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The Effect of “Ischemic” Blood on Transmembrane Potentials of Normal Porcine Ventricular Myocardium

EUGENE DOWNAR, M.D., MICHEL J. JANSE, M.D., AND DIRK DURRER, M.D.

SUMMARY “Ischemic” blood was obtained in pigs from a local coronary vein on release of coronary artery occlusion. The effects of this blood on transmembrane potentials of muscle strips taken from the same heart were compared with control blood. Whereas action potentials remained stable in control blood, ischemic blood collected after more than 15 minutes of coronary occlusion produced shortening of action potential duration, reduction of resting potential, upstroke velocity and amplitude, and finally unresponsiveness. Ischemic blood collected after shorter periods of coronary occlusion produced only mild effects (shortening of action potential and postrepolarization refractoriness).

These effects of ischemic blood could not be attributed to increased potassium concentration even in combination with acidosis, hypoxia and hypoglycemia. It appears that during ischemia unidentified factors are released which have potent depressant effects on the excitability of even normal myocardium.

THERE HAVE BEEN MANY REPORTS on the nature of biochemical changes in coronary venous blood which occur in response to myocardial ischemia.1,2 Changes in coronary venous potassium levels and their possible relationship to ventricular arrhythmias have attracted particular attention3-11 since Harris first implicated potassium as a major excitant of ventricular arrhythmias in myocardial ischemia.12 Despite such interest, both the mode of onset of early ventricular arrhythmias and their relationship to altered biochemical parameters remain matters for conjecture. This situation exists in part because there is no satisfactory model which enables study of ischemic changes in the intracellular electrophysiology of ventricular myocardium.

In an attempt to gain insight into the possible nature of such intracellular changes and their relationship to some of the altered biochemical parameters produced by ischemia, we examined the effects of coronary venous blood draining an ischemic region on action potentials recorded from an isolated strip of normal ventricular myocardium.

Methods

Thirty-two pigs weighing 20 to 25 kg were anesthetized by intravenous injection of sodium pentobarbital (20 mg/kg). Under artificial respiration the chest was opened by a midsternal incision and a pericardial cradle was constructed. A full-thickness piece of right ventricular wall from the outflow tract region was removed and the defect closed by a purse-string suture. The preparation was trimmed to about 0.5 cm² in area and 3 mm thickness. It was mounted endocardial side up in a 2 ml tissue bath superfused with running oxygenated modified Tyrode solution at a rate of 2 ml/min. The composition of the solution, in mEq/L, was: Na⁺ 156.5, K⁺ 4.7, Ca²⁺ 1.5, Mg²⁺ 0.7, H₂PO₄⁻ 0.5, HCO₃⁻ 28.0, Cl⁻ 137.0, and glucose 20 mM/L. Temperature was maintained constant at 37°C ± 0.5°C. The preparation was stimulated via a bipolar silver electrode, insulated except at the tips, at a basic cycle length of 700 msec, using rectangular current pulses with a duration of 2 msec and an intensity of

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From the Department of Cardiology and Clinical Physiology, University Hospital Wilhelmina Gasthuis, Amsterdam and the Interuniversity Cardiology Institute, The Netherlands.

Dr. Downar is a Senior Research Fellow of the Ontario Heart Foundation, Canada. His present address is Cardiovascular Unit, Toronto General Hospital, Toronto, Canada.

