Intracellular Potassium Activity in Guinea Pig Papillary Muscle during Prolonged Hypoxia

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ABSTRACT During prolonged hypoxia, intracellular potassium concentration, $[K]_i$ has been reported to fall by 70% with a concomitant decrease in the calculated potassium equilibrium potential, E_K. Nevertheless, resting membrane potential, V_m, declined only slightly. Because V_m depolarized very little in relation to the calculated E_K, it was hypothesized that electrogenic Na-K pumping contributed up to 40 mV to V_m during prolonged hypoxia. To further test this hypothesis we studied what changes prolonged hypoxia makes in the thermodynamically active fraction of cellular potassium, intracellular potassium activity, α_K^i, and how change in α_K^i affects the relationship between V_m, E_K and, by inference, the Na-K pump. Using double-barrel K-selective electrodes, V_m and α_K^i were measured in quiescent guinea pig right ventricular papillary muscles superfused for 8 h with hypoxic Tyrode's solution. Over the 8-h period both V_m and α_K^i decreased. However, the decline in V_m was paralleled by a decrease in the E_K calculated from α_K^i. At no time was there hyperpolarization of V_m beyond E_K.

After 8 h the Na-K pump was inhibited by exposing the muscles to 0.1 mM ouabain. The onset of an increase in extracellular potassium activity, measured with a double-barrel electrode, was used to mark the amount of depolarization of V_m due solely to pump inhibition. After hypoxia, V_m depolarized 8.4±4.4 mV before extracellular potassium activity (α_K^o) increased. Thus, the decrease in α_K^i during hypoxia is much less than that reported for $[K]_i$. The parallel decline in V_m and E_k and the small depolarization of V_m with ouabain suggest that after prolonged hypoxia the Na-K pump continues to contribute to V_m, but the amount of this contribution is substantially less than previously reported.

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INTRODUCTION

Hypoxia causes characteristic changes in the transmembrane action potential of ventricular muscle. Several investigators reported a shortening of the plateau and a reduction in the overshoot, both alterations occurring in the face of minimal change in the resting potential (V_m)[1] (1–3). One observation, nevertheless, remains enigmatic. McDonald and MacLeod (4, 5) exposed ventricular muscle to hypoxia for 8 h and confirmed the lack of change in V_m during this time period. They also reported that at the end of the 8-h period, intracellular potassium concentration ($[K]_i$) decreased dramatically, and from the measured potassium concentration they calculated that the potassium equilibrium potential decreased from −82 mV to −47 mV. Since the measured V_m remained at −80 mV, it appeared that during prolonged hypoxia there was sustained hyperpolarization of V_m beyond the potassium equilibrium potential (E_K) in excess of 30 mV. They attributed this deviation of V_m from E_K to increased electrogenic Na-K pumping and hypothesized that the electrogenic Na-K pump, in large measure, maintains V_m during prolonged hypoxia (4, 5).

Several studies have demonstrated that when the electrogenic sodium pump is stimulated, V_m can transiently exceed E_K (6–8). In an experiment representative of non-steady-state behavior after a period of sodium loading, Page and Storm (6) observed transient

[1] *Abbreviations used in this paper*: α_K^o, extracellular potassium activity; α_K^i, intracellular potassium activity; α_K^o, superfusate potassium activity; α_{Na}^i, intracellular sodium activity; α_{Na}^o, superfusate sodium activity; APD_{50}, action potential duration at 50% repolarization; E_K, potassium equilibrium potential; γK, K^+ activity coefficient; K_{KN_a}, selectivity coefficient; $[K]_i$, intracellular potassium concentration; OS, overshoot; V_m, resting membrane potential; V_K^o, potassium electrode reading in superfusate; V_m^o, reference electrode reading in superfusate; V_K^e, potassium electrode reading in extracellular space; V_K^i, potassium electrode reading in intracellular space.

hyperpolarization of V_m beyond E_K of 6 mV when papillary muscle recovered from prior incubation in cold, potassium-free perfusate. As summarized by Thomas and others (9, 10), this type of hyperpolarization must be temporary, because the change in the electrochemical driving force for the passive movement of potassium ($V_m - E_K$) favors a redistribution of potassium across the cell membrane. This redistribution of potassium in turn drives E_K to more negative values, which continue to approach V_m. Thus the observation that there was sustained deviation of the measured V_m from the calculated E_K during prolonged hypoxia described by McDonald and MacLeod remains problematic.

