REVISTA ESPAÑOLA DE FISIOLOGIA, 49 (1), 7-16, 1993

Lack of Effect of Acute Hypoxia and Hypercapnia on Muscle Relaxation Rate in Man

L. G. Vianna*, N. Koulouris and J. Moxham

Department of Thoracic Medicine
King's College Hospital
London (England)

(Received on January 7, 1992)

L. G. VIANNA, N. KOULOURIS and J. MOXHAM. *Lack of Effect of Acute Hypoxia and Hypercapnia on Muscle Relaxation Rate in Man.* Rev. esp. Fisiol., 49 (1), 7-16, 1993.

The effect of acute hypoxia and hypercapnia on skeletal muscle relaxation rate was investigated in man. The contractile force of limb muscles (*quadriceps femoris* and *adductor pollicis*) was studied in normal subjects using electrical stimulation. The twitch transdiaphragmatic pressure was used to investigate the diaphragm. The maximum relaxation rate was measured from contractions of the non-fatigued *quadriceps femoris*, *adductor pollicis* and diaphragm. Severe hypoxia (mean transcutaneous oxygen tension 40 mmHg) did not alter the maximum relaxation rate from 20 Hz stimulated contractions of the non-fatigued quadriceps. Acute hypercapnia (mean end-tidal CO_2 tension 65.1 mmHg) did not significantly change the maximum relaxation rate from 20 and 100 Hz tetanic contractions of non-fatigued quadriceps. The maximum relaxation rate from 1 Hz twitch tension and 20 Hz tetanic tension of adductor pollicis was also not altered by acute hypercapnia (mean end-tidal carbon dioxide tension 63 and 60 mmHg, respectively). The maximum relaxation rate from twitch transdiaphragmatic pressure was not altered by acute hypercapnia (mean end-tidal carbon dioxide tension 69.7 mmHg).

Key words: Hypoxia, Hypercapnia, Muscle relaxation rate.

Clinical observations support the view that the inspiratory muscle fatigue may precipitate or intensify ventilatory failure (25). The most recent technique used to detect respiratory muscle fatigue has been slowing of relaxation rate of the respiratory muscles (11, 19, 20). It is essential to know if acute hypoxia and hypercapnia consequent to ventilatory failure may slow muscle relaxation rate directly and offset the value of relaxation rate as an

* To whom all correspondence should be addressed: Faculdade de Ciências da Saúde, Departamento de Clínica Médica, Campus Universitário, Asa Norte, Brasília, CEP 70910 (Brazil).

index of fatigue in this clinical context.

The data to support that hypoxia consequent to ventilatory failure may directly slow muscle relaxation has been obtained from *in vitro* studies (6, 13, 23). The effect of acute severe hypoxia on skeletal muscle relaxation rate has not been previously investigated *in vivo*.

Most of the information concerning the relationship between respiratory acidosis and muscle relaxation has come from isolated preparations, and research on intact animals are scarce. HILL (16) pointed out that the twitch of the isolated frog sartorius muscle could be slowed by lowering the pH with carbon dioxide, although a very high concentration (80 % CO_2) was required. SAHLIN *et al.* (26) found a prolongation of the relaxation time in isolated rat limb exposed to 30 % CO_2. By contrast, recent studies reported that hypercapnic acidosis did not change half the relaxation time of frog sartorius, *in vitro* (24), and of canine diaphragm, *in vivo* (17, 27).

The aim of this study was to determine, in man, the effects of acute hypoxia upon the relaxation rate of non-fatigued *quadriceps femoris* and hypercapnic acidosis upon the relaxation rate of non-fatigued quadriceps, *adductor pollicis* and diaphragm muscles.

Materials and Methods

The index used to express relaxation rate was the slope of the tangent to the exponential phase of the force (or pressure) decay curve (maximum relaxation rate). Maximum relaxation rate (MRR) was calculated from the ratio of slope/peak force (or pressure) and was expressed as % force (or pressure) fall/10 ms (35, 36). Studies were performed on two non-fatigued limb muscles (*quadriceps femoris* and *adductor pollicis*) and diaphragm. For the limb muscles, the MRR was measured from twitch and tetanic isometric contractions and for the diaphragm from the twitch transdiaphragmatic pressure (Pdi)*.

