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RESPONSES OF REGULARLY-FIRING *APLYSIA* R_3-R_{13} NEURONES TO NORMOXIA AND HYPOXIA

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SUMMARY

1. Exposure of 10 R_3-R_{13} neurones to a 115-min period of hypoxia resulted in depolarization of their membrane potentials (E_M) from a mean of $-46{\cdot}9 \pm 3{\cdot}1$ to $-20{\cdot}8 \pm 4{\cdot}4$ mV (S.E.).

2. Intracellular potassium ion activities (a_K^i) decreased significantly from $118{\cdot}9 \pm 5{\cdot}1$ to $67{\cdot}7 \pm 8{\cdot}5$ mM-K^+. This is equivalent to a change in E_K from $-70{\cdot}9$ mV to $-54{\cdot}5$ mV, which is insufficient to account for depolarization of approximately 26 mV.

3. During reoxygenation of the saline surrounding the ganglion, there was a continued depolarization of E_M to $-11{\cdot}5 \pm 3{\cdot}2$ mV and progressive fall in a_K^i to $49{\cdot}2 \pm 4{\cdot}9$ mM.

4. Decreases in the membrane slope resistance were also observed in these depolarizing neurones. The depression in resistance remained irreversible for as long as experiments were conducted.

5. Computations of P_{Na}/P_K ratios were made using a steady-state calculation. Increases in the P_{Na}/P_K ratio from 0·030 to 0·045 were observed during hypoxic depolarization using a modification of the Goldman equation which neglects the contribution of chloride ions. Subsequent depolarization and loss of a_K^i during reoxygenation elevated this value to 0·183. Whether or not the observed depression of the membrane resistance is linked to a change in either the sodium or potassium ion permeability is unknown. Release of neurotransmitter and related permeability changes cannot be ruled out as an effect of hypoxia.

INTRODUCTION

In L_2-L_6 neurones of *Aplysia californica*, hypoxia has been observed to cause reversible increases in intracellular potassium ion activities (a_K^i) and concomitant membrane hyperpolarizations (Coyer, Halsey & Strong, 1983). It was concluded that these reversible changes seem to be brought about by augmentation of the sodium pump, possibly as a result of stimulation of ATP synthesis *via* glycolytic feedback. For regularly-firing neurones of the upper right quadrant R_3-R_{13}, Chaplain (1976, 1979), Chen, von Baumgarten & Takeda (1971), and Chen, von Baumgarten & Harth (1973) have shown that signalling patterns of these neurones are altered by compounds whose

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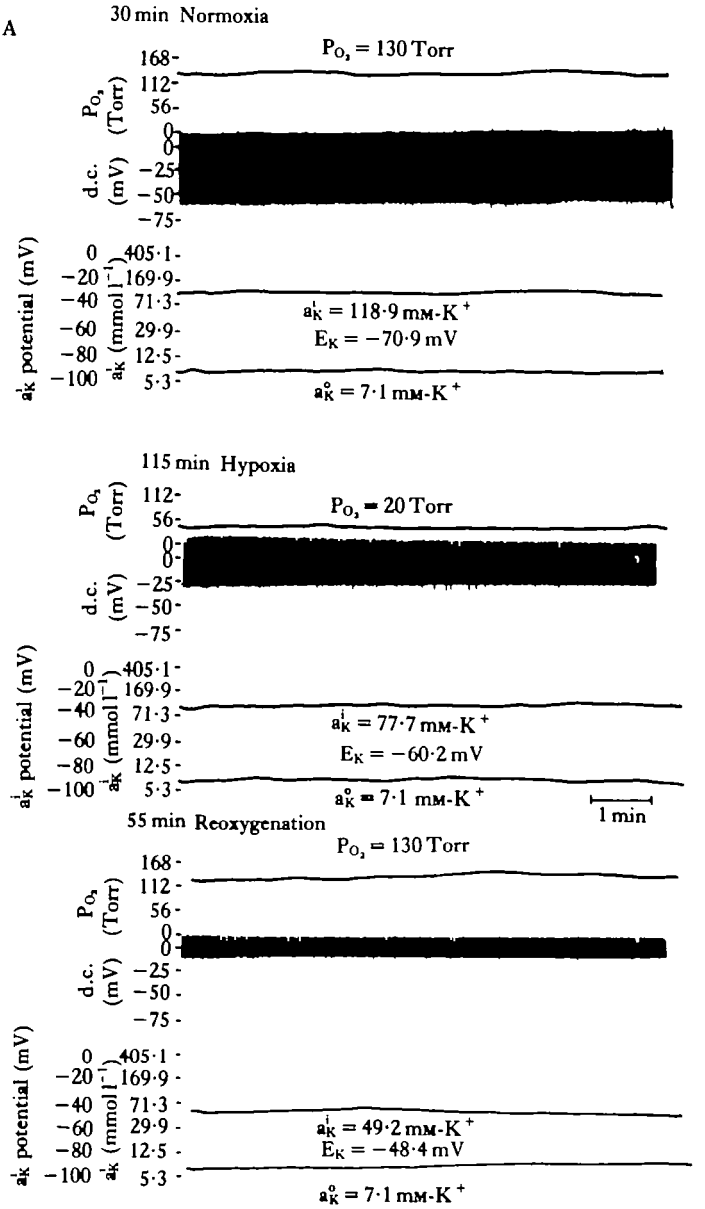

