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MECHANISMS OF BLOOD VESSEL PERMEABILITY DERANGEMENT UNDER THE INFLUENCE OF PERMEABILITY FACTORS (HISTAMINE, SEROTONIN, KININS) AND INFLAMMATORY AGENTS

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Abstract—Permeability derangement of venules may occur independent of changes in microcirculation. This was established in experiments on rat's mesentery by topical application of histamine and bradykinin. It was possible to cause venules permeability derangement using such small doses of permeability factors which did not cause any visible changes of microcirculation. Venules permeability derangement under the influence of permeability factors is probably the result of shortlived responses of endothelial cell contractile structure to injury. As has been shown by the experiments on the effect of histamine, bradykinin and serotonin on the skin vessels of rabbits, guinea pigs and rats as well as on rat's mesentery and leg vessels, this reaction is inhibited by previous topical administration of 10^{-2}–10^{-3} M solutions of NaCN. Contrary to this NaCN does not inhibit the appearance of vessel permeability derangement of rabbit's skin and rat's mesentery under the influence of inflammatory agents (burn, xylene). The results of these experiments testify against the dominating role of the three permeability factors in the pathogenesis of blood vessel permeability derangement in inflammation and underline the significance of other mechanisms independent of energy formation in aerobic tissue respiration. The possible role of leucocytes in the mechanism of early blood vessel permeability in inflammation is discussed.

RECENT electronmicroscopic investigations have shown that the increase of vessel permeability under the influence of permeability factors (histamine, serotonin, kinins) or inflammatory agents results from the shortening (rounding) of endothelial cells, with gaps forming between them. In a number of cases this is accompanied by disturbances of basal membrane integrity. The mechanisms of blood vessel permeability increase in inflammation are not widely investigated.

According to Rowley[1] the rat's venule permeability derangement under the influence of histamine, serotonin and bradykinin can be explained by the veins contraction resulting in pressure increase and in the stretching of venules. Contrary to that Majno *et al.*[2] in experiments on rat's m. cremaster did not find any connection between the venule permeability increase under the influence of permeability factors and expansion of venules. Bucley and Ryan[3] studying the influence of histamine and serotonin on rat's mesentery vessels also established the independence of venule permeability derangement and circulation disturbances.

According to Zweifach,[4] Majno *et al.*,[2] Bucley and Ryan[3] the vessel permeability increase results from the influence of permeability factors on the contractile structure

in endothelial cells. Alongside of permeability factors effects the direct vessel injury caused by inflammatory agents is mentioned.[5] However, the existence of this effect has not yet been proved. The aim of our experiments was further investigation of the mechanisms of blood vessel permeability increase in inflammation.

MATERIALS AND METHODS

Experiments were made on adult rabbits (2·5–3·8 kg), guinea pigs (500–750 g) and Wistar rats (120–240 g) of both sexes.

Microcirculation on rat mesentery was studied by Zweifach's method with minor modifications.[6] The rats were anaesthetized with 30 mg/kg sodium pentobarbital i.p. Saline solution (1:5) of Indian ink was injected i.v. (0·4 ml/100 g) to detect the mesentery vessel permeability derangement.

The investigations of skin vessel permeability state were based on Ramsdell and Menkin principles with minor modifications.[7] Unanaesthetized animals were injected i.v. with 1% saline solution of Evans blue (20 mg/kg). The results were estimated visually according to the degree of skin blueing using an arbitrary scale. Since the blueing of the outer surface of rat's skin is not clearly seen in a number of cases the appraisal was made according to inner skin surface blueing.

