# PROTECTION OF KIDNEYS AFTER PROLONGED ISCHEMIA AGAINST REOXYGENATION INJURY BY TEMPORARY INHALATION OF A HYPOXIC GAS MIXTURE

M. V. Bilenko, I. A. Volkova, and S. P. Danilov

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It was shown previously that during reperfusion of organs after prolonged ischemic lipid peroxidation (LPO) processes continue in them at a level of intensity which remains high or continues to rise further, in correlation with the severity of reoxygenation injuries in the organ, thus providing evidence of the essential role of LPO in the pathogenesis of the oxygen paradox [1-5, 7, 8, 11, 13].

Intensification of LPO during reperfusion of an ischemic organ is due to disparity between the reduced activity of protective antioxidant systems and weakened ability of the respiratory chain to utilize oxygen, on the one hand, and the relatively high concentration of oxygen reaching the organ with the blood, on the other hand, so that a situation of "relative local hyperoxia" is created [3] and this makes the usual blood oxygen concentration excessive and toxic for the organ. In fact, lowering the oxygen concentration in the blood during reperfusion of the kidneys, heart, and liver in experiments on animals inhaling a hypoxic gas mixture (HGM) [3, 5], and also administration of antioxidants [4, 8, 11], inhibit the reintensification of LPO and reduce the severity of reoxygenation injuries in the organs, whereas elevation of $pO_2$ of the salt solution during reperfusion of the heart and kidneys is accompanied by increased membrane permeability and by increased outflow of enzyme into the perfusion fluid [12].

Temporary hypoxemia as a method of protecting an organ against reoxygenation injuries could be used in cases when prophylactic antioxidant therapy has been overlooked or is impossible, but the protective effect of inhalation of HGM has been demonstrated previously in acute experiments only, and the animals were not subsequently transferred to breathing ordinary air.

In the present investigation the protective action of temporary inhalation of HGM on function of the kidneys rendered ischemic for 2 h was studied in chronic experiments, the required duration of such inhalation was determined, and correlation was established between the protective effect of HGM and the rate of resynthesis of adenine nucleotides (ADN) in the organ.

## EXPERIMENTAL METHOD

Experiments were carried out on 250 August and Wister rats weighing 120-200 g. Renal ischemia was produced under hexobarbital anesthesia (70 mg/kg) by applying microclips to the vascular pedicle of both kidneys, isolated from the perinephric cellular tissue. The clips were removed after 2 h, during which time the rats breathed ordinary air or inhaled an HGM containing 10-12% oxygen or pure oxygen through a mask. Inhalation of the gas mixtures began 5 min before the beginning of reperfusion of the kidneys. After suture of the wound in the abdominal wall the rats were kept for 4 or 24 h in a chamber ventilated with the same gas at the rate of 2 liters/min, after which they were transferred to breathing air. Stable humidity (85-90%) and temperature (23-25°C) were maintained in the chamber. The oxygen concentration in the gas mixture and chamber was recorded by an oximeter (Beckman, USA) and $pO_2$ and $HbO_2$ in the rats' blood were determined on a micro-Astrup apparatus. To compare the protective effect of HGM with the effect of antioxidants, 29 rats were given an intraperitoneal injection of 240 mg/kg of ionol (2,6-di-tertbutyl-4-methylphenol) 24 h before

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TABLE 1. Survival Rate of August Rats after Ischemia for 2 h and Reperfusion of the Kidneys during Inhalation of Gas Mixtures Differing in Oxygen Concentration and Injection of Ionol

