SUBSTANCE P AND THE MICROCIRCULATORY SYSTEM DURING STRESS

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The writers showed previously [2, 3] that stressors (immobilization, electrical stimulation) cause disturbances of the microcirculation and vascular permeability, and that mast cells play a role in their development. The property of substance P (SP) to act as mediator of antidromic vasodilatation and of disturbances of vascular permeability, which take place with the participation of mast cells, has been described [4, 6, 8]. By intravital biomicroscopy of the rat mesentery, this neuropeptide ahs been shown to cause disturbances of the microcirculation [4].

In view of the definite similarity between the pattern of changes in the microcirculation arising through the action of SP and in stress, and also the fact that mast cells participate in the formation of disturbances of the microcirculation and vascular permeability, it was decided to study whether SP can take part in the mechanisms of disturbances of the microcirculation arising in stress. A group of Soviet and East German workers has shown that SP analogs have a beneficial action on rats with chronic stress as regards normalization of their arterial pressure [7]. It was therefore also decided to investigate whether SP may also have a favorable action on the microcirculation in animals exposed to stressors.

EXPERIMENTAL METHOD

Experiments were carried out on 140 male rats weighing 180-250 g. To induce stress the rats were immobilized in the supine position for 1 or 24 h. The state of the microcirculation in the mesentery was assessed biomicroscopically on an apparatus for intravital study based on the Docuval microscope (Carl Zeiss, Jena, East Germany). The state of vascular permeability was studied by a dye method, followed by quantitative evaluation visually [1] and by a method of automatic image analysis, using the TAS texture analysis system (Ernst Leitz, West Germany) [5].

SP and antiserum (AS) against it were synthesized at the Institute for Study of Physiologically Active Substances, East German Academy of Sciences. The working concentration of SP was 7×10^{-8} M [4] and the AS concentration was calculated in a special series of experiments.

In the experiments of series I the effect of SP on the microcirculation, vascular permeability, and mast cells was determined in the mesentery of animals immobilized for 1 h. For this purpose, before or immediately after immobilization the rats were given an intraperitoneal injection of SP in 3.0 ml of physiological saline. Animals receiving an intraperitoneal injection of 3.0 ml physiological saline alone at the same times served as the control.

In series II the effect of AS against SP after immobilization for 24 h on the state of the microcirculation, vascular permeability, and mast cells of the mesentery was studied. For this purpose 0.01 ml of AS with activity of 150 ng-eq SP was injected intramuscularly in 0.5 ml physiological saline three times: 30 min before and 8 and 22 h after the beginning of immobilization.

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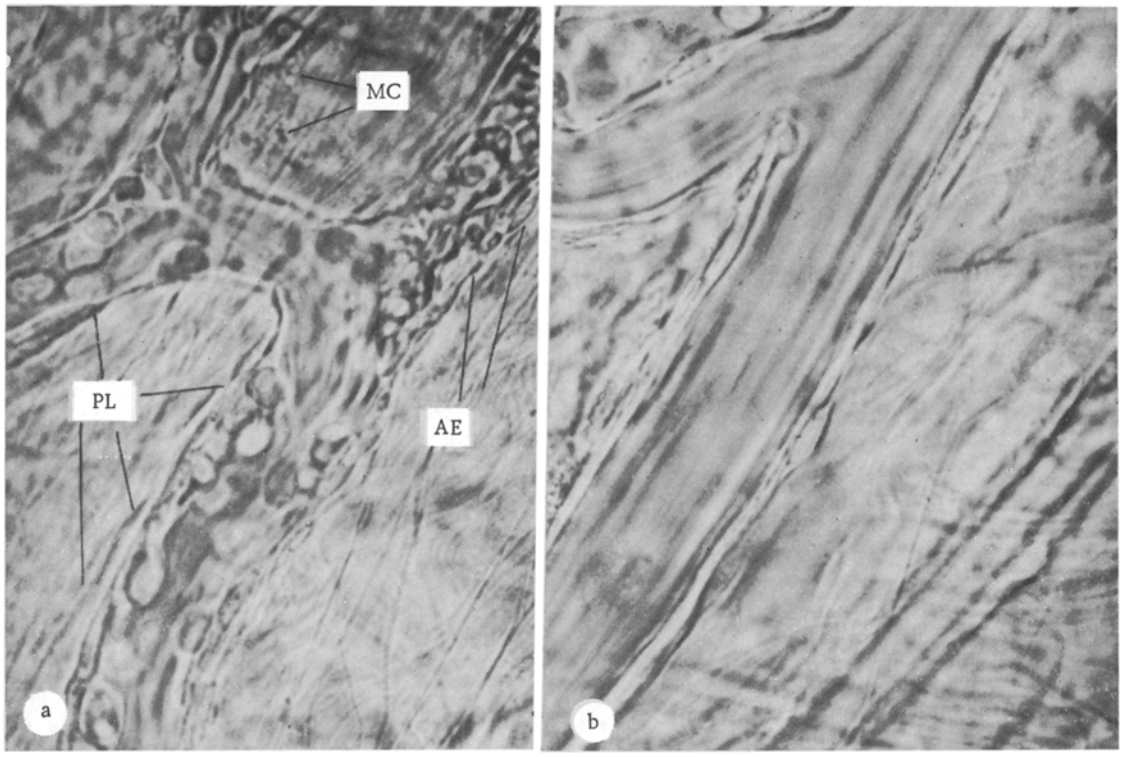

