Early Changes in Extracellular Potassium in Ischemic Rabbit Myocardium
The Role of Extracellular Carbon Dioxide Accumulation and Diffusion
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The role of local accumulation and diffusion of CO₂ to modify cellular loss and extracellular accumulation of K⁺ during the initial, reversible phase of myocardial ischemia was investigated in isolated, cylindrical papillary muscles of the rabbit. The muscles were blood-perfused through their vascular tree and placed in a (permanently flowing) humidified gas mixture with predetermined partial pressures of N₂, O₂, and CO₂. Ischemia was produced by total arrest of perfusion and O₂ withdrawal from the gas mixture. With surface Pco₂ kept constant during ischemia, [K⁺]e varied markedly with muscle geometry. After 10 minutes of ischemia, K⁺ accumulation was ~2.5 mM in muscles with a radius of 0.35 mm and ~14 mM in muscles with a radius of 0.9 mm, indicating that a large fraction of K⁺ accumulation was dependent on diffusion of a volatile metabolite. Computer simulation of CO₂ accumulation and diffusion within a tissue cylinder suggested a close phenomenological relation between Pco₂ and [K⁺]e in ischemia. This was confirmed by the finding that an increase of tissue Pco₂ in small cylinders before or during ischemia by externally applied CO₂ produced an increase in K⁺ accumulation.

The importance of CO₂ diffusion for local inhomogeneities in K⁺ within the same preparation was demonstrated by showing [K⁺], gradients with simultaneous or consecutive measurements between the papillary muscle cylinders and the adjacent septum and within 300 µm from the surface of the papillary muscle cylinders. These gradients predict an inhomogeneity of impulse conduction that might contribute to the genesis of ventricular arrhythmias. Besides the demonstration that accumulation and diffusion introduce inhomogeneities of [K⁺]e in ischemia, our results suggest that a significant component of cellular ischemic K⁺ loss is associated with production and extrusion of metabolic acid. On the basis of previous measurements of pH, and pHi in identical conditions, possible mechanisms of ischemic cellular K⁺ loss are discussed.

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Arrest of myocardial perfusion and the consequent lack of extracellular washout leads to accumulation of ischemic metabolites and ions in the extracellular space. Cellular loss of potassium and the resultant extracellular accumulation is one of the most prominent ionic shifts in early ischemia. It is closely related to the changes in membrane potential and the impairment of impulse conduction. The use of ion-selective electrodes has allowed quantification of K⁺ in the extracellular space. Thus, cellular K⁺ loss occurs in distinct phases: an initial rapid extracellular accumulation is followed by a plateau and thereafter by a secondary increase. The secondary increase is closely associated with the development of mechanical contracture and electrical cell-to-cell uncoupling. It is only partially reversible and therefore reflects the beginning of irreversible ischemic damage. The mechanism of the initial extracellular K⁺ accumulation has not yet been elucidated completely. Partial inhibition of Na⁺-K⁺ pumping and the consequent decrease of unidirectional K⁺ influx cannot fully account for the net shift of potassium ions and an increase of passive K⁺ efflux has been discussed. Both tetraethylammonium and ATP-sensitive K⁺ channels have been shown to carry this net efflux, although the mechanism of the opening of the so-called ATP-sensitive channel (in the presence of only a moderate decrease
of ATP), as well as the nature of the driving force acting on the K\(^+\) ions, is not fully understood.

In a previous study,\(^9\) measurements of extracellular pH and [K\(^+\)]\(_e\), determined on the surface of whole ischemic hearts, suggested that the local concentration of CO\(_2\) plays an important role in the accumulation of extracellular K\(^+\) in ischemia. CO\(_2\), which is displaced from HCO\(_3^-\) after the formation of lactic acid, accumulates in deep midmural tissue layers to values up to 300–400 mm Hg after 10–15 minutes of ischemia.\(^12\) This produces significant changes in net proton transfer to the extracellular space and in intracellular acidification.\(^13\) At boundaries between ischemic and surrounding tissue (ischemic border, epicardial, and endocardial tissue layers) diffusion of CO\(_2\) is predicted to lower the local concentration of CO\(_2\) in ischemia. Therefore, an interrelation between cellular K\(^+\) loss and local accumulation and/or diffusion of CO\(_2\) might have important implications for the spatial distribution of ionic and electrical changes in regional myocardial ischemia. Especially, local inhomogeneities of extracellular [K\(^+\)] during myocardial ischemia,\(^2,\(^14\) which have been implicated in the disturbances of impulse conduction and the generation of reentrant arrhythmias, may be partially related to inhomogeneities of CO\(_2\) accumulation.

The present investigation served two purposes. In a first part, we tested the hypothesis made from previous observations that diffusion of CO\(_2\) from ischemic boundaries might be a relevant factor for local gradients in [K\(^+\)], and, consequently, for the genesis of ventricular arrhythmias. In a second part, we tested the possibility that changes in PCO\(_2\) and other experimental interventions, which have been shown to change intracellular acidification and/or to influence proton extrusion in ischemia, might modify cellular K\(^+\) loss and extracellular K\(^+\) accumulation.

For the present experiments, we used a previously developed experimental model\(^6,\(^7\) that consists of a cylindrically shaped, arterially perfused papillary muscle that is surrounded by an artificial humidified gaseous atmosphere. In such an experimental system, it is possible to keep the ischemic conditions (arrest of vascular flow, total lack of oxygen) constant and to use the beating rate, the diameter of the muscle, and the partial pressure of CO\(_2\) in the surrounding atmosphere as controlled variables. Moreover, the cylindrical tissue enables the application of relatively simple diffusion mathematics to model the accumulation and diffusion of CO\(_2\) and K\(^+\) from the core to the surface.

Our results suggest that accumulation and diffusion of CO\(_2\) cause the significant inhomogeneities in [K\(^+\)] in early myocardial ischemia, which might be important for the genesis of early ventricular arrhythmias.

