EFFECT OF SUBSTANCE P ON THE MESENTERIC MICROCIRCULATION

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KEY WORDS: substance P; mast cells; microcirculation; vascular permeability.

Substance P is a physiologically active compound with a broad spectrum of action. Effects of this polypeptide on neurogenic processes, on smooth muscle tone, the secretory function of the digestive glands, and activity of certain hormones have been described [1]. The hemodynamic effects of substance P have received much less study although its probable role in the regulation of the microhemodynamics has been described [3]. Substance P is regarded as a mediator of antidromic vasodilatation and of changes in the local blood flow, vascular permeability, and nociceptive sensations arising as a result of this effect [1, 8, 9]. The mast cells (MC) of the skin may also be involved in these processes [6-8].

Since substance P is constantly found in the circulating blood [10] and its concentration is increased in some chronic diseases [11], participation of this polypeptide in the regulation of the microcirculation in health and disease may be of wider significance. The present investigation was undertaken to study changes in the blood flow, permeability of the microvessels, and degranulation of MC of the rat mesentery under the influence of various doses of exogenous substance P.

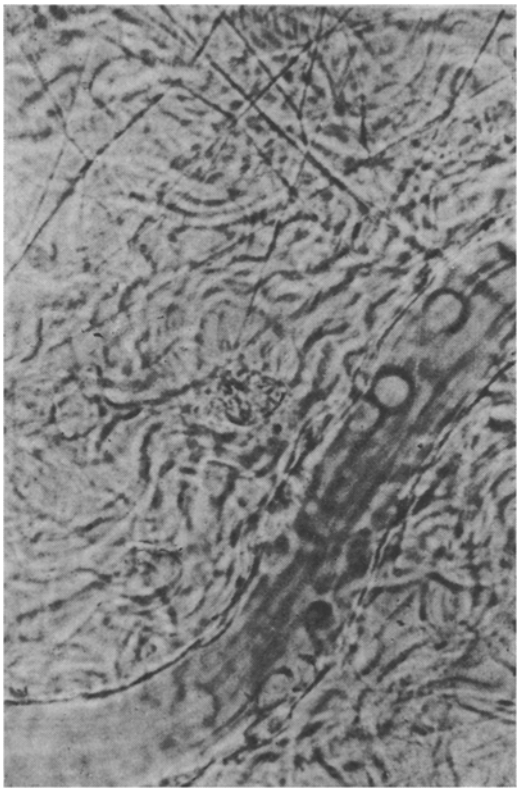

Fig. 1. Pavementing of leukocytes in venule and degranulation of MC following application of substance P in a concentration of $7 \cdot 10^{-8}$ M. Biomicroscopy, 180 X.

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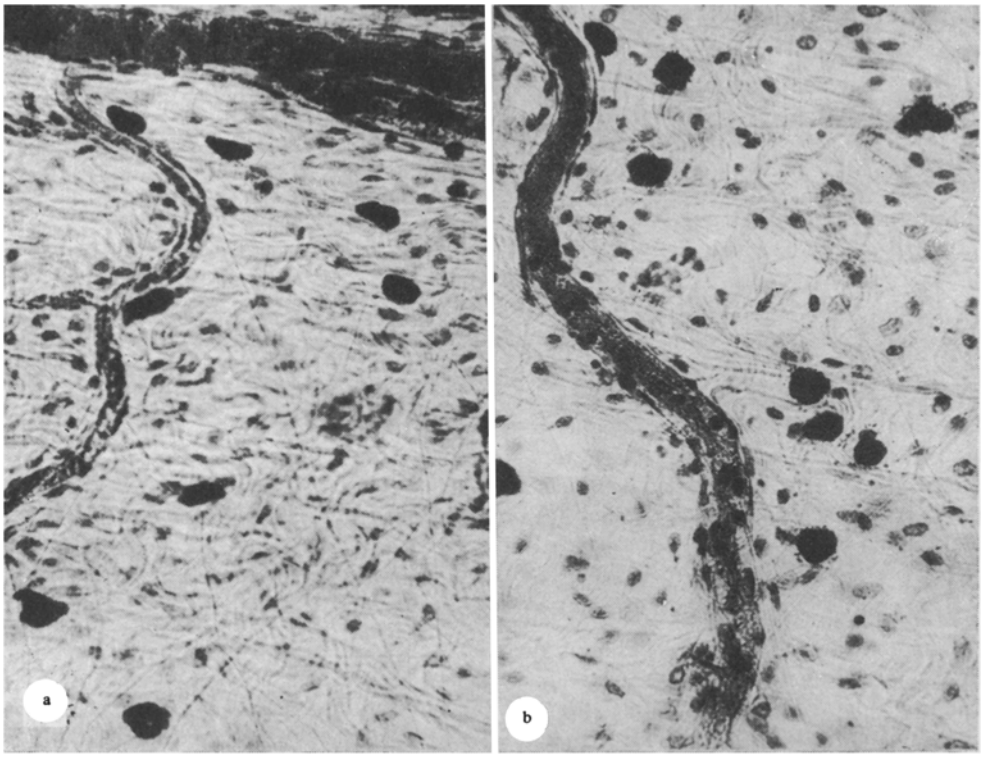