Potassium ion-selective electrodes can determine the thermodynamically active fraction of cellular potassium, intracellular potassium activity (α_K^i) (11–13). These electrodes avoid the potential pitfalls of measuring total potassium concentration in whole muscle bundles, particularly bundles exposed to the insult of hypoxia. Moreover, they are capable of monitoring changes in α_K^i in a given cell over time. Consequently, potassium-selective electrodes were used to investigate the hypothesis that the Na-K pump maintains V_m during prolonged hypoxia. We studied the changes that severe hypoxia causes in α_K^i and how these changes affect the relationship between V_m, E_K and, by inference, the Na-K pump.

To further investigate what role the pump plays in maintaining V_m during prolonged hypoxia, additional corroborative experiments were performed. During ouabain inhibition of the Na-K pump in both normoxic and hypoxic muscles the onset of an increase in extracellular potassium activity, α_K^e, was used to mark the upper limit of the amount of depolarization due solely to pump inhibition (14). We used this amount of depolarization as another index of pump contribution to V_m.

METHODS

Hartley guinea pigs (Camm Research Lab Animals, Wayne, N. J.) weighing 250–400 g were sacrificed by a blow to the base of the skull. Right ventricular papillary muscles were dissected and immersed in Tyrode's solution. Only muscles having diameters <0.5 mm were used.

Muscles were superfused in a Gadsby (15) fast-flow perfusion bath (4 mm × 10 mm × 15 mm) with either normoxic or hypoxic Tyrode's solution of the following composition in mM: NaCl, 135.4; KCl, 4.0; NaH_2PO_2, 1.8; $CaCl_2$, 2.7; $MgCl_2$, 0.5; $NaHCO_3$, 18.0; and dextrose, 5.5. With this concentration of potassium the activity of the bulk potassium (α_K^e) is ~3.0 mM (16). Normoxic solutions were bubbled with 95% O_2 and 5% CO_2. Solutions were made hypoxic by bubbling with 95% N_2 and 5% CO_2 in a glass reservoir with a one-way valve and by using a glass-enclosed perfusion system. During hypoxia the partial pressure of oxygen sampled near the muscle was 11.4±1.3 mm Hg. Temperature was 33°–34°C and bath flow was 10–11 ml/min.

The transmembrane action potential was simultaneously displayed on a Tektronix 5103 oscilloscope (Tektronix, Inc., Beeverton, Oreg.) and a Gould 2400 strip chart recorder (Gould Instruments Div., Inc., Cleveland, Ohio). The oscilloscope signals were photographed with a high-speed Grass Kymograph camera (Grass Instrument Co., Quincy, Mass.) for later determination of overshoot (OS) and action potential duration at 50% repolarization (APD_{50}). The latter was obtained by digitizing the magnified image of an action potential photograph using a SAC GP-3 graph pen digitizer (Science Accessories Corp., Southport, Conn.) interfaced with a DECLab PDP 11/03 computer (Digital Equipment Corp., Marboro, Mass.).

Ion-selective electrodes. Intracellular and extracellular potassium activities were measured with single- and double-barrel liquid ion exchanger microelectrodes as previously described (17–19). In the double-barrel electrodes the reference barrel was filled with 1.0 M NaCl. The barrel that contained the potassium-selective resin (Corning resin 477317; Corning Medical, Medfield, Mass.), was filled with 0.5 M KCl. Electrodes were calibrated according to the method of Walker and Brown (20), where the electrode's response in pure potassium solutions is compared to its response in the superfusate, Tyrode's solution (Fig. 1). This generates a value, α selectivity, which is given by the following formula: α selectivity = $\alpha K_{Tyrode} + K_{KNa}\ \alpha Na_{Tyrode}$. The selectivity, K_{KNa}, obtained from this expression was >50/1 potassium over sodium. As can be seen from Fig. 1, the electrode's response to pure potassium chloride was linear between 1,000 mM and 1 mM, and produced slopes of >50 mV/

