Effect of the Calcium-Channel Blockers Verapamil and Nifedipine on the Systemic Hemodynamics and Hepatic Macro- and Microcirculation in Rats with Acute Massive Hemorrhage

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Ischemic disturbances of calcium homeostasis and the excessive uncontrolled entry of calcium into the cell during shock and acute hemorrhage result in metabolic damage, disruptions in energy production, and cell dysfunctions, making for irreversible shock-induced hemodynamic and metabolic failure [1,7-10]. The emergence of a new class of Ca^{2+} antagonists, which are capable of blocking the injurious effects produced in the organism by an increase in the intracellular calcium content, has made it possible to study the therapeutic effect of calcium-channel blockers in shock and acute hemorrhage. The data obtained have proved to be very contradictory [6-8]. The present study was aimed at investigating the effect of verapamil (VP), a reference Ca^{2+} antagonist, and nifedipine (NF) on the course of acute massive hemorrhage in rats. We also studied their effect on the cardiovascular system of intact rats.

MATERIALS AND METHODS

The experiments were carried out on 76 urethane-anesthetized (1.25 g/kg, intraperitoneally) male Wistar albino rats weighing 200-250 g. The hemodynamics was studied with the aid of ultrasonic equipment [3].

Throughout the experiment the systemic arterial pressure (AP) was measured in intact animals. The blood flow rate in the ascending aorta (V) was determined with an ultrasonic catheter 0.6 mm in diameter which was inserted into the vascular bed. Miniature piezocrystals operating at a frequency of 26.8 MHz were used as sensor elements of the ultrasonic transducers [4]. The values of the blood flow rate in the ascending aorta and AP were entered in an analog computer for determination of the heart power (W, mm Hg×m/sec) and total peripheral resistance (TPR, mm Hg×cm/sec). The stroke (SV) and minute (MV) volumes of the heart were also computer-calculated. The heart rate (HR) (per min) was recorded with the aid of a cardiotachometer triggered by the pulse wave of blood flow in the carotid artery. VP, 0.25 mg/kg (Orion), and NF, 0.03 mg/kg (Bayer), were injected in a volume of 0.1 ml/100 g body weight, intravenously. The dynamics of the hepatic microcirculation was studied in animals by contact luminescence biomicroscopy. Fluorescein isothiocyanate (FITC)-labeled bovine albumin and globulin were used in *in vivo* studies of the vessel-tissue

