EFFECTS OF VASOACTIVE AGENTS ON THE CANINE FORE-LIMB VENOUS PERFUSION PREPARATION AND THEIR MODIFICATION BY ANTI-INFLAMMATORY DRUGS

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Abstract—Responses of the perfused forelimb paw small vein in anesthetized dog to vasoactive agents including serotonin, histamine, bradykinin, acetylcholine and epinephrine were investigated together with their modification by anti-inflammatory drugs. Venous perfusion with blood frequently caused venospasms which could not be attenuated by anesthetics, gallamine or phentolamine. In perfusion experiments both with blood and with Krebs-bicarbonate solution, close i.v. injections of epinephrine, serotonin and acetylcholine caused marked venoconstrictions (threshold doses in blood perfusion experiments, about 1, 3 and 300 ng, respectively), whereas bradykinin and histamine produced only weak constrictive responses even in large doses of more than 3 μ g. Close i.v. injections of non-steroidal anti-inflammatory drugs, i.e. phenylbutazone, 150 μ g/min, sodium salicylate, 500 μ g/min, indomethacin, 50 μ g/min, aminopyrine, 300 μ g/min and benzydamine, 10 μ g/min, nonspecifically depressed the venoconstrictor responses to epinephrine, serotonin, acetylcholine, bradykinin and histamine. A steroidal anti-inflammatory drug, hydrocortisone, however, tended to enhance the effect of epinephrine with no influence on the effects of the other vasoactive agents. The results suggest that the inhibitory effects of non-steroidal anti-inflammatory drugs on the venoconstrictor responses to the vasoactive agents may contribute to their anti-inflammatory activity through the inhibition of facilitated vascular permeability due to the rise in hydrostatic pressure of the fine blood vessels.

Most of the mediators on the acute inflammatory process are potent vasoactive substances, and some are considered to initiate an inflammatory reaction by their actions on the local peripheral blood vessels. Rowley proposed that the formation of edema is a secondary result from a rise in blood pressure of the fine blood vessels produced by a dilatation of the precapillary vessels and a constriction of the postcapillary vessels (1). Northover reported the inhibitory effect of anti-inflammatory drugs on the drug-induced vasoconstrictor responses of the isolated anterior mesenteric vein of the rat or guinea-pig (2). There is, however, no direct evidence of such action on the venous system *in situ*.

The present experiments were therefore conducted to investigate the responses of perfused vein to the vasoactive agents including the mediators of inflammation, such as histamine, serotonin, bradykinin and some neurohumoral substances, and to demonstrate the influence of anti-inflammatory drugs on the effects of the agents in anesthetized dogs.

MATERIALS AND METHODS

Venous Perfusion Preparation

Seventy-seven male mongrel dogs weighing 6 to 17 kg were anesthetized with α -chloro-

ralose, 100 mg/kg i.v. They were immobilized with 2 mg/kg i.v. of gallamine and ventilated artificially through an endotracheal tube connected to a positive respiratory pump (Natsume, KN-58). The third superficial dorsal metacarpal vein (inner diameter 0.4–0.6 mm) (noted below as the paw small vein) of the left forelimb paw was dissected about 5 mm long and a polyethylene catheter was inserted downstream 1 cm apart from the crosspoint of 3rd and 4th metacarpal bones. The vein was perfused through the polyethylene catheter with the dog's own blood delivered from the left femoral artery or with Krebs-bicarbonate solution (NaCl, 118.2, KCl, 4.6, NaHCO_3 , 24.8, KH_2PO_4 , 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.5, and glucose, 10.0 mM/liter) saturated with 95% O_2 –5% CO_2 at 37°C. Constant flow perfusion was carried out with a Sigma-motor pump (T-8), which was adjusted at the beginning of each experiment so that perfusion pressure was 15 to 20 mmHg, and was kept constant throughout the experiment. The perfused flow volume had no effect on the systemic blood pressure. When perfused with blood, dogs were given heparin sodium in an initial dose of 500 U/kg i.v. and supplemental doses of 200 U/kg i.v. every hour. The venous perfusion pressure was measured between the pump and the perfused vein via a pressure transducer (Nihon Kohden, LPU-0.1), and was used as an index of the change in venous resistance. The cephalic vein was cannulated through the median cubital vein, and blood pressure in this vein was also measured via a pressure transducer (Nihon Kohden, LPU-0.1). The systemic arterial blood pressure was monitored from the catheterized right femoral artery via a pressure transducer (Nihon Kohden, MPU-0.5). These recordings were made on a polygraph (Nihon Kohden, RM-150). Gallamine, 2 mg/kg, was given every hour via the cannulated right cephalic vein. Close i.v. injections of the vasoactive agents in volumes of 0.02 ml into the perfused paw small vein were given in a random fashion through the rubber tubing. Infusions of anti-inflammatory drugs were made in the same way at the rate of 0.09 ml/min using a syringe pump (Natsume KN-202). Responses to agonists during the infusion of an anti-inflammatory drug were obtained from approximately ten minutes after the onset of infusion.