Rat hind leg oedema was studied by injecting investigated agents under sole surface. In an hour the rats were killed, their hind legs were amputated at the ankle joint and weighed.[8]

Burns were produced by applying for 1 min a copper disc, 10 mm diameter, maintained at a temperature 54 $\pm$ 0·1° by circulating water from an ultrothermostat. The mesentery burns were caused by applying a metal V-shaped tube, 2 mm diameter, for 15 sec maintained at a temperature 54 $\pm$ 0·1° by circulating water. The following preparations were used. Histamine (Chemical Works, Lvov, U.S.S.R.). Serotonin creatinine sulphate (Lawson, England). Bradykinin (BRS 640, Sandos LTD, Basel). Cyanide sodium (Chemapol, Czechoslovakia). Evans blue (Reanal, Hungary). Indian ink (Krasny Khudozhnik, Moscow).

Indian ink was first freed of large conglomerations by filtering. The size of particles in Indian ink used was 45–70 mμ.

RESULTS

The influence of bradykinin and histamine on blood circulation and vessel permeability of rat mesentery

Experiments were carried out on 25 Wistar rats. Microscopic observation of mesentery with small doses of histamine (0·2 μg) and bradykinin (0·5 μg) applied to its parts did not detect any changes in vessel lumen and the character of blood flow. The appearance of carbon particles in venule walls as well as in arteriovenous anastamoses was observed in 30–60 sec. The application of bigger doses of bradykinin (1 μg) or histamine (5 μg) on the mesentery caused small expansion of venules and small mesentery veins (from 28 to 32 μ for histamine and from 29 to 31 μ for bradykinin). The appearance of carbon particles in the walls of venules, arterio-venous anastamoses and small veins in these experiments was also observed 30–60 sec after application of either histamine or bradykinin.

The participation of tissue respiration in the mechanism of the influence of histamine, serotonin and bradykinin on vessel permeability

0·1 ml NaCN solutions (10^{-2}–10^{-4} M) in 0·1 M tris–HCl buffer (pH 7·4, made isotonic by addition of NaCl) was introduced i.c. into the shaved parts of rabbits, guinea pigs and rats flancs. 0·1 ml of solutions of bradykinin (0·5 μg), histamine (10 μg) or serotonin (0·5 μg) was injected into the same parts of skin with different time intervals. In control experiments permeability factors were introduced into the parts of skin with tris–HCl buffer previously injected. NaCN injections served as additional control. Immediately after the injections Evans blue was given i.v. In all the cases of control tests permeability factors increased vessel permeability. NaCN did not disturb either vessel permeability or microcirculation (the measurement of skin temperature). The results are shown in Tables 1, 2 and 3.

TABLE 1. NaCN INFLUENCE ON THE DEVELOPMENT OF RABBIT VESSEL PERMEABILITY DERANGEMENT CAUSED BY BRADYKININ AND HISTAMINE

NaCN solutions	Time (min) after NaCN administration			
	5	10	15	30
	Bradykinin			
10^{-2} M	2/8	0/8	1/8	1/8
10^{-3} M	1/8	1/8	3/8	6/8
10^{-4} M	7/8	8/8	8/8	8/8
	Histamine			
10^{-2} M	1/8	0/8	0/8	1/8
10^{-3} M	2/13	0/13	1/13	6/13
10^{-4} M	10/12	6/12	9/12	10/12

The numerator: the number of tests with vessel permeability derangement, the denominator: the total number of tests.

TABLE 2. NaCN INFLUENCE ON THE DEVELOPMENT OF VESSEL PERMEABILITY DERANGEMENT OF GUINEA PIGS CAUSED BY BRADYKININ AND HISTAMINE

NaCN solutions	Time (min) after NaCN administration			
	5	10	15	30
	Bradykinin			
10^{-2} M	4/7	3/7	2/7	4/7
10^{-3} M	6/7	7/7	5/7	4/7
	Histamine			
10^{-2} M	3/7	2/7	2/7	3/7
10^{-3} M	7/7	7/7	6/7	5/7

The numerator: the number of tests with vessel permeability derangement; the denominator: the total number of tests.

