CO_2 Inhibition of the Generation of Reactive Oxygen Species in Cells of Internal Organs and Its Biological Importance

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Carbon dioxide is shown to inhibit the generation of superoxide anion radical in bioptates both directly exposed to CO_2 and after preliminary exposure of the whole organism to CO_2. A similar effect is noted for gas mixtures similar in composition to arterial and venous blood. The results substantiate the important evolutionary role of CO_2 for the maintenance of life on Earth with the appearance of potentially toxic oxygen.

Key Words: *carbon dioxide; superoxide anion radical; tissue cells; antioxidants; evolution*

The inhibitory effect of CO_2 on the generation of reactive oxygen species (ROS) by cells of some internal organs (heart, liver, brain, pylorus, and skeletal muscles) has been reported earlier [2] in experiments which were based on direct exposure of the bioptates to CO_2. However, the effect of CO_2 on the whole organism and the effect of CO_2-oxygen mixtures in ratios close to those in arterial and venous blood ("arterial" and "venous" mixtures, respectively) remain unclear, as does as the direct effect of CO_2 on the cells of some organs (kidneys and lungs). These are the topics of the present study.

MATERIALS AND METHODS

The experiments were carried out on 76 random-bred male mice weighing 23-35 g. NADPH-stimulated generation of ROS by cells was determined after exposure of either tissue bioptates (series I, n=34) or the whole organism (series II, n=42) to CO_2.

The technique of measurement of ROS generation by cells (parenchymal and interstitial together) developed by A. Kh. Kogan and co-workers [5] is based on the fact that NADPH is a substrate for NADPH-oxidase, one of the main enzymes catalyzing the generation of superoxide anion radical ($O_2^{\cdot-}$), so that NADPH stimulates the formation of $O_2^{\cdot-}$ in cells. The latter may be evaluated by the reaction with nitroblue tetrazolium (NBT) by measuring spectrophotometrically the amount of products of interaction between $O_2^{\cdot-}$ and NBT-formazans. $O_2^{\cdot-}$ represents the parent ROS which may be converted to H_2O_2 in the reaction catalyzed by superoxide dismutase; in turn, in the Haber-Weiss cycle H_2O_2 in the presence of Fe^{2+} gives rise to singlet oxygen ($^1O_2$) and hydroxyl radical ($^{\cdot}OH$). The experimental procedure was as follows: a small tissue fragment (1.6-2.5 mm) excised from the organ of interest of a decapitated or anesthetized animal was washed with a buffer mixture (2.5 ml phosphate-alkaline buffer+7.5 ml isotonic 0.9% NaCl), dried on filter paper, and cut into two approximately equal parts (hereafter referred to as bioptates). Each bioptate was precisely weighed to an accuracy of 10^{-5} g, placed in separate test tubes (control and experimental) with a diameter of 10 mm and height of 50-60 mm, and minced in 0.2-0.3 ml buffer mixture. Then 0.5-1.0 ml of 0.2% Triton X-100 in water-ethanol solution was added to the control and experimental test tubes (to loosen the mem-

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branes and facilitate the penetration of superoxide dismutase or, in the control, albumin into the cells). The suspension was incubated for 3-5 min and centrifuged at 1000 rpm for 5-7 min, the supernatant was decanted, and the pellet was thrice washed free of Triton with the buffer mixture, after which either superoxide dismutase (250 μg in 0.2 ml buffer mixture) or albumin (an equal amount in the same volume) was added to the resultant pellet. The samples were incubated for 20 min at 36.9-37.0°C and then 0.1 ml of 3% NADPH and 0.2 ml 0.2% NBT (both dissolved in the buffer mixture) were added. After a 40-50-min incubation at 36.9-37.0°C the reaction was terminated by adding 0.5 ml 5 N HCl. The samples were centrifuged at 3000 rpm for 7 min, the supernatant was removed, and the concentration of formazans was measured spectrophotometrically at λ=560 nm against the buffer during the first 10-15 min, since later the fine formazan suspension settled out. Formazans were extracted from the precipitates with a dimethyl sulfoxide:chloroform mixture (2:1) at 55-59°C for 15 min and centrifuged at 3000 rpm for 10 min, the supernatant-extract was collected, and the procedure was repeated. In the collected extracts the concentration of formazans was determined spectrophotometrically against the dimethyl sulfoxide:chloroform mixture (2:1). The data of all measurements were summarized separately for the control (with superoxide dismutase) and experimental (with albumin) samples with a correction for dilution and standardized to a 1-mg bioptate. The difference between the control and experimental samples reflects the content of O_2^- according to the optical density of formazans (products of interaction between O_2^- and NBT).