The contraction force of the quadriceps femoris was recorded using an adjustable straightbacked chair and the muscle was activated by electrical stimulation, as described by EDWARDS *et al.* (10). The left *adductor pollicis* was studied using a handboard and, for electrically stimulated isometric contractions, the ulnar nerve was stimulated proximal to the wrist joint (10). Recording of adductor pollicis electrical activity (EMG) was performed by Disa Surface Recording Electrodes type 13 K 60 (Danted Electronics Ltd.). The experiments with the *adductor pollicis* were always preceded by an immersion of hand and forearm in a bath at 45 °C, for 10 min (18). Throughout the experiments, heating of the hand was provided by a 100 W lamp, to maintain the skin temperature at 38-39 °C (34).

Simultaneous measurements of oesophageal (Poes) and gastric pressures (Pg) were obtained using the method described by AGOSTONI and RAHN (1). The differential measurement of gastric and oesophageal pressures determined the Pdi, which was assessed with two commercially available balloons (P. K. Morgan Ltd., Rainham, Kent, UK). Each balloon catheter was connected to a Validyne MP-45 pressure transducer (Validyne Engineering Corp., Northridge. CA). The Pg and the Poes catheters were also connected to opposite ports of a third transducer which indicated the Pdi. The maximal frequency response of each balloon catheter-recorder system was 10.96 Hz. The Pdi

---

* *Abbreviations:*
MRR: maximum relaxation rate; MVC: maximum voluntary contraction force; pCO_2av: arterialized venous CO_2 pressure; pCO_2v: venous CO_2 pressure; pdi: transdiaphragmatic pressure; $PETCO_2$: end-tidal CO_2; pg: gastric pressure; pHav: arterialized venous blood pH; pHv: venous blood pH; Poes: oesophageal pressure; $tcPCO_2$: transcutaneous CO_2 tension; $tcPO_2$: transcutaneous O_2 tension.

was recorded during stimulation of the left phrenic nerve, at the neck. The electrical stimulation was performed with a Bipolar Nerve Stimulating Electrode type EC 225 (Medelec Ltd., Surrey, UK). The smooth rectified surface EMG of the costal left hemidiaphragm was recorded with DISA 13 K 60 Surface Electrodes. The raw EMG signal was recorded, rectified and integrated, using a Neurolog System Electromyograph type 14 A II (Digitimer Ltd.). Twitch Pdi was measured at functional residual capacity, with the glottis closed. The constancy of end-expiratory lung volume was achieved with a commercially available Respiratory Inductive Plethysmography System - Respitrace (Respitrace Corp., Ambulatory Monitoring Inc., New York, USA).

The hypoxic gas mixture contained 11 % O_2 and a balance per cent of nitrogen; the hypercapnic mixtures had 8 and 9 % CO_2, 21 % O_2 and a balance per cent of nitrogen. The gas mixtures were stored in Douglas bags. They were inspired by the subjects via a conventional mouth-piece, using a noseclip, and expired to room atmosphere. The concentrations of oxygen and CO_2 in the Douglas bags, and the end-tidal CO_2 (P_{ET} CO_2) at the mouth were measured with a Taylor Servomex Oxygen Analyser type OA 272 (Servomex Controls, Sussex, UK) and a Carbon Dioxide Gas Analyser type 901 MK2 (P. K. Morgan Ltd., Kent, UK). During the hypoxic studies, transcutaneous oxygen ($tcPO_2$) and CO_2 tensions ($tcPCO_2$) were continuously measured using Radiometer Transcutaneous Oxygen and Carbon Dioxide Electrodes model E 5240 (Radiometer, Copenhagen, Denmark). During the hypercapnic studies, venous and arterialized venous blood pH (pH_v and pH_{av}, respectively) and pCO_2 (pCO_{2v} and pCO_{2av}, respectively) were measured at 5 min intervals using an Acid-Base Analyser type ABL 30 (Radiometer, Copenhagen, Denmark). When arterialized venous blood was used as a substitute for arterial blood, the hand was covered with a surgical glove and immersed in a water bath, with water continuously circulating to keep the temperature at 45 °C (15).

