Short communication

Electrophysiological Changes Associated with Right Ventricular Hypertrophy Induced in Adult Rat Exposed to a Simulated High Altitude

C. CHOUABE[1], P. MEGAS[2], A. CHAKIR[2], O. ROUGIER[1], A. FREMINET[2] and R. BONVALLET[1]

1 *Laboratoire de Physiologie des Eléments Excitables (URA CNRS 180)*
2 *Laboratoire de Physiologie du Métabolisme Intermédiaire et Energétique, Université Claude Bernard, 69622 Villeurbanne, France*

Many models of cardiac hypertrophy have been used in the past (for a review see Bugaisky et al. 1992). Whatever the type of chronic overloading used (pressure versus volume), the most consistent electrophysiological modification observed either in cardiac tissues or in isolated cardiomyocytes is an increase in the transmembrane action potential duration (for a review see Ten Eick and Bassett 1984). In growing rats adapted to chronic hypoxia corresponding to a high altitude of 3500 m for about 4 weeks, Turek et al. (1972), in a morphological study, have reported an increase of the muscle fibre diameter in the right ventricle corresponding to a true hypertrophy rather than hyperplasia. Unfortunately no results concerning the electrical activity are available with this model of hypertrophy. In the present study, we have examined whether this form of hypertrophy can be induced in adult rats exposed to a simulated altitude of 4000 m and if it is associated with electrophysiological changes.

The experiments were performed on male Sprague-Dawley rats. On delivery from the breeder, they were kept for one week under controlled temperature (25 ± 1 °C) and light (7–19 hours) conditions and with standard food available ad libitum. Then they were divided into two groups: one exposed to hypoxia (exposed rats = E), the other serving as control (C), i.e. maintained under normoxic conditions. As continuous exposure to hypoxia led to a slower growth than in normoxia, the control animals were also divided into two groups corresponding to the same body weight at the beginning (C1) and at the end (C2) of the experiments, respectively (animals of C2 group were younger than those of C1 and E groups). The body weight values range approximately from 250 to 450 g on the day of the experiment (see scale of Fig. 1). The exposed rats were placed in a hypobaric chamber built in the laboratory (Mégas 1990) allowing an exposition equivalent to an altitude of 4000 m without any noticeable accumulation of carbon dioxide as

verified with an i.r. analyzer owing to a sufficient flux of air through the chamber. After a five days period of intermittent exposure (2 × 2 hours per day) performed as already described (Fréminet et al. 1990), the animals were exposed continuously for 5, 10, 20 or 30 days. An interruption of 2 hours was carried out every other days for cleaning, food and drinking renewal and weighting of the animals; the decompression or ascent and the compression or descent being performed progressively in 30 min. The electrophysiological experiments were done on enzymatically isolated ventricular cells with the whole-cell patch-clamp technique (Hamill et al. 1981) in current clamp condition at room temperature on 20 days exposed rats and corresponding controls (C2). The internal solution in the patch electrode (1–3 MΩ) contained (mmol/l) NaCl 7, K-aspartate 110, KCl 30, $MgCl_2$ 2, EGTA/KOH 0.2, HEPES/KOH 5, pH 7.2. The cells were superfused in a tissue culture dish by gravity with a solution containing (mmol/l) NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2, glucose 10, HEPES/NaOH 10, pH 7.2.

The membrane capacitance of the cell was determined by measuring the membrane time constant and the input resistance from the time course and magnitude of steady-state hyperpolarization elicited by 100 ms duration square pulses of inward current (200 pA) at the resting potential. The results were statistically analysed by t-test. A difference was assumed to be statistically significant when $p < 0.05$. Values are expressed as mean ± SD.

The protocol used in this study led up to the well-known hematological response to hypoxia. Indeed hematocrit values (%) were 52.3±1.1, 53.3±0.5, 55.3±

