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Vascular Pharmacology 40 (2003) 119–125

Vascular Pharmacology

www.elsevier.com/locate/vph

Oxidative metabolism of peripheral blood neutrophils in experimental acute hypercapnia in the mechanically ventilated rabbit

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Received 26 November 2001; accepted 8 December 2001

Abstract

Background and aim: The aim of this study was to investigate the effect of acute hypercapnia due to the mechanical hypoventilation on the oxidative metabolism of peripheral blood neutrophils in the rabbit. *Methods:* The study was performed on 24 Chinchilla rabbits, randomized into normo- and hypercapnia groups (P_aCO_2 between 9 and 11 kPa over 180 min). At the baseline point and after 180 min of mechanical ventilation, a neutrophil count, luminol-dependent chemiluminescence of the neutrophils stimulated with opsonized zymosan (OZ) or PMA (phorbol myristate acetate), and the hydrogen peroxide production of the cells upon the PMA stimulation were measured. Serum cortisol levels were additionally determined. *Results:* In both studied groups, a significant neutrophilia after 180 min of mechanical ventilation was observed ($P<.05$). Neither chemiluminescence nor hydrogen peroxide production of peripheral neutrophils was changed ($P>.05$). *Conclusions:* Hypercapnia lasting for 180 min did not affect the oxidative metabolism of circulating neutrophils but a rise in a neutrophil count was observed in the mechanically ventilated rabbit.

Keywords: Hypercapnia; Artificial respiration; Neutrophils; Chemiluminescence; Rabbit

1. Introduction

Hypercapnia may complicate many clinical conditions (e.g., postoperative period) and may be associated with side effects due to surgical and anaesthetic procedures (Murdock et al., 2000; Pakulski and Drobnik, 1998). In recent years, however, its clinical significance has been reevaluated. It has been hypothesized that, in acute organ injury, carbon dioxide retention may be protective (Laffey and Kavanagh, 1999). There is evidence that patients receiving ventilatory support for some kinds of pulmonary disease (e.g., in adult acute respiratory distress syndrome), controlled hypoventilation with permissive hypercapnia results in better survival rate (Hickling et al., 1994). It has been also shown that CO_2 retention itself may be protective against tissue damage in the myocardium and brain (Kaplan et al., 1995; Vannucci et al., 1995). In the rabbit experimental model, increased partial pressure of carbon dioxide was demonstrated to be an important protective factor against the lethal cell injury after reperfusion (the concept of the "pH paradox") (Kaplan et al., 1995). The beneficial effect of hypercapnia was shown to be connected with the mechanisms involving oxidative stress: hypercapnic acidosis results in the inhibition of xanthine oxidase, which plays a key role in reperfusion injury (Shibata et al., 1998; Bulkey, 1994).

Enhanced release of reactive oxygen species (ROS) by polymorphonuclear cells (PMNs) constitutes an important link between oxidative stress and tissue injury (Burns and Doerschuk, 1994). Whether CO_2 directly affects the potentially harmful neutrophil respiratory burst is not clearly elucidated. There are studies indicating that hypercapnia may diminish the neutrophil activity and oxygen metabolism; however, some of these reports described mixed conditions of hypercapnia and hypoxia (Beachy and Weisman,

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1537-1891/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
doi:10.1016/S1537-1891(02)00338-5

1993; Baev and Kuprava, 1997). Kogan et al. (1997) suggested that CO_2, at physiological and elevated levels, was a potent inhibitor of the ROS generation by human cells and mitochondrial fractions, attributing these effects, partly at least, to the inhibition of the NADPH oxidase activity. A convincing evidence on the inhibitory ability of CO_2 on neutrophil respiratory burst was provided by Kopernik et al. (1998), who showed that exposure to high P_aCO_2 in laparoscopy–gas pressure-like conditions resulted in striking suppression of neutrophil superoxide production lasting for several hours. The observed inhibition of the ROS formation by CO_2 was accounted to acidosis (Kopernik et al., 1998; Allen et al., 1997). However, there are also studies denying any significant influence of hypercapnia on the neutrophil respiratory burst (Kantorova et al., 1999). In view of the conflicting reports, we decided to clarify whether isolated acute CO_2 retention itself would influence oxygen metabolism of circulating neutrophils. In clinical conditions, hypercapnia is most commonly a result of hypoventilation, so we decided to mimic this situation using an experimental model of isolated acute hypercapnia (with normoxia) due to hypoventilation in the mechanically ventilated rabbit.

