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The effect of hypoxia and ouabain on the muscle resting potential of *Periplaneta americana L.*

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Abstract. We have studied the effect of hypoxia and ouabain on the muscle resting potential (RP) of the cockroach *Periplaneta americana L.* The experiments were done in insects reared either in normoxic or hypoxic conditions and treated, or not treated, with ouabain. Hypoxic conditions decreased the RP by about 11.6 mV. Ouabain decreased the RP by about 8.3 mV (ouabain 10^{-5} mol/l) or 12.3 mV (ouabain 5 x 10^{-5} mol/l). Hypoxia and ouabain decreased the RP by about 28.5 mV (ouabain 10^{-5} mol/l) or 31.3 mV (ouabain 5 x 10^{-5} mol/l).

Key words: hypoxia, ouabain, resting potential (RP), cockroach

INTRODUCTION

In its original definition, hypoxia represents a state of reduced oxygen availability when the partial pressure of ambient oxygen has fallen below normooxic values. Under severe hypoxia, when the partial pressure of oxygen in the environment becomes increasingly reduced, animals will not be able to transfer oxygen to tissues at a rate sufficient to meet the oxygen requirements of mitochondria for continuous reoxidation of NADH and to produce ATP using the respiratory chain (Grieshaber et al. 1994). Hypoxia as a factor disturbing the respiration processes may cause electrophysiological abnormalities (Weiss and Shieh 1994), including:

1. membrane depolarization,
2. reduction of excitability,
3. reduction of conduction, and
4. altered refractoriness.

Many inhibitors of cell respiration, e.g., ouabain, show simillar effects, such as low oxygen levels. Therefore, it was of interest to see if the effects of both factors (hypoxia and ouabain) applied concomitantly would be additive.

METHODS

The animals used in our experiments were adult cockroaches *Periplaneta americana L.* The experiments were done in four groups of insects:

-the first group (control) - insects were reared in normal content of oxygen (21%) and RP was measured in standard physiological saline;

-the second group - RP was measured in standard physiological saline after 2 h rearing in hypoxic conditions (10% oxygen);

-the third group - insects were reared in normal content of oxygen and RP was measured in physiological saline containing ouabain;

-the fourth group - RP was measured in physiological saline containing ouabain after 2 h rearing in hypoxic conditions.

In the third and fourth experimental groups two concentrations of ouabain in physiological saline were used: 10^{-5} mol/l and 5 x 10^{-5} mol/l.

The resting potential was studied for 90 min using conventional microelectrode methods. Glass microelectrodes filled with 3 mol/l KCl were used for experiments. RP was recorded from dorso-ventral (flight) muscle.

The standard physiological saline contained (in mmol/l): NaCl - 200; KCl - 3.1; $CaCl_2$ - 5.4; $MgCl_2$ - 5; phosphate buffer pH 7.2. (Pelhate and Sattelle 1982).

The insects were reared in insectaria at room temperature. The cockroaches which were reared in hypoxic conditions were kept at room temperature in a chamber gassed with a 1:1 air/N_2 mixture (final oxygen content 10%) for 2 h.

The number of measurements in each group was 60.

RESULTS

Figures 1, 2 and 3 summarize the results obtained.

The resting potential of the control group just after the equilibration time was -66.8±0.3 mV and did not change significantly within 90 min of experiment duration.

Two hours of keeping animals in hypoxic conditions caused a decrease of RP to -55.2±0.6 mV. Ouabain in the third group of insects caused a de-

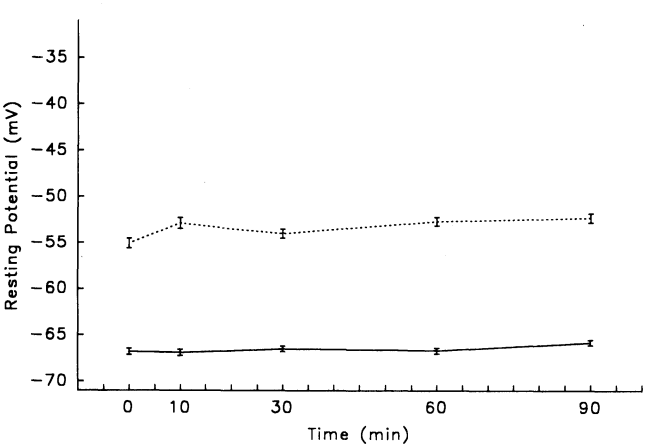

Fig. 1. Effect of hypoxia (2 h) on the muscle resting potential of cockroach *Periplaneta americana L.* (—), control; (- - - -), 2 h of hypoxia.

