ANTI-ISCHEMIC ACTION OF THE 1-HYDROXY DERIVATIVE OF 2,2,6,6-TETRAMETHYLPIPERIDINE, A NITROXYL BIOANTIOXIDANT

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An essential mechanism of ischemic damage to organs is lipid peroxidation (LPO), which develops in cell membrane structures during ischemic and reperfusion periods. The protective action of various antioxidants also has been demonstrated during ischemia and reperfusion of a wide range of organs (kidneys, liver, heart, brain, limbs) [1, 8, 11]. However, the widespread use of most antioxidants is limited by their poor solubility, their toxicity, and the need to use large doses. Hence the urgency of a continued search for new classes of effective antioxidants. One such promising class recently discovered consists of nitroxyl radicals [6]. In experiments in vitro (on models of LPO in biomembranes) we found that 1-hydroxy derivatives of 2,2,6,6-tetramethylpiperidine are no less effective bioantioxidants than nitroxyl radicals themselves, but the corresponding amines do not possess antioxidant properties [2]. These findings were subsequently confirmed by other workers [10]. There is reason to suppose that during LPO, besides certain chemical systems for oxidation of polymers [5], an oxidation-reduction cycle may be present in biological membranes, and may function effectively through equilibrium between the nitroxyl group and its 1-hydroxy derivative, each of which possesses antioxidant properties. Because of several advantages the 1-hydroxy derivatives are more convenient for use as therapeutic agents: the hydrochlorides of these hydroxylamines dissolve better in water than nitroxyls and are less toxic [12].

The aim of this investigation was to study the anti-ischemic action of the most effective of the hydroxylamine-hydrochlorides investigated in vitro, namely the 1-hydroxy derivative of 2,2,6,6-tetramethylpiperidine (I), and to compare this action with that of a structurally similar analog, namely the corresponding amine (II), which possesses no antioxidant properties.

EXPERIMENTAL METHOD

Experiments were carried out on models of long-term keeping of isolated liver, total thermal ischemia of the liver and isolated heart followed by reperfusion, and also on models of ischemic shock. Altogether 216 female Wistar rats weighing 200-300 g were used. All operations were performed under hexobarbital (70 mg/kg) anesthesia. In experiments with long-term keeping of the isolated liver (36 rats) the liver was perfused with physiological saline immediately after decapitation of the animal, after which it was removed and kept for 5 h (37°C) in physiological saline without additives (control) or with the addition of compounds I or II in a concentration of 10^{-4} M. Concentrations of TBA-active products of malonic dialdehyde (MDA) were measured by the method in [9] with the addition of the antioxidant ionol ($4 \cdot 10^{-4}$ M) to the samples during determination. Total thermal (37°C) ischemia of the liver (25 rats) was created by applying microforceps to the central and left lateral lobes for 2.5 h, after which the blood flow was restored, and the remaining lobes (about 30% of the total mass of the liver) were resected. During the next 5 days the percentage of animals which survived

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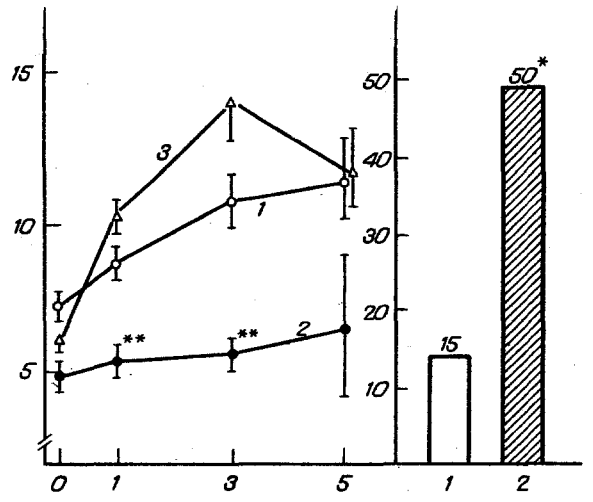