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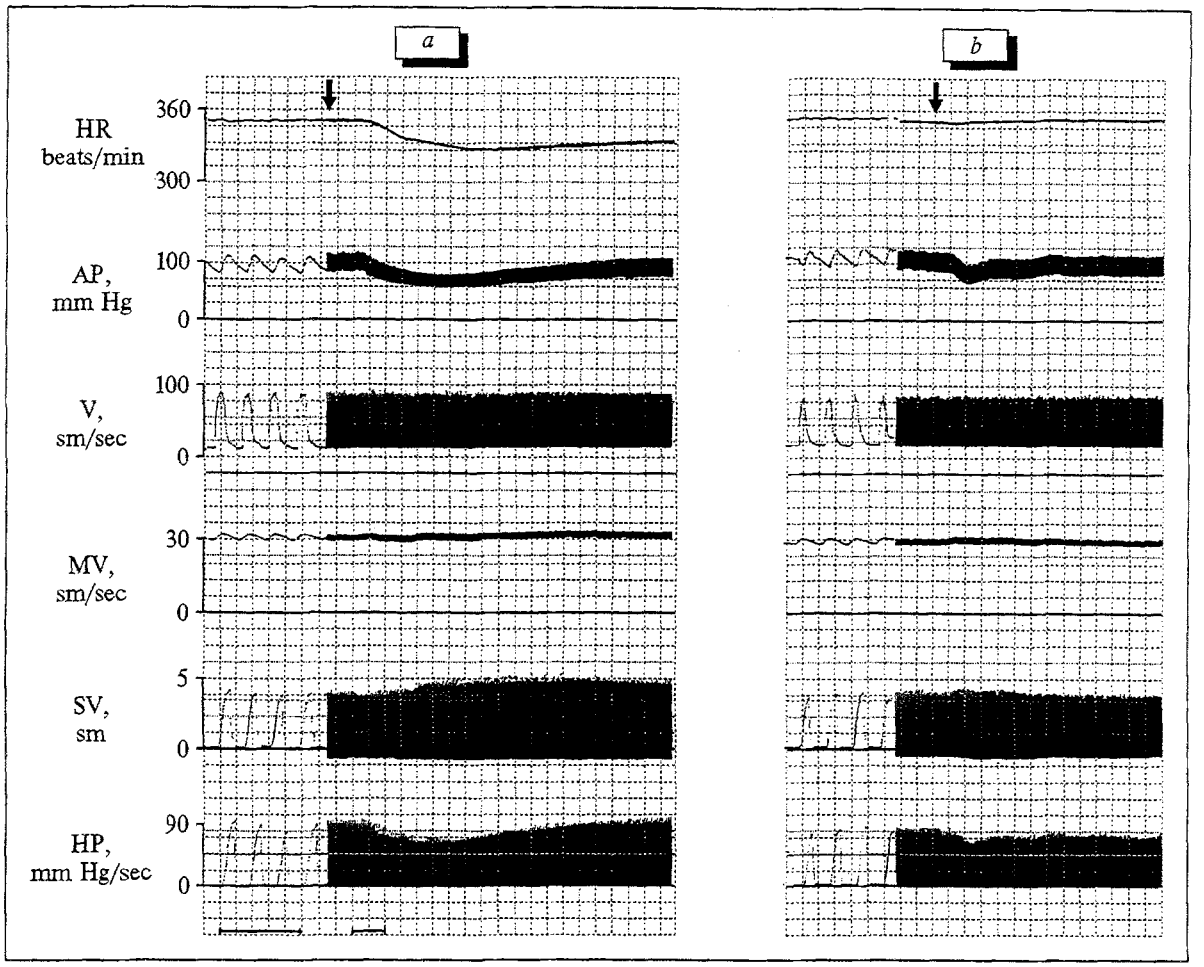

Fig. 1. Effect of 0.25 mg/kg VP (*a*) and 0.03 mg/kg NF (*b*) on hemodynamics and cardiac activity of intact rats at the moment of intravenous injection. Arrow shows the beginning of injection. HP: heart power. Time marks: 0.5 and 10 sec.

permeability [5]. Simultaneously, the AP in the femoral artery was recorded with a micromanometer and the blood flow rate in the portal vein with an ultrasonic transducer. Acute hemorrhage was induced by a single 5-min bleeding (2.5% body weight) from the femoral artery. VP (0.25 mg/kg), or NF (0.03 mg/kg) in a volume of 0.1 ml/100 g body weight, or 0.9% NaCl solution was intravenously injected 15 min after the hemorrhage was completed. The follow-up on the animals was performed 2 hours postinjection. The data were statistically processed after Fisher-Student.

RESULTS

We identified two phases of action of the calcium-channel blockers under investigation on the cardiovascular system of intact rats: 1) short-term vascular effects arising at the moment of intravenous injection of the preparation; 2) delayed stable effects lasting no longer than 1 hour. Immediately upon injection, VP lowered the AP by 25-30 mm Hg and HR by 20-30 beats/min; TPR and W dropped 17.86±4.88 and 39.29±10.18%, respectively; the blood flow rate in the aorta changed insignificantly; SV and MV increased by 24.29±14.50 and 14.28±9.05%, respectively (Figs. 1 and 2). The first phase lasted no longer than 1 min. A characteristic feature of the second phase was that all the hemodynamic parameters studied gradually reverted to the initial level. At the same time, in contrast to the other parameters, the AP, HR, and TPR attained the control values only 1 hour after injection of preparation (Fig. 2).