Materials and Methods

**Preparation and Perfusion of Papillary Muscles**

The method for the preparation of the isolated arterially perfused rabbit papillary muscle has been previously described and illustrated in detail.\(^7,\(^15\) In brief, rabbits of either sex weighing 2–3 kg were anticoagulated with heparin (200 units/kg i.v.) and killed with pentobarbital (50 mg/kg i.v.). The hearts were rapidly excised, placed in cold Tyrode’s solution (4°C), and transported to a dissection tray. The atria, left ventricular free wall, and nonperfused portion of the right ventricle were removed. The left ventricular septal surface of the tissue was secured to a wax platform that contained the ground electrode. The septal artery was cannulated and perfused with a solution of the following composition: insulin (1 unit/l), heparin (400 units/l), albumin (2 g/l), dextran (M, 70,000; 40 g/l), and Tyrode’s solution (mM: Na\(^+\) 149, K\(^+\) 4.5, Mg\(^++\) 0.49, Ca\(^++\) 1.8, Cl\(^-\) 133, HCO\(_3^-\) 25, HPO\(_4^-\) 0.4, and glucose 20). The total ischemic time before perfusion was less than 5 minutes in each experiment.

After the preparation was placed in the recording chamber,\(^7\) it was perfused through its proper microvascular bed with a solution composed of the previous perfusate plus washed bovine erythrocytes (hematocrit, 35–40%) and surrounded by an artificial gaseous atmosphere (Figure 1). In this preparation, the papillary muscles emerging from the septal surface had a diameter of 0.5–2 mm. As verified in histological sections, the endocardial endothelium and the adjacent connective layer had a thickness of about 20 μm, which amounts to 2–8% of fiber radius (W. Cascio, personal communication). Perfusion pressure was measured with a transducer (Gould, Cleveland, Ohio) and maintained between 40 and 45 mm Hg by adjustment of the perfusion flow rate (0.8–1 ml×min\(^{-1}\)×g\(^{-1}\)). This perfusion pressure can be considered normal for small arteries in the rabbit heart (diameter at the site of cannulation, ~150 μm), because about 50% of peripheral vascular resistance is located in the arteries with >150 μm diameter.\(^16\)

**Measurements of the Perfusate pH and PCO\(_2\) and PO\(_2\) in the Artificial Atmosphere Surrounding the Cylindrical Muscle**

Perfusate. The partial pressures of O\(_2\), N\(_2\), and CO\(_2\) were controlled with a membrane gas exchanger. The pH of the perfusate was continuously monitored during the experiment by a pH glass electrode connected to the perfusion line. The relative amounts of N\(_2\) and CO\(_2\) (O\(_2\) constant at 100 mm Hg during normoxic perfusion) were adjusted to yield the required pH of the perfusate. The PCO\(_2\) and [HCO\(_3^-\)] in the perfusate were measured with a blood gas analyzer (Ingold, Urdorf, Switzerland). Stainless-steel tubing was introduced between the membrane gas exchanger and the recording chamber to prevent diffusional losses.

Artificial atmosphere of the recording chamber. The PCO\(_2\) surrounding the preparation was set to the PCO\(_2\) of the perfusate. Consequently, the initial pH value on the surface of the muscle corresponded to the pH of the perfusate, and a homogeneous concentration of HCO\(_3^-\), H\(^+\), and PCO\(_2\) was achieved along the
radius of the muscle before arrest of perfusion (ischemia). The flow of the gas mixture was directed through the recording chamber in such a way that a steady convective flow of water-saturated gas around the muscle was achieved. The total chamber volume was exchanged within 30–45 seconds by the continuous gas flow.

Before the arrest of flow (45 seconds), the atmosphere in the recording chamber was changed from a mixture of CO₂, N₂, and O₂ (constant at 100 mm Hg) to a mixture of CO₂ and N₂. The volume fractions of O₂, N₂, and CO₂ in the recording chamber were accurately adjusted with individual gas flowmeters (Voegtlin, Basel, Switzerland). Before and during ischemia the volume fractions of O₂, N₂, and CO₂ were measured close to the muscle (at a 0.5-mm distance above its surface) by a gas adsorption method. The oxygen contamination during ischemia was <5 mm Hg in most experiments. In the rare cases when PO₂>5 mm Hg, the experiments were discarded. The various settings of the CO₂ concentration in the artificial atmosphere during ischemia are described below in the results section.

**Measurement of [K⁺]₀ and pH₀**

Extracellular K⁺ activity (a_K⁺) and extracellular H⁺ activity (a_H⁺) on the surface of the artificially perfused and ischemic papillary muscle were calculated from the potential difference between an extracellular reference electrode and a miniature extracellular K⁺- or H⁺-sensitive electrode (constructed, tested, and calibrated as previously described in detail). For conventional reasons, a_K⁺ is expressed as [K⁺]₀. An activity coefficient of 0.748₁⁴ was taken for conversion of a_K⁺ to [K⁺]. The change in a_H⁺ is directly expressed as changes in pH₀. This assumes that the activity coefficient for H⁺ is the same in the extracellular fluid as in the calibration solution.

Measurements of a_K⁺ in the intramural layers of the cylindrical papillary muscle were determined by ion-selective electrodes constructed from fine silver wire as previously described. The tip diameters of these electrodes averaged 110 μm. These electrodes could be introduced from the surface into the intramural layers of the papillary muscle without damaging the muscle by first penetrating the endocardium with a fine-tipped tungsten electrode and then inserting the ion-selective electrode through the small opening.

**Experimental Protocol**

In most experiments, [K⁺]₀ and pH₀ were measured simultaneously. The preparations were stimulated by application of a constant current pulse (0.5–1-msec duration, double threshold strength) between the apex and the base of the muscle. In each experiment, control measurements were obtained after the preparation had stabilized in the chamber for 30 minutes. During ischemia, measurements were obtained continuously for up to 30–40 minutes. Because the ionic and electrical changes are only partially reversible on reperfusion after 15 minutes, only a single ischemic period was induced in each heart, with the exception of the experiments shown in Figure 7, upper panel.

**Statistical Analysis**

The relation among the change of [K⁺]₀ during ischemia, muscle diameter, and heart rate was determined statistically by using a multivariable linear regression hypothesis model (Systat, Inc., Evanston, Ill.). Residuals were homogeneously distributed around zero, which justified the application of this technique to the data. Statistical comparison of values during the control and test periods was made by analysis of variance or Student’s paired t test as appropriate. All results are given as the mean±SEM. Statistical significance was considered to be p≤0.05.