Fig. 1 Effect of tetramethylpiperidine derivatives on accumulation of TBA-active products during keeping of isolated liver (a) and on survival rate of rats after ischemia of the liver for 2.5 h followed by reperfusion (b). 1) Without addition of compounds (control); 2) compound I; 3) compound II. Abscissa: a) duration of keeping isolated liver (in h); ordinate: a) MDA concentration (in nmoles/g wet weight of tissue), b) survival rate on 5th day after ischemia (in %); *$p < 0.05$, **$p < 0.01$ compared with control.

was recorded. Compound I was injected intraperitoneally in a dose of 12 mg/kg 1 h before ischemia. The isolated spontaneously contracting heart was perfused (45 rats) by the Langendorff–Fallen method [7], using the isovolumic balloon technique (Statham SP1400 pressure monitor, USA) Ischemia was produced by completely stopping the flow of perfusion fluid in the heart for 30 min at 37°C. During the experiments contractility of the myocardium was judged from the heart rate (HR, beats/min), the maximal developed pressure (P_{max}, mm Hg), and the end-diastolic pressure (EDP, mm Hg) in the left ventricle. Ischemic (tourniquet) shock was produced by applying tourniquets for 6 h to both hind limbs and then removing them. Next, in one groups of experiments (60 rats) the severity of ischemic shock was estimated from the survival rate of the animals in the course of 5 days In another groups of experiments (40 rats) contractility of the myocardium (as P_{max}) of hearts removed from animals subjected to shock, 6 h after removal of the tourniquets, was determined by the Langendorff–Fallen method, following perfusion for 30 min (preliminary experiments showed that the greatest changes in P_{max} are observed after 6 h of reperfusion of the limbs) Ionol and compound I were injected intraperitoneally. Since previous results showed that ionol and some other antioxidants, in certain concentrations, can damage biomembranes and, in particular, can inhibit Ca-ATPase and make transport of calcium ions in fragments of the sarcoplasmic reticulum of skeletal muscles less efficient [9], we undertook preliminary experiments by a pH-metric method [10] to assess the ability of compound I to exert this kind of damaging action. The results showed that compound I, even in a concentration of $5 \cdot 10^{-4}$ M, has no effect on Ca-ATPase activity or on the efficiency of calcium transport (the ratio Ca^{2+}/ATP), whereas ionol, in a concentration as low as $5 \cdot 10^{-5}$ M, depressed Ca-ATPase activity by 50% and reduced the Ca^{2+}/ATP ratio to zero.

EXPERIMENTAL RESULTS

Experiments on a model of long-term keeping of the isolated liver showed that addition of compound I to the medium used in preliminary perfusion and subsequent keeping significantly reduced the rate of accumulation of LPO products compared with the control. Compound II did not possess this property (Fig. 1a).

Intraperitoneal injection of compound I into the rats significantly increased the survival rate of the animals after sublethal periods (2.5 h) of total ischemia of the liver followed by reperfusion (Fig. 1b). Comparative assessment of the anti-ischemic action of compounds I and II on a model of total ischemia followed by reperfusion of the heart showed that