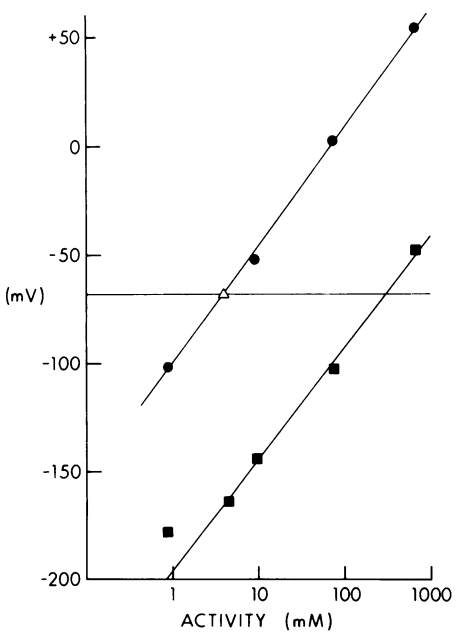

FIGURE 1 Calibration curve. Selectivity is calculated by comparing the response of the electrode in pure KCl solution (●) with the response in Tyrode's solution (Δ), where total activity = $\alpha K_{Tyrode} + K_{KNa}\ \alpha Na_{Tyrode}$. Selectivity was greater than 50/1 over sodium. Also shown is the electrode's response to pure NaCl solution (■), which is linear between 5 mM and 1,000 mM, but which tends to be nonlinear at Na concentrations, less than ~1–5 mM.

decade change in potassium activity. In solutions of pure sodium chloride, the electrode's response tended to be nonlinear at sodium concentrations less than ~1 mM, which would not interfere with measuring changes in α_K^i. The minimal detectable change in potassium concentration against a background concentration of 150 mM was 1.5 mM. Potassium activity was calculated by the following derivation of the Nikolskii equation (21), which we have previously reported (17, 18):

$$\alpha_K^x = (\alpha_K^o + K_{KNa}\,\alpha_{Na}^o)\exp\left\{\frac{2.303}{S} \times \left[\left(V_K^x - V_K^o\right) - \left(V_m^x - V_m^o\right)\right]\right\} - K_{KNa}\,\alpha_{Na}^i,$$

where

α_K^x = α_K^e (extracellular potassium activity) or α_K^i (intracellular activity)
α_{Na}^i = intracellular sodium activity
α_K^o = superfusate potassium activity
α_{Na}^o = superfusate sodium activity
K_{KNa} = selectivity coefficient
S = slope
V_K^x = V_K^e (K electrode reading in the extracellular space) or V_K^i (K electrode reading in the intracellular space)
V_K^o = K electrode reading in the superfusate
V_m^x = V_m^e (reference electrode reading in the extracellular space) or V_m^i (reference electrode reading in the intracellular space)
V_m^o = reference electrode reading in the superfusate.

Tip resistance ranged between 10^9–10^{10} Ω. In α_K^i determinations, $K_{KNa}\,\alpha_{Na}^i$ was neglected because its value is <0.1 mM.

Protocols. Muscles were quiescent in all protocols except for impalement verification. In the first set of experiments, muscles were switched to hypoxic perfusate for 3 h during which multiple impalements with single-barrel potassium-selective electrodes and conventional 3M KCl electrodes were made every 30 min. Each determination of α_K^i consisted of the average of between three and six separate V_m and V_K measurements. Browning and Strauss (17) have shown that this technique of multiple impalements is important in determining α_K^i because of the wide variation in the range of values of α_K^i.