2. Method

2.1. Animals

Experimental rabbits were treated according to the recommendations of Helsinki Declaration and to the regulations of Polish Animal Protection Law. After approval of the Medical University Ethical Commission, the study was performed on 24 Chinchilla rabbits (weight 4.1±0.7 kg, either sex), randomized into two groups, normocapnia group (n=12) and hypercapnia group (n=12).

2.2. Experimental protocol

A 19-gauge intravenous butterfly needle was inserted into the right ear marginal vein. Anesthesia was induced intravenously by pentobarbital (40 mg kg^{-1} Vetbutal, Biowet) and maintained with subsequent continuous 25 mg kg^{-1} h^{-1}. Rabbits were intubated with a cuffed endotracheal tube (Ch.-B., ID 3.5 mm, Ruesch, Germany). Muscle relaxation was achieved using intravenous pancuronium (1 mg, Pavulon, Organon) with subsequent hourly supplementation.

Rabbits were ventilated using controlled mechanical ventilation (CMV, Zimmerman pump, WGL, Germany). Tidal volume (TV) was 10 ml kg^{-1} and ventilation frequency was adjusted to achieve a P_aCO_2 of 4.7–6 kPa (35–45 mm Hg) and F_iO_2 of 0.30. After 30 min of stabilization, one group of rabbits (hypercapnia group) was hypoventilated, TV was reduced to 5 ml kg^{-1}, and respiration frequency was adjusted to keep P_aCO_2 between 9 and 11 kPa (68–83 mm Hg). In the second group (normocapnia group), ventilatory baseline parameters were not changed. To avoid hypoxia in hypoventilated hypercapnic animals, F_iO_2 was increased to 0.35–0.40. End-tidal CO_2, inspiratory O_2 fraction (Anaesthetic Gas Monitor Type 1304, Brueel & Kijaer, Denmark), and ECG were monitored continuously. An arterial access to the right ear median artery (Venflon 17G, Ohmeda, Sweden) was established allowing arterial pressure to be continuously recorded via Statham transducer and to draw the blood samples for blood gases periodical analysis and cellular studies. (We measured the arterial pressure in peripheral vessel in order to avoid additional stress and ischemic sequela while cannulating the aorta.)

2.3. Analytical determinations

2.3.1. Blood gases analysis

Arterial blood P_aCO_2, P_aO_2, and pH were analyzed at the baseline point (T_0) and at 30, 60, 120, and 180 min of hypercapnic or normocapnic CMV rabbits (T_1, T_2, T_3, and T_4) using an AVL compact, 995S (ALV, Austria).

2.3.2. Cortisol levels

Cortisol was estimated in order to evaluate a stress level after stabilization period (T_0) and at the end of the experiment (T_4). We applied a luminometric method using Chiron compact ACS 180 (Chiron, Great Britain).

2.4. Examination of neutrophils

Neutrophils were studied at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4). (We were not able to repeat the measurement and study kinetics of the observed phenomena because with greater amounts of the drown blood we would result in changes due to the induced hypovolemia.)

2.4.1. Cell preparation

The neutrophils were separated from the whole peripheral blood processed by using hydroxyethylcellulose (HEC) and Percoll gradient according to Issekutz and Issekutz (1988). Briefly, the whole blood samples with 2% EDTA were mixed with 1% HEC (Fluka Chemicals) and red blood cells were allowed to sediment for 1 h. Leukocyte-rich plasma was then harvested, layered over 68% Percoll (Sigma) and spun down at 400×g for 20 min (4 °C). Neutrophils were washed and the remaining erythrocytes were lysed with 0.84% NH_4Cl. Then the PMNs were repeatedly washed and resuspended in Tyrode's solution, pH 7.2. Cell preparations contained about 97% of viable neutrophils (trypan blue exclusion).