| Experimental conditions | Type of gas mixture | Duration of inhalation, h | Number of rats | Number of rats surviving | | |
|---|---|---|---|---|---|---|
| | | | | 3 days | 7 days | 30 days |
| Mock operation | Air | Whole expt. | 15 | 15 (100) | 15 (100) | 15 (100) |
| | Oxygen | 4 | 10 | 10 (100) | 10 (100) | 10 (100) |
| | | 24 | 10 | 10 (100) | 10 (100) | 10 (100) |
| | HGM | 24 | 15 | 15 (100) | 15 (100) | 14 (94) |
| Ischemia for 2 h | Air | Whole expt. | 24 | 5 (21) | 2 (8) | 1(4) |
| | Oxygen | 4 | 9 | 2 (22) | 0 | 0 |
| | HGM | 4 | 23 | 6 (26) | 3 (13) | 3 (13) |
| | | 24 | 26 | 19 (73)* | 17 (65)* | 13 (50)* |
| | Air preceded by injection of ionol | Whole expt. | 12 | 8 (67)* | 8 (67)* | 7 (58)* |

Legend. Number of surviving rats given in parentheses. *) Differences between experiments with inhalation of gas mixtures and experiment with inhalation of air significant at the $P < 0.05$ level.

TABLE 2. Concentrations of Adenine Nucleotides in Kidneys of August Rats after Ischemia for 2 h and Reperfusion during Inhalation of Gas Mixtures differing in Oxygen Concentration

| Experimental conditions | Type of gas mixture | Number of rats | Statistical parameters | Parameters studied (μmoles/g wet weight of tissue) | | | |
|---|---|---|---|---|---|---|---|
| | | | | ATP | ADP | AMP | Σ ADN |
| Without ischemia (norm.) | | 4 | $M \pm m$ | 1,54±0,17 | 0,66±0,06 | 0,92±0,06 | 3,11±0,26 |
| Ischemia for 2 h without reperfusion | | 5 | $M \pm m$ | 0,11±0,06$^{oo}$ | 0,20±0,05$^{oo}$ | 1,52±0,11$^{oo}$ | 1,92±0,05$^{oo}$ |
| Ischemia for 2 h + reperfusion for 4 h | Air | 6 | $M \pm m$ | 0,10±0,03 | 0,42±0,12 | 0,51±0,12 | 1,02±0,14 |
| | | | $P_1$ | — | — | <0,01 | <0,01 |
| | Oxygen$_1$ | 5 | $M \pm m$ | 0,24±0,05 | 0,51±0,20 | 0,38±0,07 | 1,12±0,24 |
| | | | $P_1$ | — | — | <0,01 | <0,02 |
| | HGM | 5 | $M \pm m$ | 0,60±0,11** | 0,35±0,03 | 0,98±0,06** | 1,93±0,43 |
| | | | $P_1$ | <0,01 | — | <0,05 | — |
| | | | $P_2$ | <0,01 | — | <0,01 | — |
| Ischemia for 2 h + reperfusion for 24 h | Air | 6 | $M \pm m$ | 0,34±0,07 | 0,24±0,04 | 0,23±0,01 | 0,80±0,10 |
| | | | $P_1$ | <0,05 | — | <0,01 | <0,01 |
| | | | $P_3$ | — | — | <0,05 | — |
| | Oxygen$^{\Delta}$ | 6 | $M \pm m$ | 0,11±0,03* | 0,27±0,03 | 0,32±0,01 | 0,70±0,07 |
| | | | $P_1$ | — | — | <0,01 | <0,01 |
| | HGM$^{\Delta}$ | 5 | $M \pm m$ | 0,45±0,04** | 0,76±0,05** | 0,66±0,04 | 1,80±0,04** |
| | | | $P_1$ | <0,01 | <0,01 | <0,01 | — |
| | | | $P_2$ | <0,02 | — | <0,01 | — |
| | | | $P_3$ | | | | |
| | HGM | 6 | $M \pm m$ | 0,62±0,12** | 0,37±0,12 | 1,05±0,03 | 2,04±0,14** |
| | | | $P_1$ | <0,01 | — | <0,01 | — |
| | | | $P_2$ | <0,01 | — | <0,01 | <0,01 |