In the second protocol the duration of hypoxia was extended to 8 h, similar to the protocol used by McDonald and MacLeod (4, 5). In these experiments a single impalement with a double-barrel electrode was made during the control period and maintained during 8 h of hypoxia or continued normoxia. The values of V_m determined by the reference barrel of the double-barrel electrode ranged between −76 mV and −84 mV, while the value of the OS was >20 mV. This protocol had the advantage of allowing us to follow both V_m and α_K^i in a single cell over time. The double-barrel electrodes, however, have the disadvantage of larger tip diameter and the use of NaCl as a filler in the reference barrel, both of which tend to lower the control measurements for V_m and α_K^i compared with the single-barrel electrodes (17). During long-term maintained impalements there is inevitable signal drift due, in part, to change in tip potentials and variation in input bias current. Correction for drift was made with the assumption that this drift was linear over the 8-h period. The drift rate was 0.5–1.5 mV/h.

To complement the experiments described above, we performed an additional set of experiments designed to further estimate the contribution of the Na-K pump to V_m (14). We inhibited the Na-K pump by exposing the muscles to 0.1 mM ouabain (Sigma Chemical Co., St. Louis, Mo.) after 8 h of hypoxia or normoxia and used the onset of an increase in α_K^e to mark the amount of depolarization due to pump inhibition. A double-barrel electrode was placed in the extracellular space as previously described (19, 22) to monitor change in α_K^e, while a 3M KCl electrode was placed intracellularly to monitor V_m during ouabain exposure. The left-hand part of Fig. 3 shows the double-barrel electrode impaling the cell and advancing to the extracellular space. Displayed are the traces from the V_K barrel of the double-barrel electrode, the reference barrel (V_{ref}), and their electronically subtracted difference signal (V_D), which provides the substrate for the calculation of α_K^e and which is so labeled. Also shown is a separately impaled 3M KCl electrode that measures V_m during ouabain exposure. After a few minutes in the extracellular space, V_K, V_{ref}, and α_K^e return to bath values. The electrodes were left in place for an additional 20 min after which the recorder gains were doubled and 0.1 mM ouabain added to the superfusate. We used the onset of an increase in α_K^e to distinguish the amount of depolarization in V_m, resulting solely from pump inhibition from that due to extracellular K^+ accumulation.

Data reduction. Data are expressed as means and standard errors of the mean. Student's *t* test for paired and unpaired data was used to test for significance (23).

RESULTS

Table I shows the changes in V_m, α_K^i OS, and APD_{50} that occur during exposure to hypoxia. At the end of 3 h of exposure to severe hypoxia there were small changes in both V_m and α_K^i. V_m decreased from −89.3±0.7 mV to −84.6±0.5 ($P < 0.01$) mV while α_K^i decreased from 104.6±4.8 mM to 97.8±3.2 mM. Assuming that α_K^e equalled the potassium activity in the bulk solution (α_K^o), the calculated potassium equilibrium potential ($E_K = RT/F \ln \alpha_K^o/\alpha_K^i$) decreased from −93.2±1.3 mV at control to −89.0±1.6 mV at 3 h, such that over this 3-h period the difference between V_m and E_K was unchanged.

As seen in previous studies (1–3), there were significant changes in both the plateau phase, manifested by a shortening of the APD_{50}, and in the OS (Table I). The shortening of the APD_{50} was seen in some experiments to begin within a few minutes of hypoxic exposure and decreased by a further 80% over 3 h. The OS of the action potential decreased about 50% in 3 h from 37.7±1.6 mV to 15.7±1.9 mV ($P < 0.01$) (Table I).