Gas mixtures with the required concentration of CO_2 [3,4] were prepared in a 6-liter miniature pressure chamber, where either control and experimental samples (minced bioptates and the above-mentioned reagents) or intact mice were preliminarily placed. The gas mixtures were prepared by replacing air with CO_2 (and, if necessary, other gases). The pressure was controlled with a manometer. Both the isolated bioptates and intact mice were exposed to a gas mixture consisting of 8.2% CO_2 (Pco_2=60 mm Hg) and 91.8% air (670 mm Hg), Po_2=153 mm Hg; the total pressure of the gas mixture was 730 mm Hg. This preserved a residual rarefaction of 30 mm Hg necessary for hermetic sealing of the chamber. The isolated bioptates and intact mice were exposed to the mixture for 40 and 50 min, respectively. The content of formazans in the samples after incubation with the gas mixture was determined by the NBT test as described above. The animals were taken out of the chamber and decapitated. The bioptates were prepared and the formazans formed in the NBT reaction were measured. In addition to the 8.2% CO_2 mixture, we also studied the effect of gas mixtures similar in composition to arterial and venous blood on the generation of O_2^- by cells of the isolated bioptates. The "arterial" mixture consisted of 5.4% CO_2 (Pco_2=39.4

TABLE 1. Effect of CO_2 on NADPH-Stimulated Generation of O_2^- by Cells of Various Organs (*M±m*)

Bioptate	Air (control)	Air+8.2% CO_2 (experiment)	Air/Air+8.2% CO_2
Liver (*n*=8)	1.116±0.140	0.337±0.27**	3.31
Brain (*n*=10)	0.770±0.086	0.165±0.025**	4.7
Heart (*n*=9)	0.659±0.090	0.193±0.023**	3.4
Pylorus (*n*=3)	0.862±0.144	0.248±0.044*	3.5
Skeletal muscles of the femur (*n*=3)	1.083±0.105	0.338±0.041**	3.2

Note. Here and in Table 2: O_2^- is superoxide anion radical. The table presents the concentration of products of interaction between O_2^- and NBT-formazans (in optical density units) per milligram bioptate (λ=560 nm). The complete composition of the gas mixture in the experiment: 8.2% CO_2 (Pco_2=60 mm Hg)+91.8% air (enriched with oxygen to 22.8% instead of 21% in the norm) (P_{air}=670 mm Hg); Po_2 in air=153 mm Hg. In the experiment and control P total=730 mm Hg. *p<0.01, **p<0.001 in comparison with the control.

TABLE 2. Changes in NADPH-Stimulated Generation of O_2^- in Cells of Various Organs after Exposure of the Whole Organism to 8.2% CO_2 (*M±m*)

Bioptate	Air (control)	Air+CO_2 (experiment)	Air/Air+CO_2
Liver (*n*=15)	0.988±0.06	0.402±0.04**	2.5
Brain (*n*=15)	0.886±0.06	0.378±0.02**	2.3
Heart (*n*=3)	0.935±0.12	0.345±0.02**	2.7
Pylorus (*n*=4)	0.917±0.02	0.322±0.04*	2.8
Skeletal muscles (*n*=4)	1.103±0.09	0.270±0.04**	4.1

TABLE 3. Effect of Arterial and Venous Gas Mixtures on NADPH-Stimulated Generation of O_2^- by Cells of Various Organs ($M \pm m$)

