EFFECT OF THE N-TERMINAL FRAGMENT OF SUBSTANCE P ON THE MICROCIRCULATORY SYSTEM

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One of us (M. P. G.) showed previously [1, 2] that substance P (SP), if applied to the rat mesentery in concentrations of $7 \cdot 10^{-6}$-$7 \cdot 10^{-10}$ M, causes pavementing of the leukocytes, swelling and degranulation of the mast cells, and increased permeability of the venules for globulin labeled with fluorescein isothiocyanate (FITC), and colloidal carbon particles.

Investigations have shown [4, 5, 7] that the N-terminal fragment of SP (SP_{1-4}) has a marked antistress action. Accordingly, in the investigation described below the effect of this fragment on the microcirculatory system and on permeability of the values was studied in biomicroscopic experiments and the morphological and functional state of the mast cells also was investigated.

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar rats (41 animals) weighing 250-280 g. The N-terminal fragment (SP_{1-4}-COOH) which was used was synthesized in the Institute of Physiologically Active Substances, East German Academy of Sciences; it was used in concentrations of 10^{-4}-10^{-10} M.

The microcirculation in the rat mesentery was studied biomicroscopically by means of a system based on the "docuval" microscope (Carl Zeiss, Germany). The permeability of the mesenteric microvessels was estimated quantitatively on an apparatus for intravital investigation, based on the LYUMAN KF-1 microscope (Leningrad Optical Equipment Combine). Rabbit globulin labeled with FITC was used as marker of disturbances of vascular permeability.

Morphological and functional states of the mast cells was studied after intravital fixation of areas of the mesentery with ethanol (96%) and staining with toluidine blue.

EXPERIMENTAL RESULTS

The intravital study of the mesenteric microcirculation of rats after application of SP_{1-4} showed that 2-3 min after application of the peptides in a concentration of 10^{-4} M and in avolume of 0.15 ml to a mesenteric window pavementing of the leukocytes appeared in venules with a diameter of 20-40 μ close to swollen or degranulated mast cells. If SP_{1-4} was applied in a concentration of 10^{-5} M pavementing of the leukocytes near the changed mast cells appeared in the venules after 5-10 min. Lower concentrations of the peptides had no effect on the microcirculation.

Comparison of the action of SP_{1-11} and SP_{1-4} on the microcirculatory system revealed that the ratio of concentration (C) with equal activity is as follows:

$$\frac{C_{SP_{1-11}}}{C_{SP_{1-4}}} = \frac{7 \cdot 10^{-8}\,M}{1 \cdot 10^{-5}\,M} = 0{,}007,$$

i.e., if activity of SP_{1-11} is taken to be 1, activity of SP_{1-4} is 0.007.

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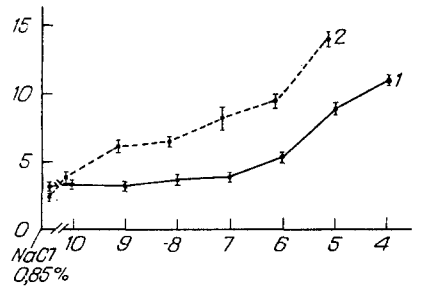

Fig. 1

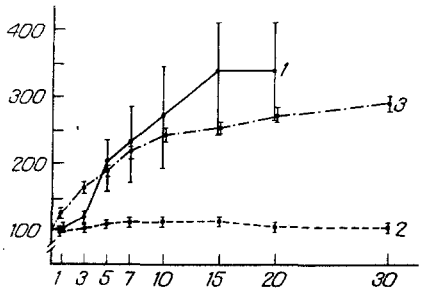

Fig. 2

Fig. 1. Effect of various concentrations of SP_{1-4} (1) and SP_{1-11} (2) on degree of degranulation of mast cells. Abscissa, concentration of peptides (C; −log M); ordinate, percentage of degranulated mast cells.

Fig. 2. Effect of SP_{1-4} and SP_{1-11} on permeability of mesenteric venules of rats for globulin-FITC. Abscissa, time after intravenous injection of FITC-labeled globulin (in min); ordinate, increase in intensity of fluorescence in % (initial background fluorescence taken as 100%). 1) $7 \cdot 10^{-8}$ M SP_{1-11}, 2) 10^{-7} M SP_{1-4}, 3) 10^{-5} M SP_{1-4}.

Application of SP_{1-4} caused a significant increase in degranulation of the mast cells in the mesentery only in concentrations of 10^{-4}-10^{-6} M, whereas the SP_{1-11} fragment had a similar action when applied in much smaller concentrations (Fig. 1). A threefold increase in mast cell degranulation was produced by SP_{1-11} in a concentration of $7 \cdot 10^{-9}$ M and by SP_{1-4} in a concentration of 10^{-5} M, whereas an increase in mast cell degranulation by 3.6 times was produced by SP_{1-11} and SP_{1-4} in concentrations $7 \cdot 10^{-8}$ M and 10^{-4} M, respectively. Thus activity of SP_{1-4} in relation to mast cells was 0.0007 compared with activity of SP_{1-11}, taken as 1.

On the basis of these data, to study the effect of the N-terminal fragments of SP on permeability of the venules, concentrations of 10^{-5} M led to a significant increase in outflow of FITC-labeled globulin through the wall of the venules at all times of the investigation (1-30 min after injection of luminescent serum; Fig. 2), whereas application of SP_{1-4} in a concentration of 10^{-7} M had no effect on the state of permeability of the venules. Similar effects as regards permeability of the venules were produced by SP_{1-4} in a concentration of 10^{-5} M and by SP_{1-11} in a concentration of $7 \cdot 10^{-8}$ M, i.e., activity of the SP_{1-4} fragments was 0.007 of that of SP_{1-11}, taken as 1.

These results are evidence that SP_{1-4} has much weaker damaging activity on components of the microcirculatory system. These findings are in agreement with the results of investigations [3, 6] which revealed that the histamine-releasing activity of SP_{1-4} is weaker than that of SP_{1-11}. For instance, according to data published in [3], it was 0.0064 relative to the activity of SP_{1-11}, taken as 1. These workers found no hyperemia of the skin after intradermal injection of SP_{1-4} in a concentration of 10^{-6} M and they did not observe the development of edema when the same fragment was used in a concentration of 10^{-4} M.

To prevent stress-induced damage to organs and systems, the N-terminal fragment of SP_{1-4} can thus be recommended, for it possesses antistress properties [4, 5, 7] but it differs from SP_{1-11} in having only a weak damping action on the microcirculatory system.

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