Enhancement of the Anti-Ischemic Kidney Resistance by Adaptive Hypoxic Preconditioning and Drug Therapy

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Hypoxic preconditioning and pretreatment with pentoxifylline, α-tocopherol, or their combination promotes stabilization of Po_2 in rabbit kidney cortex in the early reperfusion period (in experiments with α-tocopherol at a significantly lower level), while after 30-min reperfusion, the protective effect of α-tocopherol (especially in combination with pentoxifylline) markedly surpasses that of pentoxifylline and hypoxic preconditioning. α-Tocopherol sharply inhibits LPO in the reperfusion period, while pentoxifylline exhibits only weak antioxidant activity and hypoxic preconditioning was ineffective.

Key Words: *hypoxic preconditioning; pentoxifylline; α-tocopherol; reperfusion*

The possibility of reducing postischemic functional and metabolic disturbances with pharmacological agents is well studied [2,9]. The protective effect of some membranotropic drugs involves activation of metabolic processes improving the resistance of cell structures to various stress factors, in particular ischemia (glycolysis and protein synthesis, phosphorylation of membrane proteins, *etc.*) [9]. Adaptation to hypoxia produces similar effects [7]. It not only improves animal survival and reduces metabolic shifts under conditions of oxygen deficiency, but also diminishes damaging effects of local circulatory disturbances, in particular, impairment of coronary blood flow [8]. However, the effect of adaptation to hypoxia on anti-ischemic resistance of isolated organs is little studied.

The aim of the present study was to evaluate the effect of hypoxic preconditioning (HP) on kidney resistance to ischemia and to compare this effect with effects of some anti-ischemic drugs.

MATERIALS AND METHODS

Experiments were carried out on 30 Chinchilla rabbits of both sexes weighing 3.5-4.5 kg.

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Group 1 comprised intact rabbits (n=4). In group 2 rabbits (n=6) heat ischemia of the left kidney was modeled by ligation of renal vessels and the ureter for 40 minutes. In group 3 rabbits (n=5) renal ischemia was preceded by a 12-day HP; the content of O_2 in gas mixture was gradually decreased and the exposure was prolonged in each next session [4].

In groups 4, 5, and 6 (5 animals per group) ischemia was modeled against the background of pharmacological protection with pentoxifylline (PF), α-tocopherol (TP), or their combination. Similarly to HP, these drugs modify cell metabolism by activating energy production and induce structural rearrangement in cell membranes in the preischemic period, thus improving mobilization of energy reserves during ischemia [9].

Moreover, PF promotes normalization of microcirculation by regulating the tone of microvessels and improving blood rheological properties [12], while α-TP possesses potent antioxidant and membrane-stabilizing activities [11], which also contribute to the anti-ischemic effect of these preparations [1,5]. In group 4, PF was injected in a loading dose of 2.5 mg/kg 10 min before ischemia and then infused (0.1-0.15 mg/kg/min) throughout the ischemia and reperfusion periods (a total of 10 mg/kg). α-Tocopherol (oil solu-

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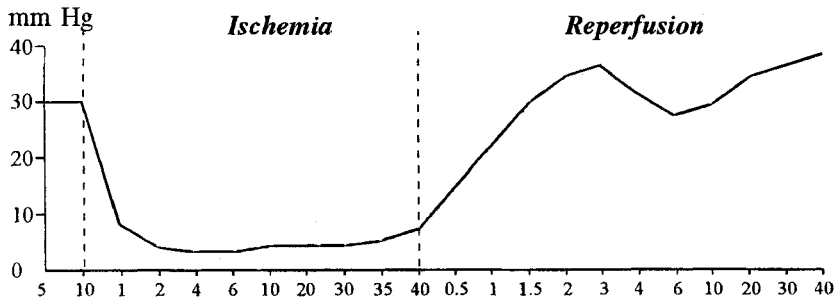

Fig. 1. Dynamics of Po_2 in kidney cortex during ischemia and reperfusion.

tion) was injected intramuscularly in a dose of 50 mg/kg 24 and 2 h before ischemia (group 5).

To evaluate functional and metabolic impacts of ischemia, Po_2 in the kidney cortex was measured in the preischemic, ischemic, and in the early reperfusion periods [6]; the content of LPO products (MDA and diene conjugates) in the kidney cortex was assessed after 30-min reperfusion [3]. Additionally, endogenous creatinine clearance and sodium reabsorption were measured [10].

The data were processed statistically using Student's *t* and Wilcoxon—Mann—Whitney *U* tests.

RESULTS

The dynamics of Po_2 in the kidney cortex in different groups showed that HP and PF had no effect on oxygenation in the preischemic period (Table 1), while α-TP (alone and in combination with PF) significantly decreased this parameter.

