dria must be less than the total and is probably only a few tens of milliTorr. These values will, of course, increase with respiratory rate per cell and with the size of the cells.

*Conclusion*

In suspensions of normally respiring human neuroblastoma cells, respiration has an oxygen dependence similar to that of suspensions of isolated mitochondria in medium with a comparable phosphorylation state ratio. When mitochondrial oxidative phosphorylation is uncoupled, the metabolically imposed oxygen dependence is very small. The respiration of uncoupler treated cells at limiting oxygen pressures is indicative of the diffusion induced oxygen pressure difference between the extracellular medium and the mitochondria. This $P_{50}$ is proportional to the cellular respiratory rate, with a value of 0.15 Torr for the respiratory rate of normal neuroblastoma cells. The oxygen pressure difference between the cytoplasm surrounding the mitochondria and the mitochondria is probable only a few tens of milliTorr.

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## Consequences of acute ischemia for the electrical and mechanical function of the ventricular myocardium. A brief review

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*Summary.* Reduction or interruption of the blood supply to the myocardium leads to marked disturbances of electrical and mechanical function within a few seconds. Electrical dysfunction is characterized by an initial depolarization of the resting membrane, and a decrease of the amplitude, the upstroke velocity and the duration of the action potential. Both depolarization and depression of the action potential are closely associated with intracellular metabolic acidosis. After this initial phase, electrical cell-to-cell uncoupling develops, probably as a consequence of increased cytosolic free $[Ca^{++}]$.

Mechanical dysfunction is characterized by a dissociation of the initial decrease of active force development from the subsequent ischemic contracture. Active force development in acute ischemia is inhibited by the accumulation of ischemic metabolic products ($H^+$, inorganic phosphate ($P_i$), $Mg^{++}$) but not by a marked decrease of [ATP]. The subsequent ischemic contracture is probably initiated by release of $Ca^{++}$ from intracellular stores. This release causes rapid consumption of ATP and the development of rigor within 1–2 minutes.

*Key words.* Myocardial ischemia; ischemic depolarization; cell-to-cell uncoupling; mechanical failure; ischemic contracture.

*Introduction*

Reduction or interruption of blood supply to the myocardium leads to marked disturbances of electrical and mechanical function within a few seconds. Both the electrical and mechanical changes have been studied over the past two decades in a considerable number of experimental investigations. These studies are summarized in review articles [2, 29].

These investigations are of special importance because of the high morbidity and mortality rates for ischemic heart disease. For example, the mortality from sudden cardiac death is estimated at 600,000 cases a year in the United States [15].

This brief review article summarizes the main pathophysiological aspects of the disturbances of electrical and mechanical function in acute ischemia at a cellular level. The mechanisms of the acute malignant ventricular arrhythmias, which occur as a consequence of the electrical disturbances, have been discussed in a recent review article in this journal [38].

*Changes of active and passive electrical properties of acutely ischemic tissue*

The main electrical changes leading to circulating excitation with reentry and related ventricular arrhythmias are: 1) slowing of propagation velocity of the electrical impulse, and 2) changes of the refractory periods of the ischemic cells [4, 29]. At a cellular level, a depression of the transmembrane ionic currents producing the action potential leads both to conduction slowing and to a change of the refractory period of the ischemic action potential. In addition, an increase of cell-to-cell coupling resistance ('electrical uncoupling') contributes to a decrease of conduction velocity and to the formation of a conduction block. Since the time course of changes of the action potential is different from that of the changes of electrical cell-to-cell coupling [40], these events will be discussed separately.

*Changes of the membrane potential in acute ischemia*

The main cause for the depression of membrane excitability in acute ischemia is the rapid positive shift of the resting membrane potential. Within 5–10 min, the resting potential depolarizes from about −80 mV to −50 mV. Concomitantly with depolarization, the amplitude of the action potential, the upstroke velocity and the duration decrease until inexcitability develops after approximately 5–10 min [14, 34].