Drugs

The drugs used were 5-hydroxytryptamine creatinine sulfate (Serotonin, Wako Pure Chemicals), epinephrine hydrochloride (Sankyo), acetylcholine chloride (Ovisot, Daiichi Seiyaku), phenylbutazone (Fujisawa), indomethacin (Inteban, Sumitomo Chemicals), sodium salicylate (Wako Pure Chemicals), aminopyrine (Sanko), benzydamine hydrochloride (Takeda), hydrocortisone sodium succinate (Solu-Cortef, Upjohn), papaverine hydrochloride (Iwaki Seiyaku), phentolamine mesylate (Regitine, CIBA-Geigy), sodium pentobarbital (Nembutal, Abbot) and gallamine triethiodide (Flaxedil, Teikoku Kagaku). All doses are expressed in terms of their salts. Phenylbutazone and indomethacin were dissolved in 0.1 N NaOH aqueous solution, diluted with saline, and neutralized to pH 7 with 0.1 N HCl aqueous solution. It could not be confirmed that the solvent solutions had any effect on perfused venous and systemic arterial blood pressures.

RESULTS

Basic consideration of perfusion with blood and with Krebs-bicarbonate solution

In the blood perfusion experiments, the average mean perfusion pressure adjusted at the beginning of the experiment and the cephalic venous pressure were 17.7 ± 0.8 (S.E.M.) mmHg and 3.6 ± 0.3 mmHg ($N=20$), respectively. The average flow maintained constant was 0.35 ± 0.6 ml/min. In the perfusion experiments with Krebs-bicarbonate solution, the corresponding parameters mentioned above were 13.9 ± 0.5 mmHg, 3.7 ± 0.2 mmHg, and 0.45 ± 0.04 ml/min ($N=57$), respectively. Thus in the Krebs-bicarbonate perfusion experiments, the venous tone was lower than that in the blood perfusion experiments.

FIG. 1. Fluctuations of venous perfusion pressure in the paw small vein when blood was perfused into the anesthetized dog.

FAP : femoral arterial pressure, VPP : venous perfusion pressure and CVP : cephalic venous pressure.

Twenty of the thirty-nine blood perfusion experiments were discarded, the reason being that in those experiments, perfusion pressure fluctuated greatly, as shown in Fig. 1, and a constant perfusion pressure could not be obtained. The fluctuations of perfusion pressure was not inhibited by the systemic i.v. injection of α -chloralose, pentobarbital or gallamine. Attenuation by the change in temperature of perfusing blood from 37°C to 28°C or to 44°C was also absent. After being pretreated with phentolamine, $300 \mu\text{g}$ close i.v., or sections of the radial, median, ulnar and musculocutaneous nerves in the forelimb, the fluctuations were transiently inhibited in some degree. Papaverine, $150 \mu\text{g}$ close i.v., abolished the fluctuations of perfusion pressure. Perfusion with venous blood from the femoral vein instead of arterial blood from the femoral artery failed to attenuate these fluctuations. Perfusion with Krebs-bicarbonate solution never caused such a change in perfusion pressure, but a close i.v. injection of a small volume of venous blood produced abrupt venospasms accompanied with an increase in basal venous tone. Accordingly, a large part of the following venous perfusion experiments were conducted using Krebs-bicarbonate solution.

Effects of vasoactive agents on perfusion and cephalic venous pressures

Typical recordings of the changes in venous perfusion pressure of the paw small vein,

FIG. 2. Responses to acetylcholine (ACh), histamine (Hist), bradykinin (Bk), serotonin (5HT) and epinephrine (Epi) in a dog paw venous perfusion experiment with Krebs-bicarbonate solution.

Drug solutions were injected close i.v. into the paw small vein.