In all experimental series Po_2 declined exponentially during ischemia and attained the minimum (3-9 mm Hg) after 4-6 min, the intergroup differences being insignificant.

In the reperfusion period we observed a biphasic dynamics of Po_2 in the kidney cortex. In control rabbits, the first phase was characterized by a rapid (within 1-2 min) increase in Po_2 to the preischemic level or higher followed by its decrease (postischemic reactive hyperoxia); during the second phase Po_2 increased again but more slowly (Fig. 1).

In rabbits protected with HP, no reactive hyperoxia developed and Po_2 rapidly returned to a near-preischemic level. Similar dynamics of Po_2 was observed in PF-treated animals, but Po_2 was stabilized at a lower level. In experiments with α-TP alone and in combination with PF, Po_2 recovered more slowly and remained below normal. In α-TP-protected rabbits, reactive hyperoxia was practically absent and Po_2 was stabilized as soon as 3-5 min after the start of reperfusion.

In the control group (without protection), the content of diene conjugates significantly increased during reperfusion, the concentration of MDA being decreased. In experiments with PF and especially with α-TP, the content of both LPO products was significantly lower than in the control (Table 2). The dynamics of LPO products in rabbits protected with HP practically did not differ from the control, while combined administration of α-TP and PF was associated with a more pronounced accumulation of diene conjugates.

All variants of protection reduced ischemia-induced shifts in the functional parameters (Table 2); however, HP and PF were less effective than α-TP alone or in combination with PF.

Table 1. Effect of Different Variants of Anti-Ischemic Protection on Po_2 in Kidney Cortex during Ischemia and Reperfusion (mm Hg, *M*±*m*)

Group	Before ischemia	Ischemia	First phase of reperfusion		Second phase of reperfusion	
			peak Po_2	time of peak Po_2, min	Po_2 max	time of Po_2 stabilization, min
1	29.6±3.1	—	—	—	—	—
2	31.2±2.4	3.8±0.4	38.6±2.1*	3.2±0.1	40.1±1.9*	>40
3	29.4±2.7	4.2±0.2	30.9±2.3+	1.3±0.1+	27.4±1.8+	5.4±0.8+
4	30.1±2.9	3.4±0.3	29.7±1.9+	1.9±0.2+	21.8±2.2*+	6.3±0.5+
5	16.7±1.8*	2.7ë0.3	16.1±0.9*+	2.6±0.1+	20.3±1.5*+	24.1±2.4+
6	16.9±1.6*	5.1±0.2	19.5±2.0*+	1.8±0.2+	23.9±2.7+	10.4±1.5+

Note. Here and in Table 2: $p<0.05$: *compared with intact kidney (group 1), +compared with 40-min ischemia without protection (group 2).

Table 2. Effect of Different Variants of Anti-Ischemic Protection on Renal Function and LPO Intensity in Kidney Cortex during the Early Postischemic Period (mm Hg, *M±m*)

Group	Creatinine clearance, ml/min	Sodium reabsorption, %	Concentration, nmol/mg lipids	
			MDA	diene conjugates
1	3.45±0.27	97±1	1.62±0.18	0.46±0.04
2	0.59±0.08*	83±3*	1.02±0.11*	0.71±0.06*
3	0.91±0.10*+	92±1*+	1.14±0.15	0.68±0.05*
4	0.62±0.11*	89±2*	0.55±0.09+	0.24±0.04*+
5	2.60±0.24*+	96±3+	0.16±0.02*+	0.06±0.01*+
6	3.42±0.29+	96±2+	1.86±0.24+	1.32±0.14*+

Thus, our experiments demonstrated that HP improves the resistance of isolated organs, in particular kidney, to ischemia and diminishes postischemic functional disturbances. At the same time, HP does not improve antioxidant defense and does not prevent accumulation of LPO products in the kidney during reperfusion.

The positive effect of HP is probably associated with prevention of energy deficit in kidney parenchymal cells during ischemia. This assumption is indirectly confirmed by the absence of reactive hyperoxia in the early reperfusion period resulting from reactive hyperemia determined by accumulation of adenine nucleotide degradation products and stimulation of prostaglandin synthesis [2]. The energy-sparing effect of HP can also be realized via enhancement of glycolytic energy production. This mechanism was demonstrated in previous experiments with adaptation to high-altitude hypoxia [7].

Thus, although HP is less effective that α-TP, its effect is comparable with that of other anti-ischemic drugs without direct membranotropic activity (PF). It should be noted that apart from the adaptive component, direct membrane-stabilizing and antioxidant activities of α-TP greatly contribute to its anti-ischemic effect.

Interestingly, pronounced antioxidant effects of α-TP and PF disappeared in their combined administration, whereas the anti-ischemic effect of this combination was maximum. This additionally indicates the complexity of mechanisms of ischemia-induced damage and anti-ischemic protection.

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