The effects of acute hypoxia upon the MRR of *quadriceps femoris* and acute hypercapnia upon the MRR of quadriceps, *adductor pollicis* and diaphragm were investigated with different protocols, as follows.

Study 1: Hypoxia and MRR from tetanic tension of limb muscle. — MRR was measured in 3 normal male subjects, 29, 35 and 40 years of age, from 20 Hz stimulated isometric contractions of the non-fatigued right quadriceps. Square wave pulses of 0.5 ms were used, with voltage set to produce 40-50 % maximum voluntary contraction force (MVC), each train of impulses lasting 1.5 s. Each subject performed one contraction on 4 different occasions breathing room air and, on a fifth occasion, after 20 min of breathing 11 % O_2. A mean taken from the 4 measurements obtained during the normoxic tests was compared with the value from the hypoxic test. The MRR reproducibility was calculated with data obtained from the 4 normoxic tests.

Study 2: Hypercapnia and MRR from twitch tension of limb muscle. — The MRR was measured from twitch tension of *adductor pollicis* in 4 normal subjects (3 males, 1 female), ranging in age from 30 to 42 years. Each subject was studied on one occasion, breathing room air followed by 8 % CO_2. The ulnar nerve was stimulated using 0.5 ms duration square wave pulses, with the voltage being varied to produce submaximal and supramaximal stimulations. Twenty-one sets of 5 twitches at 1 Hz were obtained with 15 s intervals, during 5 min, breathing air. Following the breathing of 8 % CO_2 for 10 min, 21 sets of 5 twitches were recorded during 5 min, with 15 s intervals, still breathing CO_2. Thirty sets of twitches, 15

sets recorded with normocapnia and 15 with hypercapnia, matched for equal amplitude, were analized. A mean MRR was calculated from twitches with normocapnia and compared with a mean MRR with hypercapnia.

Study 3: Hypercapnia and MRR from tetanic tension of limb muscles. — The effect of hypercapnia on MRR from tetanic isometric contractions was studied in two limb muscles, *quadriceps femoris* and *adductor pollicis.*

Quadriceps. The MRR was measured from stimulated tetanic contractions of the left quadriceps, at 20 and 100 Hz, in 4 normal subjects (3 males, 1 female), ranging in age from 29 to 41 years. Square wave pulses of 1 ms duration were used, each train of impulses lasting 1.5 s. The voltage was adjusted at the start of each study, to obtain 50 % MVC with 20 Hz stimulation. Each subject was studied in one occasion with stimulations at 20 Hz followed, 30 a later, by stimulations at 100 Hz, at 5 min intervals, for 15 min breathing room air. Following the breathing of 9 % CO_2 for 20 min, one contraction at 20 Hz and another at 100 Hz were performed, under CO_2 breathing. A mean MRR taken from the 4 contractions at 20 and 100 Hz when breathing air were compared with the MRR obtained from the forces at 20 and 100 Hz, recorded at 20 min of 9 % CO_2 breathing.

Adductor Pollicis. MRR measured from 20 Hz stimulated tetanic contractions of the adductor pollicis was studied in a 38-year-old female subject, breathing room air and 8 % CO_2. The highest of 4 MVC's was measured. Then, contractions at 20 Hz were recorded, using square wave pulses of 0.5 ms duration, each train of impulses lasting 1.5 s. Variable voltage was applied to obtain contractions ranging from 5 to 75 % MVC. The subject was studied in one single occasion. Twelve tetanic contractions at 20 Hz were recorded, at 1 min intervals, breathing air. Following the breathing of 8 % CO_2 for 10 min, 12 contractions were obtained, at 1 min intervals, with the subject still breathing CO_2.

Study 4: Hypercapnia and MRR from twitch Pdi. — As a consequence of the high levels of ventilation, it was not possible to obtain reliable data for twitch Pdi during the hypercapnic gas mixture breathing. Reliable data were obtained in all subjects for 1 min during the initial recovery period, immediately after breathing carbon dioxide.

