EFFECT OF SUBSTANCE P AND ITS FRAGMENTS ON THE MICROHEMODYNAMICS: EXPERIMENTAL STUDY

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UDC 612.135.06:612.8.018:577.175.82

KEY WORDS: substance P; microcirculation; microhemodynamics.

Data on the character of the action of substance P (SP) on blood vessels are contradictory. This substance is considered to be a powerful vasodilator, but its vasoconstrictor effect also has been described [5-7]. There has been much less study of the effect of this substance on the microhemodynamics [1, 2]. For SP to realize its effects on smooth muscles of blood vessels and on the cardiovascular system as a whole, the whole molecule of it must be present; these effects, in some cases, more strongly, can also be obtained by means of the N-terminal fragments SP_{3-11}, SP_{4-11}, and SP_{5-11} [3]. However, the C-terminal pentapeptide SP_{7-11} possesses negligible smooth-muscle activity.

The aim of this investigation was to study the effect of SP_{1-11} on the state of the microvessels and the blood flow in them, and also to compare the activity of a modified fragment of this substance, namely SP_{1-4}—$NH(CH_2)_{11}$—CH_3 (MSP_{1-4}) on the microhemodynamics.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g under pentobarbital anesthesia (6 mg/100 g, intramuscularly). The animals were prepared for biomicroscopy by the usual method. The catheter was introduced into the left carotid artery of the rats to record the systemic blood pressure (BP). A cannula also was introduced into the caudal artery for injection of the test substances. The state of the macro- and microhemodynamics was studied with the aid of an apparatus for intravital microscopy, consisting of an orthoplan microscope with thermostatically controlled stage for the animal, a PRIM apparatus for measuring gauge ("Ernst Leitz," GFR) [4]. The systemic BP and velocity of the micro-blood flow (MBF) were recorded simultaneously on a Mingograf-82 ("Siemens-Elema," Sweden). The microvascular bed of the mesentery of the rat small intestine served as the test object. Arterioles 17-34 μ in diameter were investigated. The velocity of MBF was measured with the aid of an SW# 50x/1.0 saline immersion objective. SP_{1-11} (mol. wt. 1600, seven animals) and SP_{1-4} (mol. wt. 745, eight animals) were synthesized at the Institute of Biologically Active Substances, Academy of Sciences of the GDR. The substances were diluted in 0.9% NaCl to concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M and were injected into the animals in a volume of 0.5 ml of solution. Animals of the control series were given an injection of 0.5 ml of physiological saline (four animals).

EXPERIMENTAL RESULTS

In the preliminary series of experiments the active concentrations of the test substances were determined. For SP_{1-11}, this was 10^{-6} M and for MSP_{1-4} it was 10^{-4} M (Table 1). On this basis the doses of the substances for further study were calculated: SP_{1-11} 0.3 μg and MSP_{1-4} 16 μg/100 g body weight.

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Institute for Biologically Active Substances, Academy of Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 113-115, February, 1990. Original article submitted January 12, 1989.

 0007-4888/90/0002-0128$12.50

TABLE 1. Character of Response of BP and Velocity of MBF Depending on Concentration of Test Substances

Concentration, M	Type of response	
	SP_{1-11}	MSP_{1-4}
10^{-8}	C	C
10^{-7}	C	C
10^{-6}	Fall of BP and MBF	C
10^{-5}	Fall of BP, arrest of MBF	C
10^{-4}	—	Fall of BP and MBF

Remark: C denotes character of changes in BP and MBF similar to that in control series.

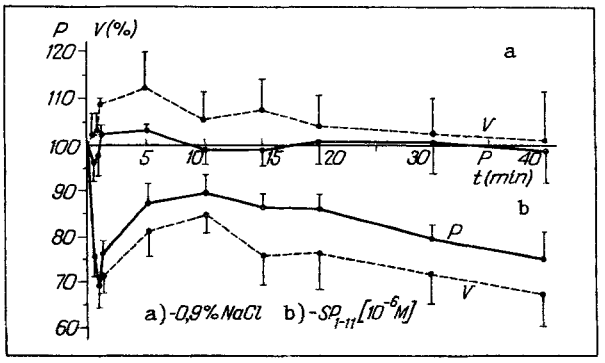

Fig. 1. Dynamics of changes in systemic BP (P) and velocity of MBF (V) (in % of initial level) after injection of 0.5 ml of 0.9% NaCl solution (a) or of a solution of substances P (SP_{1-11}) in a concentration of 10^{-6} M (b).

