Effect of hyperoxic and hyperbaric conditions on the adenosinergic pathway and CD26 expression in rat

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Rats and a murine Chem-3 cell line that expresses A2AR were exposed to 0.21 bar O2, 0.79 bar N2 (terrestrial conditions; normoxia); 1 bar O2 (hyperbaric hyperoxia); 2 bar O2 (hyperbaric hyperoxia); 0.21 bar O2, 1.79 bar N2 (hyperbaria). Adenosine plasma concentration, CD73, ADA, A2AR expression, and CD26 expression were addressed in vivo, and cAMP production was addressed in cellulo. For in vivo conditions, 1) hyperoxia decreased adenosine plasma level and T cell surface CD26 activity, whereas it increased CD73 expression and ADA level; 2) hyperbaric hyperoxia tended to amplify the trend; and 3) hyperbaria alone lacked significant influence on these parameters. In the brain and in cellulo, 1) hyperoxia decreased A2AR expression; 2) hyperbaric hyperoxia amplified the trend; and 3) hyperbaria alone exhibited the strongest effect. We found a similar pattern regarding both A2AR mRNA synthesis in the brain and cAMP production in Chem-3 cells. Thus a high oxygen level tended to downregulate the adenosinergic pathway and CD26 activity. Hyperbaria alone affected only A2AR expression and cAMP production. We discuss how such mechanisms triggered by hyperoxgenation can limit, through vasocostriction, the oxygen supply to tissues and the production of reactive oxygen species.

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Adenosine acts via four G protein-coupled receptors that are conserved among vertebrates (22). The A2AR receptor (A2AR) is of particular interest because it is expressed in the brain, where it inhibits dopaminergic pathways of the basal ganglia, and in the cardiovascular system, where it controls the heart rate and the blood pressure by its vasodilator capacity (18, 23, 39, 55, 63, 64). It is known that A2AR expression increases in hypoxic conditions (11, 13, 22, 37). Regarding hyperoxia, the adenosinergic response has been already addressed in the lung, and the supplemental use of high oxygen levels to treat the acute respiratory distress syndrome was found to weaken the local tissue hypoxia-driven and adenosine-mediated anti-inflammatory mechanism, hence further exacerbating lung injury (16, 48, 61). Recently, the effects of hyperoxia on the CD39/CD73-driven production of adenosine and A2AR expression were addressed from a cancer therapy perspective. Indeed, there is a need for reversing the immunosuppressive effects mediated by A2AR on T and NK cells in the hypoxic adenosine-rich and tumor-protecting microenvironment and for converting the latter into a normoxic and adenosine-poor environment that induces tumor regression (29, 30). These pioneer studies have evaluated the effects of hyperoxia in the lung or in cancer/inflammation models, and there is therefore a need for addressing the situation in the brain using normal animals/tissues.

ADA and surface CD26 are other major components of the response to blood oxygen level. Plasma ADA degrades adenosine into inosine and therefore controls the extracellular level of the nucleoside that, besides its anti-inflammatory effect, exhibits cytotoxicity (20). In higher mammals, ADA interacts with CD26 at the surface of mononuclear cells and the capacity of the aminohydrolase to bind CD26 was interpreted as a means to protect, via adenosine degradation, cells from cytotoxic effects of high adenosine concentration in the immediate vicinity of the cell surface (1, 19, 33, 46). In rodents, it is considered, however, that ADA does not bind cell-surface CD26 (19).

In mammals, CD26 also exhibits the dipeptidyl peptidase-IV (DPP-IV) activity. DPP-IV cleaves NH2-terminal dipeptides from peptides containing proline or alanine in the penultimate position to control the concentration of their active intact form (7, 41). CD26 substrates include peptides produced in response to oxidative stress or abnormal oxygen supply to tissues (41). These peptides act on the redox stress and energetic metabolism, in particular in high-energy-consuming tissues such as the skeletal muscle, as well as on brain function and oxygen supply to tissue (11, 13, 22, 37). Changes in CD26 expression are documented following ischemia/reperfusion and hypoxia (20, 41, 46). CD26 activity during hyperoxia and hyperbaria remains unclear.

For these reasons, we sought to address here the effects of high blood oxygen level and hyperbaria on various components of the adenosinergic pathway, from changes in the extracellular adenosine concentration up to cAMP production following A2AR activation. CD26 was also addressed.

