ROLE OF PROSTAGLANDINS IN THE PATHOGENESIS OF THE SCHWARTZMANN PHENOMENON

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Interest of investigators in the Schwartzmann phenomenon is due, on the one hand, to the fact that it is a convenient model with which to reproduce the disseminated intravascular coagulation (DIC) syndrome and, on the other hand, to the fact that the latter is the main cause of development of endotoxic shock [3, 4, 6, 7]. The most marked changes in this condition have been found by the majority of workers in the renal microcirculation.

In recent years much evidence has accumulated in support of the pathogenetic role of prostaglandins (PG) in the development of various systemic disturbances of homeostasis, including in endotoxin shock [5, 2, 8].

The writers showed previously that endotoxin (ET) of Gram-negative bacteria *in vitro* potentiates enzymic transformation of arachidonic acid into PG [1]. Accordingly the concept of a pathogenetic role of PG, synthesized *in vivo* under the influence of specific endotoxinemia, was put forward. In our view elevation of the PG level regularly causes changes primarily in the cardiovascular and excretory systems and, at the same time, in the blood clotting system.

The aim of this investigation was to study the effect of indomethacin (a powerful inhibitor of PG biosynthesis) on the development of the local and generalized Schwartzmann reactions (LSR and GSR, respectively).

EXPERIMENTAL METHOD

LSR and GSR were induced in chinchilla rabbits weighing 1.7-2.5 kg by two injections of ET of *Salmonella typhimurium*, obtained by Boivin's method. To reproduce the LSR, 0.1 mg/kg of ET was injected intradermally, and the same dose was given 24 h later into the marginal vein of the ear. The GSR was induced by two intravenous injections of ET in a dose of 0.2 mg/kg with an interval of 24 h.

The animals were divided into four groups (12 rabbits in each group): group 1) LSR; group 2) during reproduction of LSR, before the injection of ET the animals were given indomethacin in a dose of 10 mg/kg by the intragastric route; group 3) GSR; group 4) GSR with administration of indomethacin by the same scheme as in group 3.

The state of hemostasis was characterized by study of the prothrombin time (Quick, 1943), thrombin time (Szirmai, 1967), fibrinogen concentration (Rutberg, 1961), level of soluble fibrin-monomer complexes (SFMC) (Lipinski and Worowski, 1967), platelet count (Fonio, 1920), and thromboelastogram (Hartert, 1951). The parameters of hemostasis were studied before the beginning of the experiments and 30 min and 3 and 24 h after each injection of ET. For light and electron microscopy of the kidneys the animals were killed by injection of air into the auricular vein.

EXPERIMENTAL RESULTS

Intradermal injection of ET into animals of group 1 caused an increase in the clotting potential of the blood. The thrombin time 3 h after injection of ET was significantly reduced

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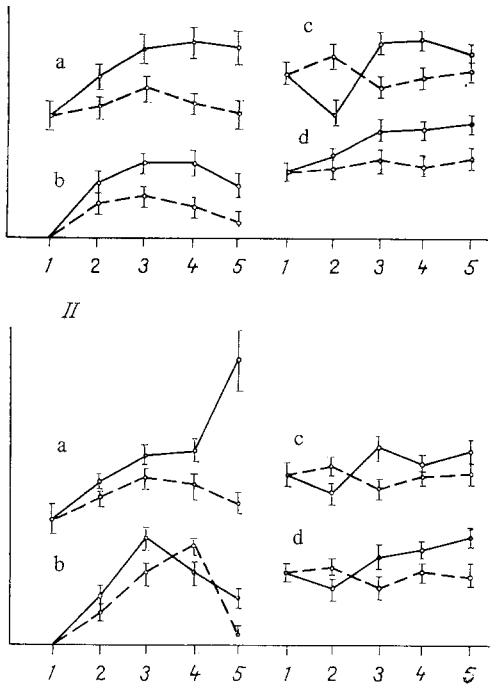

Fig. 1. State of hemostasis in animals with LSR (I) and GSR (II). Abscissa: 1) initial values of parameters, 2) 3 h after first injection of ET, 3) 30 min after second injection of ET, 4) 3 h, 5) 24 h after second injection of ET; ordinate: fibrinogen concentration (in mg/liter), thrombin and prothrombin time (in sec), SFMC level (in nominal units). a) Fibrinogen concentration; b) plasma SFMC level; c) thrombin time; d) prothrombin time. Broken line indicates time course of parameters of hemostasis during indomethacin therapy.

compared with the initial value; the fibrinogen concentration and SFMC level were raised ($P < 0.05$; Fig. 1, I). The platelet count was reduced from 354,800 ± 5100/mm^3 to 338,700 ± 11,300/mm^3 ($P < 0.05$). Lengthening of the prothrombin time during this period of the investigation was not significant (Fig. 1, I).