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2 × diastolic threshold strength. Premature stimuli of 4 × diastolic threshold strength could be applied after every eighth basic stimulus, at any desired interval with msec precision. Transmembrane potentials were recorded using conventional glass microelectrodes having a resistance of 10 to 20 megohm, and high impedance capacity-compensated amplifiers. The preparation was kept in running Tyrode solution for 30 minutes of equilibration before the next stage of the procedure. Meanwhile, the distal part of the great cardiac vein was cannulated with an 18 G polyethylene catheter. Ligatures were placed around the left anterior descending coronary artery, distal to the first diagonal branch. A 2 ml sample of coronary venous blood was taken from the catheter, using a heparinized syringe, and was introduced into the tissue bath after removal of the Tyrode solution. In some cases a 4 ml sample was collected, half of which was used for biochemical analysis. The blood remained stagnant in the bath up to 20 minutes. To prevent evaporation, the surface was covered with a thin layer of paraffin oil. Action potentials remained stable within the first 15 minutes in incubation. Thereafter some shortening of action potential duration occurred, while the general configuration remained essentially unchanged. For these reasons, control action potentials were recorded in the first 12 minutes of incubation. Having established that action potentials from a given preparation remained stable during the control period, and after determining the refractory period duration with premature test stimuli, the blood was removed and the preparation maintained in running Tyrode solution for at least 15 minutes. During this period, coronary artery occlusion was applied for 5 to 40 minutes. After release of coronary artery occlusion, the first 2 ml of coronary venous blood which appeared were collected for the tissue bath. The first 4 ml were collected when biochemical analysis was planned. The time taken to collect these samples varied from 5 to 15 seconds, taking less when there was some residual flow from the coronary venous catheter during the period of occlusion.

Biochemical analysis included the determination of potassium and sodium concentrations by flame photometry, calcium and magnesium concentrations titrometrically with EDTA, glucose by O-toluidine method, lactic acid concentrations spectrophotometrically, pH with a glass electrode (Astrup), PO2 polarographically with a Clarke type electrode and PCO2 tonometrically. On several occasions the occurrence of ventricular fibrillation either during the period of coronary artery occlusion or on release necessitated defibrillation. In these instances no venous samples were taken until control circumstances were again established. Action potentials were recorded during the first 12 minutes of incubation in this blood, which we will refer to as "ischemic" blood. After this period of observation, the ischemic blood was removed and in most cases used for potassium determinations. The tissue was again superfused with running Tyrode solution until action potentials returned to control configuration. The preparation could then be used for further observations either during incubation in a second sample of ischemic blood, or in artificially abnormally coronary venous blood for comparison. Normal coronary venous blood was abnormally to simulate ischemic blood by raising the potassium concentration, adding lactic acid to produce acidosis and equilibrating with nitrogen to produce hypoxia.

Results

Effects on Action Potential Configuration and Refractory Period

Typical changes in action potential configuration produced by ischemic blood are shown in figure 1. These changes initially included a gradual shortening of action potential duration, a loss of upstroke velocity and the appearance of a notch in the upstroke suggesting the separation of the action potential into two components. Finally, only a small local response is left. Throughout these changes there is a gradual loss of resting membrane potential, until at levels of about -50 mV the tissue becomes virtually unresponsive. Unresponsiveness developed by a fairly gradual diminution of the action potential as described, or by a period of alternation of action potential amplitude leading to 2:1 responses to the basic stimulation which then faded out (fig. 2 top). At this stage large amplitude responses could be re-evoked for one or two beats by an increase in stimulation strength. Within a few beats, however, thresholds rose...
rapidly, leading finally to inexcitability (fig. 2 bottom). Removal of the ischemic blood followed by superfusion of the preparation with flowing Tyrode's solution always restored action potentials to control configuration. Five minutes of superfusion was usually sufficient to reverse unresponsiveness. On a few occasions a longer period was necessary to restore normal action potentials, but this never exceeded 20 minutes.

Estimates of refractory period duration were made throughout the period of incubation by using test stimuli of 4 × diastolic threshold intensity, applied within 2 to 3 mm from the recording site after every eighth basic stimulus, and determining the shortest interval at which the test stimulus elicited a response. In the initial stages of incubation with ischemic blood, the time course of recovery of excitability closely followed repolarization, and the refractory period shortened commensurate with the action potential duration. Subsequently, however, despite continued shortening of action potential duration, the refractory period began to prolong, resulting in the development of postpolarization refractoriness (fig. 3). The refractory period in some instances exceeded the control value and went on to be hundreds of msec longer than the action potential, even encroaching on the next basic stimulus at cycle lengths of 700 msec. In these final stages, the exact determination of refractory period duration became impossible because diastolic threshold level began rising rapidly to high values. As these changes in excitability developed there was a partial separation of the action potential into two components. This separation was exacerbated by the application of premature stimuli. As can be seen in figure 4, the premature stimulus evoked only a relatively fast, short spike. Its effect on the next basic response, however, was to prevent the occurrence of the second component, which only reappeared in the second basic stimulus. In other instances, a similar premature beat could prevent a response to the next basic stimulus altogether (fig. 4 bottom).