MATERIALS AND METHODS

Animals and reagents. Rats (male Sprague-Dawley rats; Charles River, FR 300-350 g) were treated according to the European guidelines (Brussels, Belgium; 86/609/EEC), the French law (decree 87/848), and the rules established by our Institutional Animal Care and Use Committee (agreement number B13.0005.8). Rats were kept at 22 ± 1°C under a 12-h:12-h light/dark cycle (lights on at 7:00 AM) with food (A03, UAR) and water available ad libitum. Rats (n = 8 per condition) were exposed in a pressure chamber for 4 h (25°C; H2O: 40–60%; CO2 < 300 ppm) as follows: normoxia at atmospheric pressure (210 mbar O2, 790 mbar N2), a condition known to generate a partial pressure of oxygen in the arterial blood (Pao2) of ~90 mmHg, i.e., the control situation (10, 58) (normoxia thereafter); hyperoxia at atmospheric pressure (1 bar O2), a condition generating a Pao2 of ~450 mmHg (10, 58) (hyperoxia thereafter); hyperoxia in hyperbaric conditions (2 bar O2), a condition generating a Pao2 of ~900 mmHg (hyperbaric hyperoxia thereafter); and normoxia in hyperbaric conditions (2 bar with 210 mbar O2, 1790 mbar N2), a condition generating a Pao2 of ~90 mmHg and that is often considered to experimentally reproduce authentic normoxia under such hyperbaric conditions (9) (hyperbaria thereafter). Isoflurane was used as an anesthetic agent before brain collection. Other reagents were purchased from Sigma-Aldrich.

Cell line expressing A2AR. We used a murine Chem-3 cell line expressing the functional A2AR (HTS048C; Millipore). Suspension cells were cultured in RPMI 1640, 10% FCS, 0.8 mg/ml geneticin (G418), 100 IU/ml penicillin/streptomycin, and 1/100 dilution of the Chem-3 growth supplement (Millipore) under various pressure and gas conditions for 4 h before storage of cell pellets at −80°C.

Adenosine plasma level. Blood samples (1 ml) were obtained following cardiac puncture using a syringe containing 0.25 ml of cold stop aqueous solution [0.2 mM dipyridamole, 4.2 mM Na2-EDTA, 5 mM (9-erythro-3 nonyl) adenine, 79 mM αβ-methylene adenosine 5′diphosphate, 1 UI/ml heparin sulfate, and 0.9% NaCl] to prevent subsequent degradation and uptake of adenosine (28). Samples were then deproteinized using 5% perchloric acid (1 vol:4 vol), and adenosine was identified and quantified using high-performance liquid chromatography coupled with a diode array detector (Chrom Sys tem) as described (28). Adenosine was identified by spectrum and elution time (detection threshold: 5 pmol in plasma; coefficient of variation: <5%).

ADA. After cardiac puncture and centrifugation, serum ADA was measured as described previously (28); adenosine (750 µl, 28 mM) was incubated with serum (125 µl) in saline (125 µl, 7% BSA) for 37 min at 37°C. The reaction was started by adding adenosine and stopped in ice water. Ammonia resulting from adenosine degradation by ADA was measured using a Synchron LX 20 analyzer (Beckman Coulter) and expressed as international units per liter.

A2AR and CD73 expression. A2AR expression was assessed using a semiquantitative Western blot procedure (13). Frozen brains or Chem-3 cells were quickly thawed and treated using a 4% SDS aqueous solution supplemented with a protease inhibitor cocktail (Sigma-Aldrich) and a 15-min sonication treatment at 47 kHz. After protease quantification (microBCA assay; Pierce Biotechnology), samples (10 µg) were diluted in loading buffer (65.2 mM Tris HCl buffer, pH 8.3, containing 10% glycerol, 0.01% bromophenol blue, and 5% 2-mercaptoethanol) and analyzed using 12% SDS-PAGE before electrophoretically transferred to PVDF membrane. Filters were incubated for 20 min with Adonis (1 µg/ml), a mouse monoclonal antibody directed against human A2AR (12) that can readily detect and semiquantify in denaturing conditions A2AR antigens from various origins (e.g., human, mouse, rat, heart, peripheral blood mononuclear cells, kidney, brain). Filters were then incubated with horseradish peroxidase-labeled anti-mouse antibodies and a chemiluminescence substrate (SuperSignal West Femto, Pierce Biotechnology). Densitometry analysis of the 45-kDa species corresponding to the A2AR antigen was performed.
using the ImageJ software (NIH). CD73 expression was similarly studied in brain samples (100 µg) using Western blot analysis and the H-300 rabbit polyclonal IgG (Santa Cruz Biotechnology; 1:200) before densitometry analysis of the corresponding band (70 kDa).