Parameters of the thromboelastogram (TEG) also demonstrated a hypercoagulation shift. Values of the parameters r, K, and Ma 3 h after the beginning of the experiments differed significantly from initially (Table 1).

Intravenous injection of the "reacting" dose of ET significantly changed the parameters of hemostasis. Values of the prothrombin time, fibrinogen concentration, and SFMC were significantly increased ($P < 0.05$), but the platelet count was significantly lower than initially. Most parameters of the TEG were characteristic of the state of hypocoagulation (Table 1).

Light and electron microscopy of the kidneys of the animals of group 1, killed 24 h after the second injection of ET, revealed concentrations of fibrin in the lumen of the glomerular capillaries (Fig. 2a). The peritubular capillaries were strongly dilated and blood cells were found in their lumen. The cytoplasm of the nephrocytes in neighboring capillaries was swollen and vacuolated, and the mitochondria were swollen, with reduced cristae. The medulla was edematous. The cytoplasm of the interstitial cells was vacuolated.

In the animals of group 2 (LSR with indomethacin therapy) the dynamics of the parameters of hemostasis in response to the first injection of ET was different (Fig. 1, I and Table 1). Most parameters of hemostasis 3 h after injection of ET, by contrast with their values in the rabbits of group 1, were characteristic of hypocoagulation and were indistinguishable from

TABLE 1. Parameters of TEG in Animals with LSR and GSR Depending on Period of Investigation and of Administration of Indomethacin

Period of investigation	Group of animals	Parameter of TEG			
		r	K	Ma	$<\alpha$
Initial data	—	6,64±0,6	5,85±0,8	55,0±1,5	25,1±1,6
After injection of ET:					
3 h after first injection	1	4,33±0,7*	8,33±0,9*	47,3±3,7*	27,3±3,8
	2	6,28±0,5	8,78±3,1*	51,5±4,3	23,0±4,2
3 h after second injection	1	11,3±0,6*	12,1±5,4	41,5±3,5*	10,0±0,9*
	2	8,35±0,9	5,10±1,0	54,5±5,7	25,2±3,7
3 h after first injection	3	5,33±0,6	3,83±0,9	69,3±9,9*	46,3±5,3*
	4	8,75±0,5*	28,5±6,1*	47,3±5,3	21,8±3,7
30 min after second injection	3	8,8±1,0	15,4±1,7*	42,5±3,1*	14,4±2,3*
	4	12±1,1*	31,7±7,8*	35,7±5,8*	11,3±3,3*
3 h after second injection	3	12,25±0,9*	39,7±5,8*	19,6±2,2*	3,8±0,13*
	4	8,60±1,0*	24,0±6,2*	35,3±4,1*	11,8±1,5*
24 h after second injection	3	9,0±0,3*	30,0±2,3*	39,0±3,1*	7,33±0,96*
	4	7,70±1,2	12,3±3,4	49,7±3,2	14,9±2,0

Legend. *$P < 0.05$ compared with initial data.

the initial values. After injection of the "reacting" dose of ET no significant changes took place in the state of hemostasis with the exception of a further increase in the fibrinogen concentration, a decrease in the platelet count (326,700 ± 8900/mm^3, $P < 0.05$), and a decrease in the thrombin time (24.9 ± 0.9 sec; $P < 0.05$).

Administration of indomethacin in LSR completely prevented the appearance of fibrin in the capillaries. However, polymorphonuclear leukocytes with sequestrated lysosomes were found in the capillary lumen (Fig. 2b).

In the animals of group 3 (GSR) intravenous injection of ET was accompanied by a distinct tendency for the clotting potential of the blood to rise with simultaneous activation of fibrinolysis (elevation of the SFMC level). The platelet count fell ($P < 0.05$, Fig. 1, II). Most parameters of the TEG reflected a state of hypercoagulation (Table 1).