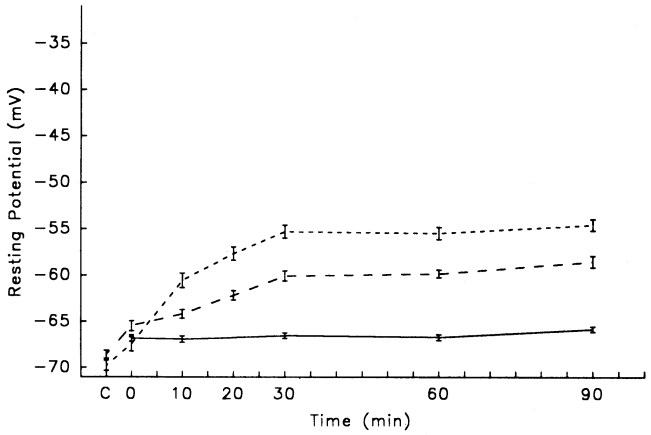

Fig. 2. Effect of ouabain (10^{-5} and 5 x 10^{-5} mol/l) on the muscle resting potential of cockroach *Periplaneta americana L.* (——), control; (– – –), ouabain 10^{-5} mol/l; (- - -), ouabain 5 x 10^{-5} mol/l.

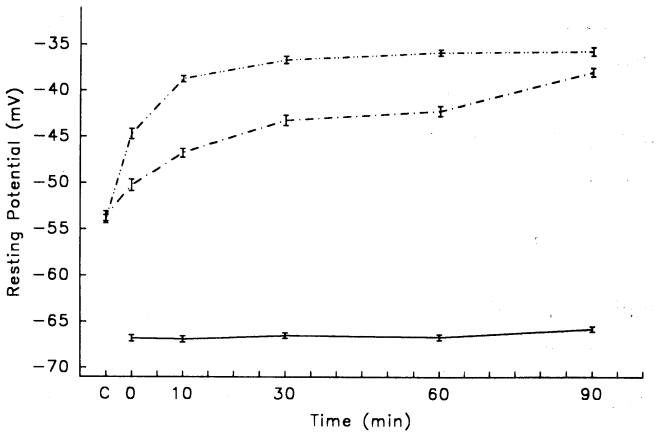

Fig. 3. Effect of hypoxia (2 h) and ouabain (10^{-5} and 5 x 10^{-5} mol/l) applicated concomitantly on the muscle resting potential of cockroach *Periplaneta americana L.* (——), control; (– . –), 2 h of hypoxia and ouabain 10^{-5} mol/l; (– .. –), 2 h of hypoxia and ouabain 5 x 10^{-5} mol/l.

crease in RP to -58.5±0.6 mV (10^{-5} mol/l) and -54.5±0.6 mV (5 x 10^{-5}mol/l) .

After 2 hours of hypoxia and application of ouabain (10^{-5} and 5 x 10^{-5} mol/l) the RP decreased to -38.3±0.5 mV and -35.5±0.5 mV respectively.

DISCUSSION

The effect of hypoxia and ouabain was investigated in the above series of experiments. Two hours of keeping cockroaches in hypoxic conditions caused a decrease of their muscle resting potential. Ouabain, the selective inhibitor of Na-K-ATPase, had a simillar effect. Ouabain applied immediately after a 2 h period of hypoxia intensified the depolarizing effect of hypoxia. There are many reports indicating that resting potential is affected by hypoxia and application of metabolic inhibitors like KCN and DNP (Kerkut and York 1969, Wareham et al. 1974, Mony et al. 1986, Fujiwara et al. 1987, Mubagwa et al. 1994). Our previous studies also confirmed those data (Grajpel et al. 1992, Katkowska et al. 1993). There are also many studies indicating the depolarizing effect of ouabain on the resting potential (Kerkut and York 1969, Wareham et al. 1974). Both hypoxia and ouabain affect the resting potential by inhibition of Na-K-ATPase (Kerkut and York 1969, Wareham et al. 1974). However hypoxia may have a more general effect. Kerkut presented two effects of oxygen on the resting potential: (1) a major one on the electrogenic Na-K-ATPase and (2) a minor one on the membrane or on membrane permeability (Kerkut and York 1969). In ischemic muscle preparation an abnormal decrease of the ratio between the permeability to K^+ and depolarizing ions (Na^+, Ca^{++}) was observed (Mubagwa et al. 1994).

In our experiments hypoxic conditions were obtained *in vivo*, i.e. the whole animal was kept in changed oxygen conditions. Moreover, decreasing the oxygen content in respired air to half of its normal value did not cause complete inhibition of Na-K-ATPase. It may be the reason for the smaller decrease of RP in our experiments compared with the experiments of Wareham (Wareham et al. 1974). The supply of oxygen by saline superfusion differs from the normal oxygen supply *via* the trachea (Mony et al. 1986).

From our experimental results it can be concluded that: (1) under hypoxia the rate of oxygen transfered to the cells is not sufficient to meet the ATP requirements. In such conditions the ionic homeostasis is disturbed; (2) the decrease of RP by ouabain indicates the role of Na-K-ATPase in maintaining the RP in *Periplaneta americana L.;* (3)

ouabain, influencing Na-K-ATPase, intensifies the effect of hypoxia. The mechanisms underlying the intensified depolarizing effect of hypoxia and ouabain remain unclear and require further investigations.

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