After 3 h of severe hypoxia there was a small but definite depolarization in V_m, which in that respect is unlike most previous studies (1–3). However, the decline of V_m was accompanied by a parallel decline in E_K. To test whether longer periods of exposure to hypoxia, similar to those employed by McDonald and MacLeod (4, 5), could stimulate the Na-K pump to hyperpolarize V_m beyond E_K, we exposed muscles to hypoxic superfusate for 8 h. In this protocol, rather

TABLE I
V_m, α_K^i, ADP_{50} and OS during 3 h of Hypoxia

	Control	60 min	120 min	180 min
V_m, *mV*	−89.3±0.7	−86.2±0.7	−95.0±1.0	−84.6±0.5*
α_K^i, *mM*	104.6±4.8	101.5±4.9	104.3±2.4	97.8±3.2*
ADP_{50}, *ms*	222.8±12.1	117.0±10.2	39.2±4.2	24.0±4.2*
OS, *mV*	37.7±1.6	22.7±1.1	21.8±1.9	15.7±1.9*

Data represent mean±SE; $n = 10$.
* $P < 0.01$.

than using multiple and separate impalements to measure V_m and V_K, we used a double-barrel electrode impaled in a single cell over the experimental period. This afforded us continuous measurements of α_K^i and V_m in a given cell over time and allowed us to confirm the changes seen in V_m and α_K^i by the technique of multiple electrode impalements. Fig. 2 illustrates a representative tracing of a continuous impalement with a double-barrel electrode and shows the depolarization of V_m and the decline in α_K^i over 8 h of hypoxia.

After the 8 h there were significant declines in the values of V_m and α_K^i (Table II). V_m depolarized from −81.4±0.8 mV to −71.4±0.6 mV ($P < 0.01$) while α_K^i decreased from 96.1±4.1 mM to 65.1±2.9 mM ($P < 0.01$). Over this period the calculated E_K decreased from −92.3±1.2 mV to −83.6±0.5 ($P < 0.05$). Although there were significant decreases in V_m and E_K, the rates of decline in V_m and E_K were not different, suggesting that the relationship between V_m and E_K was unchanged.

As can be seen from Tables I and II, the control values for V_m and α_K^i obtained with the double-barrel electrode were somewhat lower than those obtained by averaging multiple impalements with single-barrel electrodes. As demonstrated previously (17), this represents differences in tip diameter and in the use of 1.0 M NaCl as a filler in the reference barrel of the double-barrel electrode, as compared with 3M KCl in a single-barrel reference electrode. Using double-barrel electrodes it was noted that after the first 3 h of hypoxia in the 8 h protocol, V_m declined to −76.9±1.0 mV and α_K^i decreased to 84.5±3.9 mM. These data suggest that, although the control values for the single- and double-barrel electrodes are somewhat different, both techniques record similar rates of change in V_m and α_K^i during hypoxia, and because one technique samples many cells over time while the other records from one cell over time, they form a strong complement.

The parallel decline in V_m and E_K after 8 h of hypoxia stands in contrast to the findings after 8 h of control normoxic superfusion. During this control experiment there were no significant changes in V_m, α_K^i, or E_K (Table II), and the relationship between V_m and E_K was unchanged.

As a corroborative experiment we inhibited the pump with 0.1 mM ouabain after the 8 h of hypoxia or normoxia to further test the contribution of the Na-K pump to V_m (14). Using a double-barrel electrode placed in the extracellular space, we detected an increase in α_K^e after the normoxic muscle depolarized by 4.8±1.1 mV (Fig. 3). In the normoxic muscle this detection was noted after 2.8±0.1 min of ouabain exposure. In the hypoxic muscle, the muscle depolarized by 8.4±2.2 mV before an increase in α_K^e was detected. In these muscles the increase in α_K^e was detected after 3.4±0.2 min of ouabain exposure, which was a longer period than after normoxia ($P < 0.05$).

DISCUSSION

Our data do not support the hypothesis that electrogenic Na-K pumping maintains (up to 30 mV) V_m during prolonged hypoxia. Rather, these data suggest that during oxygen deprivation the resting membrane potential of ventricular muscle continues to be primarily determined by the passive distribution of potassium and that the contribution of the electrogenic Na-K