Repeated intravenous injection of ET caused hypocoagulation changes. The thrombin and prothrombin times 30 min after the second injection of ET were increased, but not statistically significantly. The platelet count was greatly reduced (291,300 ± 6900/mm^3, $P < 0.05$). At the same time the fibrinogen concentration and SFMC level were significantly higher than initially (Fig. 1, II). The state of hypocoagulation also was reflected by the TEG parameters (Table 1).

The parameters of hemostasis 3 h after the second injection of ET demonstrated continuing hypocoagulation changes in the coagulogram and TEG (Fig. 1, II, and Table 1). The signs of hypocoagulation were still present after 24 h.

During GSR marked vacuolation and swelling of the endothelium were observed in the glomerulus and peritubular capillaries, with the appearance of fibrin (Fig. 2d) and polymorphonuclear leukocytes with sequestrated lysosomes and blood cells (Fig. 2b). Marked edema of the medulla and vacuolation of the interstitial cells were seen (Fig. 2c).

In the animals of group 4 (GSR with indomethacin therapy) the first injection of ET was accompanied by the development of hypocoagulation changes (Fig. 1, II and Table 1). Injection of the reacting dose of ET caused changes in different directions in the coagulogram and TEG (Fig. 1, II and Table 1). The prothrombin and thrombin times were reduced 30 min after injection of ET, but the fibrinogen concentration and SFMC levels, on the other hand, were increased. Most of the parameters of the TEG indicated a tendency toward hypocoagulation. The thrombin and prothrombin times showed a distinct tendency to increase after 3 h, and this coincided with the parameters of the TEG, evidence of continuing hypocoagulation. After 24 h most parameters of hemostasis were indistinguishable from normal.

In the animals of group 4 microscopic examination revealed no fibrin in the capillary lumen, and much less edema of the medulla and swelling of the glomerular capillary endothelium than in the rabbits of group 3. Only polymorphonuclear leukocytes with sequestrated lysosomes were found in the peritubular capillaries (Fig. 2e). Accumulation of lipid granules (Fig. 2f) was observed in the interstitial cells of the medulla, possibly in association with inhibition of prostaglandin synthetase activity and accumulation of PG precursors.