The MRR from supramaximal stimulated twitch Pdi of the left hemidiaphragm was measured in 3 normal subjects (2 males, 1 female), aged 32, 39 and 42 years. Each subject was studied on one occasion breathing room air followed by 9 % CO_2. During a control period breathing air, the MRR from 10 twitches with square wave pulses of 0.5 ms, at 1 Hz frequency, were obtained. Subsequently, the subjects breathed 9 % CO_2 for 12 min. MRR was measured from 1 twitch Pdi recorded during the first minute of recovery, when breathing air. In each subject, the mean MRR obtained during normocapnia was compared with the measurement taken at the recovery period following hypercapnia.

Statistical analysis. — The coefficient of variation and the paired Student's test were used in the statistical analysis.

Results

Study 1: Hypoxia and MRR from tetanic tension of limb muscle. — The MRR from 20 Hz tetanic isometric contractions of non-fatigued quadriceps with normoxia was 12.48 ± 0.54, 13.35 ± 0.70 and 14.35 ± 0.95 %, in each subject; the coefficients of variation were 4.33, 5.24 and 6.62 %,

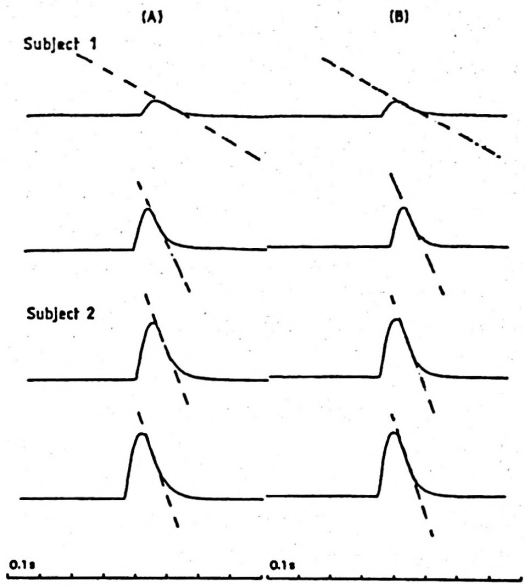

Fig. 1. *Recordings showing MRR from twitch tension of the adductor pollicis, matched for amplitude, breathing room air (A) and 8 % CO_2 (B).* Records from 2 subjects. MRR was not significantly changed by hypercapnia.

respectively. The MRR values with hypoxia were 12.21, 14.90 and 15.10 %. The difference between the values of both groups was not significant.

During the normoxic and hypoxic tests, the mean $tcPO_2$ was 98 and 33 mHg (range 28-36 mmHg) respectively, and the mean $tcPCO_2$ was 40 mmHg (range 37-43 mmHg) for both of them.

Study 2: Hypercapnia and MRR from twitch tension of limb muscle. — The 4 subjects were able to tolerate the breathing of 8 % CO_2 for 15 min. However, all of them experienced intense physical distress, marked hyperventilation and severe headache.

The twitch tension was 7.96 ± 4.71 N with air and 7.89 ± 4.48 N with CO_2, the MRR obtained from them was 13.10 ± 1.50 % with air and 13.28 ± 1.54 % with 8 % CO_2. There was no significant difference between these values. Recordings with representative examples of the MRR are in figure 1.

When breathing 8 % CO_2, pH_{av} fell from 7.41 ± 0.03 to 7.30 ± 0.02, PCO_{2av} rose from 39.24 ± 1.30 to 56.20 ± 1.50 mmHg, and $P_{ET}CO_2$ increased from 40.0 ± 2.5 to 63.0 ± 2.7 mmHg.

Study 3: Hypercapnia and MRR from tetanic tension of limb muscles. — *Quadriceps.* The MRR measured from stimulated contractions at 20 Hz was 13.13 ± 2.09 % with air and 13.51 ± 1.45 % with 9 % CO_2. The values obtained at 100 Hz with air and 9 % CO_2 were similar. Differences were not significant.