A2A R mRNA. Total mRNA was extracted from brain samples (PAXgene, Blood RNA kit, Pre Analytix, Qiagen). Real-time quantitative mRNA expression analysis was performed using 250 ng of cDNA encoding the A2A R sequence (Light cycler, Roche). The forward primer sequence was 5’-GAGGACGTGGTCCCATGAAAT-3’, and the reverse primer sequence was 5’-TGACGGTGAGGGACCGAGT-3’. β-Actin was used as housekeeping gene.

cAMP production. Measurement of total cAMP in Chem-3 cells was performed using the Biotrak kit (GE Healthcare Biosciences) according to the nonacetylation procedure described by the manufacturer. Cells (10 6 cells/well) were incubated with/without various concentrations of the A2AR agonist CGS-21680 for 60 min at 37°C under various gas and pressure conditions. The incubation period was stopped by adding the dodecyltrimethyl ammonium bromide acetate lysis buffer provided with the kit. Production of cAMP in each well was then determined (n = 3) using the competitive enzyme immunoassay according to the manufacturer’s instructions (11). Results were given either as a percentage of cAMP produced in the condition of interest vs. the basal production level or as pg/10 6 cells.

CD26/DPP-IV. Blood samples (5–10 ml) were obtained following intracardiac puncture using a syringe containing calciparine. Mononuclear cells were isolated using a separation gradient-based procedure; blood was diluted (vol/vol) using 0.9% NaCl and 0.05% NaN 3 and mixed (vol/vol) with the lymphocyte separation medium Lymphoep (MP Biomedicals). Cells were isolated (21°C, 400 g, 40 min) before being washed (n = 3) using RPMI 1640 culture medium. Cells (2 × 10 6/ml) were then incubated with a colorimetric substrate of DPP-IV, Gly-Pro-P-nitroanilide (3 mM, 2 h in 75 mM glycin buffer, pH 8.7) (46). Signal background was determined using incubation in 1 M acetate buffer, pH 5, a condition in which DPP-IV is inactive. Optical density was measured at 405 nm, and the results were expressed as international units.

Statistical analysis. Data were expressed as means ± SD. A two-way ANOVA was used for intergroup comparisons. A P value of <0.05 was considered significant.

**RESULTS**

Adenosine plasma concentration (in vivo). Hypoxic conditions promote accumulation of adenosine inside and outside the cell, notably in the blood (22). Here, we quantified adenosine in blood sampled from animals submitted to hyperoxia combined, or not, with hyperbaric conditions. Hyperoxia alone (1 bar O 2) reduced the adenosine plasma concentration compared with normoxia, the control situation (means ± SD: 0.40 ± 0.18 vs. 0.77 ± 0.15 µM, respectively; Fig. 1A). Hyperbaric hyperoxia (2 bar O 2) further affected this parameter (0.15 ± 0.02 µM). No significant difference was found between the adenosine level obtained using hyperbaria alone (0.65 ± 0.25 µM) and normoxia. These data show that the adenosine plasma level was mainly decreased by the oxygen level, hyperbaric treatment lacking significant influence per se under normoxic conditions.

CD73 regulation (in vivo). CD73 expression is a marker used to address the regulation of adenosine synthesis (29, 52). CD73 expression in brain samples was analyzed by Western blotting. The 70-kDa species corresponding to the CD73 antigen (Fig. 1B) was semiquantified using densitometry analysis. Hyperoxia alone increased CD73 expression compared with normoxia (means ± SD: 3.43 ± 0.64 vs. 1.10 ± 0.19 AU, respectively; Fig. 1C), hyperbaric hyperoxia exhibiting a similar effect (2.95 ± 0.72 AU). Hyperbaric treatment alone had no significant effect vs. normoxia (1.49 ± 0.27 AU). These results show that hyperoxia increased CD73 expression, hyperbaric conditions having no significant influence.