**Results**

**Theory: Diffusion of CO₂ and K⁺ in a Cylinder**

Production of CO₂ in an ischemic tissue cylinder. After interruption of myocardial flow, metabolic acid
is produced by anaerobic metabolism.\(^9\) The released protons are buffered mainly by the protein and carbonic buffer systems. In such a way, CO\(_2\) is formed from HCO\(_3^-\) and distributes between the intracellular and extracellular compartments. At boundaries between ischemic and adjacent tissue, CO\(_2\) will diffuse along a concentration gradient. In contrast, CO\(_2\) will accumulate (with a very small or an absent diffusional component) in midmural layers in the center of an ischemic region, provided that the ischemic ventricle is of a large thickness. Therefore, it is reasonable to assume that the Pco\(_2\) measurements in midventricular layers of the ischemic dog heart\(^12\) provide a reliable estimate for the CO\(_2\) accumulation curve. The curve for CO\(_2\) formation, Qco\(_2\), (millimolars per minute), is obtained by differentiation of the accumulation curve. For analytical reasons, the measurements of Pco\(_2\) (millimolars of mercury) obtained by Case et al\(^{12}\) were fitted by (Figure 2, upper panel)

\[
P_{CO_2} = \frac{1}{\alpha_{CO_2}} \frac{a}{b^2} (1 - be^{-bt} - e^{-bt})
\]

which yields a relatively simple function for Qco\(_2\)(t) (Figure 2, lower panel)

\[
Q_{CO_2} = ate^{-bt}
\]

where \(a\) (0.9 mM×min\(^{-1}\)) is an empirical parameter determining the increase of CO\(_2\) formation after the onset of ischemia, \(b\) (0.245 min\(^{-1}\)) is an empirical rate constant describing self-inhibition of intracellular CO\(_2\) formation, \(\alpha_{CO_2}\) is the solubility coefficient for CO\(_2\) (0.0422 mM×mm Hg\(^{-1}\))\(^{20,21}\) and \(t\) (minutes) is the time after coronary occlusion. The production function, Qco\(_2\)(t), is very similar to measurements of early lactate production in ischemic rat heart.\(^22\) Its shape reflects the initial acceleration and subsequent inhibition of anaerobic glycolysis in early myocardial ischemia.\(^23\)

**Diffusion of CO\(_2\) from the ischemic tissue cylinder into the surrounding atmosphere.** For the case of a cylindrical muscle surrounded by an artificial gaseous atmosphere of known composition, total CCO\(_2\) (or Pco\(_2\)) along the radial distance \(r\) (millimeters) will be determined by the CO\(_2\) concentration present in the tissue before ischemia, \(C_{CO_2}\), and by the concentration of CO\(_2\) added after the onset of metabolic acidosis, \(C^*_{CO_2}\):

\[
CCO_2 = C^*_{CO_2} + CCO_2
\]

\(C^*_{CO_2}\) is determined by CO\(_2\) production \(Q_{CO_2}(t)\) and CO\(_2\) diffusion in the cylinder (diffusion coefficient \(D_{CO_2}\), 6.8×10\(^{-4}\) cm\(^2\)×min\(^{-1}\)). The relation between the change of CCO\(_2\)(r,t) or Pco\(_2\)(r,t) at any site along the radial distance \(r\) (radial symmetry) and at any time \(t\) is given by the differential equation for cylindrical diffusion

\[
\frac{\partial}{\partial t} C^*_{CO_2}(r,t) = D_{CO_2} \frac{1}{r} \frac{\partial}{\partial r} \left( \frac{\partial}{\partial r} \right) C^*_{CO_2}(r,t) + Q_{CO_2}(t)
\]

with the initial and boundary conditions

\(C^*_{CO_2}(r,0) = 0\)

and

\(C^*_{CO_2}(r=R,t) = 0\)

where \(R\) (millimeters) is the radius of the cylindrical muscle and

\[
P_{CO_2} = \frac{1}{\alpha_{CO_2}} C_{CO_2}
\]

**Hypothetical relation between accumulation and diffusion of CO\(_2\) and extracellular K\(^+\) accumulation in ischemia.** As mentioned in the introduction, an increase of Pco\(_2\) at a diffusion boundary during ischemia, by externally applied CO\(_2\), has a significant effect on \(K^+\) accumulation in ischemia.\(^9\) The hypothesis to test theoretically and experimentally was whether local Pco\(_2\) that is, the intrinsic CO\(_2\) accumulation in the ischemic tissue, would affect local \(K^+\) accumulation. The simplest hypothetical relation is a direct influence of Pco\(_2\) on \([K^+]_o\) during ischemia; that is, \(P_{CO_2} - [K^+]_o\). Qualitatively, such a
relation is in accordance with the two experimental observations available at present: 1) when major diffusional components are absent (midmural layers of pig or dog ventricles), the curves relating \( \text{PCO}_2 \) and \([K^+]_o\) to time (during reversible ischemia) are of similar shapes, and 2) during ischemia of subepicardial muscle layers, \([K^+]_o\) can be changed by concordant changes in PCO\(_2\). For the present model, this simple linear relation yields a proportionality between the first time derivatives of PCO\(_2\) (or CCO\(_2\) and \([K^+]_o\)). This can be expressed as

\[
QK(r,t) = q \frac{\partial}{\partial t} \text{CCO}_2(r,t) \tag{5}
\]

where \(QK\) is the net \(K^+\) transfer rate (millimolar per minute), \(q\) is an arbitrary scaling factor (see “Discussion”), and \(\text{CCO}_2(r,t)\) is the local CO\(_2\) concentration given by the diffusion equation for CO\(_2\). \(CK\) at the surface of the cylindrical muscle equals the \([K^+]\) present in the extracellular space before ischemia (\(CK\) is constant) plus the \([K^+]_o\) added during ischemia \((CK(R,t))\). \(CK(R,t)\) is obtained by solving the differential equation for cylindrical diffusion of \(K^+\) (diffusion coefficient \(D_k\), \(7.38 \times 10^{-4}\) cm\(^2\)x min\(^{-1}\))

\[
\frac{\partial}{\partial t} CK(r,t) = DK \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial}{\partial r} \right) CK(r,t) + q \frac{\partial}{\partial t} \text{CCO}_2(r,t) \tag{6}
\]

with the boundary conditions

\[
CK(r,0) = 0
\]

and

\[
\frac{\partial}{\partial r} CK(r=R,t) = 0
\]

The second condition is explained by the fact that the surface of the muscle represents a diffusion limit for \(K^+\) (in contrast to the volatile CO\(_2\), where CCO\(_2\)=0 at \(r=R\)). It is important to note that the assumed relation between PCO\(_2\) and \([K^+]\), does not implicate a direct causal relation between the two parameters. In addition, eventual hydration of CO\(_2\) (secondary acidification) was neglected in this model (see “Discussion”).