Depolarization of the resting membrane is associated with an increase of $[K^+]_o$ from 4 mM up to 15–20 mM [34]. The mechanism of this marked net $K^+$ displacement from the intracellular to the extracellular space has only been partially clarified [35, 39]. A small component is explained by a partial inhibition of active $Na^+/K^+$ pumping (decrease of energy-dependent unidirectional $K^+$ influx [62]) and a larger component by an increase in unidirectional $K^+$ efflux [35, 39]. The intracellular acidification has a direct effect on net $K^+$ efflux and extracellular $K^+$ accumulation. This has been shown in ischemic cylindrically papillary muscles which were kept in an artificial oxygen-free gaseous atmosphere. In the small muscles (diameter < 1 mm), where the $CO_2$ (formed from bicarbonate anions in metabolic acidosis [11]) remained low because of diffusion into the surrounding atmosphere, extracellular $K^+$ accumulation [8] and depolarization [40] were only minor. By contrast, extracellular $K^+$ accumulation and depolarization were marked in muscles with a diameter > 2 mm, where $CO_2$ accumulated in the core of the cylindrical muscle [8]. The mechanism which relates intracellular acidification and $CO_2$ accumulation to net cellular $K^+$ loss in myocardial ischemia has not been entirely clarified (for discussion see Kléber [35, 39]). Coupling of $K^+$ efflux to (pH-dependent) lactate efflux may play a role, because the effect of $O_2$ can be mimicked by inhibition of lactic acid transport [9]. It can be predicted that, independently of the underlying mechanism, the effect of $CO_2$ diffusion will be important. This is because at any site in an ischemic region where $CO_2$ can diffuse along a concentration gradient (epi- and endocardial ischemic boundaries, ischemic border zone), the diffusional inhomogeneities in local $[CO_2]$ are predicted to produce (within a few mm) inhomogeneities in $[K^+]_o$ and resting potential distribution. This is likely to be followed by inhomogeneities in refractory periods and to favor the formation of conduction block, as discussed below.

Intracellular acidification depresses the depolarizing ionic currents, in addition to affecting $K^+$ homeostasis and resting potential. For a given level of resting membrane potential and elevated extracellular $[K^+]$, the reduction of action potential amplitude and upstroke velocity is larger in simulated ischemia (combination of acidosis, elevated $[K^+]_o$ and hypoxia) than with elevated $[K^+]_o$ alone [41, 53]. Accordingly, at a given level of increased extracellular $[K^+]$ and resting membrane depolarization, conduction velocity in globally ischemic hearts is lower than in hearts perfused with normoxic solutions containing elevated $[K^+]_o$ [36].

One of the most characteristic features of the ischemic action potentials, which is mainly responsible for the occurrence of conduction block [29], is the time dependency of recovery of excitation. In cells depolarized by elevated $[K^+]_o$, the recovery of the fast $Na^+$ inward current becomes markedly prolonged, leading to prolongation of the relative and absolute refractory periods [19]. This effect is enhanced by acidosis and hypoxia [41, 53] or ischemia [14]. In ischemia, the absolute refractory period outlasts the duration of the action potential, and excitation during the subsequent (prolonged) relative refractory period will produce graded responses. As a consequence, the action potential during a beat with a small coupling interval to the preceding beat will be of small amplitude, whereas the action potential of the same cell after a long coupling

interval will be of a larger amplitude. Accordingly, conduction velocity in ischemia, which is related to the amplitude and upstroke velocity of the action potential, becomes *markedly dependent on beating rate*[36]. This prolongation of the recovery of excitability (so-called 'postrepolarization refractoriness') is only present in cells depolarized close to the threshold of the rapid $Na^+$ inward current[19,41]. Within these ranges of resting potential a very small change of resting membrane potential (by only 1–2 mV) will cause marked changes in refractoriness. Therefore, the inhomogeneities in distribution of extracellular $K^+$, and the associated inhomogeneities of resting potential (see above) increase the physiological local dispersion of refractoriness[20]. The increased dispersion of refractoriness underlies the formation of unidirectional conduction block and circulating excitation in acute ischemia[29,36].