A sharp fall of BP by 25-30% of its initial value was observed 10-15 sec after injection of SP_{1-11} into the animal's bloodstream (Fig. 1). Changes in the velocity of MBF at this time virtually coincided with those of BP. Slowing of the blood flow could be seen visually in this case, and was uniform throughout the vessels studied, remaining homogeneous in character.

By the 5th-10th minute after injection BP rose to 80-90% of its initial level, and the velocity of MBF to 75-85% of its level. This increase was evidently connected with the hypervolemic effect, for it coincided in time and character of the changes in the corresponding curves in the control series with injection of physiological saline (Fig. 1a). Later, when this effect had weakened, the test parameters fell again. BP 40 min after injection of MSP_{1-4} fell to 70-80%, and the velocity of MBF to 60-75%.

After injection of MSP_{1-4} the character of changes in both the systemic BP and the microhemodynamics differed appreciably from those described above (Fig. 2). During the first 40 sec after the beginning of injection an increase in both parameters was observed to 115-120% of the initial values, followed by a gradual fall to 35-45% for BP and to 15-35% for the velocity of MBF after 15 min. Later, two parameters remained at this same or an even lower level until 30 min.

As in the case of SP_{1-11}, so also after injection of MSP_{1-4} the changes in the velocity of MBF were more marked than those in BP in the corresponding time intervals. The essential fact is that the diameter of the arterioles investigated remained unchanged.

Thus, SP_{1-11} and its fragment MSP_{1-4} give rise to a marked hypotensive effect, as well as to an abrupt change in the microhemodynamics in the part of the regional microcirculatory bed investigated. However, the character of these changes differed for each of them. Whereas SP_{1-11} caused a virtually immediate response, development of a complete response to injection of MSP_{1-4} required up to 10-15 min. It can accordingly be postulated that the whole SP molecule has a direct action on vascular tone, whereas realization of the dilator effect of its fragment requires the inclusion of certain intermediate parameters. This is evidently connected with differences in the concentration of the substances tested, necessary in order to obtain the reactions observed.

Although the presence of numerous nerve fibers containing substance P has been demonstrated in the arterial walls of the rat small intestine by immunocytochemical methods [6], injection of exogenous SP caused no appreciable reaction of the mesenteric microvessels. The decrease in the velocity of MBF observed under these circumstances was connected with lowering

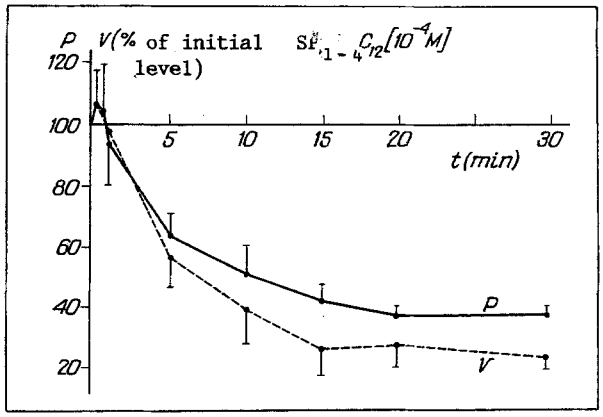

Fig. 2. Dynamics of changes in systemic BP (P) and velocity of MBF (V) after injection of 0.5 ml of a solution of modified fragment of substance P (MSP_{1-4}) in a concentration of 10^{-4} M. *t*) Time, min.

of the systemic BNP, due evidently to vasodilation in other organs. A similar fall of BP in response to intra-arterial injection of SP was described in [5], in which a direct vasodilator action of SP was observed in the limb vessels in dogs. Absence of such a reaction in the mesenteric vessels, which we established, may perhaps be connected with organ specificity of the action of SP.

On the whole, the vascular effects of SP and its fragment, MSP, expressed as lowering of the systemic BP and a corresponding decrease in the velocity of the blood flow in the mesenteric microvessels, are qualitatively similar to each other and are identical in direction. Essential differences are, however, observed in the dynamics of development of the vascular reaction under the influence of each of these two test substances.

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