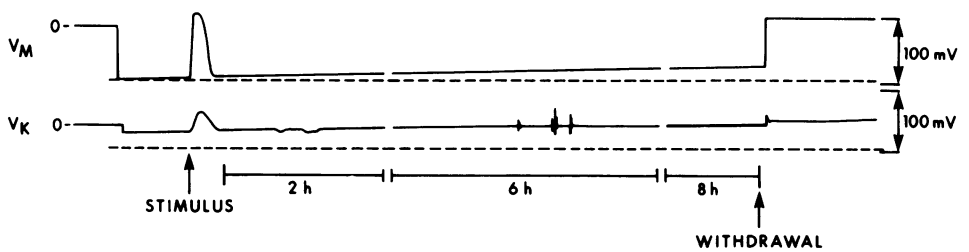

FIGURE 2 Decrease in V_m and V_K measured with a double-barrel electrode over the 8-h period. A stimulus to demonstrate impalement is seen on the left-hand part of the trace. The top of the action potential from the reference barrel has been abbreviated at this scale to focus on resting potential. In this muscle, V_m depolarized from −82 mV to −73 mV, while α_K^i decreased from 94 mM to 67 mM. The zero drift in this study was ~0.5 mV/h.

TABLE II
V_m, α_K^i, and E_K over 8 h of Hypoxia or Normoxia

	Control	2 h	4 h	6 h	8 h
Hypoxia (n = 8)					
V_m, mV	−81.4±0.8	−78.5±0.9	−76.3±0.9	−73.8±0.9	−71.4±0.6*
α_K^i, mM	96.1±4.1	85.2±4.9	82.8±4.5	73.4±3.7	65.1±2.9*
E_K, mV	−92.3±1.2	−87.1±1.4	−87.1±1.1	−85.2±1.3	−83.6±0.5‡
Normoxia (n = 6)					
V_m, mV	−79.5±0.9	−82.0±0.6	−75.8±1.0	−79.0±0.2	−80.1±0.9
α_K^i, mM	95.8±6.0	97.1±4.1	91.4±3.5	92.8±6.6	97.0±6.6
E_K, mV	−92.3±1.6	−95.0±1.7	−90.8±1.7	−90.8±1.6	−92.3±1.3

Data represent mean±SE.
* $P < 0.01$.
‡ $P < 0.05$.

pump changes little, if at all. Our data are in agreement with some preliminary data of Weir (24) but differ somewhat from the data of Baumgarten et al. (25) who showed evidence of muscle heterogenicity during hypoxia. The potassium equilibrium potential, E_K, that we calculated from the measured α_K^i and the bulk α_K^o during prolonged hypoxia parallels the decline in the measured V_m. The absolute value of E_K, however, must be interpreted with caution, as small increases in α_K^e in the transverse tubules or clefts may tend to decrease E_K. Cleft α_K^e would uniformly have to increase by over 50% for V_m to be more negative than E_K, and this seems unlikely particularly with the use of Gadsby's (15) fast flow bath. We found no evidence of a relative hyperpolarization of V_m beyond E_K and thus cannot support the concept that stimulated electrogenic Na-K pumping maintains V_m during hypoxia in the degree previously suggested.

Our data also demonstrate that severe hypoxia will cause significant depolarization. The lack of significant depolarization described in previous studies may have been due to less severe reduction of pO_2 (1–3). As demonstrated by Schubert (26) and also by Grunewald (27), the normoxic pO_2 in myocardial tissue may range as low as 20–30 mm Hg. The pO_2 sampled at the muscle surface in our experiments was substantially lower and probably explains the decrease in V_m seen in our studies.