The Effect of Duration of Ischemia

The intensity of the effects described above could be related to the duration of the ischemic period. As shown in figure 5, ischemic blood collected after a period of occlusion of five minutes only produced shortening of the action potential and some degree of postpolarization refractoriness. Similar effects were seen when blood was taken from the

**FIGURE 3.** Postpolarization refractoriness (two different experiments). Left panel: Basic and earliest induced premature action potentials. In control blood, time course of recovery of excitability closely follows time course of repolarization (S1S2 200 msec). After six minutes of incubation in ischemic blood the action potential has shortened and the shortest successful S1S2 is 160 msec. After eight minutes, the test pulse given after 170 msec only gives rise to a small response, and after eight and one-half minutes the refractory period lags considerably behind completion of repolarization. Right panel: Graph from another experiment indicating the changes in action potential duration and refractory period (ordinate) during incubation in an ischemic blood sample (abscissa).

**FIGURE 4.** Effects of induced premature beats on subsequent basic beats in experiments A and B. Basic cycle length 700 msec. In A, after 10 minutes of incubation in ischemic blood, test stimulus evokes only a short, relatively fast spike, as does the next basic stimulus. The second component reappears only at the second basic stimulus. In B, after six minutes of incubation in ischemic blood, the test pulse results in a very small response and prevents the response to the next basic stimulus altogether.
same vein after 10 minutes of coronary artery occlusion, although these occurred after a shorter period of incubation. The preparation became virtually unresponsive after 7½ minutes of incubation in blood collected after a 15 minute period of occlusion, and the same effects were produced by blood sampled after 30 minutes of ischemia. Longer periods of up to one hour of occlusion did not produce more marked effects, suggesting that the factors responsible were released in the first 15 to 30 minutes of ischemia. Periods of occlusion of five minutes or so usually produced only the mild effects shown in figure 5. On occasion, however, five minutes of coronary artery occlusion produced more marked effects resulting in very short, low amplitude responses.

The depressant effect of ischemic blood was not confined to the first few milliliters to emerge on release of occlusion. In several instances a second sample of ischemic blood was collected two minutes after release, during the hyperemic phase, and compared with the initial sample. Such hyperemic samples were able to produce the same effects as the ischemic samples, but took longer.

### Altered Biochemical Parameters

Table 1 shows the results of biochemical determinations in 29 ischemic blood samples, all of which produced unresponsiveness. The numbers in brackets indicate corresponding values of nonischemic coronary venous blood. Because of the limited quantity available of each sample, it was not possible to determine more than a few parameters in each sample. Potassium was determined in every case because a sufficiently high concentration could account for the observed effects. Using Tyrode solutions with different potassium concentrations, we found that $[K^+]$ had to be raised to 14 to 15 mEq/L in order to produce unresponsiveness in a similar way to that of ischemic blood. Figure 6 shows in histogram form the potassium concentrations, and where available the corresponding pH of ischemic samples which led to unresponsiveness and those which produced mild

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from figure 6, the majority of ischemic samples which showed mild effects had potassium levels between 5 and 7 mEq/L, whereas samples which produced unresponsiveness most frequently had K⁺ concentrations between 7 and 9 mEq/L. Although there was a trend for ischemic blood samples to produce the most marked effects at higher K⁺ concentrations, the fact that unresponsiveness was produced at K⁺ concentrations of about 7 mEq/L, and on occasion even at levels within the limits of normal, suggested that potassium was not the only factor responsible for the observed effects.