All subjects became acidotic when breathing 9 % CO_2, with a fall in the pH_v

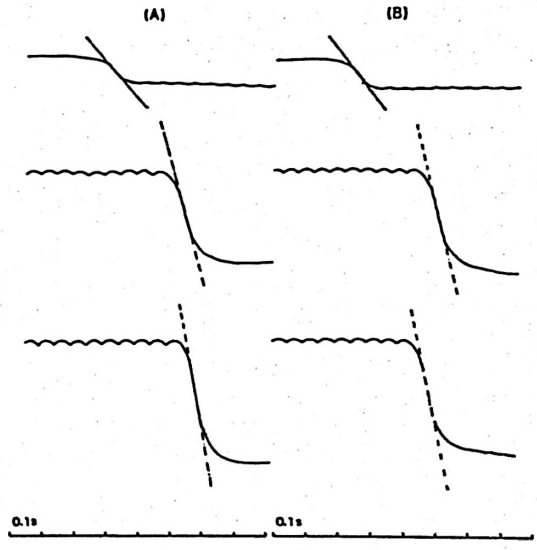

Fig. 2. *Recordings showing MRR from contractions at 20 Hz on the adductor pollicis, matched for amplitude, breathing room air (A) and 8 % CO_2 (B).*
Records from one subject. MRR in (A) was 13.7. MRR was not significantly changed by hypercapnia.

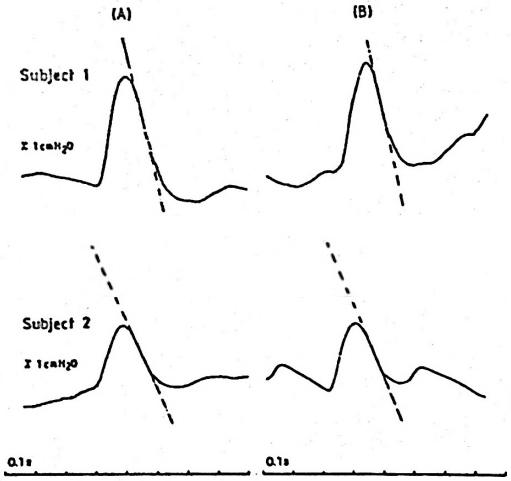

Fig. 3. *Recordings showing MRR from supramaximal stimulated twitch Pdi at 1 Hz, before (A) and during the first minute of recovery (B) following the breathing of 9 % CO_2.*
Records from 2 subjects. MRR in (A) was 11.8 (subject 1) and 10.4 (subject 2). MRR was not significantly changed by hypercapnia.

from 7.37 ± 0.02 to 7.26 ± 0.02 ($p < 0.01$). The pCO_{2v} rose to 61.30 ± 3.20 mmHg ($p < 0.001$) and the $P_{ET}CO_2$ to 65.1 ± 3.3 mmHg ($p < 0.001$).

Adductor pollicis. The MVC recorded with normocapnia was 84.86 N. The tetanic contractions ranged from 5.02 to 64.85 N. The MRR measured from 20 Hz stimulated contractions was 13.77 ± 0.29 % with air and 13.61 ± 0.26 % with 8 % CO_2 (figure 2). The plateau $P_{ET}CO_2$ was 60 mmHg.

Study 4: Hypercapnia and MRR from twitch Pdi. — The mean twitch Pdi was 6.3, 11.5 and 18.0 cm H_2O, and the MRR during the control period breathing air, was 9.31 ± 0.71, 11.09 ± 1.18 and 11.73 ± 0.69 % for each subject. The values were similar, after hypercapnia. Differences were not significant (figure 3).

The mean $P_{ET}CO_2$ was 69.7 mmHg (68.0, 70.5 and 70.5 mmHg, in each subject).

Discussion

The results of the present study showed that neither acute hypoxia nor hypercapnia *per se* altered the MRR from electrically stimulated isometric contractions of non-fatigued skeletal muscles in man.