FAP : femoral arterial pressure, VPP : venous perfusion pressure and CVP : cephalic venous pressure. Transient increase in VPP in each case is an artifact due to injection of the drug solution. Time line marks indicate minutes.

FIG. 3. Dose-response curves for epinephrine (Epi), serotonin (5HT), bradykinin (Bk), acetylcholine (ACh) and histamine (Hist) on venous perfusion pressure in dog paw venous perfusion experiments.

A : blood perfusion experiments and B : Krebs-bicarbonate perfusion experiments. Ordinate : the changes in venous perfusion pressure and abscissa : the doses of agents administered close i.v. Each point represents mean value obtained from 7 to 19 dogs in A and 7 to 35 dogs in B. Vertical bars represent S.E.M.

cephalic venous pressure and femoral arterial pressure produced by rapid close i.v. injections of acetylcholine, histamine, bradykinin, serotonin and epinephrine in Krebs-bicarbonate perfusion experiments are illustrated in Fig. 2. Serotonin, 1 μ g, and epinephrine, 0.3 μ g, caused a marked increase in perfusion pressure with no substantial change in cephalic venous and systemic arterial pressures. Acetylcholine, 30 μ g, also caused a considerable rise in venous perfusion pressure and a moderate rise in cephalic venous pressure, but acetylcholine in the dose used produced a concomitant marked fall in systemic arterial pressure. Histamine, 30 μ g, having only a slight pressor effect on perfusion pressure also caused a marked fall in systemic arterial pressure and a moderate rise in cephalic venous pressure. Bradykinin, 3 μ g, had an effect similar to that of histamine, although its effect on perfusion pressure was less potent than that of histamine. Each corresponding dose of acetylcholine, histamine and bradykinin administered systemically did not produce any significant change in perfusion pressure.

The durations of responses on perfusion pressure to epinephrine, acetylcholine and histamine were relatively short, being about two to three minutes, while that to serotonin was up to several minutes. On the other hand, in the blood perfusion experiments, the dura-

FIG. 4. Dose-response curves for epinephrine (Epi), serotonin (5HT), bradykinin (Bk), acetylcholine (ACh) and histamine (Hist) on cephalic venous pressure (solid line) and femoral arterial pressure (dotted line) in dog paw venous perfusion experiments.

Notations as in Fig. 3.

tion of response to each agent tended to be longer than that in Krebs-bicarbonate perfusion experiments.

Fig. 3 shows the dose-response curves for changes in perfusion pressure caused by epinephrine, serotonin, bradykinin, acetylcholine and histamine given by close i.v. injection.

In blood perfusion experiments, epinephrine, serotonin and acetylcholine produced a marked rise in perfusion pressure. Bradykinin and histamine produced a mild rise in perfusion pressure. The order of venoconstricting potencies was as follows: epinephrine > serotonin > acetylcholine > bradykinin > histamine. The threshold dose required to cause the response in blood perfusion experiments was as follows: epinephrine, 1 ng, serotonin, 3 ng, bradykinin, 30 ng, acetylcholine, 0.3 μ g, and histamine, 3 μ g. It appeared that on the ceiling effect produced by a drug, epinephrine, serotonin and acetylcholine were relatively high, while bradykinin and histamine were low. The slopes of the dose-response curves for the relatively active substances, that is, epinephrine, serotonin and acetylcholine, were almost all parallel.

In Krebs-bicarbonate perfusion experiments the dose-response curves for epinephrine, serotonin and histamine were similar to those in blood perfusion experiments, while responses to acetylcholine and bradykinin were strikingly smaller in Krebs-bicarbonate perfusion than those in blood perfusion experiments.

FIG. 5. Effects of indomethacin on the changes in venous perfusion and cephalic venous pressures induced by epinephrine (Epi) and serotonin (5HT) in a dog venous perfusion experiment with Krebs-bicarbonate solution.

Epi and 5HT were injected and indomethacin, 50 μ g/min, was infused close i.v. into the paw small vein. Upper record: before infusion, and lower record: during infusion of indomethacin.

FAP: femoral arterial pressure, VPP: venous perfusion pressure and CVP: cephalic venous pressure. The agents were injected at the arrows in the doses indicated.