*Changes of cell-to-cell coupling resistance*

Recently, our laboratory developed a new method by which the electrical longitudinal resistance of the intracellular space can be assessed in arterially perfused rabbit papillary muscle, not only under conditions of physiological perfusion[37] but also during ischemia (arrest of flow)[40] and hypoxia[51]. In ischemia, these experiments show that the intracellular resistive elements (intracellular resistance = $r_i$) remain initially constant until a steep increase in $r_i$, which indicates cell-to-cell uncoupling, occurs after about 15 min. The process of uncoupling leads to total conduction block approximately 2 min after its onset, because the flow of local electrotonic current is inhibited[10]. Before the occurrence of the block, conduction velocity increases transiently. This apparent paradoxical increase of velocity has been explained by an inhomogeneous onset of cell-to-cell uncoupling, and is a further indication that, in multicellular tissue, the rate of the metabolic, electrical and mechanical changes is not equally distributed at a cellular level. Electrical cell-to-cell uncoupling is closely related to the onset of mechanical contracture (see below), an increase of free cytosolic $Ca^{++}$[46,56] and to a secondary rapid cellular $K^+$ loss[10]. Out of the many metabolic changes occurring during ischemia, the increase of cytosolic $[Ca^{++}]$ is probably the causative agent for cell-to-cell uncoupling. This is suggested by the above-mentioned coincidence of uncoupling with the rise in $[Ca^{++}]$ and the observation that $Ca^{++}$ is a major uncoupler in normoxic cells[13,50]. Intracellular acidosis, which has been reported to increase coupling resistance in normoxic cells in addition to $Ca^{++}$[50], plays a minor role. This conclusion was drawn from the fact that cell-to-cell uncoupling is similar in ischemia and hypoxia[40,51], whereas the changes in intracellular pH differ markedly[1,18] between the two conditions.

*Decrease of active force generation and development of ischemic contracture*

After coronary occlusion, a decrease of active force generation is observed within seconds[25,40,61]. Active force generation ceases completely after approximately 3–5 min and the muscle remains in its resting state until, after 10–15 min, ischemic contracture develops. Although the exact time course of the decrease in active tension development in no-flow ischemia may vary with the experimental conditions and the animal species, there is a general agreement that the decrease in force development occurs during a first phase, and the ischemic contracture during a subsequent second phase. This suggests that these two basically different changes in mechanical behavior are brought about by different mechanisms, which have in common the withdrawal of oxygen and deprivation of chemical energy derived from oxidative phosphorylation.

*Mechanisms of the decrease in active force generation*

Over the past years, two hypotheses for the decrease in force generation have been discussed and experimentally challenged: 1) the *limitation of active force generation* by a decrease in the concentration of energy-rich phosphate compounds, and 2) the *inhibition of excitation-contraction coupling and/or active force generation* by accumulated ischemic metabolites.

With the first mechanism, the force-producing machinery would be maximally activated during ischemia and the decrease in force would reflect the decreasing availability of chemical energy from the hydrolysis of ATP. With the second mechanism, accumulation of ischemic products would inhibit the contractile machinery, thereby preventing energy consumption by the contractile proteins and protecting the cell from further damage.

*Energy-rich phosphate compounds and 'inotropic reserve':* The experimental results available at present indicate that the contractile mechanism is inhibited by ischemia, i.e. that there is a negative feedback interaction between the products of ischemic metabolism and active force production during an initial period of time termed 'reversible ischemia'. During this time period, the decrease of cellular [ATP] and of the cytosolic phosphorylation potential[17,30] does not limit the generation of active force. The presence of an 'inotropic reserve' in early ischemia has been demonstrated by the increase of contractile force after application of extrastimuli (post-extrasystolic potentiation), $Ca^{++}$[31,32] or norepinephrine[52].

*Excitation contraction coupling:* The inhibition of active force generation by ischemic metabolism may be caused by changes of excitation contraction coupling, in addition to the changes at the level of the contractile proteins. The rapid depolarization of the ischemic membrane and the development of inexcitability in the center of the ischemic zone (see above) will exclude the activation of

$Ca^{++}$ release and subsequent contracture after 4–7 min of ischemia. Before this period, $Ca^{++}$ release from the SR appears to depend on the availability of energy from glycolysis. When glycolysis is taking place, $Ca^{++}$ transients of almost normal amplitudes are observed with inhibition of oxidative phosphorylation, simulated ischemia or anoxia[3,45,57]. If glycolysis is inhibited, the amplitudes of the $Ca^{++}$ transients decrease rapidly. This change is not caused by a depletion of the $Ca^{++}$ stores in the SR, but by a shortening of the transmembrane action potential[44,57]. Intracellular injection of ATP reverses this shortening (by closure of ATP-dependent $K^+$ channels[49]) and restores contracture[44]. In ischemia in vivo, $Ca^{++}$ transients of increased amplitudes are found in surface cells loaded with fluorescent or luminescent $Ca^{++}$ indicators during the first five minutes of ischemia. This has been taken as evidence that *in acute ischemia in vivo* the main inhibitory effects of ischemic metabolites are exerted at the level of the contractile proteins.