We considered whether a combination of a moderately elevated potassium concentration with alterations in other parameters might account for the effect of ischemic blood. As shown in table 1 most ischemic samples had a low pH, although the scatter was wide and the values ranged from 6.70 to 7.60. In all cases where control pH was measured there existed alkalosis in combination with a low pCO₂, indicating that the animal was in respiratory alkalosis due to overventilation. There was no definite trend for the lowest pHs to be associated with the lowest K⁺ concentrations (fig. 6).

All samples had a low pO₂ (range 17 to 44) and often a high pCO₂ (range 32 to 130). Ca⁺⁺ and Mg⁺⁺ concentrations were not significantly altered in ischemic blood.

Finally, glucose concentrations tended to be low (range 1.4 to 5.4).

In order to evaluate the effect of low glucose levels, 5 mM glucose/L was added to three ischemic blood samples which had produced unresponsiveness (experiments 27, 28 and 29). After sufficient time had elapsed for the preparation to recover during superfusion with Tyrode solution from the incubation in the original ischemic blood sample, the high glucose samples were tested. These samples, in which glucose concentration was now 8.3, 7.8 and 8.3 mM/L, respectively, still produced unresponsiveness in exactly the same way and after the same period of incubation as did the original sample.
In order to test whether a combination of elevated $K^+$, low pH and low $pO_2$ could account for the effects of ischemic blood, normal coronary venous blood was rendered abnormal in six experiments by raising lactic acid to a concentration of about 25 mM/L in order to produce a pH of about 6.9, and by equilibrating with a gas mixture of $N_2$ (1400 ml), $CO_2$ (110 ml) and air + 5% $CO_2$ (350 ml) to achieve a $pO_2$ of about 25 mm Hg and a $CO_2$ of about 45 mm Hg. The potassium concentration in these blood samples was raised to three different levels: about 8, 12 and 16 mEq/L. The abnormal blood with a $K^+$ concentration of about 8 mEq/L did not produce the effect of ischemic blood with a similar $K^+$ level (fig. 7).

Abnormal samples with $K^+$ concentrations of 12.9 and 13.0 mEq/L only produced mild effects, whereas samples with $K^+$ levels of 13.6, 15.8 and 16.0 mEq/L produced unresponsiveness. Thus, the effects of ischemic blood cannot be ascribed to the combined effects of acidosis, hypoxia, elevated lactate and hyperkalemia in ischemic blood.

Discussion

Our findings demonstrate that coronary venous blood draining an ischemic area can produce striking electrophysiologic changes in healthy myocardium. These changes range in severity from shortening of action potential duration, the development of postrepolarization refractoriness, loss of upstroke velocity, loss of resting potential, the fractionation of the action potential into two components, increase in diastolic threshold and finally total unresponsiveness.

All of the effects observed could be caused by a sufficiently elevated extracellular potassium concentration, as was shown by the experiments with Tyrode solutions containing 16 mEq/L of $K^+$. In a perfused preparation of the pig's moderator band, Gettes and Surawicz19 reported that potassium concentration of 10 to 12 mEq/L produced in both muscle and Purkinje cells shortening of action potential duration and refractory period, and decrease of its magnitude and maximal diastolic potential. However, at these $K^+$ levels, their preparations were far from being unresponsive. $K^+$ concentrations in the ischemic blood samples were usually 7 to 8 mEq/L. In our experiments such $K^+$ concentrations in Tyrode or blood produce only mild changes such as action potential shortening, slight loss of dV/dt and some degree of postrepolarization refractoriness.

The possibility of this mild degree of hyperkalemia having potent depressant effects when combined with acidosis, hypoxia and hypoglycemia was considered. Each of these derangements alone has significant effects on the action potentials of in vitro heart preparations. Acidosis has been reported to lengthen action potentials in Purkinje fibers,14 frog ventricular strips18 and rabbit atria.18 It also lengthened ventricular action potentials in perfused guinea pig hearts but shortened ventricular action potentials in perfused rat hearts.17 Using $CO_2$-induced acidosis, Coraboeuf et al.18 produced depolarization in some canine Purkinje fibers and repetitive humps in the repolarization phase. We do not feel that acidosis played an important role in producing the ischemic effect for two reasons.

