the concentrations used, indicates that affinity of hormone for receptor is similar in the animals of these two groups, the differences consisting only of an increase (mainly of 1.6 times) in the number of insulin receptors during the crush syndrome compared with the control. A tendency for insulin-receptor interaction in the hepatocytes was found 2 h after removal of the limb from the press, despite the fact that the insulin concentration continued to rise and the glucose concentration remained high. Hyperinsulinemia after removal of the press is evidently connected not only with reduction of sensitivity of the cells to insulin, but also with a defect at both receptor and postreceptor levels.

Thus in the crush syndrome insulin-receptor binding is disturbed in MN, erythrocytes, and hepatocytes, as is shown by the insulin-resistance, hyperglycemia, and hyperinsulinemia which we recorded. Stress-induced insulin resistance is connected with reduction of specific binding with insulin, i.e., with a defect at the receptor level, whereas in the later stages – during the period of toxemia – insulin resistance is evidently due also to a defect, but at the postreceptor level, i.e.., a disturbance of glucose utilization by the cells.

The absence of a complete analogy in insulin-receptor characteristics which we found in MN, and hepatocytes can be explained to some degree by tissue specificity, and for that reason it is not always legitimate to extrapolate data obtained on insulin receptors of some cells to others.

LITERATURE CITED

1. L. N. Kobylyanskii, "Lysosomal mechanisms of the crush syndrome," Author's abstract of dissertation for the degree of Doctor of Medical Sciences, Moscow (1985).
2. N. P. Mikaelyan, M. I. Ul'yanov, and O. F. Murashov, Byull. Eksp. Biol. Med., No. 5, 551 (1984).
3. N. P. Mikaelyan, Vopr. Med. Khimii, No. 5, 96 (1988).
4. S. A. Morenkova, A. A. Karelin, and É. G. Dvletov, Patol. Fiziol., No. 1, 26 (1985).
5. A. Böyum, Scand. J. Clin, Lab. Invest., Suppl. 97, 77 (1968).
6. C. R. Kahn, P. Freychet, J. Roth, and D. M. Neville, J. Biol. Chem., 249, 2249 (1974).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
8. M. K. Markwell, S. M. Haas, J. Bieber, and N. E. Tolbert, Anal. Biochem., 87, 206 (1978).
9. P. M. Meyts and J. Roth, Biochem. Biophys. Res. Commun., 66, 1118 (1975).
10. J. M. Olefsky, Diabetes, 30, 148 (1981).

ACTION OF SP_{1-11} AND ITS N-TERMINAL FRAGMENT SP_{1-4} ON SOME PARAMETERS OF THE MICROCIRCULATORY SYSTEM DURING STRESS

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The problem of prevention of stress-induced damage to organs and systems is an urgent task at the present time. Disorders arose in the microcirculatory system in different kinds of stress. It has been shown that if these can be prevented tissue hypoxia is reduced, so that damage to organs either does not develop or is reduced to a minimum [2].

It has now been established that the vasoactive peptide substance P (SP_{1-11}) and its N-terminal fragment SP_{1-4} possess antistressor properties [1, 7, 9, 10, 11]. Application of SP_{1-11} to intact rats causes an increase in degranulation of the mast cells in their mesentery, an increase of venular permeability, and adhesion of leukocytes to the walls of

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the venules. SP_{1-4} possesses the same properties, but much higher concentrations of it are required to produce these disturbances [4].