Fig. 1. State of microcirculation in rat mesentery: a) injection of SP after immobilization for 1 h. AE) Aggregation of erythrocytes, PL) pavementing of leukocytes, MC) degranulated mast cells; b) injection of AS against AP after immobilization for 24 h. 180 ×.

TABLE 1. State of Vascular Permeability of Rat Mesentery after Injection of SP Preceded by Immobilization for 1 h (results of evaluation by TAS method)

Experimental conditions	Number of measurements	Area of mast cell in % of area of measured fields	Intensity of label
1. Physiological saline + immobilization (n = 8)	63	0,855±0,076 $P_{1-2}<0,001$ $P_{1-3}<0,005$	0,115±0,003 $P_{1-2}<0,001$ $P_{1-3}<0,001$
2. SP + immobilization (n = 8)	109	1,978±0,135 $P_{2-4}<0,001$	0,230±0,004 $P_{2-4}<0,001$
3. Immobilization + physiological saline (n = 5)	49	0,605±0,098 $P_{3-4}<0,01$	0,178±0,006 $P_{3-4}<0,05$
4. Immobilization + SP (n = 5)	92	1,017±0,084	0,245±0,005

EXPERIMENTAL RESULTS

The biomicroscopic investigations showed that in response to preliminary injection of SP (before immobilization for 1 h) the rats developed disturbances of their microcirculation characteristic both of immobilization stress and of the action of SP itself. Slowing of the blood flow and aggregation of erythrocytes were observed, plasmatized vessels appeared, pavementing of leukocytes took place in the venules, and the mast cells underwent degranulation (Fig. 1a). Large areas of prestasis and stasis were found in these animals. Extravasation from the venules occurred in 50% of cases.