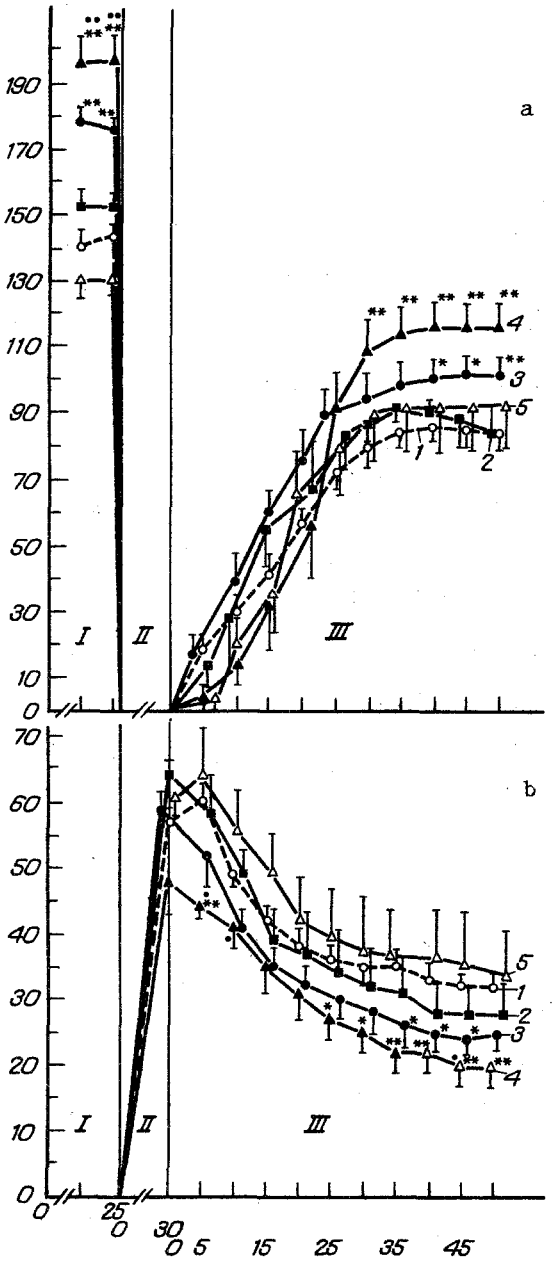

Fig. 2. Effect of tetramethylpiperidine derivatives I and II on P_{max} (a) and EDP (b) in left ventricle of isolated rat heart, under normal conditions and after ischemia for 30 min. 1) Without addition of compound (control); 2) compound I (10^{-6} M); 3) the same ($5 \cdot 10^{-6}$ M); 4) the same (10^{-5} M); 5) compound II (10^{-5} M). Abscissa, duration (in min) of initial perfusion (I), ischemia (II), and reperfusion (III). **Ordinate: a) P_{max}** (in mm Hg); b) EDP (in mm Hg), *p < 0.05, **p < 0.01 compared with control; °p < 0.05, °°p < 0.01 for comparison of 4 with 5.

addition of compound I to the perfusion fluid in concentrations of $5 \cdot 10^{-6}$ and 10^{-5} M gives a significant dose-dependent effect of an increase in P_{max}, when depressed through the action of ischemia (Fig. 2a). In a concentration of 10^{-5} M compound I also caused a significant decrease in EDP in the post-ischemic period (Fig. 2b), (the EDP level characterizes the degree of contractural changes of the myocardium induced by ischemia). Injection of compound II into the perfusion

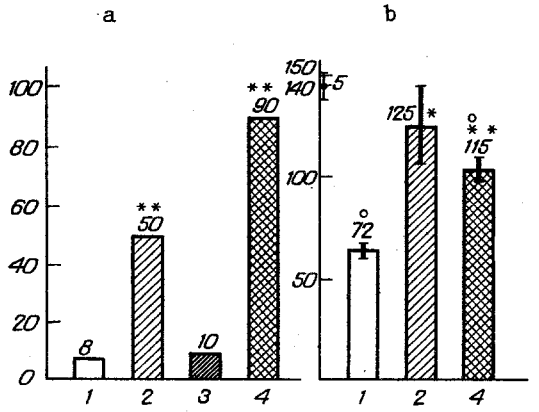

Fig. 3. Survival rate of rats (a) and contractility (P_{max}) of heart of rats (b) subjected to ischemic shock, and receiving injections of ionol and compound I. 1) Without compounds (control); 2) compound I (1 h before removal of tourniquets, 36 mg/kg); 3) ionol (30 min before removal of tourniquets, 120 mg/kg); 4) ionol (4 h before application of tourniquets, 120 mg/kg); 5) intact rats. Ordinate: a) survival rate on 5th day (in %); b) P_{max} (in mm Hg). *$p < 0.05$, **$p < 0.01$ compared with control; °$p < 0.01$ compared with intact rats (4).

fluid caused no changes in the parameters tested (Fig. 2a, b). In all the series of experiments HR did not differ significantly from its values in the control (when no compounds were injected).