We studied the effect of prophylactic administration of these peptides on the state of various components of the microcirculatory system during exposure to stress, and in that way shed light on the role of SP_{1-11} and SP_{1-4} in mechanisms of regulation of the microcirculatory system during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 66 male Wister rats weighing 200-225 g. As the extraordinary stimulus, the animals were immobilized for 5 h (immobilization stress), in the supine position on a special table. The animals were fixed by their teeth and upper and lower limbs. After the end of immobilization the microcirculation was studied in the mesentery of the rats, which were anesthetized with pentobarbital sodium (5 mg/100 g body weight). A system for intravital investigation based on the "Docuval" microscope ("Carl Zeiss," GDR) was used for this purpose. The morphology and functional state of the mast cells in the mesentery were assessed after intravital fixation with 96° alcohol and staining with 0.05% toluidine blue solution. The permeability of the microvessels in the mesentery was determined by the "labeled vessels" method [3]. The peptides (SP_{1-11} or SP_{1-4}) were synthesized in the Institute of Physiologically Active Substances, Academy of Sciences of the GDR (Berlin) and injected intraperitoneally immediately after the beginning of immobilization. The doses of the peptides were: SP_{1-11} 125 mg/kg, SP_{1-4} 53 mg/kg equimolar to the dose of SP_{1-11}. The results were subjected to statistical analysis [5, 6].

EXPERIMENTAL RESULTS

The experiments showed that immobilization of the rats for 5 h led to definite disturbances of the microcirculation: slowing of the blood flow in venules 20-60 μm in diameter, aggregation of erythrocytes in the capillaries and venules, and the appearance of "plasmatic" vessels and stasis (Fig. 1). Degranulation of the mast cells increased (Fig. 2). Permeability of the walls of the venules for colloidal carbon particles increased, as regards both area of spread and intensity (Table 1). Contractility of the lymphatic microvessels increased (Table 2).

Prophylatic injection of SP_{1-11} prevented stress-induced involution of the thymus. This peptide also potentiated the anesthetic effect of pentobarbital sodium.

Biomicroscopy of the mesentery showed that SP_{1-11} potentiated disturbances of the microcirculation in the immobilized animals. The number of animals with aggregation of erythrocytes in the capillaries ("rouleaux"), with adhesion of leukocytes in the venules, and with "plasmatic" vessels was increased. Compared with the control, there was considerable extravasation

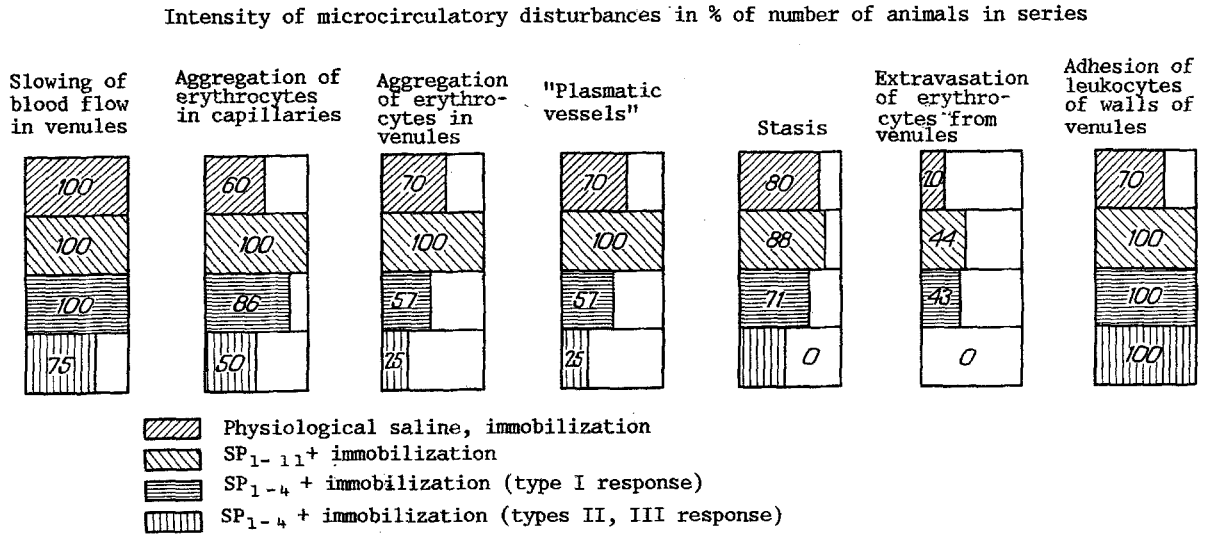

Fig. 1. Symptoms of microhemocirculatory disturbances in mesentery of rats immobilized for 5 h and receiving prophylactic injection of SP_{1-11} and SP_{1-4}.