Fig. 1. (A) Records obtained from an R_3-R_{13} neurone during normoxia (top), hypoxia (middle) and reoxygenation (bottom), showing changes in P_{O_2} (upper trace), membrane potential (E_M, middle trace), and the intracellular and extracellular (a_K^i and a_K^o) potassium ion activities (lower traces). The potassium equilibrium potential, E_K, was calculated from the relative values of the potassium ion activities. Membrane depolarization and a concomitant loss of a_K^i are shown in the middle panel following 115 min of hypoxia. The irreversibility of this response is shown in the bottom panel in which the membrane potential and a_K^i continue to decrease following reoxygenation. (B) Dynamic current voltage curves for 1 of the 10 neurones during normoxia (curve 1), after 60 min hypoxia (curve 2), 115 min hypoxia (curve 3), and after 55 min reoxygenation (curve 4). Membrane slope resistance is indicated by the slope of the line tangent to the curve, and shows a progressive decrease during hypoxia and reoxygenation.

function is to regulate cellular metabolism either by inhibiting glycolysis or by disrupting certain steps in this process such as the catalysis of fructose-6-phosphate (F-6-P) by phosphofructokinase (PFK).

In the present paper, hypoxia is shown to cause irreversible decreases in intracellular potassium ion activity (a_K^i), membrane potential (E_M), and membrane slope resistance of R_3-R_{13} neurones. Computations of the relative permeability ratios (P_{Na}/P_K), based upon a modification of the Goldman equation, are also expressed for the responses of these neurones to normoxia and hypoxia (see Coyer, 1981; Coyer *et al.* 1983). Irreversible effects of hypoxia upon membrane potential and input resistance have been given in a preliminary report (Coyer, Halsey & Strong, 1981).

MATERIALS AND METHODS

Neurones within the right rostral 'white cell' quadrant were recognized visually as R_3-R_{13} using established criteria (Frazier *et al.* 1967; Kandel, Frazier, Waziri & Coggeshall, 1967; Koester & Kandel, 1977). Standard electrophysiological techniques were employed to measure the membrane potentials of 10 cells during normoxia, hypoxia and reoxygenation. These neurones were exposed to 30 min of