ADA regulation (in vivo). High adenosine concentration can exhibit cytotoxic effects, and its extracellular level is regulated by an adaptive response that includes degradation into inosine by the serum aminohydrolase ADA. We addressed here the effects of hyperoxic and hyperbaric treatments on serum ADA. We found that hyperoxia alone tended to increase ADA com-

![Fig. 1. Plasma adenosine concentration, CD73, and serum adenosine deaminase (ADA) activity in vivo. Blood was collected by cardiac puncture following the stay of the rats in the pressure chamber in the various conditions. Plasma adenosine was quantified using high-performance liquid chromatography (A).Brains were also sampled, and the CD73 antigen was detected using Western blotting and an anti-CD73 antibody before semiquantitation using densitometry analysis of the 70-kDa band. Western blot analysis of the sample that generated the densitometry value that was the nearest to the mean value of expression of the 70-kDa species detected in the rats tested in the condition of interest is shown (B). The data resulting from densitometry analysis of all samples are given (C). Serum ADA was quantified using incubation with adenosine and measurement of ammonia production (D). Data are given as means ± SD; P < 0.05 vs. normoxia, hypoxia, and hyperbaric hyperoxia.](http://jap.physiology.org/2017/10220-0023.1/)
hyperbaric conditions. Overall, these results show that hyperoxic and hyperbaric conditions downregulated A2AR expression, the regulation being exerted at the transcriptional level.

Regulation of A2A expression and cAMP production (in vitro). Adenosine binding to A2A stimulates the adenyl cyclase pathway and Gs proteins, which induces a rise in the intracellular cAMP level and activates a plethora of biological processes (36). We addressed here the influence of hyperbaric and/or hyperoxic treatments on A2A expression in brain, dissociating heart tissue into its component cells being a long and difficult process that affects the integrity of protein components, hence their biochemical characteristics/characterization. We studied mRNA expression of A2A (Fig. 3A). Hyperoxia alone decreased mRNA expression compared with normoxia (means ± SD: 1.9 ± 0.6 vs. 3.0 ± 0.6 AU, respectively). Hyperbaric hyperoxia further decreased the expression (1.3 ± 0.8 AU), hyperbaric treatment alone inducing a similar effect (1.5 ± 0.3 AU). The protein expression of A2A was also semiquantified, and a similar pattern was found (Fig. 3, B and C; normoxia: 9.9 ± 1.5 AU; hyperoxia: 8.2 ± 1.5; hyperbaric hyperoxia: 6.9 ± 1.2 AU; hyperbaria: 6.1 ± 2.3 AU). Overall, these results show that hyperoxic and hyperbaric conditions downregulated A2AR expression, the regulation being exerted at the transcriptional level.

Fig. 2. CD26/dipeptidyl peptidase-IV (DPP-IV) activity associated with the blood mononuclear cell surface in vivo. Blood was collected by cardiac puncture after the stay of the rats in the pressure chamber in the various conditions. Monocytic cells were then isolated using a density gradient and incubated with Gly-Pro-P-nitroanilide, a colorimetric substrate of DPP-IV. Optical density was measured at 405 nm. Data are given as means ± SD; 1P < 0.05 vs. normoxia.

CD26 expression on blood mononuclear cells sampled from animals using the chromogenic peptide substrate Gly-Pro paranitroanilide. Hyperoxia alone significantly decreased DPP-IV activity compared with normoxia (means ± SD: 0.87 ± 0.16 vs. 1.08 ± 0.11 U) (Fig. 2). Hyperbaric hyperoxia further decreased the activity (0.78 ± 0.16 U). Hyperbaric treatment alone did not significantly modify DPP-IV (1.01 ± 0.17 U) compared with normoxia. These data show that CD26 expression was mainly downregulated by the oxygen level, hyperbaric conditions having no significant influence per se under normoxic conditions.

CD26/DPP-IV regulation (in vivo). CD26 expressed on blood mononuclear cells displays the DPP-IV activity, which controls by endoproteolysis hypoxia-regulated peptide substrates with proline in penultimate position (7). We monitored CD26 expression on blood mononuclear cells sampled from animals using the chromogenic peptide substrate Gly-Pro paranitroanilide. Hyperoxia alone significantly decreased DPP-IV activity compared with normoxia (means ± SD: 0.87 ± 0.16 vs. 1.08 ± 0.11 U) (Fig. 2). Hyperbaric hyperoxia further decreased the activity (0.78 ± 0.16 U). Hyperbaric treatment alone did not significantly modify DPP-IV (1.01 ± 0.17 U) compared with normoxia. These data show that CD26 expression was mainly downregulated by the oxygen level, hyperbaric conditions having no significant influence per se under normoxic conditions.