Central to our hypothesis is the observation that the decrease in α_K^i after prolonged hypoxia is much less than the decrease in $[K]_i$ reported by McDonald and MacLeod (4, 5). The discrepancy in the decrease in α_K^i that we observed and the decrease in $[K]_i$ reported by McDonald and MacLeod (4, 5) during exposure to hypoxia may be attributed to at least three mechanisms. First, as noted by Page and Storm (7), the determination of $[K]_i$ is done on whole muscle bundles where, depending on thickness, there may be large [K] gradients between the cells in the center and cells on the surface. This would tend to substantially lower the average K concentration made on the whole bundle. Because of the presence of these potassium gradients, it cannot be assumed that the potassium content of a surface cell, where V_m is measured, is the same as the potassium content derived from analysis of the total muscle bundle. Our measurements of α_K^i, particularly as followed in a given cell, were obtained from cells on the surface of the preparation, which are in contact with rapidly exchanging perfusate and where gradients would tend to be less. Second, the determination of $[K]_i$ requires estimation of the intracellular volume, usually determined with the use of extracellular markers. These may be inaccurate after prolonged hypoxia because of changes in membrane permeability, as for instance those suggested by the morphological studies of Ganote or Hatt (28, 29). The determination of α_K^i with potassium-selective electrodes requires no estimate of cell volume. Third, hypoxia may cause a shift in the K^+ activity coefficient (γ_K), the ratio of α_K^i to $[K]_i$. Changes in the activity coefficient were reported by Lee and Armstrong (30) when skeletal muscle fibers were loaded with sodium and it is conceivable that the sodium loading, which is thought to occur in hypoxia, may also affect γ_K.

Regardless of the mechanism of the low $[K]_i$, it is difficult to accept that V_m could hyperpolarize beyond E_K by 30–40 mV for such prolonged periods under such hypoxic stress. This is so because the new electrochemical driving force for K^+ ($V_m - E_K$) would tend to continually drive K^+ into the cell and drive E_K toward V_m. The maintenance of this reversal of driving force would, in the steady-state, actually require active extrusion of potassium, which has not been described.