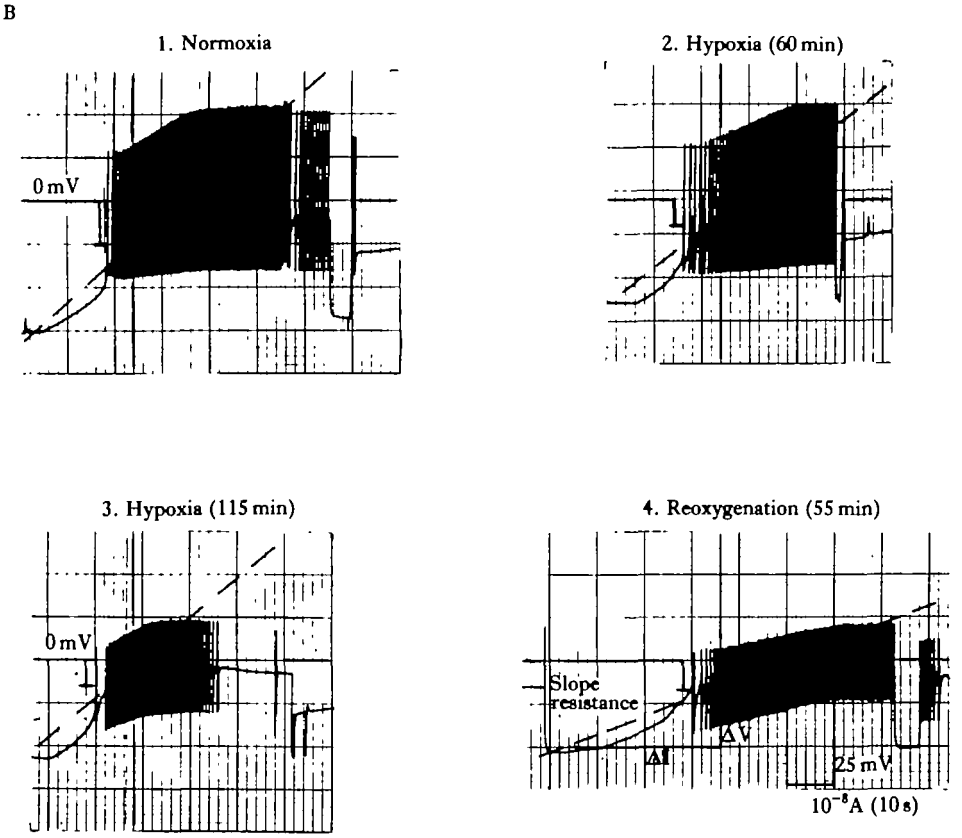

Fig. 1B

normoxia corresponding to a P_{O_2} between 130 and 150 Torr, 115 min of hypoxia (P_{O_2} = 12–20 Torr), and 55 min of reoxygenation. Intracellular potassium ion activity was measured using double-barrelled, K^+-selective microelectrodes (Walker, 1971; Khuri, Hajjar & Agulian, 1972; Vyskočil & Kříž, 1972). The construction of these microelectrodes has been adequately described by Vyskočil & Kříž (1972), Schlue & Deitmer (1980) and Deitmer & Schlue (1981), and their usage in making intracellular measurements in *Aplysia* neurones has been described by Coyer *et al.* (1983). Dynamic current voltage curves were constructed by passing a linear, depolarizing current across the neural membrane (see Coyer *et al.* 1983). The outputs of high input impedance d.c. amplifiers were displayed on high fidelity penwriters (Gould) and recorded on a four-channel FM magnetic tape recorder (Hewlett-Packard). Oxygen microelectrodes and P_{O_2} determinations were made following the techniques described by Coyer *et al.* (1983). Normal *Aplysia* saline consisted of: 425 mM-NaCl, 10 mM-KCl, 10 mM-$CaCl_2$, 22 mM-$MgCl_2$, 26 mM-$MgSO_4$, 2·5 mM-$NaHCO_3$, 10 mM-Tris-HCl (pH = 7·3). Saline was delivered constantly at a rate of 10 ml min^{-1} to the experimental bath from a reservoir located above it. Hypoxia was arbitrarily defined as a suffusate P_{O_2} below 20 Torr, which probably corresponds to an intracellular P_{O_2} of 5–7 Torr (Coyer *et al.* 1978). The calculation used in determining the relative permeability ratios (P_{Na}/P_K) from the values of the intracellular