Legend. $\Delta$) Inhalation of oxygen or HGM for 4 h, inhalation of air for the remaining 20 h; $^{oo}$) significance of differences between experiments with ischemia for 2 h without reperfusion and experiments without ischemia ($P < 0.01$); *, **) significance of differences between experiments with reperfusion preceded by inhalation of gas mixtures and by inhalation of air, * $P < 0.05$, ** $P < 0.01$; $P_1$) significance of differences between experiments with reperfusion for 4 or 24 h and experiments without reperfusion; $P_2$) significance of differences between experiments with reperfusion preceded by inhalation of HGM and by inhalation of oxygen; $P_3$) significance of differences between experiments with reperfusion for 24 h and reperfusion for 4 h preceded by inhalation of the same gas mixture.

ischemia. The protective action of HGM on integral kidney function was estimated as the percentage of rats surviving 30 days after the operation. The ADH concentration in the kidneys was determined before and 4 and 24 h after reperfusion. ATP was determined by the hexokinase method, ADP and AMP by the pyruvate kinase method, and the total ADN pool (ΣADN) was calculated. The results were subjected to statistical analysis by Student's t test for small samples and by the chi-square test.

## EXPERIMENTAL RESULTS

Estimation of the blood oxygen concentration in 12 intact rats 5 min after the beginning of inhalation of the gas mixtures showed that HGM led to a fall of $pO_2$ to 47±1.7 mm Hg and of $HbO_2$ to 82±1.7%, whereas

TABLE 3. Concentrations of Adenine Nucleotides in Kidneys after Ischemia for 2 h and Reperfusion Preceded by Injection of Ionol

| Experimental conditions | Preparation | Number of rats | Statistical parameters | Parameters studied (μmoles/g wet weight of tissue) | | | |
|---|---|---|---|---|---|---|---|
| | | | | ATP | ADP | SMP | Σ ADN |
| Without ischemia (norm.) | Without ionol | 6 | $M \pm m$ | 1,65±0,09 | 0,63±0,05 | 0,38±0,08 | 2,66±0,11 |
| | With ionol | 5 | $M \pm m$ | 1,14±0,08 | 1,24±0,12 | 0,56±0,05 | 2,94±0,20 |
| | | | $P_2$ | <0,01 | <0,01 | — | — |
| Ischemia for 2 h without reperfusion | Without ionol | 5 | $M \pm m$ | 0,12±0,04$^{00}$ | 0,20±0,05$^{00}$ | 0,84±0,05$^{00}$ | 1,6±0,08$^{00}$ |
| | With ionol | 5 | $M \pm m$ | 0,11±0,01$^{00}$ | 0,24±0,02$^{00}$ | 1,00±0,06$^{00}$ | 1,35±0,04$^{00}$ |
| | | | $P_2$ | — | — | — | — |
| Ischemia for 2 h + reperfusion for 24 h | Without ionol | 8 | $M \pm m$ | 0,42±0,05 | 0,24±0,05 | 0,31±0,05 | 0,97±0,12 |
| | | | $P_1$ | <0,01 | — | <0,01 | — |
| | With ionol | 7 | $M \pm m$ | 0,56±0,05 | 0,32±0,04 | 0,68±0,05 | 1,56±0,12 |
| | | | $P_1$ | <0,01 | — | <0,01 | — |
| | | | $P_2$ | | — | <0,01 | <0,01 |

Legend. $^{00}$) Significance of differences between experiments with ischemia for 2 h and experiments without ischemia ($P < 0.01$); $P_1$) significance of differences between experiments with reperfusion of the kidneys and without reperfusion, $P_2$) significance of differences between experiments without and with ionol.

inhalation of oxygen led to a rise of these values to 258±7.5 mm Hg and 100% respectively, compared with 102±1.8 mm Hg and 98±0.2% with inhalation of air, i.e., a state of moderate hypoxemia or of marked hyperoxemia developed in rats inhaling gas mixtures, in the same way as in dogs [5].