2.4.2. Analytical procedures

Luminol-dependent chemiluminescence reflecting hydrogen peroxide production was measured using a 1250 LKB luminometer (Bioorbit, Denmark). Chemiluminescence studies (cells stimulated with zymosan or phorbol myristate acetate, PMA) were performed according to Allen (1986)

Table 1
Arterial blood pressure (kPa): systolic (Pa sys), diastolic (Pa diast), and mean (Pa mean) measured in right ear median artery at the baseline point (T_0) and at 30 (T_1), 60 (T_2), 120 (T_3), and 180 min (T_4) of CMV in normo- (NC) and hypercapnia (HC) subgroups (mean ± S.D.)

NC/HC	T_0	T_1	T_2	T_3	T_4
Pa sys (kPa)					
NC (n=12)	9.5±2.5	9.1±2.5	9.4±3.4	9.8±3.3	9.7±3.4
HC (n=12)	10.1±1.5	10.5±1.7	10.5±1.9	9.7±1.7	9.1±2.2
Pa diast (kPa)					
NC (n=12)	7.0±2.4	6.4±2.2	6.5±2.5	7.1±3.0	7.1±3.3
HC (n=12)	7.4±1.4	7.5±1.8	7.4±1.7	7.1±1.8	6.8±2.3
Pa mean (kPa)					
NC (n=12)	8.0±2.2	7.6±2.1	8.1±3.1	8.3±3.0	8.3±3.2
HC (n=12)	8.7±1.4	8.8±1.5	8.9±1.8	8.3±1.7	7.8±2.1

No significant changes were noted (two-way ANOVA test; P>.05) between the time points as well as the studied subgroups.

with our own minor modifications. The reaction mixture contained of 5×10^5 cells, 0.15 mM luminol (Sigma) and either serum (FCS, Sigma) opsonized zymosan (OZ, 1 mg ml^{-1}, Sigma) or PMA (100 ng ml^{-1}, Sigma). Chemiluminescence was recorded for 90 min and data integrated. Serum OZ was prepared according to Labedzka et al. (1989).

To confirm the results obtained by chemiluminescent method, hydrogen peroxide production by intact and PMA (50 ng ml^{-1}, Sigma)-stimulated cells was measured by the phenol red–peroxidase micromethod of Pick and Mizel (1981). Hydrogen peroxide generation was calculated from a standard curve with titrated H_2O_2 solution and was expressed in nM min^{-1}/10^6 cells.

2.5. Statistical analysis

Data of blood pressure measurements, blood gas analysis, and cell counts are shown as mean ± S.D. Cortisol, chemiluminescence, and H_2O_2 production values are presented as median±average deviation with minimal and maximal values. Statistical analysis was performed by means of two-way ANOVA test (blood pressure measurements, blood gas analysis) and Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test (cellular studies and cortisol levels). Significance was accepted as P<.05 (*P<.05 between the time points; $^{\dagger}P$<.05 between the two groups).

Table 3
Serum cortisol levels (μg dl^{-1}) at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4) under normo- (NC) and hypercapnia (HC) conditions (median±average deviation; minimal and maximal values)

NC/HC	T_0	T_4
NC (n=7)	0.4±0.3 (0.0–1.6)	0.6±0.4 (0.0–1.9)
HC (n=7)	0.9±0.3 (0.2–1.1)	0.7±0.3 (0.5–1.4)

No statistically significant changes were noted (Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test; P>.05).

3. Results

3.1. Hemodynamic monitoring

Results of peripheral arterial pressure, systolic, diastolic, and mean, which were measured in the right ear median artery (mean±S.D.), are presented in Table 1. No significant changes between time points and groups were observed.

3.2. Blood gas analysis

Results of blood gas measurements (mean±S.D.) are presented in Table 2. Hypercapnia was associated with moderate acidosis, which tended to normalize after 180 min. This could be attributed to buffering mechanisms. However, the difference between the pH values at T_0 and T_4 still remained statistically significant.

Table 2
Arterial blood P_aCO_2, P_aO_2 (kPa) and pH at the baseline point (T_0) and at 30 (T_1), 60 (T_2), 120 (T_3), and 180 min (T_4) of CMV in normo- (NC) and hypercapnia (HC) subgroups (mean±S.D.)