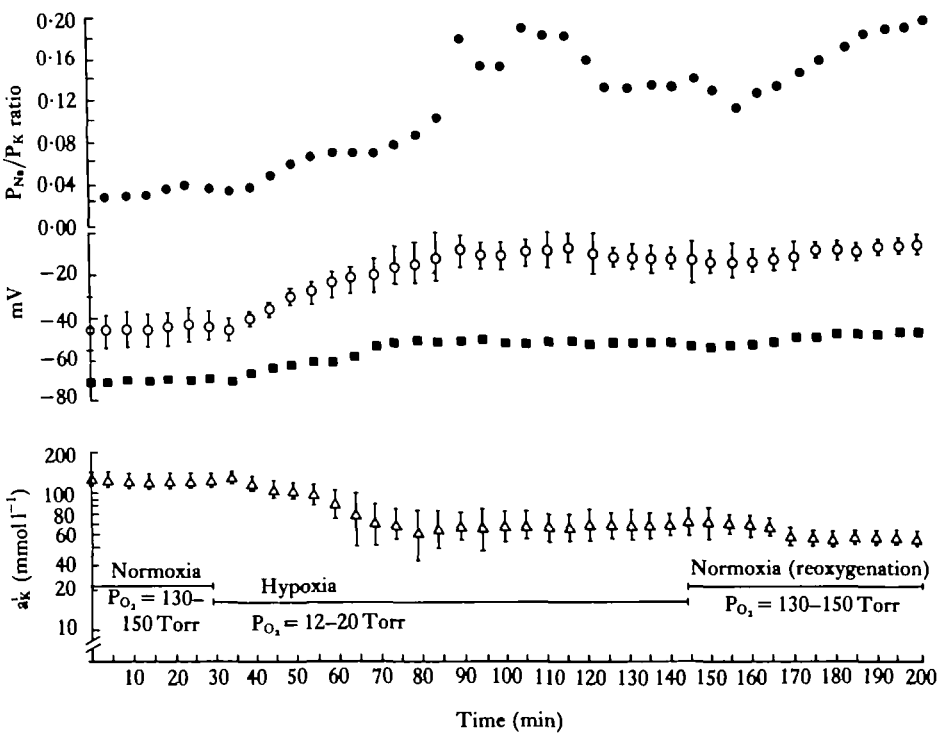

Fig. 2. Means ± standard errors (x±S.E.) corresponding to E_M (○), a_K^+ (△), and the computed values for E_K (■) and the P_{Na}/P_K (●) ratios are presented graphically for seven R_3-R_{13} neurones whose responses to normoxia, hypoxia and reoxygenation were studied. Moreover, a_K^+ decreased thus making E_K more positive. The computed P_{Na}/P_K ratios also increased. These changes in E_M, a_K^+, E_K and P_{Na}/P_K were irreversible upon subsequent reoxygenation.

potassium ion activities was as described elsewhere (Coyer, 1981; Coyer *et al.* 1983). Means and standard errors (x±S.E.) of E_M and a_K^i were computed using sample statistical procedures. Mean values of these variables were used in solving the equations for E_K, the potassium equilibrium potential and the P_{Na}/P_K ratio.

RESULTS

Determinations of E_M, a_K^i and P_{Na}/P_K ratios in R_3-R_{13} neurones during normoxia, hypoxia and reoxygenation