1) The depressant effect was seen once during alkalosis and four times when there was little or no acidosis (pH 7.6, 7.34, 7.33, 7.26, 7.25) as a consequence of a respiratory alkalosis from overventilating the animals.

2) Our abnormal bloods with a lactic acid-induced pH of 6.9 failed to have even a mild ischemic effect.

Anoxia has long been known to reduce action potential duration and amplitude16 but it has little effect on the resting membrane potential.20 Our ischemic blood samples had a major effect on the resting potential and in seven of 12 instances had a $pO_2$ at least as high as the control coronary venous blood which had no depressant effects. The effects of hypoxia on action potential configuration can be prevented by high glucose concentrations and can be exaggerated in glucose-free solutions.21, 22 Although glucose levels in ischemic blood were always lower than the controls, the reduction was not always very marked and the depressant effects could not be prevented by elevating glucose above control values.

It is possible that the ischemic blood induced abnormalities in ATP production which resulted in suppression of electrogenic pumps. The electrogenic sodium pump maintains the resting potential of anoxic ventricular muscle at a more negative level than can be accounted for by the ionic distribution of potassium across the myocardial membrane.23 Inhibition of the sodium pump by cooling and ouabain results in a reduction in the resting potential to levels predicted by the potassium gradient. Ischemic blood, unlike anoxia, rapidly produced severe depolarization at $[K^+]$ levels that were usually only slightly elevated above normal (on three occasions it even remained within the upper limit of normal). It seems unlikely therefore that suppression of an electrogenic pump could account for one of the main features of the ischemic blood effect. Reduced ATP levels may have been responsible in part for the observed shortening in action potential duration.24

In view of the fact that the effects of ischemic blood cannot be accounted for by the combination of hyperkalemia, acidosis, hypoxia and hypoglycemia, the possibility arises that an additional factor or factors are present in ischemic blood, that also have depressant effects on the electrical behavior of normal ventricular myocardium. As yet we have no indication as to which of the many metabolites released by ischemic myocardium may be implicated, alone or in combination.

The appearance of two-component action potentials in the ischemic blood may have been due to a true separation of fast and slow components as a result of depolarization. If so, the slow component was the first to be inhibited at a time when the remnant of the faster component was able to persist for some considerable time longer. However, at a stage prior to total unresponsiveness, it was possible to evoke the second component by either increasing the stimulus strength or introducing a sufficiently long diastolic pause. Also, the amplitude of this second component varied with stimulus strength. These are characteristics of the secondary depolarizations, described by Carmeliet and Vereecke25 and the slow responses of Cranefield et al.26 observed in partially depolarized Purkinje fibers under the influence of epinephrine.

We were unable for technical reasons to measure catecholamine levels of our ischemic blood samples. If they were sufficiently elevated then the second slower component that we observed prior to the onset of unresponsiveness should
have persisted at the terminal resting potential of about $-55$ mV. This did not occur and we never saw the spontaneous emergence of typical slow responses after the tissue had become inexcitable. The addition of epinephrine and calcium gluconate on the one occasion it was tried did evoke such responses with a $35$ mV positive overshoot — a feature we never saw as part of the ischemic blood effect. In considering the nature of the two components of these action potentials, however, it must be stated that we cannot exclude as a cause electrotonic interaction between tissue close to the stimulating electrode and more remote tissue at a time when regenerative conduction was at the point of failing. However, as far as it was possible to tell by using a second exploring microelectrode, the changes observed were representative of the electrical activity throughout the small piece of tissue. It is well known that refractory period duration is shortened in ischemic heart muscle. More recently, however, it has been reported that although refractory period duration shortens in the initial phase of myocardial ischemia, it lengthens again in later stages. In Purkinje fibers, isolated some hours after coronary occlusion, El Sherif et al. found that the recovery of excitability outlasted action potential duration, and in later papers the phrase “postpolarization refractoriness” was used to describe this phenomenon in the ischemic canine His bundle. The very marked postpolarization refractoriness observed by us in healthy myocardium exposed to ischemic blood may simply be secondary to loss of resting potential, which is known to retard recovery from inactivation of the fast current; or it may be an expression of structural derangement of the cell membrane. In favor of the first possibility is the fact the postpolarization refractoriness could be evoked by superfusion with Tyrode containing $15$ mEq/L of K$^+$.