TABLE 3. NaCN INFLUENCE ON THE DEVELOPMENT OF VESSEL PERMEABILITY DERANGEMENT OF RATS CAUSED BY HISTAMINE

NaCN solutions	Time (min) after NaCN administration			
	5	10	15	30
10^{-2} M	1/9	0/9	0/9	3/9
10^{-3} M	2/9	3/9	5/9	4/9

The numerator: the number of tests with vessel permeability derangement; the denominator: the total number of tests.

Experiments have shown that the most pronounced inhibition of permeability derangement was observed 10–15 min after administration of NaCN. In experiments on guinea pigs and rats the influence of cyanide was significant though less pronounced than in the experiments on rabbits. NaCN solution (10^{-2} M) significantly inhibited the effect of serotonin on skin vessels of rats. Analysing the results of the experiment described it is necessary to take into account the fact that the true cyanide concentration in endothelial cells was much lower than that administered due to continuous transfer of cyanide into circulation.

In inflammation several mediators are likely to form. Therefore in experiments on rabbits bradykinin (0·25 μg) and histamine (5 μg) were simultaneously injected i.c. The mediators were administered 10 min after injecting NaCN 10^{-2} M solution. Permeability derangement developed in two out of 26 tests while in control (the mediators were introduced after injecting saline) it took place in all the 26 cases.

In the second series of experiments the influence of cyanide on the development of vessel permeability derangement of mesentery was studied on 56 rats. NaCN (10^{-2}–10^{-3} M) solutions in tris–HCl buffer in a volume of 0·1 ml were dropped on parts of the mesentery. In 3 min Indian ink was introduced i.v. and in 2 more min 0·1 ml solutions of bradykinin (1 μg), histamine (5 μg) or serotonin (20 μg) was applied on the parts of mesentery affected by cyanide. In control experiments tris–HCl buffer was used instead of NaCN. In all the cases of control experiments a pronounced increase of venule permeability occurred which was seen from the blackening of the vessel walls (Indian ink was trapped in the gaps between adjacent endothelial cells). The application of NaCN 10^{-2} M solution almost fully suppressed venule responses to mediators. The inhibition was significant though less pronounced in experiments with 10^{-3} M NaCN.

In the third series of experiments the influence of cyanide on the development of rat leg oedema was studied on 30 rats. NaCN solution (10^{-2} M) in tris–HCl buffer in a volume of 0·1 was injected into the hind leg plantar surface. 0·1 ml of solutions of histamine (25 μg), bradykinin (25 μg) or serotonin (2·5 μg) 10 min later was introduced. In control experiments tris–HCl buffer in a volume of 0·1 ml was injected instead of NaCN solution. Tris–HCl buffer in a volume of 0·1 ml injections and 10 min later an injection of saline which served for solving permeability factors were used for additional control. In 60 min the oedema intensity was determined by weighing the leg amputated at the ankle joint (Table 4).

TABLE 4. NaCN INFLUENCE ON THE DEVELOPMENT OF RAT LEG OEDEMA CAUSED BY BRADYKININ, HISTAMINE AND SEROTONIN (LEG WEIGHT IN GRAMS)

Permeability factors	Groups of experiments		
	Buffer and saline (control 1)	Buffer and permeability factor (control 2)	10^{-2} M NaCN and permeability factor
Bradykinin	1·33	1·44*	1·28†
Histamine	1·10	1·25*	1·09†
Serotonin	1·10	1·50*	1·27†

* $P < 0.05$ as compared to control 1.
† $P < 0.05$ as compared to control 2.

As the experiments have shown cyanide fully inhibited bradykinin and histamine caused oedema and by 50 per cent inhibited serotonin oedema.

In the experiments that followed we have investigated the possibility of inhibiting vessel responses to usual inflammatory agents by cyanide.

The participation of tissue respiration in the mechanism of the influence of inflammatory agents on vessel permeability

The experiments were carried out on eight rabbits and ten rats.