Hypoxic depolarization and decreases in the intracellular potassium ion activity

Hypoxia produced a depolarization of the membrane potential (E_M) and loss of intracellular potassium activity (a_K^i) that continued during reoxygenation (Fig. 1A). In 10 cells, a 115-min period of hypoxia produced a depolarization of their membrane potentials (E_M) from a mean of $-46{\cdot}9 \pm 3{\cdot}1$ to $-20{\cdot}8 \pm 4{\cdot}4$ mV (S.E.). In addition, intracellular potassium ion activities (a_K^i) decreased significantly from $118{\cdot}9 \pm 5{\cdot}1$ to $67{\cdot}7 \pm 8{\cdot}5$ mM-K^+. Computed values of the potassium equilibrium potential (E_K) were $-70{\cdot}9$ mV and $-54{\cdot}5$ mV respectively. During reoxygenation of the saline surrounding the ganglion, there was a continued depolarization of E_M to $-11{\cdot}5 \pm 3{\cdot}2$ mV and progressive fall in a_K^i to $49{\cdot}2 \pm 4{\cdot}9$ mM. During hypoxia, there was a depolarization of approximately 26 mV as opposed to a change in E_K of 16 mV. Therefore, the extent of the depolarization is greater than could be accounted for by the change in

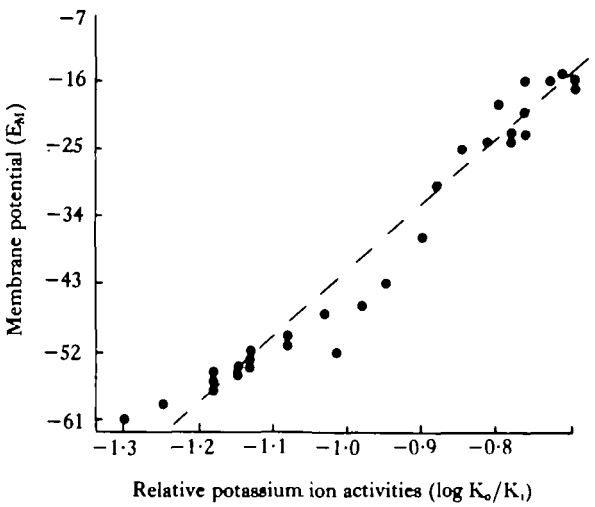

Fig. 3. The data for E_M and a_K^i were plotted in the form of E_M as a function of the logarithm of K_o/K_i assuming K_o remained constant having a value equal to 7·1 mM-K^+. Since a high perfusion rate of 10 ml min^{-1} was utilized, no fluctuations in a_K^i were observed with hypoxia. This method is similar to that of Coyer, Halsey & Strong, (1983). A slope of 89·3 mV indicates that the observed hypoxic depolarization could be accounted for not only by decreases in a_K^i but also changes in the P_{Na}/P_K ratio since a Nernstian slope was not observed.

E_K. The computed P_{Na}/P_K ratios (Fig. 2, solid circles) increased from a value of 0·028–0·031 to 0·045. The irreversibility of this increase in the P_{Na}/P_K ratio and the continuing depolarization and loss of a_K^i are shown in the right portion of Fig. 1A as the P_{Na}/P_K ratio reaches a value of 0·183.

Values for E_M were replotted as a function of a_K^i (Fig. 3). Again the calculated slope of 89·3 mV shows that the depolarization cannot be explained by changes in E_K only.

Decreases in the membrane slope resistance of R_3-R_{13} neurones during hypoxia

Membrane slope resistance decreased during hypoxia and reoxygenation (Fig. 1B).

DISCUSSION

The results suggest that hypoxia causes irreversible depolarization of R_3-R_{13} neurones occupying the *Aplysia* abdominal ganglion, together with a decrease in a_K^i and E_K. The calculated P_{Na}/P_K ratio increased as the neurones continued to depolarize at a level which was not commensurate with changes in E_K. Thus, the depolarization could be caused by a change in a_K^i together with a change in P_{Na}/P_K. An important, alternative explanation which could account for the depolarizations could be that these cells have an electrogenic sodium pump whose contribution is important in maintaining the resting membrane potential (see Discussion below). Similar changes in membrane potential, which were found to exist in R_2 by Carpenter & Alving (1968) during inhibition of the sodium pump, may account for the discrepancy between changes in membrane potential and E_K in the R_3-R_{13} neurones.