Fig. 2. MC in preparation of rat mesentery: a) after intraperitoneal injection of physiological saline; b) after intraperitoneal injection of substance P in a concentration of $7 \cdot 10^{-8}$ M. Stained with 0.05% toluidine blue solution. 180 X.

EXPERIMENTAL METHOD

Experiments were carried out on 42 noninbred male albino rats weighing 230-250 g. The effect of substance P on the state of the microcirculation, on vascular permeability, and on the degree of degranulation of MC was assessed by means of a biomicroscopic technique and on histological preparations of the mesentery. The apparatus for intravital study of the microcirculation was based on a Docuval (East Germany) microscope. The preparation of substance P used in the investigation was synthesized in the Institute for the Study of Physiologically Active Substances, East Germany Academy of Sciences. Substance P, made up in physiological saline, was applied in concentrations of $7 \cdot 10^{-11}$-$7 \cdot 10^{-6}$ M in a volume of 0.15 ml to a window of the mesentery. The state of the blood flow was assessed visually and photographically. To determine the degree of degranulation of MC after application of substance P the mesentery was fixed with 96° alcohol and stained with 0.05% toluidine blue solution. The change in vascular permeability and the state of MC also were estimated on preparations of the mesentery. In this series of experiments substance P was injected intraperitoneally in the corresponding concentration in a volume of 5.0 ml, and a solution of purified ink in a dose of 0.2 ml/100 g was injected intravenously simultaneously into the anomals. Intravital fixation of the mesentery was carried out 20 min later by intraperitoneal injection of 15 ml of Carnoy's solution and histological preparations of the mesentery were made and used to assess the state of vascular permeability by the method described previously [2]. The degree of degranulation of MC was determined in the same preparations after staining with toluidine blue. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Application of substance P in concentrations of $7 \cdot 10^{-6}$ and $7 \cdot 10^{-7}$ M led to the appearance of pavementing of the leukocytes in the venules after 2-3 min, together with swelling and degranulation of MC (Fig. 1). In a concentration of $7 \cdot 10^{-8}$ and $7 \cdot 10^{-9}$ M substance P caused less marked changes which appeared 5-6 min after its application. When substance P was applied in a concentration of $7 \cdot 10^{-10}$ M pavementing of the leukocytes was not observed, and the signs of incipient degranulation of MC did not appear sooner than after 15 min. Assessment of the degree of degranulation of MC in this series showed significant changes in the process for substance P in concentrations of $7 \cdot 10^{-6}$ and $7 \cdot 10^{-10}$ M (Fig. 2a, b; Table 1).

Intraperitoneal injection of substance P ($7 \cdot 10^{-7}$-$7 \cdot 10^{-8}$ M) caused an increase in vascular permeability, which was expressed as deposition of particles of colloidal carbon in the walls of venules measuring 20-30 μ in diameter and in the venular part of the capillaries (Fig. 3). Lower concentrations of substance P caused no changes in permeability. In this series of experiments degranulation of MC was observed only with concentrations of $7 \cdot 10^{-7}$ and $7 \cdot 10^{-8}$ M (Table 1). With respect both to degranulation of MC and disturbances of vascular permeability, the effective doses of substance P when given by intraperitoneal injection were thus similar.

TABLE 1. Effect of Substance P on Degranulation on MC in Mesentry (M ± m)

Concentration of substance P, M	Number of degranulated MC, %	
	intravital assessment	assessment on histological preparations
Control (physiological saline)	2,3±0,52	0,7±0,03
$7 \cdot 10^{-11}$	3,5±0,28	—
$7 \cdot 10^{-10}$	6,1±0,45*	0,7±0,08
$7 \cdot 10^{-9}$	6,7±0,45*	0,6±0,06
$7 \cdot 10^{-8}$	8,3±1,10*	1,0±0,10*
$7 \cdot 10^{-7}$	9,7±0,46*	1,1±0,08*
$7 \cdot 10^{-6}$	14,4±0,75*	—

*Difference from control for the same series significant (P = 0.01).