Regulation of A2AR expression (in vivo). Adenosine acts following binding to the surface A2AR of numerous cell types, notably in the cardiac and nervous systems (22, 63, 64). We chose here to monitor the effects of hyperoxic and/or hyperbaric treatments on A2AR expression in brain, dissociating heart tissue into its component cells being a long and difficult process that affects the integrity of protein components, hence their biochemical characteristics/characterization. We studied mRNA expression of A2AR (Fig. 3A). Hyperoxia alone decreased mRNA expression compared with normoxia (means ± SD: 1.9 ± 0.6 vs. 3.0 ± 0.6 AU, respectively). Hyperbaric hyperoxia further decreased the expression (1.3 ± 0.8 AU), hyperbaric treatment alone inducing a similar effect (1.5 ± 0.3 AU). The protein expression of A2AR was also semiquantified, and a similar pattern was found (Fig. 3, B and C; normoxia: 9.9 ± 1.5 AU; hyperoxia: 8.2 ± 1.5; hyperbaric hyperoxia: 6.9 ± 1.2 AU; hyperbaria: 6.1 ± 2.3 AU). Overall, these results show that hyperoxic and hyperbaric conditions downregulated A2AR expression, the regulation being exerted at the transcriptional level.
evaluate in samples derived from in vivo experiments because the protocol used to isolate cell samples from animals (e.g., length of procedure, use of chemical reagents, and physical effects) does not comply with the requirements of a reliable cAMP quantitation. The cell line we used readily expresses the functional A2AR, and it is therefore a useful tool to address cAMP production resulting from activation of the receptor (6). To validate this cell line as a model to test the effect of hyperbaric and/or hyperoxic conditions on the cAMP component of the adenosine response, we examined whether A2AR expression responded to hyperbaric and hyperoxic treatments. Changes in A2AR expression observed in the cell line submitted to the various conditions used to treat animals were found to be similar to those observed in brain samples (normoxia: 13.1 ± 0.7 AU; hyperoxia: 11.2 ± 0.1 AU; hyperbaric hyperoxia: 10.1 ± 0.4 AU; hyperbaria: 7.1 ± 0.2 AU). Regarding CGS-21680, its use instead of adenosine was attributable to its stability, the natural ligand being rapidly (few seconds) degraded in the cell environment. First, the concentration of CGS-21680 that induced 50% of the maximal production of cAMP (EC50) in the condition of interest was studied (Fig. 4A). The EC50 value reflects the ability of a ligand to activate a receptor and to subsequently cause transduction (its efficacy). Hyperoxic and/or hyperbaric treatments did not modify the EC50 values found in normoxia. Second, cAMP production was examined (Fig. 4B). Hyperoxia alone decreased the production compared with normoxia (means ± SD: 2,990 ± 130 vs. 3,990 ± 250 pg/106 cells). Hyperbaric hyperoxia had a similar influence (3,200 ± 260 pg/106 cells). Hyperbaric conditions alone exhibited the strongest effect (2,150 ± 180 pg/106 cells). Overall, these results show that hyperoxia and hyperbaria downregulated cAMP production in a context where the ligand efficacy remained unchanged compared with normoxia.

**DISCUSSION**

This study adds to the line of research that was started by demonstration that A2AR is critically involved in the down-regulation of hypoxia/CD73/A2AR-mediated inflammatory damage (50). The present findings are that increasing the oxygen level in inhaled air 5- to 10-fold, and hence the partial pressure of oxygen in the arterial blood, compared with the terrestrial conditions decreased in a dose-dependent manner adenosine plasma concentration, A2AR expression, and CD26 level, whereas it increased both CD73 expression and ADA activity. The consequence of hyperbaric treatment alone was dual: the treatment lacked significant effect on adenosine, ADA, CD73, and CD26, whereas it downregulated A2AR expression.