The numerical solutions of the equations were calculated on a personal computer (MacIntosh IIcx) by using a program written in TURBO-PASCAL and transcribed to a mathematical package (Mathematica, Wolfram Research, Champaign, Ill.). The program used a box model with a stepwise integration procedure for solving Equations 4 and 6. The box was divided into radial compartments \(dr=R/15\), and integration steps of \(dt=(dr)^3D_{(CO_2,K)}^{-1}0.1\) were taken for the time domain.\(^{20}\)

\(CO_2\) and \(K^+\) Diffusion and Accumulation in Ischemic Tissue: Comparison of Simulated and Experimental Results

Simulation of PCO\(_2\) and \([K^+]_o\) in ischemic papillary muscles. The profiles of simulated PCO\(_2\) along the radial distance \(r\) are shown in Figure 3 by means of three-dimensional plots for two cylinders of different radius (R). There is little accumulation in the cylinder with a radius of 0.5 mm (upper panel). With a
dependence of sectional minutes, radius coronary flow. After large cylinders on 414 Circulation Research values of at the surface of muscle cylinders with radius R varying from 0.3 to 1.5 mm. Lower panel: Simulated dependence of [K+]o at the surface of the cylinder on cross-sectional area or fiber radius 5 and 12 minutes after arrest of coronary flow. After 5 minutes, there is only a relatively flat dependence of [K+]o on cross-sectional area >2 mm²; after 12 minutes, there is an almost linear and steep dependence of [K+]o on cross-sectional area.

PCO₂ at the surface of the cylinder of 65 mm Hg, maximal values of 95 mm Hg are obtained in the core (r=0) 5 minutes after the onset of simulated CO₂ production. The maximal gradients along the radial distance r are 30 mm Hg after 5 minutes and decrease subsequently. After 11 minutes, the gradients are very small (<15 mm Hg). The simulation for a cylinder with a large R (1.5 mm) is shown in the lower panel of Figure 3. The maximal PCO₂ in the core of the cylinder is obtained after 10 minutes and amounts to 244 mm Hg; the corresponding gradient along the radial distance r is 179 mm Hg. The subsequent decrease of PCO₂ is small. After 15 minutes, PCO₂ in the core of the cylinder is still 205 mm Hg. Comparison of the diffusion profiles of the small versus large cylinders shows that the differences in PCO₂ along the radial distances r are relatively small during the initial minutes of ischemia and become large after 10 minutes. In experimental ischemia, because secondary accumulation of extracellular K⁺ occurs after −15 minutes, the simulations were not extended over this period.

**Figure 4.** Upper panel: Simulated [K+]o between 0 and 15 minutes of ischemia at the surface of muscle cylinders with radius R varying from 0.3 to 1.5 mm. Lower panel: Simulated dependence of [K+]o on the surface of the cylinder on cross-sectional area or fiber radius 5 and 12 minutes after arrest of coronary flow. After 5 minutes, there is only a relatively flat dependence of [K+]o on cross-sectional area >2 mm²; after 12 minutes, there is an almost linear and steep dependence of [K+]o on cross-sectional area.

For the simulation of the diffusion profiles of [K+]o (Figure 3), the scale for [K+]o was selected in such a way that the maximal values of [K+]o obtained in the large cylinder (lower panel) reached 18 mM at the surface of the cylinder. This was close to the maximal values of [K+]o measured in guinea pig hearts or in the present experiments after application of a lactic acid transport inhibitor (see Figure 10). This was made by adjusting the value of q (a pure scaling factor). Moreover, the initial [K+]o value was chosen to be the same as in the experimental study (4.5 mM).

Two main observations can be made from the diffusion profiles shown in Figure 3. First, for any given diameter, the [K+]o profiles are relatively flat along the radial distance r. In the cylinder with the large R (≥1.5 mm), the increase of [K+]o becomes faster and larger in the core than at the surface (difference of 3 mM after 10 minutes). Second, the time course of surface [K+]o is largely dependent on the cylinder radius R. In small cylinders the maximal increase of K⁺ is achieved relatively early (7 minutes versus 10 minutes in the large cylinder) and is about three times less than in the large cylinder (7 versus 21 mM).

One of the main purposes of this simulation was to calculate K⁺ accumulation on the muscle surface. This is shown in Figure 4 (upper panel) for muscles with a radius R ranging from 0.3 to 1.5 mm in increments of 0.1 mm. In the small muscles, there is an initial small increase of [K+]o during the period when production of CO₂ (and hypothetically coupled to net cellular K⁺ loss) is not compensated by diffu-
sion. In the large muscles, the longer radial distance favors accumulation of CO₂ and K⁺ in the core of the muscle. Consequently, a higher surface [K⁺]₀ is observed after 5–10 minutes of simulated production. In the lower panel of Figure 4, the [K⁺]₀ at the cylinder surface is plotted versus radius and cross-sectional area of the muscle at 5 and 12 minutes of ischemia. At 5 minutes (and similarly at 2, 3, and 4 minutes), there is a relatively flat relation between [K⁺]₀ and R beyond areas of 2 mm². This reflects the fact that the rate of CO₂ production, QCO₂, dominates over the rate of CO₂ diffusion. Later, during simulated ischemia (i.e., between 10 and 12 minutes), the [K⁺]₀ at the surface is almost linearly and steeply related to cross-sectional area. This reflects the fact that, during this period, QCO₂ is relatively small and radial CO₂ diffusion dominates over production.

Experimental determination of [K⁺]₀: Dependence of surface [K⁺]₀ on radius R and heart rate. The effect of muscle diameter on [K⁺]₀ is shown in Figure 5 for four different experiments. These experiments were matched as closely as possible for stimulation rate (500–550 msec). [K⁺]₀ increased initially in muscles with a diameter of 1 mm or below to values of only 7–8 mM; these values are similar to those obtained for the simulated profiles. Subsequently, a secondary decrease of [K⁺]₀ was observed. In muscles with a large diameter, the [K⁺]₀ followed more closely the time course described for large hearts; that is, there was a steady increase to a so-called “plateau phase,” which amounted to 15–20 mM. In the largest papillary muscles, no secondary decrease of [K⁺]₀ after 10–12 minutes was observed. The [K⁺]₀ after 10 minutes of ischemia amounted to 2.2 mM in the smallest and 14.1 mM in the largest muscles. This showed that after 10 minutes at least 80% of [K⁺]₀ accumulation was dependent on fiber geometry. This dependence on geometry suggests an association between [K⁺]₀ accumulation and diffusion of an ischemic metabolite that, in the present experimental conditions, was able to diffuse into the gaseous atmosphere surrounding the preparation.