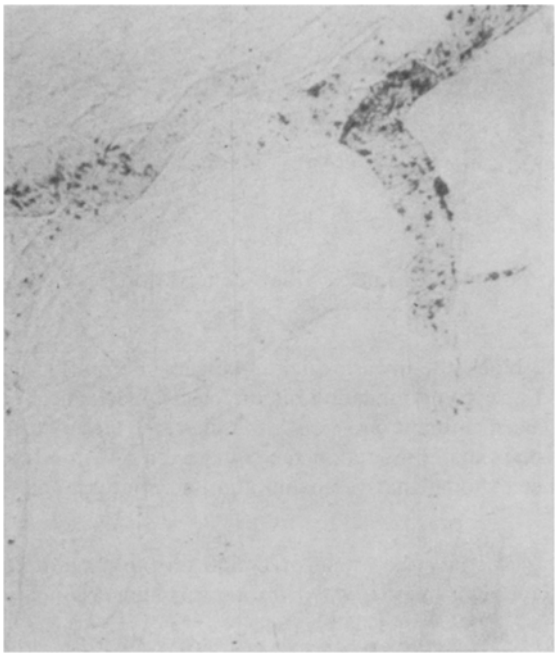

Fig. 3. Deposition of colloidal carbon in wall of mesentric microvessels after intraperitoneal injection of substance P in a concentration of $7 \cdot 10^{-8}$ M in a rat. Preparation of mesentery, 50 X.

It can be concluded from these results that substance P acts effectively on the microhemodynamics, permeability of the wall of the microvessels, and on degranulation of MC. In the writers' view, the effect of this polypeptide on the microcirculation and vascular permeability is mediated through the mast cells, for pavementing of the leukocytes in the venules is observed mainly at sites of degranulation of MC, and the two processes coincide in time. Disturbance of vascular permeability after injection of substance P occurs in concentrations which give rise also to degranulation of MC.

These results are in agreement with data in the literature [5, 6], indicating that on incubation of MC in a solution of substance P (concentrations $1 \cdot 10^{-4}$ and $1 \cdot 10^{-5}$ M) histamine is liberated into the incubation medium. In the present experiments, under conditions of intravital microscopy, however, signs of degranulation of mesenteric MC could be observed with much lower concentrations of substance P.

The results of these experiments draw attention to new aspects of the physiological action of substance P. Changes in tone and permeability of the microvessels arising under the influence of substance P, directly or through MC, may be a significant factor in the regulation of the microcirculatory vascular component of various organs.

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EFFECT OF CALCIUM ANTAGONISTS ON THE ACTION POTENTIAL OF MUSCLE FIBERS OF WARM-BLOODED ANIMALS

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Verapamil and its derivative D 600 block the inward calcium current more effectively than polyvalent cations and are used to study calcium channels [6]. These compounds inhibit the electromechanical coupling system, which triggers the inflow of calcium through the outer cell membrane in smooth muscles and the myocardium of mammals [6, 9, 10]. They have been used as calcium antagonists in experiments on the squid axon [4], molluscan neurons [11], and other objects. More recently, however, it has been shown that verapamil and its structural analogs can also inhibit the current formed by sodium ions [4, 5, 13].

The object of this investigation was to study the effect of D 600 on the magnitude, duration, and rate of rise and fall of the action potential (AP) of the diaphragm muscle, when the motor innervation is preserved or absent.

EXPERIMENTAL METHOD

Preparations of mouse diaphragm were used. To compare the results, innervated and denervated halves of the diaphragm from the same animal were used. Denervation was carried out under ether anesthesia by dividing the left phrenic nerve inside the thorax. The experiments were carried out 4 days after operation. The isolated muscle preparation was placed in a constant-temperature chamber (28-30°) with continuously flowing oxygenated Lilly's solution of the following composition: Na^+ 152 mM, K^+ 4 mM, Cl^- 149 mM, Mg^{2+} 1 mM, Ca^{2+} 4 mM, $H_2PO_4^{2-}$ 0.9 mM, HCO_3^- 16.2 mM, glucose 11 mM; pH 7.2-7.4. APs were evoked by intracellular stimulation. The recording electrode and the stimulating electrode filled with potassium citrate were inserted into the muscle fiber 50 μ apart. To compare APs obtained under different experimental conditions, the membrane potential (MP) of the recorded muscle fiber was kept strictly constant, by inducing local hyperpolarization of the membrane by passing a current through the stimulating electrode. To assess the maximal rate of rise of AP, a RC-differential circuit with time constant of 10^{-5} sec was used. In the course of the experiment the original solution was replaced by a solution containing D 600 in a concentration of $5 \cdot 10^{-6}$-$1 \cdot 10^{-4}$ g/ml. The muscle fiber was stimulated either by a single pulse or by a series of pulses with a following frequency of 1 and 2 Hz. Usually the characteristics of the first and 10th responses, when any further change in AP ceased, were compared in a series of reactions.

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