These results were obtained using wild-type animals although transgenic mice and rats deficient in A2AR expression or overexpressing A2AR are commonly used to study the adenosinergic pathway. We considered, however, that the use of such models had no interest here because manipulating the expression of A2AR induces pleiotropic effects, including high blood pressure, altered heart rate, changes in blood cell properties, metabolic changes, and chronic inflammation (65), which all display patently obvious influences on the variables we studied, thus precluding valuable conclusions regarding authentic mechanisms.

A large amount of literature demonstrates the importance of high adenosine concentration and high A2AR expression in pathophysiological mechanisms resulting from ischemia/hypoxia. It is also known that adenosine and A2AR are important factors in the hyperoxia-induced lung pathophysiology: on one hand, recent studies further described how pulmonary inflammation and edema resulting from hyperoxia are associated with an increase in adenosine concentration and A2AR expression, a hypothesis being that these changes occur to protect the vascular barrier function (16, 17); on the other hand, it was also shown that hyperoxia alters the tissue-protecting mechanism in acute inflammatory lung injury by decreasing the A2AR signaling pathway, and inhalative application of A2AR agonists during oxygen therapy was proposed as treatment (61). Although the interest of examining the effect of hyperoxia on the lung pathophysiology is obvious, the situation was poorly examined for other organs such as the brain and in normal animals/tissues.

However, besides providing knowledge on the impact of systemic high oxygen level on the brain, addressing brain A2AR is greatly needed because the receptor recently emerged as a potential therapeutic target to implement the treatment of acute ischemic stroke (42, 43). Evidence suggests, however, that A2AR has additional roles during stroke: in the first phase of ischemia, it promotes excitotoxicity mechanisms, whereas, in the second phase, immune blood cell-associated A2AR
promotes cell adhesion mechanisms and infiltration in the ischemic parenchyma (14, 27, 42, 43, 67). Thus, in addition to induction of reactive oxygen species production, systemic hyperoxia may be detrimental to the brain integrity and function through A2AR expression, and we addressed here the receptor and its ligand following hyperoxegenation.

Measuring the intracerebral adenosine level faces major difficulties (its half-life <10 s, its compartmentalization by the various cell populations, and its release induced by in situ sampling microprocedures). Although the intravascular adenosine concentration may only imperfectly represent the extracellular, active, concentrations found in the various regions of the brain, we proceeded to study blood adenosine level. In contrast with recent studies in the lung (16, 17) but in agreement with conclusions reached from other studies (29, 61), we observed that hyperoxegenation induced a decrease in both plasma adenosine concentration and A2AR expression in the brain in a concentration-dependent manner. Regarding CD73, the present work and a previous study (16) show that its expression increased following hyperoxia in the brain and in the lung. However, hypoxia upregulates CD73 expression (59), and one can expect that hyperoxia does the reverse. The increased expression of CD73 we found here in hyperoxic conditions suggests that the corresponding low adenosine plasma concentration did not result from a decreased synthesis of adenosine by CD73 but from an increased degradation by ADA. Consequently, the high CD73 expression observed here in hyperoxic conditions where adenosine plasma concentrations were low may result from a feedback mechanism to restore the plasma level of the nucleoside.

With consideration that lack of A2AR activation promotes vasoconstriction (47), the low plasma adenosine concentration as well as the low A2AR expression level we found here in the brain following hyperoxia may also constitute an adaptive response by inducing vasoconstriction to diminish the oxygen supply to tissue and the deleterious consequences of production of reactive oxygen species. In this respect, it is worthwhile to note here that 1) the hyperoxic conditions achieved in our experiments mimic conditions that are used in human, inasmuch as 1-bar O2 is a common treatment of CO poisoning and 2-bar O2 is used notably by combat divers (31), suggesting that the effects we observed here can be found in humans; 2) the way hyperoxia regulates the adenosine response, as shown here, contributes to explain how and why hyperoxia promotes vasoconstriction (24); 3) our data are consistent with the observation that a low oxygen level induces adenosine release and high A2AR expression, in particular in peripheral blood mononuclear cells (13, 22, 35, 56, 63).

The hyperbaric condition alone potently decreased A2AR expression in the brain and in the cell line we used even when the oxygen supply to tissue was maintained at an ambient level (normoxia). The decrease in the surface expression of A2AR following hyperoxic and hyperbaric treatments is consistent with the decrease in mRNA production we observed here. Various receptors are affected by the hydrostatic pressure, notably in brain tissues (54). Pressure alters protein expression via a transcription/translation regulation process (4, 34, 57), and this mechanism may have pathophysiological relevance. For instance, with the use of hydrostatic pressure in the range of glomerular capillary hydraulic pressure observed in experimental glomerulosclerosis (uninphrectomized spontaneously hypertensive rats), it was shown that discrete pressure changes increase cell proliferation via DNA synthesis (34).