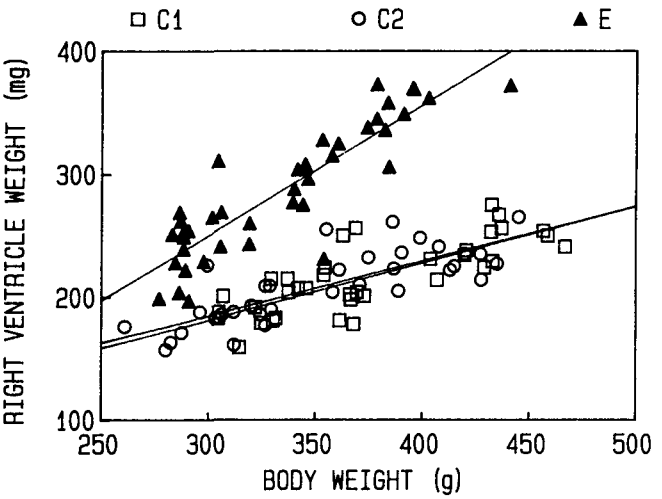

Figure 1. Weight of right heart ventricle of control (C1 and C2) and high altitude exposed rats (E) plotted against their body weight. Note that the slope of the regression line is higher for the right ventricle of the exposed rats as compared with the controls. See text for more explanations.

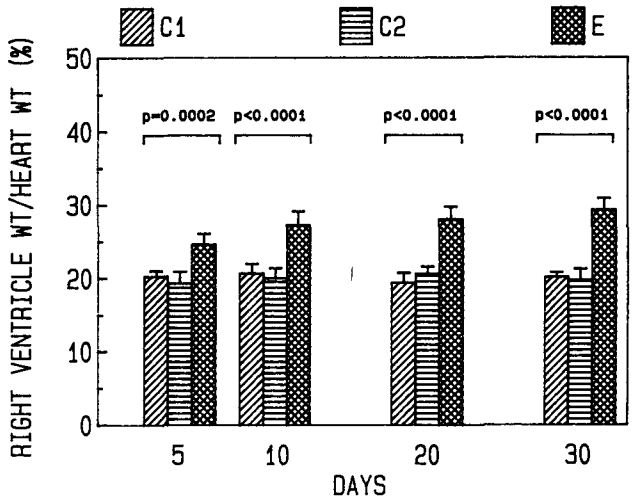

Figure 2. Evolution of right ventricular weight to heart weight ratio in controls (C1 and C2) and high altitude exposed rats (E). Note the increase in the right ventricle weight in the exposed animals. For C1, C2 and E groups n varied between 9 to 15 for each period. Bars correspond to SD.

0.5 and 57.5 ± 1.0 in E group after 5, 10, 20 and 30 days of exposure whereas the corresponding pooled values (owing to the similarity between them) in C1 and C2 groups were 41.1 ± 0.6, 42.3 ± 0.3, 43.8 ± 0.4 and 44.8 ± 0.35. Fig. 1 shows the weight of the right ventricle as a function of the body weight for the two groups of rats. The increase of this parameter is greater for the right ventricle of the exposed rats (E) as compared with the controls (C1, C2). The slope of the regression lines are 1.05, 0.46 and 0.44 for the E, C1 and C2 groups, respectively. Fig. 2 shows the ratio of right ventricle weight to heart weight plotted against the duration of the exposure of rats to simulated altitude. The weight of the right ventricle was significantly larger in the exposed animals as compared to controls with an increase of 28, 32, 40 and 46% after 5, 10, 20 and 30 days of exposure, respectively. This increase in right ventricle weight can be due to cell proliferation and/or enlargement of the individual cells. The estimate of myocyte membrane capacitance can give an insight into these two possibilities. This was tested on isolated cells from right and left ventricles taken from 20 days control (C2) and exposed rats. Fig. 3 shows the results of the membrane capacitance; the mean values for the right ventricular cells were 161 ± 37.9 pF ($n = 16$) and 223.2 ± 55.6 pF ($n = 14$) for control and exposed groups, respectively, while those for left ventricular cells were 176.0 ± 50.8 pF ($n = 11$) and 164.8 ± 57.2 pF ($n = 7$) for control and exposed groups, respectively.

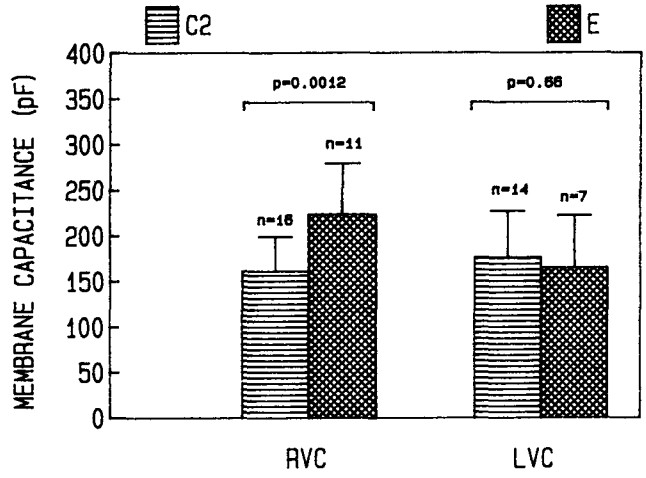

Figure 3. Membrane capacitance of right (RVC) and left ventricular cells (LVC) in 20 days control and exposed rats. Note the increase of membrane capacitance only in the right ventricular cells of high altitude exposed rats. Bars correspond to SD. See text for more explanations.