Hypotheses concerning the effects of hypoxia on R_3-R_{13} neurones

The depression in the membrane resistance and associated decreases in a_K^i and E_M indicate that the membranes of these cells become more permeable to either sodium, chloride, potassium or a combination of these ions during hypoxia. Substantial increases in the P_{Na}/P_K ratios during hypoxia are shown in Fig. 2 (closed circles). The decrease in membrane resistance during hypoxia is consistent with these observed increases in the P_{Na}/P_K ratios. The observed irreversible nature of membrane depolarization, loss of a_K^i, and depression of the membrane resistance might be expected from changes in ionic permeabilities.

Normally, the metabolically-dependent sodium pump contributes to the membrane potential by maintaining the asymmetric distribution of ions across the neural membrane. It has been suggested that molluscan neurones have ATP reserves sufficient for 20–30 min of normal sodium pumping providing the intracellular P_{O_2} is higher than 20 Torr, as exists under normal, aerated conditions of intracellular recording (Kerkut & York, 1969; Junge & Oritz, 1978). Kerkut & York (1969) demonstrated the oxygen sensitivity of the electrogenic sodium pump in brain neurones of the snail *Helix* by injecting cells with sodium and potassium ions. Finding that sodium-injected neurones had membrane potentials which were more dependent upon P_{O_2} than did potassium-injected neurones, Kerkut & York (1969) concluded that the sodium pump relies heavily upon the process of oxidative phosphorylation to supply energy in the form of ATP or another high-energy phosphate containing compound. More than likely, the metabolic reserves of the R_3-R_{13} neurones are

consumed during a 115-min period of hypoxia under correspondingly low intracellular P_{O_2} conditions.

Tonic release of neurotransmitter has been mentioned as a possible effect of hypoxia on the L_2-L_6 neurones (Coyer *et al.* 1983). Pinsker & Kandel (1969) have demonstrated the sensitivity of the pump current in L_5 to the presynaptic influence of interneurone L_{10}. It is possible either that the sodium pump of R_3-R_{13} neurones is modulated by presynaptic sources and neurotransmitter release during hypoxia, or that these permeability changes result from synaptic inputs. In addition, Mayeri, Brownell, Branton & Simon (1979*a*) and Mayeri, Brownell & Branton (1979*b*) have shown the tonic influences resulting from release of egg-laying hormone (ELH) contained in bag cells on these and other neurones within the ganglion. Since the bag cells were not isolated from these R_3-R_{13} cells, this may be a factor worthy of consideration. However, during current stimulation of the bag cells (i.e. to release ELH), only slow inhibition and some instances of transient excitation were observed (Mayeri *et al.* 1979*b*). Depolarization and release of intracellular potassium were observed in these studies of hypoxia which is in opposition to those which were shown to be mediated by ELH (Mayeri *et al.* 1979*b*).

Possible differences between R_3-R_{13} and L_2-L_6 neurones accounting for the disparate responses to hypoxia

Both groups of neurones are oxygen sensitive although prolonged hypoxic exposure has opposite effects on the membrane potentials and intracellular potassium ion activities of these two groups of cells. One can only speculate that the R_3-R_{13} neurones have a greater pump dependence for the membrane potential and that hypoxia steadily upsets the ionic imbalance of sodium, potassium and chloride ions across the neural membrane which contributes to the membrane potential. This difference may reflect an intrinsic difference in the active pump sites or possibly the metabolism of each cell type and its capability to provide ATP for operation of the pump. As suggested in an earlier paper (Coyer *et al.* 1983), activity of the sodium pump in L_2-L_6 neurones may be stimulated by decreasing oxygen conditions through glycolytic feedback and ATP synthesis. In R_3-R_{13} neurones this phenomenon may not occur due to differences in the metabolic machinery of these cells. Future experiments are planned to test this hypothesis by testing their pump sensitivities to ouabain and also by injecting intermediary compounds, such as ADP and citrate, into R_3-R_{13} and L_2-L_6 neurones using a pressure microinjection system. In each of these groups of neurones, these experiments will test how prevalent the sodium pump is and whether or not it can be stimulated by intermediary compounds which may be thought to stimulate ATP synthesis through glycolytic feedback mechanisms.

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