The majority of the papillary muscle preparations were spontaneously active. Therefore, it was not possible to overdrive all preparations at the same rate (unless a rapid rate had been chosen), and the effect of heart rate on [K⁺]₀ had to be considered in addition to the effect of muscle diameter. The interdependence among [K⁺]₀, muscle cross-sectional area, and heart rate at 5 and 12 minutes of ischemia (i.e., at the times given for the simulations shown in Figure 4) is depicted in Figure 6 for all experiments. There was a distinct difference between the effect of heart rate and the effect of cross-sectional area on
A linear dependence of \([K^+]_o\) on heart rate was noted at 3 minutes \((p=0.10)\) and reached statistical significance between 4 and 6 minutes \((p=0.05)\). After 6 minutes, the dependence of \([K^+]_o\) on heart rate disappeared. A significant (linear) dependence on muscle cross-sectional area was observed after 3 minutes and throughout the measuring period (15 minutes). The main difference between the simulated and experimental results was that the increase of experimentally determined \([K^+]_o\) with cross-sectional area was more pronounced (see “Discussion”).

**Experimental determination of \([K^+]_o\): Differences between changes of surface \([K^+]_o\) on or close to the ventricular wall and on the right ventricular papillary muscle.** The plane parts of surface of the interventricular septum can be considered as a surface of a cylinder with infinite \(R\). Therefore, the relation of \(CO_2\) and \(K^+\) accumulation predicts a faster and larger increase of \([K^+]_o\) on the surface of the interventricular septum than of a papillary muscle within the same preparation. Figure 7 (upper panel) illustrates the result from a single experiment in which the \(K^+\)-sensitive and reference electrodes were placed on the papillary muscle’s surface (diameter, 1 mm) during the first and third and on the surface of the interventricular septum during the second of three successive short test occlusions (duration, 2 minutes; interval between occlusions, 30 minutes; distance between measuring sites, ~5 mm). In this and two other experiments the increase of surface \([K^+]_o\) on the ventricular septum was much faster than on the papillary muscle, the difference in \([K^+]_o\) amounting to 1.8–2.3 mM after 2 minutes. Moreover, the increase in \([K^+]_o\) was slightly smaller on the papillary muscle during the third occlusion. Figure 7 (lower panel) shows results of a similar type of experiment (mean±SEM from five muscles; cycle length, 480±16 msec; diameter, 1.3±0.2 mm; 54±5 mm Hg) in which \([K^+]_o\) was determined simultaneously at two different locations along the axis of the papillary muscle. One pair of electrodes was located within the middle or distal third of the papillary muscle (open circles), and the other pair of electrodes was immediately adjacent to the interventricular septum (closed circles). As predicted from the larger accumulation in the septum and \(K^+\) diffusion into the base of the papillary muscle, there was a larger increase of \([K^+]_o\) at the base during ischemia.

**Experimental determination of \([K^+]_o\): Difference between \([K^+]_o\) along the radial distance \(r\).** The dependence of \([K^+]_o\) in ischemia on local \(PCO_2\) predicts small \(K^+\) gradients between the core and the surface in cylinders of sufficient radius \(R\) (≥0.75 mm, Figure 2). In two muscles with \(R=0.75\) mm it was possible to introduce small (diameter, 0.1 mm) ion-sensitive and reference electrodes without detectable microscopic muscular or vascular injury. The results of a single experiment are shown in Figure 8. In this experiment, the intramyocardial electrode was placed at a distance of 300 \(\mu\)m from surface (or 450 \(\mu\)m from the core). At any instant during the 10-minute occlusion, the \([K^+]_o\) at the measuring site closer to the core exceeded the \([K^+]_o\) on the surface, the difference amounting to 0.9–1.1 mM after 7 minutes of ischemia. An almost identical result was obtained in a second experiment.

**Effects of Interventions Changing \(pH_0\) and \(pH_0\) on \([K^+]_o\): Accumulation in Ischemia.**

Effect of changes of \(PCO_2\) in the surrounding atmosphere. The results shown in Figures 5–8 suggest that \([K^+]_o\) accumulation in ischemia is dependent on a diffusible and volatile product that has production kinetics qualitatively similar to anaerobic metabolic
rate. This observation makes CO\textsubscript{2} the most likely candidate. In a previous report we have shown that changing PCO\textsubscript{2} before or during ischemia modifies intracellular acidification (pH\textsubscript{r}). Therefore, in an independent series of experiments, the identical interventions were carried out to test whether the CO\textsubscript{2} effect on K\textsuperscript{+} accumulation might eventually be explained by an effect of intracellular metabolic acidosis on net K\textsuperscript{+} efflux.

In six experiments (Figure 9, upper panel), PCO\textsubscript{2} in the surrounding atmosphere was increased constantly between 4 and 12 minutes of ischemia from 57±3 to 267±9 mm Hg (10 minutes) and 318±4 mm Hg (12 minutes) to mimic accumulation in ischemia.\textsuperscript{12} The mean values measured in these experiments are superimposed in six control experiments matched for muscle diameter and beating rate. The small diameters were selected to ensure that PCO\textsubscript{2} along r was mainly determined by diffusion, that is, by PCO\textsubscript{2} in the atmosphere (see Figure 2). At 5 minutes of ischemia, developed isometric tension had become minimal; therefore, the delayed and ramp-type increase of PCO\textsubscript{2} caused a decrease in both pH\textsubscript{r} and pH\textsubscript{i} that was not affected by the direct effect of CO\textsubscript{2} on mechanical tension. Figure 9 (upper panel) shows that increasing PCO\textsubscript{2} in the surrounding atmosphere of the recording chamber produced a significant increase of [K\textsuperscript{+}]\textsubscript{i} from control levels of 9–11 mM to 13–15 mM between 10 and 15 minutes of ischemia.

In another series of six experiments (Figure 9, lower panel), [K\textsuperscript{+}]\textsubscript{i} accumulation was measured during ischemia in the presence of an increased PCO\textsubscript{2} (56±1 versus 24±2 mm Hg). This condition has been shown to decrease pH\textsubscript{i} before ischemia from 7.03 to 6.96 and during ischemia (18 minutes) from 6.84 to 6.64.\textsuperscript{13} The test and control series were matched with regard to heart rate and muscle diameter. As for the experiments shown in Figure 9 (upper panel), only muscles with small diameters were selected to minimize diffusional gradients of pH\textsubscript{r}, PCO\textsubscript{2}, and [HCO\textsubscript{3}\textsuperscript{-}]\textsubscript{i}. Accumulation of K\textsuperscript{+} was significantly less in the muscles with a higher initial pH\textsubscript{i} and a lower PCO\textsubscript{2} than in control.