NC/HC	T_0	T_1	T_2	T_3	T_4
P_aCO_2 (kPa)					
NC (n=12)	4.9±0.7	4.7±0.9	4.9±0.8	4.7±1.0	4.3±0.4
HC (n=12)	4.6±0.5	10.2±0.9*,†	10.7±1.1*,†	10.4±0.9*,†	10.5±0.6*,†
P_aO_2 (kPa)					
NC (n=12)	15.8±3.3	15.2±3.0	13.7±2.6	16.3±2.6	16.2±2.7
HC (n=12)	15.9±4.9	12.2±2.4*,†	14.3±4.9	14.4±2.7	15.6±3.3
pH					
NC (n=12)	7.39±0.08	7.43±0.08	7.45±0.07	7.46±0.07	7.48±0.05
HC (n=12)	7.47±0.08	7.24±0.07*,†	7.24±0.06*,†	7.25±0.05*,†	7.29±0.06*,†

Differences between baseline point (T_0) and particular points of measurement remained statistically significant (two-way ANOVA test) concerning pH and P_aCO_2 values.
* P<.05 vs. T_0.
† P<.05 vs. normocapnia.

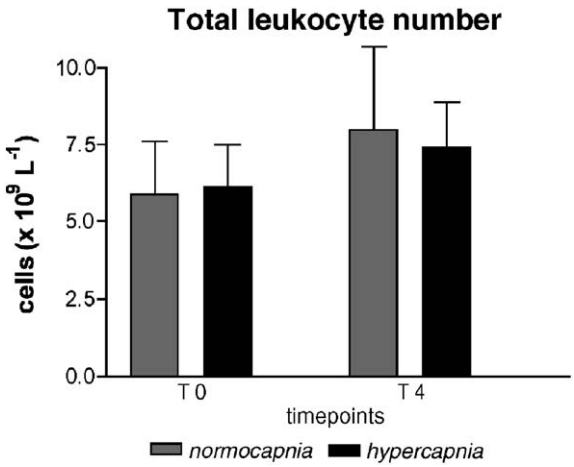

Fig. 1. Total leukocyte number at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4) under normo- and hypercapnia conditions (mean±S.D.). No significant changes were observed (normocapnia: 5.9±1.7 vs. 8.0±2.7×10^9 l^{-1}; hypercapnia: 6.1±1.4 vs. 7.4±1.5×10^9 l^{-1}; two-way ANOVA test; $P>.05$).

3.3. Serum cortisol levels

Serum cortisol levels, as shown in Table 3, did not significantly change after 180 min (T_4) of mechanical ventilation in both normo- and hypercapnia subgroups.

3.4. Leukocyte studies

3.4.1. Cell number

The total leukocyte number in the whole peripheral blood is presented on Fig. 1. There were no significant differences in total leukocyte numbers between the baseline point (T_0) and after 180 min (T_4) in both normo- and hypercapnia subgroups.

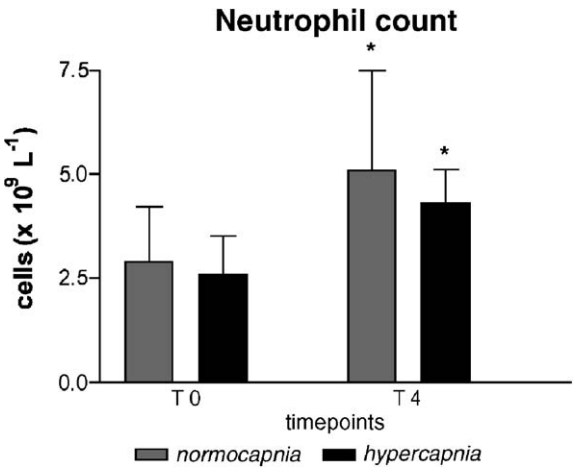

Fig. 2. Neutrophil count in the whole peripheral blood at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4) in normo- and hypercapnic animals (mean±S.D.). A significant increase in the number of neutrophils after 180 min in both studied groups (normocapnia: 2.9±1.3 vs. 5.1±2.4×10^9 l^{-1}; hypercapnia: 2.6±0.9 vs. 4.3±0.8×10^9 l^{-1}; two-way ANOVA test; *$P<.05$ between time points) can be noted. No significant changes between the studied subgroups were observed.