At the moment of injection NF caused a transient drop of AP and TPR, by no more than 15-20%; a slight decrease of W and an increase of SV and MV were also noted. The HR and the blood flow rate in the aorta were virtually unchanged (Fig. 1). During the second phase, TPR reverted to the subnormal level; AP attained the control values and then rose slightly; more marked was the rise of such hemodynamic parameters as W, SV, MV, and the blood flow rate in the aorta,

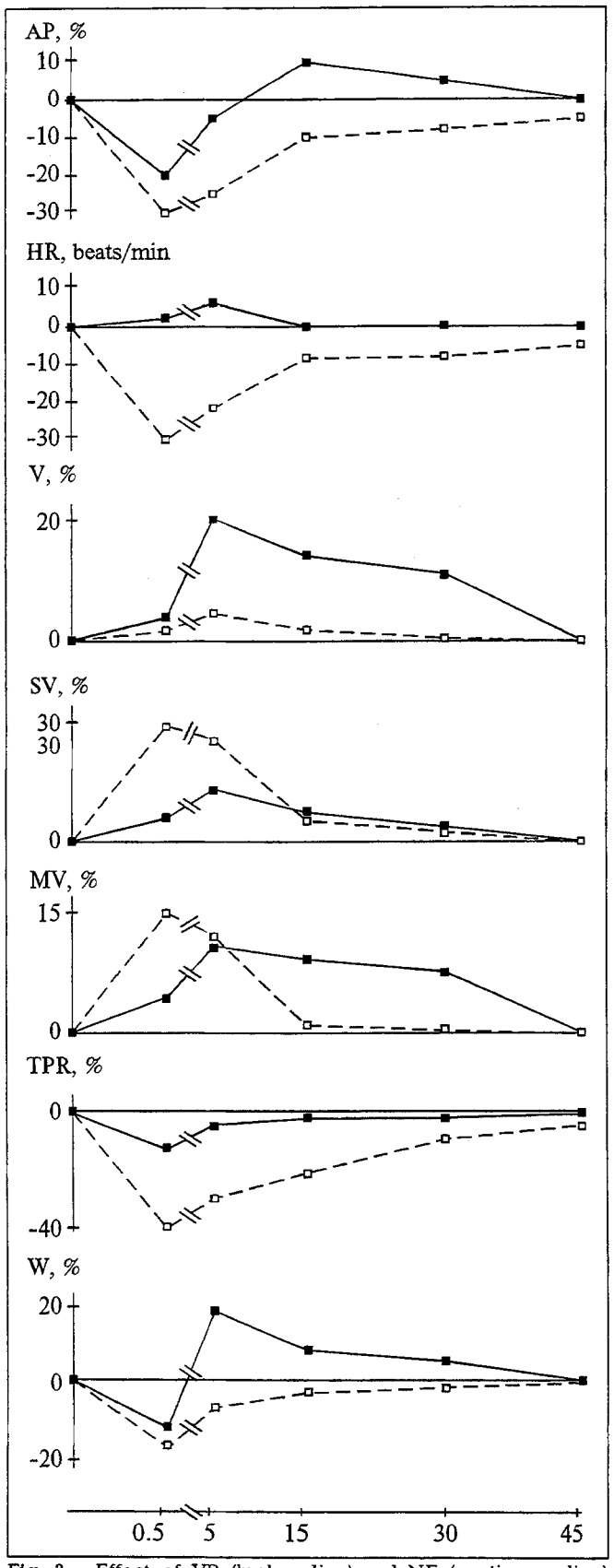

Fig. 2. Effect of VP (broken line) and NF (continuous line) on parameters of cardiovascular system of intact rats. Abscissa: time postinjection, min; ordinate: values of parameters studied, % of initial values.

the latter exceeding the initial level by 20-25%. As before, the HR remained at the control level (Fig. 2). The second phase of the vascular effect of NF lasted some 45 min.

The withdrawal of blood in a volume of 2.5% of the body weight reduced the AP and blood flow rate in the portal vein to 20-30% of the initial values. At the level of the hepatic microcirculation, constriction of microvessels was noted along with a marked drop of the blood microflow and a decrease in the total blood filling volume in the terminal vascular bed of the organ. Within the first 15 min after hemorrhage was completed, the hemodynamic parameters, which had dropped during blood withdrawal, began to recover (compensated blood loss) in most animals (60-70% of cases). On the 30th min after the completion of bleeding, the AP and the portal blood flow rate in the organ constituted 68.57±9.88 and 72.85±12.86%, respectively, of the initial values recorded prior to hemorrhage (Fig. 3). The state of the hepatic microcirculation markedly improved. The phase of temporal compensation of the parameters of the systemic and portal circulation gave way to the phase of their secondary irreversible decrease (secondary irreversible decompensation), and after 2 hours all the hemodynamic parameters studied were markedly reduced. During this stage of the investigation, marked focal disturbances of the microcirculation, manifesting themselves as microstasis, microthrombosis, and microhemmorrhage in separate sinusoids or their fragments, developed in the liver. Luminescent vacuoles with FITC-labeled albumin and globulin appeared in the cytoplasm of hepatocytes, this being clear *in vivo* evidence of the increased vessel-tissue permeability for proteins in the liver [5]. Biomicroscopically, erythrocyte aggregation and slowing of the blood microflow were observed in the terminal vascular bed of the organ.