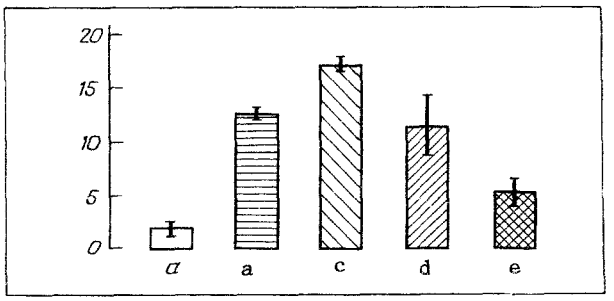

Fig. 2. Degree of degranulation of mast cells in mesentery of rats after immobilization for 5 h and prophylactic injection of SP_{1-11} and SP_{1-4}. Ordinate, percentage of degranulated mast cells. a) Control; b) physiological saline + immobilization (I); c) SP_{1-11} + I; d) SP_{1-4} + I (types I and II of response); e) SP_{1-4} + I (type III response).

TABLE 1. State of Venular Permeability during Immobilization for 5 h and Prophylactic Injection of SP_{1-11} and SP_{1-4}

Serial No. of experiment	Nature of series of experiment	Number of mesenteric windows, % of total examined			Number of rats with labels differing in their degree of intensity, % of total number of animals				
		without label	1-10 labeled vessels	over 10 labeled vessels	0	I	II	III	IV
1	Control – physiological saline (n = 10)	96	4	0	80	20	20	0	0
2	Physiological saline + immobilization (n = 10)	64*	30*	6*	10*	90*	90*	90*	3*
3	SP_{1-11} + immobilization (n = 10)	51	41	8	0	100	100	100	100**
4	SP_{1-4} + immobilization (n = 8), type I of response	80	16***	4	12	78	78	78	12***
5	SP_{1-4} + immobilization (n = 8), types II, III of response	88	10*⁴	2*⁴	40*⁴	60*⁴	60*⁴	40	0

Legend. $*p_{1-2} < 0.05$, $**p_{2-3} < 0.05$; $***p_{2-4} < 0.05$, $*^4p_{2-5} < 0.05$.

of erythrocytes from venules 30-40 μm in diameter (Fig. 1). There was a parallel increase in degranulation of the mast cells (Fig. 2), the intensity of the disturbances of venular permeability (Table 1) and contractile activity of the lymphatic microvessels were increased (Table 2).

In the case of prophylactic adminsitration of SP_{1-4}, stress-induced involution of the thymus, just as in the case of administration of SP_{1-11}, was not noted, which agreed with the data of P. Ochme et al. [11].

In the course of the experiment, attention was paid to the behavioral responses of the rats. In all the animals there were no vocal and motor reactions throughout the immobilization – a type I response (the tranquillizing effect of SP_{1-4}). In 30% of the rats no specific reactions were observed when the root of the tail was compressed – a type II response (the sedative effect of SP_{1-4}). In 20% of the cases there was no corneal reflex – a type III response. In these animals biomicroscopy could be performed without premedication with pentobarbital sodium (the narcotic effect of SP_{1-4}).

Depending on the types of response, the severity of biomicroscopic disturbances of the microcirculation varied. In animals with a type I response these changes did not differ from those in the mesentery of rats immobilized without preliminary injection of SP_{1-4}. In animals with types II and III of response the velocity of the blood flow was increased, aggregation of erythrocytes in the venules and the number of "plasmitic" microvessels and of those with stasis were reduced, and extravasation was absent (Fig. 1). The percentage of degranulated mast cells without preliminary injection of the peptide (Fig. 2). In animals with the type III response degranulation of the mast cells was reduced two-threefold. The number of labeled venules and the intensity of the label in animals with types II and III of response were reduced (Table 1). Contractile activity of the lymphatic microvessels was reduced in these same rats (Table 2).