*Accumulation of $[H^+]$, inorganic phosphate ($P_i$) and $[Mg^{++}]$*

Intracellular acidification, accumulation of $P_i$ and elevation of the free cytosolic $[Mg^{++}]$ are thought to be the main inhibitors of contraction in acute ischemia. Average intracellular pH has been measured with $^{31}P$ NMR and shown to decrease from 7.0 to about 6.8–6.6 within 10–15 min of ischemia[7,18], mainly as a consequence of lactic acid formation[18]. The breakdown of CP[17,28,30] and ATP is associated with both formation of $P_i$ and release of ATP-bound $Mg^{++}$. Thus $P_i$ increases from an initial level of about 3 mM to 10–15 mM within 10–15 min of ischemia or hypoxia[1,30,42]. Recent determinations of $Mg^{++}$ indicate that free cytosolic $[Mg^{++}]$ increases from about 0.5 mM to about 5–10 mM after 10–15 min. The time course of the increase in $[Mg^{++}]$ is inversely related to the decrease in [ATP][55].

In the setting of myocardial ischemia in vivo, it is only partially possible to separate the inhibitory effects of $[H^+]$, $P_i$ and $[Mg^{++}]$ on contracture[43]. This separation was, however, possible in the elegant experiments by Fabiatio[16] and Kentish[33] with skinned cardiac cells, and indicated an essential difference between the effects of $H^+$ and $[Mg^{++}]$ on the one hand and the effect of $P_i$ on the other. With either decreasing pH (down to about 6.6) or increasing $Mg^{++}$, there is a decrease of the sensitivity of the contractile proteins to $Ca^{++}$. A major component of this change is probably an altered binding of $Ca^{++}$ to troponin C. Consequently, for a given $Ca^{++}$ release by the SR, there is a smaller active force generation. With enough $Ca^{++}$ released, a generation of (unchanged) maximal force is still possible (shift of $Ca^{++}$ vs tension curve towards a higher $[Ca^{++}]$). In contrast, the main effect of $P_i$ is to decrease the maximum of the activated force[33]. This decrease has been explained by a change in the binding and release cycle of ATP on the myosin molecule, whereby accumulated $P_i$ inhibits dissociation of the myosin · ADP · $P_i$ complex. The action of $P_i$ is therefore direct, and not due to a decrease in the cytosolic phosphorylation potential[33].

In conclusion, both a shift of the $Ca^{++}$ vs activated tension curve towards a higher $[Ca^{++}]$, as well as a decrease of the maximum activated force, explain contractile failure which starts within seconds after the interruption of the blood supply to the myocardium.

*Mechanism of ischemic contracture*

The onset of contracture in ischemia occurs with a delay of 10–15 min after the interruption of coronary flow. This delay corresponds approximately to the duration of the reversible phase of ischemia. Accordingly, irreversible electrical cell-to-cell uncoupling[40], and breakdown of cellular homeostasis with rapid cellular $K^+$ loss[10] and increase of free cellular $[Ca^{++}]$[46,56] is observed concomitantly with ischemic contracture. If fully developed, contracture is of the 'rigor type', i.e. it is caused by fixed actin-myosin cross-bridges which, below an ATP-concentration of $< 0.1-0.3$ µM close to the contractile proteins, remain in their bound state and cannot undergo active, energy-consuming cycling. This has been demonstrated by the measurement of heat production during ischemic contracture, which is markedly below heat production during 'tetanic' or $Ca^{++}$-activated tension[27].

The important question is how the ischemic contracture, the breakdown of ionic homeostasis, electrical cell-to-cell uncoupling and the rapid secondary decrease of [ATP] are initiated. The answer is not straightforward because the experimental studies have provided different, seemingly conflicting results. Two main mechanisms for the initiation of ischemic contracture have been proposed: 1) a decrease of [ATP] below the rigor threshold, and 2) a primary increase of intracellular free $[Ca^{++}]$, leading to active energy-dependent cross-bridge cycling. In the latter case, activation of the contractile machinery would be the primary cause, and the subsequent rigor-cross-bridge formation a consequence of the energy imbalance and the rapid consumption of ATP.