Bioptate	Air	AGM	VGM	Air/AGM	Air/VGM	AGM/VGM
Liver (n=10)	0.910±0.082	0.365±0.035**	0.240±0.040**+	2.5	3.7	1.5
Brain (n=10)	0.985±0.107	0.357±0.040**	0.251±0.030**	2.8	3.9	1.4
Heart (n=10)	1.210±0.115	0.409±0.052**	0.167±0.015**++	3.0	7.3	2.4
Lungs (n=8)	1.075±0.085	0.433±0.110**	0.225±0.030**	2.5	4.8	1.9
Kidneys (n=8)	0.690±0.053	0.348±0.041**	0.237±0.030**+	1.9	2.9	1.5
Pylorus (n=10)	0.786±0.100	0.326±0.004**	0.252±0.020**	2.4	3.1	1.3
Membranous part of stomach (n=10)	0.430±0.045	0.261±0.035*	0.192±0.023**	1.7	2.2	1.3

Note. AGM and VGM are arterial and venous gas mixtures, respectively. *p<0.01, **p<0.001 in comparison with air, +p<0.01, ++p<0.001 in comparison with AGM. O_2^- is superoxide anion radical. The table presents the concentration of products of interaction between O_2^- and NBT-formazans (in optical density units) per milligram bioptate (λ=560 nm).
Content of gas mixtures used in the experiment: AGM: 5.4% CO_2 (Pco_2=39.4 mm Hg)+13% O_2 (Po_2=95 mm Hg)+81.5% N_2 (PN_2=595 mm Hg). VGM: 6.2% CO_2 (Pco_2=45 mm Hg)+5.3% O_2 (Po_2=39 mm Hg)+88.5 % N_2 (PN_2=646 mm Hg). In the experiment and control P total=730 mm Hg.

mm Hg), 13% O_2 (Po_2=95 mm Hg), and 81.5% N_2 (PN_2=595 mm Hg). The "venous" mixture contained 6.2% CO_2 (Pco_2=45 mm Hg), 5.3% O_2 (Po_2=39 mm Hg), and 88.5% N_2 (PN_2=646 mm Hg). The total pressure of each gas mixture was 730 mm Hg.

RESULTS

Our findings (Tables 1-3) suggest that CO_2 inhibited the NADPH-stimulated generation of O_2^- in cells (parenchymal and interstitial together) of all studied organs (liver, brain, heart, lungs, kidneys, skeletal muscles, and stomach). The inhibiting effect of CO_2 on the NADPH-stimulated generation of O_2^- is confirmed both in the case of direct exposure of bioptates of internal organs to CO_2 (Table 1) and when the whole organism was exposed to CO_2 (Table 2). Since under natural conditions CO_2 in the blood acts in combination with O_2, we believe that the effect of gas mixtures similar in composition to arterial and venous blood warrants special investigation. The experiments showed that both the "arterial" and "venous" mixtures inhibited the generation of O_2^-, the effect of the "venous" mixture being more pronounced (Table 3). Thus, the data presented here and previous findings [2-4] prove the inhibiting effect of CO_2 on the generation of O_2^- by many (or most) types of cells: blood phagocytes and cells of internal organs, suggesting that CO_2 is a natural inhibitor of ROS generation in various cells. This fact attests to the important evolutionary role of CO_2 in maintaining life on Earth when potentially toxic oxygen appears in the atmosphere and the anaerobic pathway of energy production replaces the aerobic one [6], and provides new understanding (in light of the role of ROS) of the physiological and pathophysiological effects (vasodilative and influence on the respiratory center). In conclusion, these data are of interest for such a serious global problem as the progressive build up of CO_2 in the atmosphere [1].

REFERENCES

1. I. I. Borzenkova, M. I. Bud'ko, E. K. Byutner, *et al.*, in: *Man-Induced Changes of the Climate* [in Russian], Leningrad (1987), p. 405.
2. A. Kh. Kogan, S. V. Grachev, and S. V. Eliseeva, *Dokl. Ross. Akad. Nauk*, **340**, No. 1, 132-134 (1995).
3. A. Kh. Kogan, B. M. Manuilov, S. V. Grachev, *et al.*, *Byull. Eksp. Biol. Med.*, **118**, No. 10, 395-398 (1994).
4. A. Kh. Kogan, B. M. Manuilov, and S. V. Grachev, *Fiziol. Cheloveka*, **21**, No. 4, 128-136 (1995).
5. A. Kh. Kogan, I. V. Khapugina, M. I. Rasulov, *et al.*, *Byull. Izobret.*, No. 21 (1996).
6. R. F. Schmidt and G. Thews, in: *Human Physiology*, Berlin (1989), p. 507.