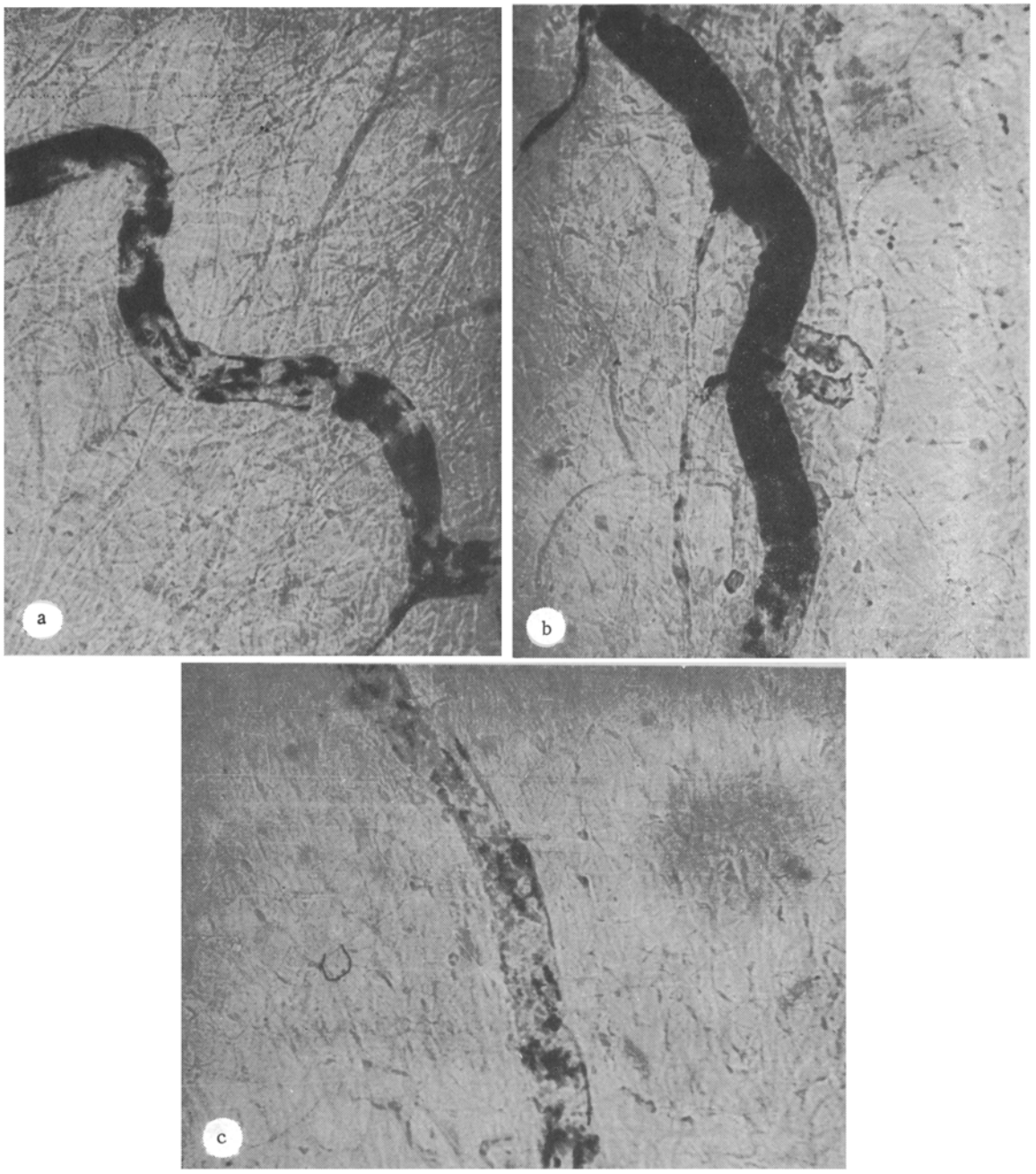

Fig. 2. Deposition of colloidal carbon particles in wall of microvessels in rat mesentery: a) after immobilization, b) injection of SP after immobilization for 1 h, c) injection of AS against SP after immobilization for 24 h. 50 ×.

Introduction of SP after 1 h immobilization resulted in the identical change in microcirculation; however, the symptoms connected with SP were less expressed. Extravasation was observed in 20% of the cases.

Evaluation of the state of vascular permeability by the visual method showed that intraperitoneal injection of SP both before and after immobilization for 1 h aggravates the disturbance of vascular permeability caused by the stressor as regards both the extent and the intensity of the lesion (Fig. 2b). Administration of SP before the stressor led to more severe disturbances than when SP was injected after immobilization. The results of analysis of the data for this series by automatic image analysis (Table 1) agreed with those of visual estimation. Injection of SP caused an increase in the degree of degranulation of the mast cells in the mesentery, but did not change their number. For instance, injection of SP before immobilization increased degranulation of the mast cells from 0.8 ± 0.06 to 1.6 ± 0.1% whereas injection of SP after immobilization increased it from 0.9 ± 0.07 to 1.8 ± 0.04%.

TABLE 2. Determination of Activity of AS against SP

Substance applied*	% of degranulated mast cells	P
1. Physiological saline	2,3±0,18	
2. SP	5,7±0,4	$P_{1-2}<0,01$
3. AS against SP	2,3±0,07	
4. SP + AS against SP	2,2±0,14	$P_{2-4}<0,01$

*Doses and concentrations of substances are given in text.

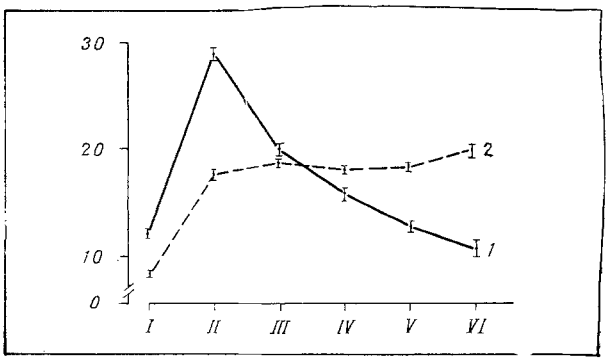

Fig. 3. Intensity and extent of spread of disturbances of vascular permeability in rats immobilized for 24 h (1) and receiving AS against SP after immobilization (2). Abscissa, intensity of labeling (in steps); ordinate, area of label (in conventional units). Estimation by automatic image analysis method.