In both series of experiments illustrated in Figure 9, pH\textsubscript{i} was determined simultaneously by means of an H\textsuperscript{+}-sensitive electrode. The changes in pH\textsubscript{i} and the calculated changes in [HCO\textsubscript{3}\textsuperscript{-}] (not shown) were very similar to those obtained by Yan and Kléber (unpublished results).

[FIGURE 8. Differences between simultaneous measurements of [K\textsuperscript{+}]\textsubscript{i} during ischemia by a pair of surface electrodes (open circles) and a pair of intramural electrodes (closed circles). As shown in the inset, the intramural electrodes were located 300 \mu m from the surface.]

[FIGURE 9. Upper panel: Comparison of the effect of a continuous increase of PCO\textsubscript{2} in the atmosphere surrounding the muscle cylinder on [K\textsuperscript{+}]\textsubscript{i} during ischemia (filled circles) with [K\textsuperscript{+}]\textsubscript{i}, measured in an ischemic control group (open circles). The two groups were matched with regard to fiber diameter (0.97±0.12 vs. 0.99±0.09 mm in control) and cycle length (500 msec). PCO\textsubscript{2} was increased from 57±3 mm Hg after 4 minutes to 318±4 mm Hg after 12 minutes and kept constant at 56±0.0 mm Hg in the control series. The increase of PCO\textsubscript{2} produced a significant increase of [K\textsuperscript{+}]\textsubscript{i}, (p<0.05 after 8 minutes). Lower panel: Comparison of the effect of different PCO\textsubscript{2} surrounding the muscle cylinder on [K\textsuperscript{+}]\textsubscript{i} during ischemia. Filled circles: PCO\textsubscript{2}, 24±2 mm Hg; initial pH\textsubscript{i}, 7.6. Open circles: PCO\textsubscript{2}, 56±1 mm Hg; initial pH\textsubscript{i}, 7.2. Both groups were matched with regard to cycle length (500 msec) and muscle diameter (1.01±0.08 vs. 0.99±0.09 mm in control). The less pronounced [K\textsuperscript{+}]\textsubscript{i} accumulation with initial alkalosis was statistically significant between 6 and 10 minutes (p=0.002). The small initial difference in [K\textsuperscript{+}]\textsubscript{i} was also statistically significant (p=0.001) and was attributed to alkalosis.]
possibility of a relation between intracellular metabolic acidosis and cellular K⁺ loss in ischemia was tested by the application of α-cyanocinnamate (2-4 mM), a blocker of lactic acid transport. The figure 10 shows the effect of α-cyanocinnamate on [K⁺], during ischemia in seven muscles. The experiments were matched with regard to heart rate and muscle diameter. In control, the mean [K⁺] reached after 10 minutes was 9.8±1.1 mM and 10.2±1.2 mM after 15 minutes. In the presence of α-cyanocinnamate, [K⁺], was 16.1±3.4 mM after 10 minutes and 18.9±2.7 mM after 15 minutes, that is, 6-8 mM higher than control (the differences were statistically significant [p=0.017] after 10 minutes). The presence of α-cyanocinnamate had no significant effect on [K⁺], during normal perfusion.

**Discussion**

**Comparison of Computer Simulations and Experiments**

In essence, the simulation was based on four parameters: 1) fiber radius, 2) diffusion coefficients, 3) CO₂ production, QCO₂, and 4) coupling between local PCO₂ and K⁺ efflux. Whereas the first two parameters are relatively well defined in the experimental setting, the values taken for the others are more speculative. QCO₂ was taken from differentiation of the CO₂ accumulation curve measured by Case. The values given for PCO₂ accumulation in literature are subject to considerable variability, and it cannot be excluded that the values taken for QCO₂ represent an underestimation. This is because diffusion of CO₂ might have influenced the measurements on which the values taken for the simulations were based. Qualitatively there is close agreement about initial acceleration and subsequent inhibition of anaerobic glycolysis, with corresponding changes of lactate, [L⁻], and PCO₂ in no-flow ischemia, however. In the model, two simple assumptions were made in the absence of experimental data. The first concerns the dependence of QCO₂ on the radial distance r. Inhibition of anaerobic glycolysis (after initial acceleration) has been described in no-flow ischemia by both accumulation of L⁻ and a decrease of pH. In contrast, the inhibitory phase is absent in hypoxia, because the pH changes and lactate accumulation are to a major part prevented by maintained perfusion. In the present experimental model, some intermediate degree of inhibition might be postulated for those tissue compartments along the radial distance r where CO₂ diffusion prevails over accumulation, and therefore, the pH changes are smaller than those toward the core of the cylinder. Moreover, net local CO₂ transfer from the extracellular to the intracellular compartment might be modulated by secondary hydration of CO₂ (secondary acidosis). These potential variabilities, which would implicate a dependence of QCO₂ on r, were not considered in our model. The second assumption concerns the purely hypothetical coupling between QCO₂ and net K⁺ efflux. The linear relation that was taken for the simulation certainly represents the simplest possibility. Especially, it does not account for the other components contributing to cellular ischemic K⁺ loss (see below).

Despite the relative simplicity of the model, there was a clear qualitative accordance between the simulated and experimental results that allows the following conclusion: extracellular K⁺ accumulation in ischemia is modulated to a significant extent by a process that is (directly or indirectly) determined by a diffusible component of ischemic metabolism. Three arguments suggest that this component is CO₂: 1) The rate of production of this component is proportional to the rate of anaerobic glycolysis; that is, it shows an initial phase of acceleration and a subsequent decrease; 2) the radial dependence of cellular ischemic K⁺ loss in the present setup (where the fibers were surrounded by a gaseous atmosphere) indicates that the metabolite is volatile; and 3) an increase of PCO₂ in the gaseous atmosphere surrounding the diffusion boundary increased [K⁺], during ischemia. This confirms earlier findings in ischemic guinea pig hearts in which externally applied CO₂ modified [K⁺]. It is important to mention that both the simulated and experimental results do not implicate a causal relation between CO₂ and [K⁺] in ischemia. Accumulation of CO₂ affects pH, and pH₀ is and is likely to affect transmembrane transfer of H⁺ and/or anions (see previous paragraph).