3.4.2. Circulating neutrophil counts

Circulating neutrophil counts are shown on Fig. 2. There was a significant increase in neutrophil number in both studied groups after 180 min of mechanical ventilation under both normo-, and hypercapnia regime (T_4). Thus, phenomenon of neutrophilia was similarly observed in normo- and hypercapnic rabbits.

3.5. Oxidative metabolism

Results of luminol-dependent chemiluminescence, reflecting hydrogen peroxide production of peripheral blood neutrophils, are presented in Table 4. There were no significant changes between values of chemiluminescence in both OZ- and PMA-stimulated cells obtained at the baseline point (T_0) and after 180 min (T_4) of mechanical ventilation under both normo- and hypercapnic regime.

Similarly, there were no significant differences in hydrogen peroxide production between groups studied as measured by direct spectrophotometric method of PMA-

Table 4
Luminol-dependent chemiluminescence (Vs) of OZ- and PMA-stimulated peripheral blood neutrophils at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4) under normo- (NC) and hypercapnia (HC) conditions (median±average deviation; minimal and maximal values)

NC/HC	T_0	T_4
Intact cells		
NC (n=5)	1.7±1.2 (0.96–4.28)	1.6±0.6 (0.9–2.1)
HC (n=5)	1.7±0.3 (1.0–2.1)	1.6±0.7 (0.4–3.0)
OZ-stimulated cells (1 mg ml^{-1})		
NC (n=12)	153.0±149.8 (24.4–704.2)	297.4±119.0 (71.3–552.4)
HC (n=12)	144.6±108.2 (34.1–389.1)	250.3±169.6 (36.2–808.8)
PMA-stimulated cells (100 ng ml^{-1})		
NC (n=12)	16.5±24.7 (2.1–103.7)	24.2±36.2 (0.8–96.3)
HC (n=12)	6.2±23.4 (1.8–107.7)	12.2±44.1 (1.9–276.3)

No significant changes were noted (Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test; $P>.05$).

Table 5
Hydrogen peroxide production (nM $min^{-1}/10^6$ cells) of intact and PMA-stimulated peripheral blood neutrophils at the baseline point (T_0) and after 180 min of mechanical ventilation (T_4) under normo- (NC) and hypercapnia (HC) conditions (median±average deviation; minimal and maximal values)

NC/HC	T_0	T_4
Intact cells		
NC (n=7)	7.0±2.5 (2.0–10.0)	4.2±5.2 (1.0–17.0)
HC (n=7)	7.1±3.3 (5.5–16.0)	7.0±5.8 (3.4–27.0)
PMA-stimulated cells (50 ng ml^{-1})		
NC (n=7)	9.5±4.2 (3.0–18.5)	16.5±3.8 (6.5–17.1)
HC (n=7)	14.3±3.5 (8.0–21.0)	12.1±5.5 (6.0–27.0)

No significant changes were noted (Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparisons test; $P>.05$).

stimulated cells obtained at the baseline point (T_0) and after 180 min (T_4) of mechanical ventilation (Table 5).

4. Discussion

In this study, we have shown that isoxic hypercapnia lasting for 180 min due to hyperventilation under CMV conditions and pentobarbital anesthesia in the rabbit:

1. does not affect the oxidative metabolism of peripheral blood neutrophils as measured by luminol-dependent chemiluminescence (Table 4) and peroxidase spectrophotometric micromethod (Table 5);
2. does not significantly differ in the peripheral blood neutrophilia observed both in hypercapnia or normocapnic conditions (Fig. 2).

4.1. Experimental model

Experimental hypercapnia was achieved by simple hypoventilation reducing TV and ventilation frequency. Under clinical conditions, hypercapnia often results from hypoventilation; this model refers also to current approaches of prevention the ventilatory-induced lung injury (Tremblay et al., 1997). However, this study only addressed the question whether acute 180-min lasting rise in CO_2 arterial blood tension would affect the ROS production by circulating neutrophils. Throughout the whole study, no changes in systematic blood pressure were noted, confirming previously reported observations considering hemodynamics in experimental hypercapnia (Table 1) (Heijden and Guiguard, 1986).

