PHYSIOLOGY

Effect of Erythropoietin on Permeability of Rat Abdominal Aortic Wall for Proteins

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The effect of erythropoietin on albumin and immunoglobulin extravasation through the wall of isolated perfused fragment of the abdominal aorta from intact rats and animals treated with parathyroid hormone is studied. It is shown that this parameter does not depend on the perfusion rate. The protein extravasation markedly increases in parathyroid hormone-treated animals in comparison with the control. Erythropoietin has practically no effect on protein extravasation in the control aorta fragments and normalizes it in fragments from animals treated with parathyroid hormone.

Key Words: *erythropoietin; abdominal aorta; protein extravasation; parathyroid hormone*

Erythropoietin (EP) is widely used for correction of anemia in patients with chronic renal failure. However, similar to other endogenous peptides, EP acts as a polyfunctional regulator: in addition to stimulation of erythropoiesis it exerts a direct effect on blood vessels. It has been shown that EP enhances vascular smooth muscle cell contractility [2,3,8] and modulates the composition of tissue fluid. The mechanism of vascular effects of EP is not yet clarified; however, these effects should modify the regulation of arterial pressure (AP) and water and macromolecule exchange through the vascular wall.

The aim of the present study was to elucidate the mechanism of local effect of EP on the contractility and permeability of blood vessels. It was found that EP markedly stimulates the spontaneous contractile activity of *vena porta* preparations from rats with moderate uremia caused by subtotal nephrectomy in comparison with intact controls [2]. This experimental model of chronic renal failure is characterized by elevated AP, hypercalcemia, and impaired barrier function of the endothelium, as evidenced by aggravation of proteinuria [1]. Published data indicate that disturbed calcium homeostasis and altered renovascular reactions typical of both essential and experimental hypertension result from an abnormal response of the target tissue to elevated blood concentration of parathyroid hormone (PTH) [10]. For instance, long-term exposure of cultured smooth muscle cells and rat mesenteric artery to PTH leads to desensitization of PTH receptors and prevents activation of the adenylate cyclase pathway and PTH-induced vasodilation [6]. The present study examines the proposed interplay of vascular effects of EP and PTH.

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MATERIALS AND METHODS

The experimental model of disturbed vascular permeability for proteins was reproduced in male Wistar rats weighing 180-200 g receiving daily intramuscular injections of PTH (20 U/ml, 0.5 ml/100 g body weight) for 7 days. The animals were decapitated under ether narcosis 1.5-2 h after the last injection. Age-matched rats served as controls. All animals were kept under similar conditions and fed identical low-protein (5-7%) chow *ad libitum*. In the control and experimental animals a fragment of abdominal

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TABLE 1. Albumin Extravasation, and Aorta Diameter as Functions of Flow Rate (V_{flow}) and Protein Concentration (C_{ini}) in Experimental and Control Series (*M±m*)

Group	Albumin concentration in perfusate, C_{ini}, mg/ml	Albumin extravasation, % of C_{ini}	V_{flow}=10 ml/min	V_{flow}=5 ml/min	Decrease in aorta diameter at a reduced V_{flow}, %
Control	*n*=10	43.6±5.5	0.62±0.11	0.64±0.13	1.5±0.6
	n=5	94.1±9.9	0.80±0.09	0.88±0.10	1.9±0.4
PTH	*n*=9	45.4±6.2	3.74±0.69*	3.78±0.94*	8.9±1.7*
	n=6	90.1±3.8	1.40±0.44**		8.0±0.9

Note. Here and in Table 2: *$p<0.01$ in comparison with the control, **$p<0.05$ in comparison with hyperoncotic perfusion.