The absolute values of MRR measured from human quadriceps, *adductor pollicis* and diaphragm muscles were similar to those reported by others (20, 35, 36). To improve the accuracy of the results whenever possible, the MRR was obtained from 3-10 contractions and the mean values were calculated. The values obtained on different occasions were considered reliable, since the coefficient of variation for MRR from 20 Hz stimulated contractions of the quadriceps ranged from 4.3 to 6.6 %, which agreed with data of WILES (33) using the differentiated force record.

Hypoxia and MRR. — Severe acute hypoxia (mean $tcPO_2$ 40 mmHg) did not alter the MRR of human non-fatigued quadriceps muscle. The effect of acute severe hypoxia on human skeletal muscle relaxation rate has not been previously investigated.

Studies of the effect of hypoxia on heart muscle relaxation rate *in vitro* have yielded conflicting results. BING *et al.* (3) observed, on isolated heart muscle, no indication of relaxation rate impairment by hypoxia. By contrast an alteration in relaxation rate of cat papillary muscle with hypoxia has been found (13), as well as that hypoxia prolongs the relaxation rate of the mammalian myocardium (6). A change in relaxation rate in the isolated rat left ventricular papillary muscles has also been reported (23), although it appeared to be less affected than active contraction.

Hypoxia *in vitro* predisposes to reduc-

tion of intramuscular pH and phosphoryl creatine concentrations and increased intracellular lactate concentrations. These metabolic alterations are associated with an increase in the time constant of frog gastrocnemius muscle relaxation (8). During hypoxia *in vivo*, the muscle must employ compensatory mechanisms to maintain the requisite level of oxidative phosphorylation. The mechanisms which may influence muscle oxygen uptake during hypoxia are: a) increased total blood flow (28); b) redistribution of flow within the microcirculation to decrease the diffusion distance (2); and c) the presence of myoglobin-facilitated oxygen diffusion (7). In our study, it seems that these compensatory mechanisms in the quadriceps muscle were sufficient to compensate for the reduction in oxygen supply.

The subjects hyperventilated during the hypoxic experiments and developed mild hypocapnia (mean $tcPCO_2$ 33 mmHg) but the hypocapnia associated with hypoxia did not influence the present results obtained on MRR. In accordance to this, SCHNADER *et al.* (27) also reported no change in half relaxation time of twitch Pdi of canine diaphragm *in vivo*, when exposed to low arterial blood CO_2 tension from 0 to 34 mmHg.

Hypercapnia and MRR.- Acute respiratory acidosis ($PETCO_2 \leq 69.7$ mmHg) did not alter the relaxation rate of human non-fatigued *quadriceps femoris*, *adductor pollicis* and diaphragm.

Intracellular pH reduction has been recognized to lead to a relaxation rate decline of cardiac muscle (14). The relaxation rates from frog gastrocnemius muscle (32) and from snail retractor pharynx (5) also decreased by exposure to acute hypercapnia. The isolated frog sartorius and rat limb muscles could be slowed by lowering the pH with 80 % and 30 % CO_2, respectively (16, 26). However, since these muscles were not superfused, a partial ischaemia could develop. Our findings are similar to those reported in other studies (17, 24, 27).

In our study, neither MRR from low frequency (20 Hz) nor MRR from high frequency (100 Hz) stimulated contractions changed with hypercapnic acidosis. With fatigue, the shape of the frequency/force curve can be altered with a reduction in the forces generated at high stimulation frequencies (high frequency fatigue) and/or at low frequencies (low frequency fatigue). High frequency fatigue rapidly recovers when the stimulation is discontinued. By contrast, low frequency fatigue is characteristically much slower to recover (9).

Any alteration in lung volume must be taken into account when performing serial measurements of MRR (20). As a consequence of the high levels of ventilation, it was not possible to obtain reliable data for twitch Pdi during the hypercapnic gas mixture breathing. The MRR from twitch Pdi was studied for 1 min during the initial recovery period, immediately after breathing CO_2. During this period, the twitch Pdi were measured from functional residual capacity judged by the Respitrace trace and pre-hypercapnic twitch Pdi values. Probably, during this period, any change in MRR due to hypercapnia would not be completely reversed. WILES (33) reported a time course of recovery of MRR up to 5 min after the restoration of circulation in quadriceps and *adductor pollicis*. VIITASALO and KOMI (31), studying the effect of fatigue on MRR, showed that the relaxation rate values returned to the pre-fatigue levels within 1 min of the recovery period. KOULOURIS *et al.* (19) found that the decrease in MRR of maximal sniffs was no longer found by the third minute of recovery. Also, it is rather unlikely that hypercapnia would affect diaphragm relaxation rate, since no effect was observed in limb muscles.