In rats in which the bleeding-reduced parameters of the systemic and portal circulation did not increase during the first 15 min of the posthemmorhagic period, the blood loss resulted in a primary decompensation of the cardiovascular system (decompensated blood loss), which caused quick death of animals over the first hour of the experiment, an irreversible drop of the AP and portal blood flow in the organ, and the development of stable pathological constriction of the hepatic microvessels. This was consistent with our previous observations [2].

Intravenous injection of 0.9% NaCl solution did not affect the pattern of the process in animals with compensated and decompensated blood loss. The AP, the hepatic blood flow rate, and the

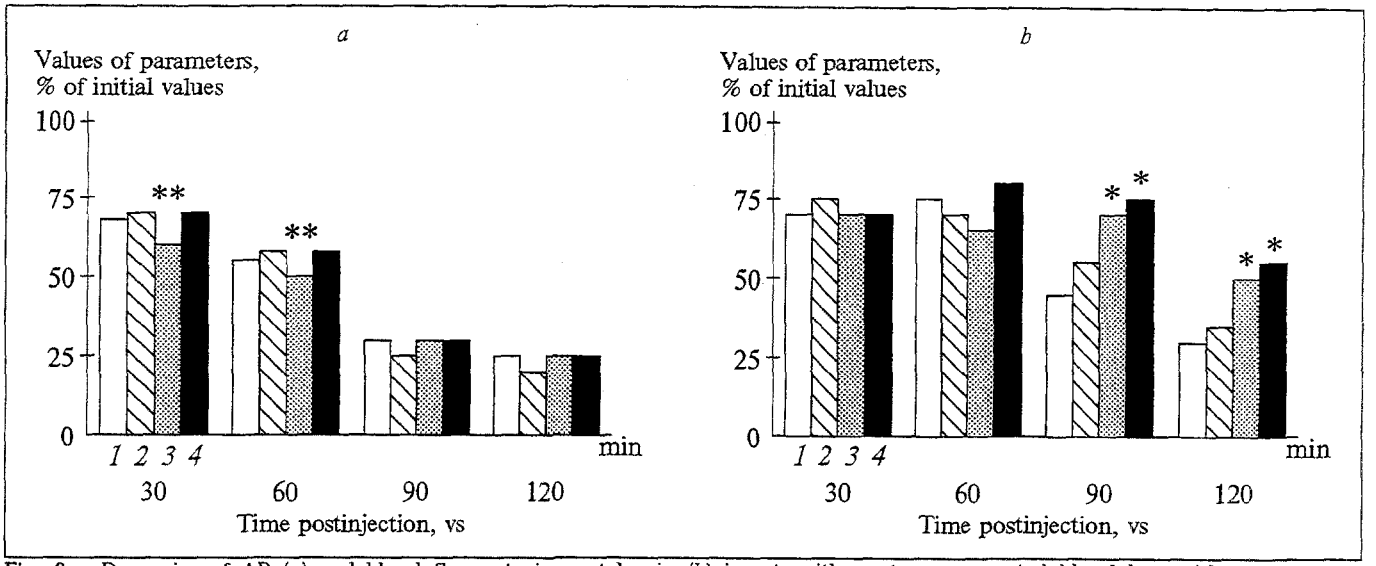

Fig. 3. Dynamics of AP (*a*) and blood flow rate in portal vein (*b*) in rats with acute compensated blood loss without treatment (*1*) and after intravenous injection of 0.9% NaCl (*2*), VP (*3*), and NF (*4*). One and two asterisks denote $p<0.01$ and $p<0.05$, respectively, vs. the group of untreated animals.

dynamics of these values during the posthemorrhagic period did not differ reliably from the same parameters in animals with acute blood loss without treatment (Fig. 3).