Actual activity of AS against SP was determined in a special series of experiments. In this part of the investigation the percentage of degranulated mast cells in the mesentery was determined *in vivo* by application of 0.5 ml physiological saline, of 0.5 ml SP in the working concentration, of 0.01 ml AS in a working concentration of 150 ng-eq SP and 0.5 ml physiological saline, and also a mixture of 0.5 ml SP and 0.03 ml AS to the mesentery.

The results of this series of experiments show that SP in a concentration of 7×10^{-8} M caused a significant increase in mast cell degranulation, and that AS against SP, with activity of 150 ng-eq SP, completely neutralized the ability of SP to degranulate mast cells (Table 2).

In the next series of experiments the effect of administration of AS against SP on the development of microcirculatory disturbances in the mesentery of rats immobilized for 24 h was determined. In 66% of cases (in 24 of 35 rats) injection of AS improved the state of the microcirculation in the mesentery. For instance, the velocity of the blood flow was restored to normal, and this effect was particularly marked in venules; aggregation of erythrocytes and the number of plasmatized vessels were reduced. Extravasation was not observed in any of the animals (Fig. 1b).

Visual analysis of the state of vascular permeability in the last series of experiments showed that the extent and intensity of labeling in rats receiving AS were a little less than in animals not receiving AS (Fig. 2c). The method of automatic image analysis showed a significant decrease in the spread of ink label in its most intensive degree (Fig. 3) in rats receiving a prophylactic injection of AS. Meanwhile low-intensity labeling in these animals showed a wider spread than in rats not receiving AS (Fig. 3). On the whole these results indicate a decrease in permeability of the microvessels for colloidal carbon particles when AS against SP was injected after immobilization for 24 h.

The results indicate that SP has an injurious effect on the microcirculation, on vascular permeability, and on degranulation of mast cells in immobilized animals. The results of another investigation [7], showing that SP has a positive effect on certain parameters, including arterial blood pressure in immobilized rats, must evidently be attributed to the adaptive action of large doses of SP when injected repeatedly before immobilization.

The investigation thus showed that injection of SP aggravated both disturbances of the microcirculation and disturbances of vascular permeability developing in immobilized rats, and this was accompanied by increased degranulation of mast cells. Injection of AS against SP after immobilization for 24 h led to improvement of the state of the microcirculation and to a significant decrease in disturbances of vascular permeability for colloidal carbon particles. The present investigations are evidence that SP takes part in mechanisms of disturbance of the microcirculatory system in stress.

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RHEOLOGIC PROPERTIES OF PURIFIED HEMOGLOBIN SOLUTIONS MIXED WITH BLOOD

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The development of blood substitute solutions able to perform a gas transporting function *in vivo* is an important development in modern transfusion science [2]. One possible method of solving this problem is by using a natural oxygen carrier, in the form of solutions of native hemoglobin or its modifications, as the transfusion medium [4, 7,8]. The blood substitute must not only perform certain physiological functions (hemodynamic, gas-transporting, etc.), but it must also be suitable from the point of view of its rheologic properties.

This paper gives the results of a study of some rheologic and viscosity parameters of concentrated hemoglobin solutions, free from stromal impurities and from procoagulant activity.

EXPERIMENTAL METHOD

Hemoglobin solutions obtained by the method described previously were used [3]. The relative viscosity of purified hemoglobin in physiological NaCl solutions was determined by means of "Ubbelohde" capillary viscometers. The rheologic characteristics were studied *in vitro* on a "Low Shear 30" rotation viscometer (from Contraves, Switzerland) at 37°C within a range of shear velocities of between 0.05 and 128.5 sec^{-1}.

To study the effect of solutions of extraerythrocytic hemoglobin on the suspension properties of blood comparative *in vitro* models were set up with replacement of 25, 50, and 75% of blood by 10%, 15%, and 20% solutions of purified hemoglobin and clinical preparations of dextran (6% polyglucin and 10% rheopolyglucin), and the rheologic properties of the resulting mixtures were investigated. The final concentrations of free hemoglobin in the blood plasma are given in Table 1.

EXPERIMENTAL RESULTS

Measurement of the relative viscosity of hemoglobin in concentrations of between 5 and 25% showed that the viscosity of even higher concentrated solutions (25%) is low, about 3.0

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