The dependency of [K⁺], on heart rate in ischemia has been a matter of controversy in earlier studies. In our experiments, the dependency of [K⁺], on rate was confined only to the very early stage of ischemia (first 4-6 minutes after arrest of coronary flow). A likely explanation for the effect of heart rate on [K⁺], can be given on the basis of the simulation linking PCO₂ to net [K⁺] efflux: it is reasonable to assume that glycolytic flow in the first few minutes of ischemia is accelerated at increased heart rate (although no experimental data are available). This initial acceleration is likely to produce an earlier
inhibition of glycolysis by accumulating metabolic products. In the present simulation model, these changes would be expressed by an increase of the coefficients a and b [leftward shift of the QCO2(t) function in Figure 2, lower panel], with either a relatively small change or no change of the time integral of QCO2. This is predicted to produce the experimentally observed variability of [K']o, that is, a rate-dependent variability at the beginning of ischemia (when the rate of CO2 formation is higher than the rate of CO2 diffusion) and an unchanged [K'], after ~6 minutes (when the rate of CO2 diffusion is higher than the rate of CO2 formation). Therefore, these theoretical considerations fit with the experimental observation illustrated in Figures 6A and 6B, and they are an additional (albeit indirect) argument in favor of a phenomenological relation between net K+ efflux, glycolytic rate, and CO2 accumulation in early ischemia. Moreover, they are in agreement with the experimental observations made by Weiss and Shine,30 which indicate that heart rate influences the steepness and the time of onset but not the plateau level of [K'] in ischemic rabbit myocardium.

CO2 Diffusion and Inhomogeneity of K+ in Regional Ischemia

Local inhomogeneities in [K']o in ischemic tissue have been implicated to be responsible for the local electrical inhomogeneities and the formation of unidirectional conduction block.5 The unidirectional block of propagation is one of the critical electrophysiological changes setting the conditions for circulating excitation with reentry to occur. In our experiments accumulation and diffusion of CO2 produced changes in [K']o among recording sites on the interventricular septum, on papillary muscles of variable diameter, and within papillary muscles along the radial distance. This offers an explanation for the finding of local inhomogeneities in [K']o.14 In regional ischemia, diffusion boundaries for CO2 will be present in the ischemic border zone, at epicardial and endocardial sites, and close to larger vessels.

The effect of local PCO2 on electrophysiological parameters may be complex. One obvious effect relates the changes in [K']o to the changes in resting membrane potential (Vm).4 In small ischemic papillary muscles, only moderate shifts in Vm were recorded in ischemia with an initial depolarization and a subsequent hyperpolarization,7 whereas depolarizations of as much as 30 mV were observed on (plane) surfaces in guinea pig hearts (in which Vm approached the equilibrium potential for K+ a few minutes after arrest of coronary occlusion).4 This corresponds to the CO2-dependent [K']o changes observed in our experiments. Because membrane excitability in ischemia in vivo and in so-called "simulated" ischemia has been shown to be extremely sensitive to minor changes of Vm and [K']o (on the order 1 mM),31 the diffusion gradients of CO2 may play an important role. Recently, several articles appeared in which models of simulated ischemia were used for the analysis of ionic changes ("air" model32 and "silicon oil" model33,34). In all these models there was a relatively modest depolarization with a preserved amplitude but a marked shortening of the action potential; that is, the electrical changes resembled more closely the classical changes seen in hypoxia35 than in real in vivo ischemia.36 Because CO2 is allowed to diffuse freely in these models, part of the different findings might have been explained by the absence of the effect of CO2 accumulation.

Additional effects of CO2 and the associated decrease of pH and pHi may include pH-dependent depression of membrane excitability37 and eventual effects of pH and CO2 on electrical cell-to-cell uncoupling.38 A simultaneous assessment of propagation velocity with electrical cable properties will be needed to separate the eventual contributions of these components.

Relation Between CO2 and K+ Accumulation: Possible Mechanisms

The present results show that a large fraction of [K']o, accumulating during the initial phase of myocardial ischemia (Figure 5) is sensitive to the local PCO2. Whether all or part of the remaining [K']o (approximately 2–2.5 mM after 10 minutes) is due to cellular K+ loss of other origin or to extracellular volume changes cannot be concluded from our experiments. Theoretically, three mechanisms may explain the observed relation between CO2 and [K']o in ischemia.

Direct effect of CO2. CO2 is known to bind amino groups of intracellular proteins (e.g., hemoglobin40), but no direct effects on membrane channel function or ionic transport have been demonstrated. On the basis of our observations that both changes of PCO2 and inhibition of lactic acid transport affect [K']o, in ischemia, an indirect effect of CO2 on cellular K+ loss seems more likely.

Decrease in pH. Both increasing PCO2 before or during ischemia13 and inhibition of lactic acid transport34 lead to an increased intracellular acidification. Thus, it may be speculated whether net cellular K+ loss in ischemia was a direct or indirect (via cytosolic Ca2+ release41) consequence of the decrease in pH. Theoretically, such an effect may be mediated through a variety of mechanisms such as an effect on Na+-K+ pumping42. Ca2+-dependent increase of inward current with secondary redistribution of K+43 formation of arachidonic acid (with eventual effects on K+ channels44), ATP-dependent K+ channels,45 and secondary effects of pH-dependent increase of amphiphilic metabolites.46

Formation and intracellular retention of metabolic acid. Two mechanisms have been proposed to relate the increased intracellular level of lactic acid to depolarization and (secondary) net K+ extrusion: 1) a decrease of fixed negative charges on the intracellular proteins (in a closed system such as ischemia, this is predicted to cause a redistribution of mobile anions and cations between the extracellular and intracellular space)47; and 2) an increase of the
transmembrane electrochemical gradient for L\(^-\) with a consequent electrogenic efflux or transfer of K\(^+\) and a secondary redistribution of K\(^+\).\(^{48}\) The difference between the two mechanisms is an unspecific transmembrane movement of permeable anions in the first and an L\(^-\) movement in the second.