It is interesting to note that, by contrast with barbiturates, which do not change or may even aggravate disturbances of the microcirculation induced by various factors [8], the N-terminal fragment SP_{1-4} was able to maintain an adequate depth of anesthesia in 20% of the experimental animals and to lead to relative normalization of the microcirculation in 50%. Under these circumstances, depending on the strength of the psychotropic action of SP_{1-4}, the severity of the disturbances of the microcirculatory system induced by immobili-

TABLE 2. Contractile Activity of Lymphatic Microvessels during Immobilization Stress for 5 h and Prophylactic Injection of SP_{1-11} and SP_{1-4}

Series No. of experiment	Nature of series of experiment	Number of contractions of walls of lymphatic microvessels (90-100 μ)	p
1	Conrol – physiological saline (n = 8)	3,0±0,7	
2	Physiological saline + immobilization (n = 7)	6,2±0,4	$p_{1-2}<0,005$
3	SP_{1-11} + immobilization (n = 15)	9,4±0,2	$p_{2-3}<0,001$
4	SP_{1-4} + immobilization (n = 8), type II, III of response	6,8±0,5	$p_{2-4}<0,005$
5	SP_{1-4} + immobilization (n = 6), types II, III of response	4,7±0,6	$p_{2-5}<0,005$

zation stress changed. In the presence of the tranquilizing effect of the peptide, disturbances of the microcirculation remained the same as without it. In animals with a sedative and, in particular, with an anesthetic action of SP_{1-4}, relative normalization of the terminal blood flow, secretory activity of the mast cells, venular permeability, and contractile activity of the lymphatic microvessels took place.

The N-terminal fragment SP_{1-4}, with a weak damaging action on the microcirculation in intact animals [4], has a protective action on the microcirculatory system if injected prophylactically before immobilization for 5 h. It can be tentatively suggested that the N-terminal fragment SP_{1-4}, by acting on mast cells, reduces the release of physiologically active substances from them. This lowers venular permeability and reduces the contractile activity of the lymphatic microvessels. This is the probable mechanism of regulation of SP_{1-4} at the level of the microcirculatory system in immobilization stress.

According to our data, unlike SP_{1-4}, SP_{1-11} does not possess this property.

LITERATURE CITED

1. V. A. Arefolov, L. A. Malikova, A. V. Val'dman, et al., Byull. Éksp. Biol. Med., No. 2, 201 (1988).
2. M. P. Gorizontova, Vest. Akad. Med. Nauk SSSR, No. 2, 44 (1988).
3. M. P. Gorizontova, O. V. Alekseev, and A. M. Chernukh, Byull. Éksp. Biol. Med., No. 3, 44 (1975).
4. M. P. Gorizontova, J. Odarjuk, and M. Bienert, Byull. Éksp. Biol. Med., No. 4, 403 (1988).
5. E. V. Montsevichyute-Éringene, Patol. Fiziol., No. 4, 71 (1964).
6. P. Oehme, Patol. Fiziol., No. 3, 57 (1984).
7. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
8. K. Hecht, P. Oehme, J. A. Kolometseva, et al., Neuropeptides and Neural Transmission, ed. by C. Ajmone-Marsan and W. T. Traczyk, New York (1980), pp. 159-164.
9. F. N. McKenzie, E. Svensjo, and K. E. Arfors, Microvasc. Res., 4, 43 (1972).
10. P. Oehme, K, Hecht, L. Pieschel, et al., Substance P in the Nervous System, London (1982), pp. 296-306.
11. P. Oehme, K. Hecht, J. Jumatov, et al., Pharmazie, 42, No. 1, 34 (1987).