The rabbits were first injected i.c. with 0·1 ml 10^{-2} M NaCN solution in tris–HCl buffer, 10 min later Evans blue solution was given i.v. After this the parts of skin injected with cyanide were painted with 0·02 ml of xylene or a copper disc (15 mm in diameter, maintained at a temperature of 54° by circulating water) was placed on them for 1 min. In control experiments xylene and heat were applied to the parts of skin with tris–HCl buffer previously injected. In these experiments an inhibitory effect of cyanide has not been detected. Permeability derangement (skin blueing) was practically identical in the parts of skin injected with solution of cyanide in tris–HCl buffer or tris–HCl buffer alone.

In experiments on rats 10^{-2} M NaCN solution in a volume of 0·1 ml was applied to mesentery. Three min later Indian ink was injected i.v. and 2 more min later a metal loop (2 mm in diameter, maintained by circulating water at a temperature of 54° was applied. In control experiments instead of cyanide tris–HCl buffer was dropped to the mesentery. Under the influence of high temperature vessel expanded and stasis originated at the place where the loop was applied. In periphery a pronounced vessel permeability derangement occurred. It was practically identical in the experiments with previously applied NaCN solution in tris–HCl buffer or buffer alone.

DISCUSSION

The experiments carried out have shown the relative independence of microvessel permeability enhancement of the state of microcirculation. They support the theory of the active and energy requiring character of the permeability enhancement of endothelial membrane under the influence of permeability factors (histamine, bradykinin, serotonin). The investigations further showed that the permeability enhancement of skin, mesentery and probably other tissue vessels in inflammation could not be

explained by permeability factors alone. The inhibition by cyanide of tissue respiration did diminish the influence of permeability factors but not of inflammatory agents (xylene, burn) upon the vessel permeability. This is in accordance with our observations which have shown a more pronounced skin vessel permeability derangement under the influence of xylene or burn (54°, 1 min) than that caused by i.c. injections of optimal doses of histamine or bradykinin. Besides, the permeability factors, in contrast to inflammatory agents, cause vessel permeability enhancement only of short duration, blood vessels soon lose the sensitivity to the influence of histamine.[14,15]

Apparently the inflammatory agents increase the blood vessel permeability with the participation of both permeability factors and some other mechanisms independent of energy liberated by aerobic tissue respiration. This point of view is in accordance with the results of numerous investigations which proved the inability of permeability factor inhibitors and antagonists to completely suppress the initial manifestation of acute inflammation.

Too much absorption in permeability factors averted the investigator's attention from the search for other permeability increasing mechanisms in inflammation.

During recent years some attention has been paid to the possible role of blood coagulation system in the pathogenesis of early manifestations of inflammation.[9] The experiments showed, however, that the development of acute normergic inflammation (enhancement of blood vessel permeability, exudation and leucocytic emigration) does not depend on the blood clotting and fibrinolytic systems. The intensity of inflammation was similar both in control animals and animals with complete blood uncoagulability.[10,11]

Direct blood vessel injury under the influence of inflammatory agents may have greater importance,[6] but the mechanism of this is unknown.

It is possible to assume as a working hypothesis that leucocytes participate not only in delayed but also in early events of inflammation. In our laboratory there were confirmed the conclusions of other investigators about the weakening of exudation and blood vessel permeability derangement in rabbits and rats made leucopenic by irradiation or treatment with cytostatic drugs. Our experiments showed that leucocyte transfusion to leucopenic rabbits and rats immediately improved the reactivity of skin blood vessel to the inflammatory agents.[12] It seems likely that leucocytes sticking to the inner surface of endothelial cells in some unknown way cause contractile reaction of cells resulting in permeability enhancement. In leucopenic animals the number of circulating leucocytes is probably not sufficient to provoke such an endothelial cells reaction. It is interesting to note that in contrast to inflammatory agents permeability factors (histamine, bradykinin) are able to cause skin vessel permeability derangement also in rabbits with experimental leucopenia.[13] The elucidation of the above working leucocytic hypothesis requires further investigations.

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