A2AR is a G protein-coupled receptor that modulates the production of the second messenger cAMP, a regulator of important cellular functions such as proliferation, differentiation, inflammation, and apoptosis (36, 40, 64). Here, cAMP production decreased during hyperoxia associated, or not, with hyperbaric treatment, the effect being maximum when the hyperbaric condition was used alone. This observation is consistent with the downregulation of A2AR in a hyperoxic or hyperbaric environment as shown here. The modulation of cAMP production we observed here is also in agreement with previous reports that used systems submitted to hyperoxia or (patho)physiological hydrostatic pressure. For example and regarding oxygen level, a decrease of cAMP production was found following hyperoxia in the carotid body of rats, a region that is enriched in A2AR and is involved, through adenosine release, in mechanisms maintaining high cAMP levels during hypoxia (45). Regarding the hydrostatic pressure, the condition decreases accumulation of cAMP in cells isolated from the proliferative region of tibia epiphysal cartilage, a zone where pressure-dependent cell signaling likely has physiological significance (8). Thus hydrostatic pressure affects per se cAMP production as part of a general regulatory mechanism (8, 15, 45) that was further observed here using hyperbaric treatment alone.

Regarding the effects of the oxygen level on ADA and surface CD26, both catalysts increase during hypoxia in humans where both proteins are interacting at the mononuclear cell surface (20, 46). This increase was interpreted as a means to protect mononuclear cells from cytotoxic effects of high adenosine concentration in the vicinity of the cell surface via binding to CD26 of the aminohydrolase that degrades adenosine, and hence as an evolutionary advantage (46). Here, in the rodent context where ADA does not bind CD26 (19, 53), we observed that hyperoxia increased the serum ADA level. That low adenosinemia and high ADA level coexisted here following hyperoxia is in apparent discrepancy with the idea that high ADA levels observed during hypoxia in humans are induced by high adenosinemia to counteract adenosine cytotoxicity. The high ADA level we measured here following hyperoxia and its consequences in terms of adenosine degradation are fully consistent, however, with the low adenosine level we found in our study. In contrast, hyperbaric conditions did not affect per se adenosine plasma level, CD73, ADA, and CD26.

Finally, we found that a high oxygen level tended to decrease CD26 expression in rat, which is in agreement with previous findings in humans where an inverse relationship was found between blood oxygen level and CD26 expression (46). Our present data also support the idea that downregulation of CD26 expression by high oxygen concentration constitutes an advantage. Besides its role in the immune response, there are various ways by which CD26 may participate in controlling the response to hyperoxia. First, a diminished degradation of CD26 substrate peptides with antioxidant capacity promotes their activity, which represents an obvious advantage in hyperoxic conditions (5, 32, 44, 49, 60). For example, a decreased degradation of the vasoactive intestinal peptide is consistent with the observation that its serum level increases following production of reactive oxygen species, which constitutes an adaptive response because the peptide has per se antioxidative...
capacities via its capacity to scavenge singlet oxygen and to reduce hydroxyl radical production (32, 41, 44). A similar conclusion can be reached for the glucagon-like peptide 1 that fights against redox-generated cell senescence (49). Second, a diminished degradation of cardio/vasoactive CD26 substrates such as the potent neuropeptide Y results in an increase of the vascular tone, which reduces the oxygen supply to tissues (41, 66). Third, CD26 controls energetic metabolism and energy supply to cells via degradation of peptides such as the glucagon-like peptide 1 (41). Last, but not least, a long-term loss of CD26 activity was reported to increase the capability against the oxidative stress and reactive oxygen species (38). Although addressing CD26 substrates was out of the scope of this work, monitoring them during hyperoxia certainly deserves further study.

In conclusion, we showed that increasing PaO2 by up to 10-fold tended to downregulate the adenosinergic pathway and CD26 activity. This mechanism may participate in an adaptive response to extreme values of oxygen supply to tissues and their deleterious consequences such as reactive oxygen species production through A2A-mediated vasoconstriction and decreased vasoactive and antioxidant peptide degradation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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