It will be clear from Table 1 that inhalation of gas mixture with different concentrations of oxygen had no effect on animals undergoing the mock operation but significantly affected the survival rate of rats with renal ischemia. In experiments with inhalation of air or oxygen most of the rats died, mainly during the first 3 days after the operation. Reperfusion of the kidneys against the background of HGM increased the percentage of surviving animals, and the protective effect was most marked and significant in the case of inhalation of HGM for 24 h in the postischemic period.

Estimation of the ADN concentration showed (Table 2) that ischemia caused a sharp fall in ΣADN in the kidneys and altered the ratio between its components: a fall in the ATP and ADP levels and an increase in the AMP concentration. Reperfusion of the kidneys for 4 and 24 h while the animals breathed air or oxygen ($O_2$ for 4 h or $O_2$ for 4 h followed by air for 20 h) was accompanied by a further decrease in ΣADN due to breakdown of AMP. By contrast, reperfusion of the kidensy against the background of inhalation of HGM by the rats for 4 and 24 h was accompanied by preservation of ΣADN and by a significant rise in the ATP concentration. Inhalation of HGM for 4 h followed by resumption of air breathing by the animals was less effective than inhalation of HGM for the whole 24 h on resynthesis of high-energy compounds, and, correspondingly, a 4-h course of HGM was less effective also according to the criterion of survival rats.

Prophylactic injection of ionol into the rats improved their survival rate by approximately the same degree as in the experiments with HGM (Table 1). In this case ionol reduced the ATP concentration in the kidneys of intact rats, did not affect the fall of ADN concentration or the change in their components during renal ischemia, but improved the restoration of ATP and ΣADN 24 h after reperfusion (Table 3). As Fig. 1 shows, the degree of restoration of ATP and ΣADN when reperfusion of the kidneys was preceded by injection of ionol was about the same as that after inhalation of HGM for 24 h, and surpassed the level observed after reperfusion of the kidneys preceded by inhalation for HGM for 4 h or inhalation of air, and was very much higher than its level in experiments with inhalation of oxygen. Positive correlation was found ($r = 0.74$) between the degree of restoration of ATP 24 h after reperfusion of the kidneys and the percentage of animals which survived.

Chronic experiments, like the acute experiments undertaken previously, thus showed that temporary moderate hypoxemia in the early reperfusion period has a protective action on kidney function after prolonged ischemia.

After 2 h of total renal ischemia a stable protective effect of the HGM, about equal to the effect of a high dose of ionol, was observed, but only when inhaled for 24 h, and not for 4 h, although with shorter periods of ischemia or during reperfusion of organs less sensitive to ischemia, a shorter period of inhalation of the HGM would probably be sufficient.

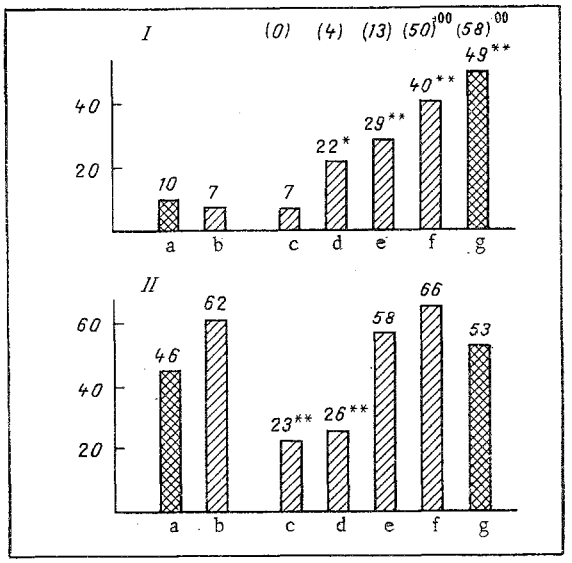


Fig. 1. Concentration (in % of initial) of ATP (I) and ΣADN (II) in rat kidneys after ischemia for 2 h, before reperfusion (a, b) and 24 h after reperfusion (c-g): a) Wister rats, b) August rats, c) inhalation of oxygen, d) inhalation of air, e) inhalation of HGM for 4 h and air for 20 h, f) inhalation of HGM for 24 h, g) injection of ionol. Numbers in parentheses give percentage of rats surviving after 30 days. *) Significance of difference between ATP and ΣADN content compared with period after ischemia: *P < 0.05, **P < 0.01. Circles) Significance of differences for survival rate of rats compared with value after inhalation of air, P < 0.05.