VP, intravenously injected in rats with decompensated blood loss, aggravated the course of the posthemorrhagic period, resulting in death in the 5th-10th min postadministration in 70% of cases, this likely being due to specificities of the drug's effect on the cardiovascular system of intact rats (a stable decrease of AP, HR, and TPR). NF did not produce a marked effect upon the course of the posthemorrhagic period in rats with decompensated blood loss.

In the animals with a compensated course of the posthemorrhagic period, VP lowered the AP by 15-20 mm Hg at the moment of intravenous injection. Within 3-5 min this value reverted to the initial level and then slightly increased. However, VP impeded the posthemorrhagic recovery, and 30 and 60 min after injection of the blocker, the AP proved to be reliably lower in the group of animals given VP as compared to the group of untreated animals (Fig. 3). At the same time, at the microcirculation level, VP inhibited the development of such microhemodynamic disturbances as an increase of permeability, local microstasis, microthrombosis, and generalized erythrocyte aggregation in the sinusoidal bed of the liver; this improved perfusion of the liver tissue, leading to a stabilization of the volume flow rate of the portal blood flow in the organ which lasted longer than in the group of untreated animals.

A similar positive effect on the hepatic microcirculation was produced by NF in the animals with compensated blood loss. On the other hand, this blocker did not affect the AP recovery either at the moment of injection or during the subsequent posthemorrhagic period, although, just like VP, it reliably prolonged the compensation phase with respect to such a hemodynamic parameter as the portal blood flow rate, the drop of which always started later than the secondary irreversible drop of AP (Fig. 3).

Our findings on the protective effect of Ca^{2+} antagonists on the hepatic microcirculation suggest that the pathogenesis of posthemorrhagic microcirculatory disturbances and of delayed secondary hepatic ischemia is associated with the posthemorrhagic disturbances of the calcium homeostasis, this providing a sound basis for using this class of substances in the treatment of acute hemorrhage. However, there is a certain inherent risk of aggravating the general course of the pathological process, which is determined, on the one hand, by the type of cardiovascular system response to acute circulatory hypoxia, and, on the other hand, by the specificities of action of the Ca^{2+} antagonist on the heart and peripheral circulation. We suggest that in the complex pathogenetic therapy of acute blood loss, in order to reduce the negative sequelae of the suppressive effect of calcium-channel blockers on the heart, these drugs must be combined with preparations with a marked ino- and chronotropic effect. At the same time, for blocking the development of such reperfusion disturbances as Ca^{2+} and O_2 paradox [1] calcium antagonists must be administered ahead of cardiotonic agents.

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Relationship between the Catecholamine and Protein Content in the Submaxillary Salivary Gland Tissues of and Mucosa over the Secretory Cycle for Chronic Inflammation of the Oral Soft Tissues

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Chronic inflammations of the oral cavity are characterized by an increased content of immunoglobulins, lysozyme, and enzymes in the saliva [1,6]. This provides for the antibacterial defense of the surface of the oral mucosa. On the other hand, the formation of defense mechanisms inside the tissue hampers generalization of the inflammatory process. Enhancement of neurotrophic effects, optimization of the blood flow, and the use of vitamins as activators of enzyme synthesis have been shown to step up the nonspecific defense of the organs and tissues [4].

In this connection it was of interest to elucidate the content of catecholamines and proteins in the salivary gland tissues (SGT) and in the oral mucosa (OM) for basal and induced secretion and in the saliva for induced secretion during chronic inflammation of the oral soft tissues.

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MATERIALS AND METHODS

The experiments were carried out on 140 nonpedigree rats of both sexes weighing 160 g. The animals were divided into the following experimental groups: 1) intact rats with basal secretion; 2) intact rats with induced secretion during 40 min (pilocarpine in a dose of 1 mg/kg, subcutaneously); 3 and 4) rats with basal and induced secretion, respectively, during chronic inflammation of the oral soft tissues. Chronic inflammation was caused by injection (under sterile conditions) of 0.1 ml of 2% carragenan solution into the submucosa of the left transitional fold of the vestibular maxillary aspect at the site corresponding to the location of the first molar.

For the synchronization of secretory activity of the salivary glands, all the animals were de-

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