One of the problems with the investigation of the mechanism of contracture is the slow and probably inhomogeneous onset in whole hearts or multicellular preparations[24]. This renders the interpretation of tension measurements and the comparison with average levels of energy-rich phosphate compounds or ions difficult. Moreover, even in oxygenated preparations, an abrupt increase of free cytosolic $[Ca^{++}]$ (e.g. during so-called $Na^+$-withdrawal contracture) leads to very rapid consumption of ATP and CP, and to secondary development of rigor[26,58].

In order to overcome the problem of inhomogeneity, experimental measurements were carried out in single cells. In a suspension of dissociated, metabolically-inhibited cells, an individual cell undergoes a change from a normal rod-shape to a state of 'hypercontracture' (with

a markedly distorted cytoarchitecture[5]) within a few seconds[24]. The interrelationship between the increase in cytosolic free [$Ca^{++}$], [ATP] and hypercontracture depends on the experimental conditions: In isolated beating chick embryonic cells (with loaded intracellular $Ca^{++}$ stores), an increase of free cytosolic $Ca^{++}$ can be produced with metabolic inhibition, if $Ca^{++}$ efflux through the $Na^+/Ca^{++}$ exchange is inhibited[6]. The initial rise of resting tension in this situation is caused by active energy-dependent cross-bridge cycling and rigor development occurs only afterwards.

In contrast, hypercontracture only develops below an [ATP] of approximately 0.5 mM in quiescent isolated rat heart cells; this concentration is almost one order of magnitude lower than the average [ATP] observed at the onset of ischemic contracture in whole tissue[24, 25]. Moreover, only a minor increase of free cytosolic [$Ca^{++}$] is observed in the same conditions (from 20 nM to 80 nM[23]). Whether or not this type of contracture is solely caused by rigor cross-bridge formation or is initiated by active cross-bridge cycling is uncertain. This is because the $Ca^{++}$-threshold for the activation of the contractile proteins by $Ca^{++}$ is markedly decreased in low [ATP] solutions, probably as a consequence of an increased sensitivity of troponin C to $Ca^{++}$[63]. The differences between quiescent and beating cells indicate that the $Ca^{++}$ content of intracellular $Ca^{++}$ stores (which is dependent on beating rate), and the exchange of $Ca^{++}$ between the extra- and intracellular space, are important determinants for the onset of contracture in ischemia; if the storage of a critical amount of $Ca^{++}$ is preserved during the initial phase of metabolic inhibition (and probably ischemia), release of $Ca^{++}$ from intracellular stores seems to trigger ischemic contracture.

The experimental results obtained in whole tissue ischemia or hypoxia are in favor of the hypothesis that the rise of free intracellular [$Ca^{++}$] observed with the onset of contracture is the cause rather than the consequence of rigor formation and rapid consumption of ATP. Measurement of $^{45}Ca^{++}$-exchange in the initial phase of hypoxia and metabolic inhibition, and determination of cellular $Ca^{++}$ content in ischemia[21, 47, 54], have shown that there is no increase or, in some experiments, a moderate decrease of cellular $Ca^{++}$ content (rapidly exchangeable $^{45}Ca^{++}$). In hypoxia, cellular $Ca^{++}$ content increases only after the onset of contracture[48]. This suggests that the increase of free cytosolic $Ca^{++}$ must originate from an intracellular source. An initiation of ischemic contracture by lack of ATP bound to myosin seems to be only thermodynamically possible with almost completely emptied intracellular $Ca^{++}$ stores[22]. Such a process, which would occur in the first minutes of ischemia (before the onset of contracture) would implicate $Ca^{++}$ release from the SR and subsequent $Ca^{++}$ extrusion to the extracellular space by $Na^+/Ca^{++}$ exchange and/or the $Ca^{++}$-pump. This, however, would be in contrast to the only moderately decreased intracellular $Ca^{++}$ pools, as measured by $^{45}Ca^{++}$ exchange (see above) and electron-probe microanalysis[59, 60]. Therefore, the initiation of ischemic contracture by release of $Ca^{++}$ from intracellular stores with a thermodynamically limited capacity seems to be the most probable mechanism.

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