Recent studies (29, 30) have shown decreased human quadriceps and *adductor pollicis* muscle force generation with hy-

percapnic acidosis. Various mechanisms were considered to explain how a low intracellular pH could decrease muscle force generation. Acidosis has been reported to decrease the amount of calcium ions initiating the contraction by inhibition of the sarcoplasmic reticulum function. A decreased intracellular pH has been associated with increased binding of calcium ions by the sarcoplasmic reticulum (22) and with decreased rate of calcium ions uptake and release per impulse (12). The inhibition of the sarcoplasmic reticulum function by acidosis would lead to a decrease in calcium ions sequestration, with slowing of muscle relaxation rate (4). The fact that a raised CO_2 tension did not alter relaxation rate in the present study suggests that inhibitions of sarcoplasmic reticulum is not the predominant effect of respiratory acidosis, under the conditions used here.

Resumen

Se investiga en humanos el efecto de la hipoxia y de la hipercapnia agudas sobre la velocidad de relajación muscular esquelética. Se estudia en sujetos normales, por estimulación eléctrica, la fuerza contráctil de los músculos de las extremidades *quadriceps femoris* y *adductor pollicis* y la presión transdiafragmática tras sacudida del diafragma. La velocidad de relajación máxima se mide a partir de las contracciones del *quadriceps femoris*, *adductor pollicis* y diafragma no fatigados. La hipoxia severa (tensión de oxígeno transcutánea media de 40 mmHg) no altera la velocidad de relajación máxima tras contracciones por estímulos de 20 Hz del cuadriceps no fatigado. La hipercapnia aguda (tensión media de CO_2 al final de la espiración normal de 65,1 mmHg) no cambia significativamente la velocidad de relajación máxima tras contracciones tetánicas de 20 y 100 Hz, del cuadriceps no fatigado. La velocidad de relajación máxima de la tensión de sacudida de 1 Hz y de la tensión tetánica de 20 Hz del *adductor pollicis* tampoco se altera por hipercapnia aguda (tensión de CO_2 media al final de la espiración de 63 y 60 mmHg, respectivamente). La velocidad de relajación máxima a partir de la tensión transdiafragmática de sacudida del diafragma no se altera por la hipercapnia aguda (tensión de CO_2 media de 69,7 mmHg).

Palabras clave: Hipoxia, Hipercapnia, Velocidad de relajación muscular.

References

1. Agostoni, E. and Rahn, H.: *J. Appl. Physiol.*, 15, 1087-1092, 1960.
2. Beer, G. and Yonce, L. R.: *Am. J. Physiol.*, 223, 492-498, 1972.
3. Bing, O. H. L., Keefe, J. F., Wolk, M. J., Finkelstein, L. J. and Levine, H. J.: *J. Clin. Invest.*, 50, 660-666, 1971.
4. Blinks, J. R., Rudel, R. and Taylor, S. R.: *J. Physiol.* (Lond.), 277, 291-323, 1978.
5. Bozler, E.: *J. Physiol.* (Lond.), 69, 442-462, 1930.
6. Brutsaert, D. L., Housmans, P. R. and Goethals, M. A.: *Circ. Res.*, 47, 637-652, 1980.
7. Cole, R. P.: *Respir. Physiol.*, 53, 1-14, 1983.
8. Dawson, M. J., Gadian, D. G. and Wilkie, D. R.: *J. Physiol.* (Lond.), 299, 465-484, 1980.
9. Edwards, R. H. T.: In «Human muscle fatigue: physiological mechanisms». (Porter, R. and Whelan, J., eds.). Pitman Medical Ltd., London, 1981, p. 1-18.
10. Edwards, R. H. T., Young, A., Hosking, G. P. and Jones, D. A.: *Clin. Sci.*, 52, 283-290, 1977.
11. Esau, S. A., Bellemare, F., Grassino, A., Permutt, S., Roussos, C. and Pardy, R. L.: *J. Appl. Physiol.*, 54, 1353-1360, 1983.
12. Fabiato, A. and Fabiato, F.: *J. Physiol.* (Lond.), 276, 233-255, 1978.
13. Frist, W. H., Palacios, I. and Powell, W. J. Jr.: *J. Clin. Invest.*, 61, 1218-1224, 1978.
14. Gaskell, W. H.: *J. Physiol.* (Lond.), 3, 48-75, 1880.
15. Harrison, E. M. and Galloon, S.: *Br. J. Anaesth.*, 37, 13-18, 1965.
16. Hill, D. K.: *J. Physiol.* (Lond.), 199, 637-684, 1968.
17. Howell, S., Fitzgerald, R. S. and Roussos, C.: *Am. Rev. Respir. Dis.*, 132, 241-247, 1985.
18. Jones, D. A., Bigland-Ritchie, B. and Ed-

