact ionic mechanism background of the modulatory effects of redox agents still awaits its explanation. However, on the basis of our recent knowledge the following conclusions can be drawn. Oxidants decrease the input, and increase the output current in atrial trabaeculae fibres. Reductants act inversely (Puppi et al. 1979). Decreasing $[Ca^{2+}]_o$ by 50 % resulted in an inhibition of the amplitude of the autorhythmic contractures and the action potentials; this inhibition was more pronounced with oxidant than with reductant application, suggesting that at physiological $[Ca^{2+}]_o$ the oxidant inhibited I_{si}, while the reductant increased it (Prager et al. 1982). This notion is in good agreement with the report of Valverde and Malaisse (1980) that GSSG markedly inhibited, while GSH increased calcium translocation through ionophores. These specific ion flux modulations caused by oxidants or reductants may serve as a good explanation for the impairment of autorhythmicity in relative oxidosis and the enhancement of this function in relative redosis. The question concerning interactions of redox agents with the channel structures was discussed elsewhere (Puppi et al. 1980; Puppi and Dely 1983).

It was shown earlier that the redox-buffer mechanisms and their regulators operate within a more restricted range in normal than in pathological hearts (Dikstein 1971; Shapiro 1972). Following aortic strangulation the application of redox agents resulted in more pronounced functional upshots and broadener amplitudes of E_o' alterations than without strangulation. This is an important aspect in the possible administration of redox agents as pharmacotherapic modulators (regulators) of heart activity.

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