aorta below the renal arteries was excised after ligation of the lateral branches. A 5-mm long isolated segment was mounted (20 mN tension) on cannulae in a 5-ml thermocontrolled chamber filled with standard Krebs solution supplemented with 0.25 g/liter sodium glutamate and 0.75 g/liter Na_2EDTA, pH 7.4±0.1. The same solution containing either hyperoncotic or near-isooncotic concentrations of bovine serum albumin (*Allergen* Research and Manufacturing Association, Stavropol) was used for perfusion. After a 30-min perfusion in a 50-ml closed system at a rate of 10 ml/min, the incubation medium was collected for protein assay. The experiment was repeated at a perfusion rate of 5 ml/min or with fresh perfusion solution containing 33.4±3.4 mg/ml immunoglobulin (IgG was obtained from the Institute of Experimental Medicine, Russian Academy of Medical Sciences). The vessel was placed into fresh incubation solution; 25 mU/ml EP (Boehringer Mannheim) was added to the perfusate. Protein concentration was measured as described elsewhere [4,12].

The experimental installation for perfusion of isolated vessels consisted of peristaltic pump, 2-channel thermocontroller, electromagnetic flowmeter, and longitudinal and transverse shift transducers with 100 μ/mm and 100 μ/cm sensitivity, respectively. Perfusion pressure was attained by raising the reservoir with perfusion solution at a height of 80 cm. On the day of experiment AP in the caudal artery was measured by indirect photoelectric method under light ether narcosis.

RESULTS

The experiments showed that in the control vessel protein extravasation through the vessel wall was about 0.6% of its content in the vessel over a 30-min perfusion and did not depend on the perfusion rate (Table 1). A slight increase in protein extravasation was noted when the vessel was perfused with isooncotic solution (Table 1).

In PTH-treated rats, protein extravasation was markedly increased (Tables 1 and 2) but still did not depend on perfusion rate despite the increase in the vessel diameter and, consequently, exchange area (Table 1). Perfusion with a hyperoncotic solution was accompanied by lower extravasation in comparison with isooncotic solution, but the aorta permeability still surpassed the control level. The observed increase in the aorta permeability in hyperparathyroid rats was not accompanied by changes in the AP, which was 124±2 and 127±9 mm Hg in the control and experimental groups, respectively.

The data summarized in Table 2 suggest that EP added to the perfusion solution in a dose of 25 mU/ml corresponding to its normal plasma concentration [5] had no effect on the transport of protein macromolecules through the vascular wall in the control animals. However, in PTH-treated rats, EP reduced pathologically enhanced permeation of albumin and IgG from the aorta caused by a long-term treatment with high doses of PTH.

The fact that EP added to perfusion solution in a low concentration that produced no vasomotor

TABLE 2. Effect of Erythropoietin on Protein Extravasation (*M±m*)

Group	Dose of erythropoietin, mU/ml	Protein concentration in incubation medium, mg/ml	
		albumin	immunoglobulin
Control	0	0.25±0.05	0.27±0.10
	25	0.16±0.04	0.18±0.06
PTH	0	1.84±0.11	2.55±0.35
	25	0.47±0.1*	0.25±0.11*

effects in the arterial bed normalized protein permeation from the abdominal aorta in rats with disturbed vascular permeability suggests that EP restored selective properties of the endothelial filter. It has been shown that cyclic adenosine monophosphate (cAMP) enhancing protein sorption on the endothelial surface activates endo- and transcytosis [13] and induces formation of aqueous channels in the luminal membrane [11]. These effects are probably caused by PTH and can be abolished by EP. However, EP exerts an opposite effect on vascular permeability and increases hydraulic conductivity of the vessel wall, which directly depends on Ca^{2+} concentration in endotheliocytes [7]. Therefore, the opposite effect of EP on vascular conductivity can be attributed to elevation of intracellular calcium concentration, which can be abolished by cAMP [9].

Thus, these data suggest that EP restores the selectivity of the endothelial barrier disturbed by high doses of PTH. Moreover, being an intrinsic endogenous regulator, EP most actively affects the vessels with altered functional activity. Therefore, further investigation of vascular effects of EP is of particular interest for physiological and clinical aspects of hypertension, atherogenesis, and other vascular pathologies.

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