4.2. ROS production

Whether and to what degree hypercapnia itself may modify inflammatory reactions, especially neutrophil function, is not well known. Hickling et al. (1998) showed in rabbit (saline lavage) that low TVs promoted a reduction of ventilatory-induced lung injury and decreased a neutrophil count in the final lavage (lung).

An important aspect of the neutrophil contribution to inflammation is its ability to release the ROS, which may play a pivotal role in a tissue injury. ROS generation following the stimulation of NADPH oxidase system is a complex phenomenon, being influenced by a number of molecules and also the other cells involved in inflammatory reaction such as endothelial cells and platelets (Klebanoff, 1988). Previous observations provided the evidence that hypercapnia may influence endothelium and coagulation system (Rounds et al., 1988; Egbrink et al., 1989). In an in vitro study, it has been shown that hypercapnia and acidosis could cause a reduction in a rabbit platelet aggregation (Egbrink et al., 1990). In view of the fact that neutrophil act in a strict relationship with endothelium and platelets, it could be expected that this cell function would be modified by hypercapnia.

To investigate the influence of hypercapnia on the ROS secretion by neutrophils, we used a luminol-dependent chemiluminescence, which reflects hydrogen peroxide production (Allen, 1986). To reconfirm the observed phenomena, we additionally assessed hydrogen peroxide production by direct spectrophotometric micromethod (Pick and Mizel, 1981). Hydrogen peroxide is a nonradical oxidant and constitutes a dismutated product of superoxide produced after activation of the enzymatic system of NADPH oxidase. It passes readily through the biological membranes and may exert its damaging effects at a distance from the production site (Klebanoff, 1988). Hydrogen peroxide has been proven to occur as the main oxidant altering a signal transduction and affecting the viability of endothelium (Vercellotti et al., 1991). It has been shown that the enzymes that scavenge H_2O_2 are the most important ones in conferring protection to the endothelium from neutrophil-derived ROS (Hoover et al., 1987). Hydrogen peroxide released by human neutrophils correlates with degranulation (Suchard and Boxer, 1994) and NO generation (Carreras et al., 1994), which may be of importance to the host defense and tissue injury.

Our results bring the evidence that hydrogen peroxide production by circulating neutrophils in the rabbit is not affected by hypercapnia due to hypoventilation lasting for 180 min. However, it should be mentioned that we were able to assess only the activity of circulating neutrophils. An alteration of oxidative metabolism in the tissue-sequestered cells cannot be excluded here.

4.3. Stress response

Under conditions of mechanical ventilation and hypercapnia, there are at least a few important factors that could influence NADPH oxidase activity and thus oxygen metabolism of neutrophils. Intubation and mechanical ventilation may elicit stress response (McCoy et al., 1995; Schmidt and Kraft, 1996). Stress reaction in turn provokes a hormonal response, i.e., elevated cortisol, catecholamine and endogenous opioid levels, and modifications of cytokine secretion, i.e., rise in granulocyte colony-stimulating factor (GCSF) (Yokota et al., 1995). We measured cortisol concentration because steroids are known to profoundly inhibit the inflammatory response and ROS production (Klebanoff, 1988). We have shown that the acute hypercapnia due to hypoventilation under conditions of general anesthesia does not involve cortisol elevation. In our study, cortisol levels after 180 min of acute hypercapnia (T_4) in an anesthetized rabbit did not change significantly in comparison to its levels determined at the baseline (T_0) (Table 3) or to values noted in normocapnic animals, which may be attributed to adequately applied anesthesia (Crozier et al., 1992). This also confirmed the previous reports on the influence of experimental hypercapnia on serum cortisol. In the horse hypercapnia model, plasma cortisol levels remained unchanged, whereas norepinephrine levels increased progressively with every increase of P_aCO_2 (Khanna et al.,

1995). In the sheep fetus, hypercapnia was associated with elevated cortisol levels only after carotid sinus denervation and bilateral vagotomy (Chen and Wood, 1993). However, possible adrenergic stimulation (not measured in our study) could lead to suppression of ROS generation by neutrophils (Weisdorf and Jacob, 1987).