The protective effect of ionol and of temporary inhalation of HGM on renal function, like the protective effect of HGM on cardiac function [5], correlates with the rate of resynthesis of ATP in the ischemic organs. Manifestation of the protective effect of LPO inhibitors by acceleration of resynthesis of high-energy compounds in the organ is reasonable, especially if it is assumed that induction of LPO in the mitochondria is accompanied by reduced activity of the respiratory chain and by uncoupling of oxidative phosphorylation [6], and that ischemia of organs leads to the more rapid accumulation of LPO products in the mitochondria than in other subcellular organelles. The question of the effect of ionol on the ADN level in the kidneys calls for special discussion. The decrease in ATP which we found under the influence of ionol in the intact kidney is in harmony with data obtained by other workers [9], who showed that most drugs with anti-ischemic action (including $\alpha$-tocopherol) give rise to an energy-deficient state in the intact organ, possibly facilitating adaptation of the organ to ischemia. The absence of effect of ionol on the ADN content of the kidney after ischemia is evidently due to the rapid cessation of their synthesis during total ischemia, superposed on their extremely rapid utilization in order to maintain ionic gradients, characteristic of the kidney (and of several other organs also). Other antioxidants ($\alpha$-tocopherol, $CoQ_{10}$) likewise protected neither ATP nor ΣADN from a fall in their level during ischemia of the kidneys and liver, but guaranteed preservation of the mitochondria according to the criterion of the respiratory control, and stimulated ADN resynthesis in the reperfusion period [13, 15].

Meanwhile antioxidants ($\alpha$-tocopherol, $CoQ_{10}$) delayed the fall of the ATP (but not the creatine phosphate) level in hypoxia of the heart [10, 14], the metabolism of which is known to differ from that of other organs, and in which both the breakdown and the reduced synthesis of ATP last longer, especially under conditions of hypoxia.

In conclusion, attention is drawn to the possible use, in principle, of temporary inhalation of hypoxic gas mixtures in the early reperfusion period to prevent reoxygenation injuries and to improve the function and

metabolism of an organ subjected to prolonged ischemia; however, the duration of inhalation, other methods of creating hypoxemia and, in particular, the search for ways of inducing local hypoxemia, require further investigation.

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# COMPARISON OF THE PROTECTIVE EFFECT OF ADAPTATION TO SHORT-TERM STRESS AGAINST INJURY TO THE HEART AND PORTAL VEIN BY LONG-TERM STRESS


E. B. Manukhina, L. S. Katkova, and F. Z. Meerson



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Long-term stress causes marked disturbances of contractility of the heart and capacitive vessels [2]. Stress injury to the myocardium has been shown to be preventable by preliminary adaptation of animals to daily short exposures to stress [3]. The protective effect of intensive adaptation of this kind is incomplete and it has its "price," i.e., adaptation itself causes the significant depression of myocardial contractility.

In the present investigation, besides the protective effect of daily (intensive) adaptation, the effect of gentle adaptation, in which short exposures to stress were given on alternate days, also was studied. Since vascular injury in long-term stress has been shown to be more severe than the disturbance of myocardial function after exposure to the same kind of stress [4] the main aim of this investigation was to compare the protective effect of preliminary adaptation on disturbance of contractile function of the right atrial myocardium and the smooth muscle of the portal vein arising during long-term stress.

## EXPERIMENTAL METHOD

Experiments were carried out on male Wister rats weighing 200-280 g in two stages. In the first stage the effect of preliminary intensive adaptation of the animals to stress on disturbance of contractile function of the myocardium and vascular smooth muscle arising during long-term stress was studied. Intensive adaptation


Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.)


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