wards, R. H. T.: *Exp. Neurol.*, 64, 401-413, 1979.
19. Koulouris, N., Vianna, L. G., Mulvey, D. A., Green, M. and Moxham, J.: *Am. Rev. Respir. Dis.*, 139, 1213-1217, 1989.
20. Levy, R. D., Esau, S. A., Bye, P. T. P. and Pardy, R. L.: *Am. Rev. Respir. Dis.*, 130, 38-41, 1984.
21. Moxham, J., Morris, A. J. R., Spiro, S. G., Edwards, R. H. T. and Green, M.: *Thorax*, 36, 164-168, 1981.
22. Nakamaru, Y. and Schwartz, A.: *J. Gen. Physiol.*, 59, 32, 1972.
23. Nakamura, Y., Wiegner, A. W. and Bing, O. H. L.: *Cardiovasc. Res.*, 20, 690-697, 1986.
24. Renaud, J. M. and Stevens, E. D.: *Am. J. Physiol.*, 240, R301-R309, 1981.
25. Roussos, C. S. and Macklem, P. T.: *N. Engl. J. Med.*, 307, 786-797, 1982.
26. Sahlin, K., Edstrom, L. and Sjoholm, H.: *Am. J. Physiol.*, 245, C15-C20, 1983.
27. Schnader, J. Y., Juan, G., Howell, S., Fitzgerald, R. and Roussos, C.: *J. Appl. Physiol.*, 58, 823-829, 1985.
28. Stainsby, W. N.: In «Limiting factors of physical performance» (Keul, J., ed.). International Symposium at Gravenbruch 1971. Keul, J. and G. Thieme Co. Stuttgart, 1973. p. 137-144.
29. Vianna, L. G., Jenkins, S. C., Soutar, S. A., Lanigan, C., Gray, B. and Moxham, J.: *Bull. Eur. Physiopathol. Resp.*, 22 (suppl), 162s, 1986.
30. Vianna, L. G., Koulouris, N., Lanigan, C., Gray, B., Jenkins, S. C., Soutar, S. A. and Moxham, J.: *Clin. Sci.*, 72 (suppl), 1P, 1987.
31. Viitasalo, J. T. and Komi, P. V.: *Acta Physiol. Scand.*, 111, 87-95, 1981.
32. Waller, A. D. and Sowton, S. C. M.: *J. Physiol.* (Lond.), 20, 16-17, 1896.
33. Wiles, C. M.: The determinants of relaxation rate of human muscle *in vivo*. University of London, London, 1980.
34. Wiles, C. M. and Edwards, R. H. T.: *Clin. Physiol.*, 2, 485-497, 1982.
35. Wiles, C. M., Young, A., Jones, D. A. and Edwards, R. W. T.: *Clin. Sci.*, 56, 47-52, 1979.
36. Wiles, C. M., Young, A., Jones, D. A. and Edwards, R. W. T.: *Clin. Sci.*, 57, 375-384, 1979.