If our observations on the occurrence of postpolarization refractoriness can be applied to ischemic myocardium in the intact heart, they have important implications. First, estimates of refractory period duration based upon measurements of Q-T intervals of local electrograms or action potential duration may be grossly misleading. Second, the extent of heterogeneity of recovery times, a key determinant of ventricular fibrillation threshold, could be greater than previously supposed. It is likely that at a certain critical stage of ischemia recovery times range from the abnormally short values of mildly ischemic border cells to those more ischemic cells on the verge of unresponsiveness that require the entire diastolic interval. The vulnerable period would indeed extend throughout diastole as has recently been suggested.

The K$^+$ concentration that we found in ischemic blood was higher than reported in the literature. Values found by Harris et al. did not exceed $25$ mg % ($6.4$ mEq/L). The highest value found by Thomas et al. in the local coronary vein draining an ischemic area in the dog’s heart was $6$ mEq/L. However, these values were found during coronary occlusion, indicating that there was still venous flow during the occlusion, presumably because of collateral flow or because the vein also was draining normally perfused myocardium. This implies that the actual interstitial K$^+$ level might have been higher. In order to get a better reflection of ischemic interstitial fluid, we required that there should be little or no local venous flow during the period of coronary artery occlusion — a requirement met more reliably in porcine than canine hearts, as we found in preliminary experiments.

We attempted to standardize ischemic samples by collecting the first $2$ ml to appear on release of coronary occlusion. However, since it took a variable time to collect these $2$ ml, an inevitable and variable dilution factor probably was present. To some extent this may have accounted for the observed variations in K$^+$ concentrations and potency of the depressant properties of the ischemic blood. In this regard it is of interest to emphasize that blood collected two minutes after release of occlusion still had potent depressant properties, albeit to a lesser extent than blood collected immediately on release. This is also a further argument that the effects were not solely determined by K$^+$ since K$^+$ in the hyperemic phase was only slightly above control values.

Some questions may be raised about the fact that our observations were made on tissue immersed in stagnant blood. It should be stressed that before each observation in ischemic blood, the preparation was monitored in stagnant normal coronary venous blood and could on every occasion be maintained in a stable condition as far as action potential configuration and excitability were concerned for a period of at least $15$ minutes. The effects of ischemic blood took variable periods of time to develop; sometimes a maximal effect was seen after two minutes of incubation, other times it took $12$ to $15$ minutes. In order to stay within safe limits, our observations were always limited to the first $12$ minutes of incubation. A second factor which might have influenced the time course of events was the depth of the cell from which recordings were made. We always tried to record from the most superficial cells to minimize differences due to concentration gradients. Most of our observations were on the subendocardial layers of the right ventricular outflow tract. This introduces some uncertainty as to the exact morphological nature of the cells impaled. However, on a number of occasions the corresponding epicardial surface was used and the same phenomena were observed. Even if some of the cells impaled were subendocardial Purkinje fibers, their response to ischemic blood did not differ qualitatively from that of ventricular myocardial cells.

In conclusion, ischemic myocardium releases agents which have a potent depressant effect on the electrical activity of normal myocardium. Although potassium contributes to these effects, it cannot completely account for them, not even in combination with acidosis, hypoxia, hypoglycemia and elevated lactate. The nature of the other depressing agents remains to be determined as well as the way in which they mediate their effects.

The extent to which our observations reflect the actual changes which occur during ischemia in the intact heart may well be questioned. However, recently we have been able to record action potentials directly from ischemic regions of the intact heart and have observed precisely similar changes.

The fact that our observations were made on normal myocardium raised the interesting possibility of the existence of nonischemic cells on the periphery of the ischemic area, which functionally behave as though they were ischemic. Such cells would respond not only to changes in perfusion but also to changes in the pattern of coronary venous drainage.
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