Thus the chronic exposure to hypoxia used in the present study seems to have an effect only on the membrane capacitance of the right ventricular cells. The significant increase in membrane capacitance (+38.6%, $p = 0.0012$) is in agreement with an hypertrophy of the right ventricular myocytes tested while the left ventricular myocytes tested do not seem modified.

Fig. 4*A* shows recordings of action potentials from control (left trace) and exposed right ventricular cells (right trace). As reported by Scamps et al. (1990) the action potentials from normal cells displayed a short duration with suppression of the late plateau due to the presence of EGTA in the patch-electrode. Fig. 4*B* shows the mean values ($n = 10$) of action potential duration measured at 25%, 50%, 75%, and 90% of repolarization. A significant increase in the action potential duration was observed at all levels of repolarization for the right ventricular cells while no modification was observed for the left ventricular cells (results not shown). Action potential amplitude was not significantly modified nor was the resting potential.

The following conclusions can be drawn :

1) on adult rats exposed to a high altitude of 4000 m an increase of the right ventricle weight takes place; this is in agreement with results obtained by Turek et al. (1972); 2) this weight increase is due all or in part to a hypertrophy; 3) the

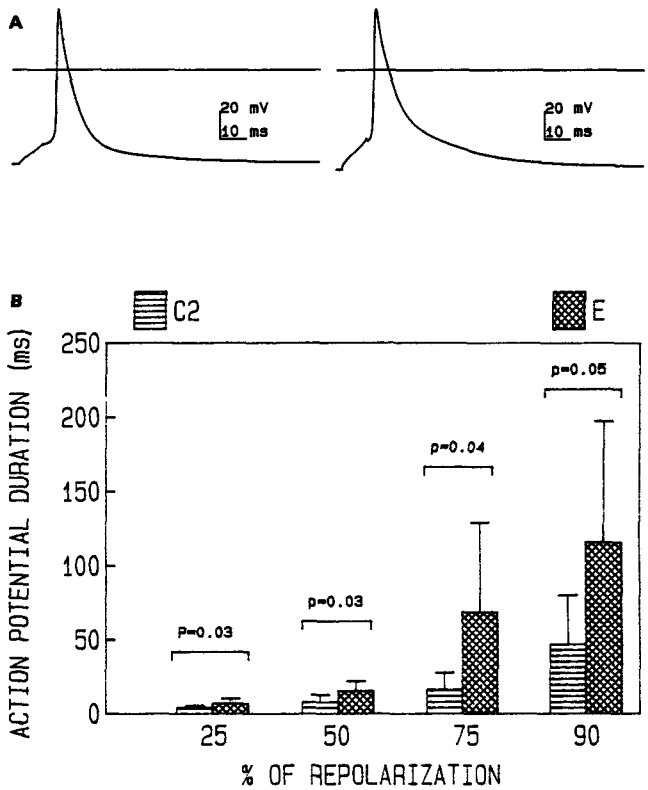

Figure 4. *A*) Action potentials recorded in single right ventricular cells from 20 days control (left trace) and exposed (right trace) rats. The horizontal lines indicate the 0 potential level. *B*) Right ventricular cell action potential duration measured at 25%, 50%, 75% and 90% of repolarization in 20 days "control" and "exposed" rats. Note the increase in the action potential duration at all levels of repolarization in high altitude exposed rats. Bars correspond to SD, $n = 10$ in each condition. See text for more explanations.

hypertrophied myocytes develop a prolonged action potential as generally reported whatever the procedure for overloading.

The identity of the ionic current(s) that is(are) altered in myocytes from exposed rats is currently under investigation.

Acknowledgements. Research was supported by the University Lyon I ("BQR" grant) and DRED (Equipe d'accueil "Métabolisme et canaux ioniques musculaires" EA 1657).

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Final version accepted December 28, 1993