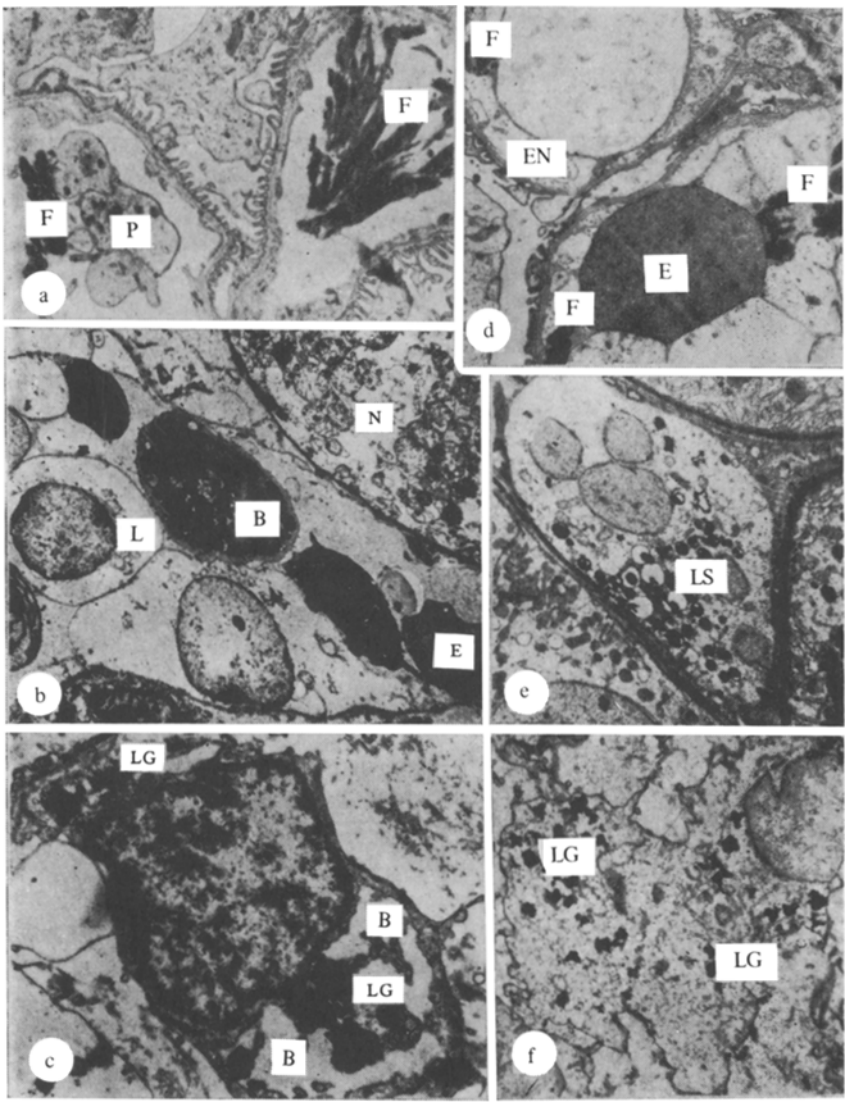

Fig. 2. Light and electron microscopy of animals' kidneys: a) fibrin (F) in lumen of glomerular capillaries after second injection of ET during LSR; P) platelets. b) Peritubular capillary in GSR greatly dilated and filled with blood cells: erythrocytes (E), lymphocytes (L), and basophils (B). Cytoplasm of nephrocyte (N) vacuolated. c) Interstitial edema of renal medulla, vacuolation (V) of cytoplasm of interstitial cells in GSR; LG) lipid granules. d) Marked edema of endothelium (EN) of glomerular capillaries, appearance of erythrocytes (E) and fibrin (F) in capillary lumen during GSR. e) Polymorphonuclear leukocyte with sequestrated lysosomes (LS) during GSR after administration of indomethacin. f) Accumulation of lipid granules (LG) in cytoplasm of interstitial cell after administration of indomethacin.

Consequently, administration of indomethacin during the LSR and GSR abolishes the action of ET, directly toward increasing the clotting potential of the blood and reducing the platelet count. Indomethacin does not affect the ET-induced increase in the fibrinogen concentration. Electron-microscopic examination of the kidneys showed that indomethacin prevents the development of the severest changes arising in the renal microcirculation after injection of ET, namely the inhibition of fibrin and injury to the peritubular capillaries.

The results of these experiments show that the genesis of the DIC syndrome and of disturbances in the renal microcirculation are largely associated with increased PG synthesis. This goes some way toward confirming previous observations on the ability of ET of Gram-negative

bacteria to potentiate the enzymic transformation of arachidonic acid into PG *in vitro*. The positive effect of indomethacin on the state of hemostasis and the microstructure of the kidneys is evidence that the genesis of these changes is linked with an increase (under the influence of ET) in PG synthesis.

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CONTRACTILE PROPERTIES OF REINNERVATED SKELETAL MUSCLE AND THEIR DEPENDENCE ON THE LEVEL OF NERVE INJURY

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The absence of selectivity of reinnervation of skeletal muscle in mammals leads to changes in the ratio between the types of muscle fibers in it, their spatial grouping, and the functional characteristics of the muscle [1, 6-8, 12]. Differences in the functional profile of motor units (MU) of the muscle with completion of the reinnervation process lasts for several years after nerve injury [9]. Several factors determining the development of a certain characteristic histochemical composition, or a distinctive kind of contractile properties in a reinnervated muscle have recently been established [6, 14].

The object of this investigation was to study the contractile properties of muscle, and changes in the mean size and number of its MU depending on the time after denervation and the level of nerve injury.

EXPERIMENTAL METHOD

Experiments were carried out under ether anesthesia and under sterile conditions on 22 mature rats weighing 150-220 g. The sciatic nerve (SN) was isolated on one side at the level of its division in the popliteal fossa and above. The peroneal nerve (PN) was crushed at the level of the lateral condyle of the femur or SN at the level of the ischial tuberosity by the method in [2], after which the wound was sutured. Depending on the time after the operation and the level of crushing the animals were divided into three groups: 1) rats taken in the experiments 2 weeks after crushing of PN (eight muscles were tested on the side of injury to PN, n = 8), 2) 4 weeks after crushing of PN (n = 5), 3) 6-7 weeks after crushing of SN (n = 6). Control experiments were carried out in some cases on the tibialis anterior muscle (TAM) on the side opposite to the operation, and also on TAM of three intact animals (n = 17).

In the animals of group 1 reinnervation of the anterior group of leg muscles after crushing of SN was found to begin on the 8th day [2] and to be largely completed after 2 weeks [15]. Consequently, in the animals of group 1 the test TAM was in the stage of incomplete reinnervation, whereas in the animals of groups 2 and 3 it was in a stage of complete reinnervation.

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