An important experimental observation made with both externally applied CO\(_2\)\(^-\) and diffusion of CO\(_2\) formed by ischemic metabolism (Figure 5) was that the first time derivative of [K\(^+\)], (as an approximate measure of ischemic net K\(^+\) efflux) was related to the rate of CO\(_2\) production, QCO\(_2\), and not to the absolute level of PCO\(_2\). This relation was used in the model to simulate CO\(_2\)-dependent accumulation of extracellular K\(^+\). This dependence is likely to speak against a direct relation between the level of pH\(_i\) and net K\(^+\) efflux and to favor the hypothesis of a relation between electrogenic extrusion of anions formed in metabolic acidosis and net K\(^+\) efflux: 1) Comparison of the time course of pH\(_i\) with [K\(^+\)], during ischemia (measured under identical experimental conditions) shows that the rate of [K\(^+\)], accumulation is high during the development of intracellular acidosis and low ("plateau phase" of extracellular K\(^+\) accumulation\(^{2}\)) when pH\(_i\) is significantly decreased. 2) Intracellular [L\(^-\)] in ischemia will be the result of both L\(^-\) production and L\(^-\) extrusion. Total L\(^-\) extrusion appears to be the sum of electroneutral (LH) transport and (to a smaller extent) transfer of L\(^-\). The mechanism of the latter has not been entirely clarified yet. Application of protein modifiers such as mersalyl blocks total net L\(^-\) uptake almost completely, indicating that both components are likely to be carrier mediated.\(^{49}\) LH transport is a function of pH\(_i\), pH\(_m\), and extracellular buffering power.\(^{50,51}\) It occurs mainly when pH\(_i\) is high and decreases at low pH\(_i\). Also, at a given pH\(_i\), LH transport decreases with decreasing extracellular buffering power. The transmembrane electrochemical L\(^-\) gradient, in turn, will be built up by intracellular accumulation of L\(^-\). Thus, any process that will accelerate intracellular L\(^-\) production (e.g., heart rate) and/or diminish LH transport (decrease of pH\(_i\) or extracellular buffering power) will favor electrogenic L\(^-\) efflux and concomitant K\(^+\) loss. In the case of a carbonic buffer system, the extracellular buffering power will be markedly lowered (and extracellular acidification accelerated) if the CO\(_2\) accumulates. In contrast, it will remain relatively high if the PCO\(_2\) changes little or remains constant. Thus, interference of CO\(_2\) with extrusion of LH and formation of an electrochemical L\(^-\) gradient will be mainly related to QCO\(_2\) and less to the absolute level of PCO\(_2\).

Interestingly, our findings for ischemic cardiac muscle are very similar to previous findings obtained for metabolic acidosis of skeletal muscle: decreasing pH\(_i\) was associated with a decrease in LH extrusion and a concomitant increase in net K\(^+\) efflux.\(^{46}\) Moreover, a diameter dependence of L\(^-\) efflux was observed in these preparations.\(^{48}\) Also, the above hypothesis implies a dependence of L\(^-\) and K\(^+\) efflux on membrane potential, V\(_m\). This has been shown for L\(^-\) efflux in skeletal muscle\(^{37}\) and for K\(^+\) efflux in the ischemic rabbit interventricular septum\(^3\) (depolarization with elevated [K\(^+\)]\(_i\)).

Several aspects inherent to the complex pathophysiological situation in myocardial ischemia will make it difficult to reach final conclusive evidence for one of the proposed mechanisms: 1) An eventual relation between transmembrane L\(^-\) and K\(^+\) movement does not predict a constant stoichiometric ratio of total outward movement of L\(^-\) and K\(^+\).\(^{11}\) This is because the contribution of the different pathways to total L\(^-\) efflux is predicted to vary during ischemia with pH\(_i\) and V\(_m\). 2) The change in [K\(^+\)]\(_i\) during ischemia is affected by an osmotic change of the extracellular compartment.\(^{39}\) 3) Different perfusion techniques affect the time course and the extent of intracellular acidification and the presence of intracellular L\(^-\) before ischemia (glibenclamide,\(^{52}\) see below) is expected to modify the rate of metabolic acid production.\(^{29}\) 4) Blockers of LH transport are not entirely specific and affect mitochondrial pyruvate and LH transport.\(^{27}\) At present it seems difficult to account for all these variables in the same experimental setting.

The observation that extracellular K\(^+\) accumulation in ischemia can be inhibited by application of blockers of tetraethylammonium- and ATP-sensitive K\(^+\) channels\(^{10,11,51}\) is not necessarily in contradiction to our experimental observations. Evidence for an involvement of ATP-sensitive K\(^+\) channels in cellular ischemic K\(^+\) loss was derived from the finding that application of sulfonylurea during ischemia lengthens the action potential (membrane potential effect) and inhibits extracellular accumulation of K\(^+\) (flux effect) to a variable degree.\(^{53}\) In addition, the flux effect was directly related to production of L\(^-\) before ischemia.\(^{53}\) The membrane potential effect of sulfonylurea in ischemia, in the presence of a variety of metabolites with adverse effects on the ATP-sensitive channels (only moderate ATP depletion,\(^{54}\) ADP accumulation,\(^{55}\) and Mg\(^{2+}\) accumulation\(^{56,57}\)) has been explained by the fact that only a very few channel openings per cell can induce repolarization. This is because of the very low membrane conductance during the plateau of the cardiac action potential.\(^{58}\) In our opinion, the mechanism of the flux effect has not been entirely clarified yet. One hypothesis can explain the effects of both CO\(_2\) and sulfonylurea on cellular ischemic K\(^+\) loss by the possibility that it is due primarily to a pH\(_i\)-sensitive opening of the ATP-sensitive K\(^+\) channels. A second hypothesis could involve the metabolic effect of sulfonylurea (L\(^-\) accumulation before ischemia up to 10 mM). This will reduce intracellular L\(^-\) formation during the subsequent ischemic period, thereby reducing associated K\(^+\) efflux, as discussed above. In simulated ischemia (combination of moderate hypoxia with restriction of extracellular medium) the very early increase of [K\(^+\)]\(_i\) (first 4 minutes of simulated ischemia) was inhibited by large concentrations of tolbutamide (1 mM) and
not influenced by application of inhibitors of LH or anion transfer. The reason for the difference between our findings and these experiments is not entirely clear. Because sulfonylurea abolished the decrease in mechanical tension development in simulated ischemia entirely, the metabolic side effect of these drugs, in addition to the effect on ATP-sensitive K⁺ channels, must be considered. Alternatively, one might argue that the association of cellular ischemic K⁺ loss with CO₂ accumulation, which is highly significant during the plateau phase of acute ischemia (at ~8–10 minutes) is due to a different mechanism. This would also explain the lack of an effect of lactic acid transport inhibition in the first 5 minutes of simulated ischemia.

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