Administration of compound I in ischemic shock (Fig. 3a) significantly increased the survival rate of the animals compared with the control. It was more effective than ionol, when injected shortly before removal of the tourniquets and in a much larger dose.

Compound I also increased P_{max} of the hearts, which was considerably reduced in the shocked animals (Fig 3b). Values of P_{max} were close to those of intact animals and did not differ from those when ionol was used, even though compound I was given in a much smaller dose and a much shorter time before removal of the tourniquets than ionol.

The results indicate that the 1-hydroxy derivative of tetramethylpiperidine (I) gives a significant anti-ischemic effect on models of total ischemia of the rat liver and heart, and also of ischemic shock In the latter case compound I, if given shortly before reperfusion of ischemic limbs, was found to be more effective than the familiar antioxidant ionol, probably due to the relatively slow assimilation of ionol Thus the hydroxylamine I may prove to be particularly promising if used immediately before the beginning of reperfusion of ischemic organs.

It must be pointed out that the analog of hydroxylamine I, namely the amine II, which does not possess antioxidant activity, gave no anti-ischemic effect in any of the series of experiments. This suggests that the anti-ischemic effect of the 1-hydroxy derivative of tetramethylpiperidine (I) is based on its marked antioxidant properties.

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EXPERIMENTAL EVALUATION OF MODIFIED POLYHEMOGLOBIN ON A MODEL OF LIMITING HEMODILUTION

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Definite progress has been made in the creation of an artificial oxygen carrier (AOC) on the basis of hemoglobin (Hb). Solutions of modified polymerized Hb, freed from stroma, have high oxygen capacity and can remain for quite a long time in the blood stream [5, 7, 8, 11]. How effectively an AOC can carry out the gas-transporting function of blood is currently under discussion in the scientific press [4, 9, 12]. The fullest answer to this question, in our opinion, can be given by the use of an experimental model of limiting hemodilution. In such a case, only 5-10% of the experimental animal's own blood remains in the blood stream, and the remaining volume is made up of the test preparation, so that the oxygen supply to the body is virtually entirely dependent on the properties of the OAC.

The aim of this investigation was to evaluate the efficacy of modified polyhemoglobin, proposed as an AOC, on a model of limiting hemodilution in dogs.

EXPERIMENTAL METHOD

Experiments were carried out on 14 mongrel dogs, male and female, weighing 11.3 ± 1.9 kg and anesthetized with pentobarbital sodium in a dose of 30 mg/kg. A blood substitute based on a modified Hb polymer, containing pyridoxal-5'-phosphate (PH-PP) as regulator of reversible oxygenation, was used as the AOC (the preparation was developed at the All-Union Research Center for Hematology and its physicochemical properties have been described previously [1]). The limiting hemodilution model was obtained by replacement transfusion with a 10% solution of PH-PP (8 dogs) or rheopolyglucin (6 dogs — control). Salts were added to both preparations before use in accordance with the electrolyte formula of Ringer—Locke solution [2]. Blood was removed from the femoral artery and the blood substitute was injected simultaneously into the femoral vein at the rate of 1.4-2.0 ml/kg/min until the hematocrit (Ht) index fell to 5% or below. The following parameters of the systemic hemodynamics were determined in all animals: blood pressure (BP), cardiac output (CO), and circulating blood volume (CBV), by the dye (cardiogreen) dilution method on a "Cardiac Output Computer" (USA). Other parameters studied included Ht, the blood Ht concentration, and the partial pressure of oxygen (pO_2) separately in plasma from arterial and mixed venous blood, by the methemoglobin-cyanide method on an AVL gas microanalyzer (Switzerland). The oxygen concentration in the blood and plasma was calculated from the data thus obtained by the equation

$$O_2 \text{ concentration} = 1.34Hb[pO_2/p^{50}]^n/1 + [pO_2/p^{50}]^n,$$

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