4.4. Acidosis

Acidosis is another important consequence of hypercapnia. Under conditions of acidosis, a decrease in the ROS formation by leukocytes has been reported (Kopernik et al., 1998; Allen et al., 1997). In our study, pH changes of arterial blood were of moderate degree (pH did not fall below 7.2; Table 2), which should not affect the function of NADPH oxidase system of which the optimal pH ranges from 7.0 to 7.5 (Rossi et al., 1986). It has been suggested that hypercapnic acidosis may inhibit endogenous xanthine oxidase (Shibata et al., 1998). It is an interesting phenomenon because in the course of the inflammatory reaction, superoxide anion initially generated by xanthine oxidase may up-regulate both endothelial and neutrophilic adhesion molecules with subsequent neutrophil activation what may normally increase a production of toxic oxygen species (Bulkey, 1994). However, acidosis due to the acid infusion was also reported to induce lung damage (Pedoto et al., 1999). The decrease in pH probably plays a role in stimulating a nitric oxide synthase. Nitric oxide was shown to be responsible for hypercapnic vasorelaxation (Carr et al., 1993) and it is also known to inhibit both platelet and leukocyte adhesion (Kanwar and Kubes, 1995). Thus, it may antagonize the biological effect of oxidants when reacting with superoxide and forming peroxynitrite (Bastian and Hibbs, 1994). It cannot be, therefore, excluded that the final effect of hypercapnia on PMN-oxidative metabolism is also influenced by nitric oxide scavenging-like effect.

4.5. Anesthesia

The modulating influence of an anaesthetic, pentobarbital, on oxidative metabolism of neutrophils cannot be also excluded (Weiss et al., 1994); however, the same dose regime in both groups diminished the possibility of influencing the differences between the groups.

4.6. Neutrophilia

In both studied groups of rabbits, whether normo- or hypoventilated, we observed a significant neutrophilia at the end of the experiment. Neutrophilia associated with mechanical ventilation may result from leukocyte redistribution in a response to stress (Dhabhar et al., 1995). It may indicate a possible GCSF level of elevation (Yokota et al., 1995) and/or to reflect the adrenergic effect on both granulocytes and endothelial cells (Weisdorf and Jacob, 1987) because of the reduced cell adherence. The observed phenomenon may be also due to spleen mobilization of neutrophils and the release of myeloid neutrophil reserve in response to cortisol (Toft et al., 1994). However, we were able to demonstrate, as previously mentioned, that hypercapnia lasting for 180 min in a mechanically ventilated rabbit under pentobarbital anesthesia did not result in cortisol levels elevation (Table 2). In view of the role of nitric oxide in hypercapnia pathophysiology, one could expect that, in hypercapnia group, the neutrophilia would be better pronounced due to the possible anti-adhesive effect of nitric oxide (Kanwar and Kubes, 1995). In our study, the quantitative differences between neutrophil count in normo- and hypercapnic rabbits were insignificant. On the other hand, it has been recently suggested that acidosis may induce CD18-mediated neutrophil adhesion despite a decrease in ICAM-1 expression (Serrano et al., 1996). There is also some evidence that mechanical ventilation may cause a change in neutrophil organ distribution and may influence a neutrophil influx and/or activation in atelectasis-prone rabbit lungs (Suigura et al., 1994). Entrapment of at least a part of these cells in lungs cannot be excluded.

In conclusion, we have demonstrated that acute hypercapnia due to the hypoventilation in CMV-ventilated rabbits did not significantly influence peripheral blood neutrophil oxidative metabolism. However, whether our results could be extrapolated on long lasting carbon dioxide retention needs further investigation.

Acknowledgements

The authors are very grateful to Prof. Dr. H. Sonntag and Dr. F. Mielck for helpful discussions and valuable suggestions and to Mrs. Holderna and Mrs. D. Mielcarek for excellent technical assistance.

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