As a corroborative experiment we used an increase

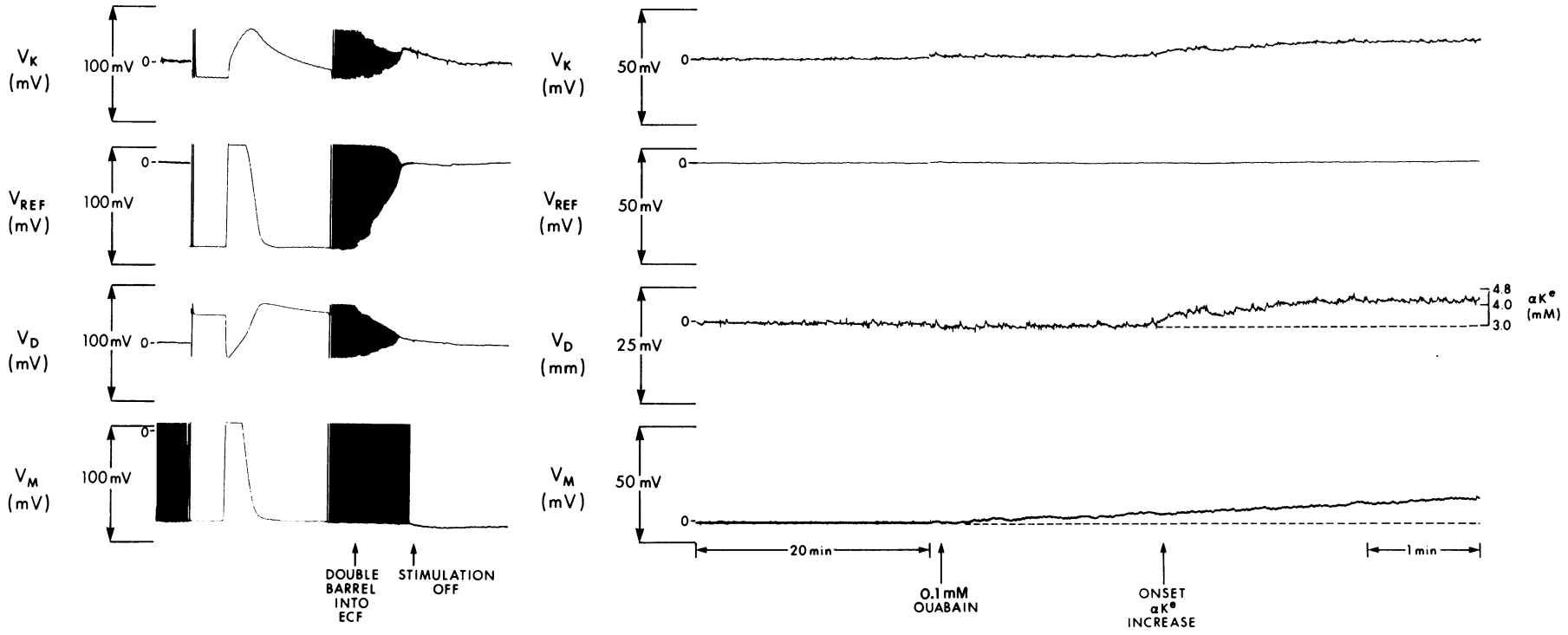

FIGURE 3 Inhibition of the pump with ouabain. Displayed are V_K and V_{ref} from the double-barrel electrode and their electronically subtracted signal, V_D, which is the substrate for the calculation of α_K^e. V_m from a separate intracellular 3M KCl electrode is shown. During impalement the tissue is paced at 1 Hz. The action potential tops of V_{ref} and V_m have been truncated to focus on the base line. After the double-barrel is advanced through the first cell layer to the extracellular space, V_K, V_{ref}, and V_D return to bath levels. After 20 min, the gain settings on the recorder were doubled and V_D is also labeled as α_K^e. At the third arrow, 0.1 mM ouabain is added. As can be seen, V_m depolarized by 5 mV before α_K^e increased, denoting pump inhibition (fourth arrow). Note that during the increase in α_K^e from K^+ accumulation no change in voltage is seen in V_{ref}, indicating that the electrode has remained extracellular.

in α_K^e during exposure to 0.1 mM ouabain as a marker for the amount of depolarization due solely to pump inhibition (17). Similar types of measurements of α_K^e using double-barrel potassium-selective electrodes have been reported by Kunze (19) in response to rapid pacing and by Kline and Morad (22) who reported changes in α_K^e during a single action potential. After prolonged normoxia, this value was 4.8±1.1 mV, which is in accord with other estimates of pump contribution to V_m (31). After prolonged hypoxia this value was 8.1±2.2 mV, which is somewhat higher than control but still considerably less than that reported by McDonald and MacLeod (4, 5). In fact, it is likely that this estimate after hypoxia is spuriously high. The double-barrel electrode almost certainly damages the hypoxic tissue and distorts the cleft to a greater extent than in normoxia. If there is poor healing-over in hypoxia, this would leave a larger space for the same of less K extrusion and makes this method a less sensitive index of change in pump rate during hypoxia. The observation that the time between ouabain exposure and the detection of an increase in α_K^e in hypoxia was greater than during normoxia is also consistent with the double-barrel electrode causing a greater distortion of the space. Because of possible changes in sodium or potassium conductances, these estimates probably set an upper limit for pump contribution to V_m. Nonetheless, these corroborative experiments will set the pump contribution to V_m considerably lower after hypoxia than previously stated.

Changes seen in this study in the duration of the action potential are consistent with those previously described (1, 2, 5). Our experiments were not designed to shed light on the controversy as to whether the plateau shortening during exposure to hypoxia represents an outward, presumably K^+ (32) current, or a decrease in the slow inward current (33), but they merely serve as the basis for comparison with other studies.

With the degree of hypoxia seen in these studies, there was also a remarkable decrease in the OS, ~50% in 3 h. This would be compatible with either changes in g_{Na} or E_{Na}. The latter almost certainly decreases as intracellular sodium activity increases. However, as noted by Coraboeuf (34), the guinea pig papillary muscle may be a poor model for correlations between E_{Na} and OS.

In summary, this study demonstrates that α_K^i decreases much less during prolonged hypoxia than $[K]_i$. The relatively higher value of α_K^i yields a calculated E_K from which the measured V_m does not hyperpolarize. The hypothesis that electrogenic Na-K pumping maintains V_m during hypoxia is not supported. Rather, these data suggest that during severe hypoxia the resting membrane potential continues to be primarily determined by the passive distribution of potassium.

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