Fig. 4 shows the dose-response curves for changes in cephalic venous and systemic arterial pressures recorded simultaneously in the same experiments described above. The changes in cephalic venous pressure were much less than those in perfusion pressure, being only a few mmHg in both blood and Krebs-bicarbonate perfusion experiments. Epinephrine and the highest dose of serotonin showed only a slight pressor effect on cephalic venous pressure, whereas bradykinin, histamine and acetylcholine showed more intense pressor effects in blood perfusion experiments.

Effects of anti-inflammatory drugs on responses to vasoactive agents

In Krebs-bicarbonate perfusion experiments, close i.v. infusions of 300 $\mu\text{g}/\text{min}$ of aminopyrine and 10 $\mu\text{g}/\text{min}$ of benzydamine caused a slight fall in perfusion pressure by 2.5 and 1.5 mmHg in the average of four experiments, respectively. Phenylbutazone, 150 $\mu\text{g}/\text{min}$, sodium salicylate, 500 $\mu\text{g}/\text{min}$, and indomethacin, 50 $\mu\text{g}/\text{min}$, caused no significant change in perfusion and cephalic venous pressures.

Typical recordings of the effect of indomethacin on the responses to epinephrine and serotonin are shown in Fig. 5. The pressor responses to close i.v. injections of 0.03, 0.1 and 0.3 μg of epinephrine and 0.3 and 1.0 μg of serotonin were abolished or greatly reduced during the close i.v. infusion of indomethacin.

Indomethacin and sodium salicylate displaced the dose-response curves for epinephrine and serotonin downward, as shown in Fig. 6.

In studying the effects of aminopyrine, phenylbutazone, benzydamine and hydrocortisone, acetylcholine, histamine and bradykinin as well as epinephrine and serotonin were used as venoconstrictor agonists. The results obtained are shown in Fig. 7. Aminopyrine, 300 $\mu\text{g}/\text{min}$, and benzydamine, 1.0 $\mu\text{g}/\text{min}$, depressed the venoconstriction produced by epinephrine and serotonin as were the cases with indomethacin and sodium salicylate. Concurrently the responses to acetylcholine and histamine were also depressed markedly. Phenylbutazone, 150 $\mu\text{g}/\text{min}$, inhibited the venoconstrictor responses to the agonists nonspeci-

FIG. 6. Effects of sodium salicylate and indomethacin on epinephrine (Epi)- and serotonin (5HT)-induced changes in perfusion pressure by both anti-inflammatory drugs in dog paw venous perfusion experiments with Krebs-bicarbonate solution.

Solid lines : before infusion and broken lines : during infusion of anti-inflammatory drugs. Each point represents mean value obtained from 4 dogs.

FIG. 7. Effects of aminopyrine, phenylbutazone, benzydamine and hydrocortisone on the changes in perfusion pressure induced by epinephrine (Epi), serotonin (5HT), bradykinin (Bk), acetylcholine (ACh) and histamine (Hist) in dog paw venous perfusion experiments with Krebs-bicarbonate solution. The anti-inflammatory drugs were infused and the vasoactive agents were injected close i.v. into the paw small vein. Solid lines : before infusion and broken lines : during infusion of the anti-inflammatory drug. For bradykinin, closed circles : before infusion and open circles : during infusion of the anti-inflammatory drug. Each point represents mean value obtained from 4 dogs.

fically. Consequently it is recognized that all the non-steroidal anti-inflammatory drugs used in the present study nonspecifically depress the venoconstrictor responses induced by the agonists in the perfused paw small vein. On the other hand, it was demonstrated that 20 $\mu\text{g}/\text{min}$ of hydrocortisone tended to enhance the effect of epinephrine and gave no demonstrable influence on the pressor responses to serotonin, acetylcholine, histamine and bradykinin.

DISCUSSION

Blood perfusion of the canine paw small vein evoked abrupt spasms in most experiments undertaken. In Krebs-bicarbonate perfusion experiments, the venous tone was lower than that in blood perfusion. The ability of normal blood or plasma to maintain vascular tone has long been recognized, although the vasoactive component in blood or plasma is yet undefined (3). The abrupt spasms observed in blood perfusion experiments occurred with high vascular tone. These venospasms were not inhibited by α -chloralose, pentobarbital or phentolamine or sections of the radial, median, ulnar and musculocutaneous nerves in the forelimb. Therefore, the spasms do not appear to be related to the "neurogenic factor". They were abolished by papaverine given close i.v. Such fluctuations in perfusion pressure were neither observed in the Krebs-bicarbonate perfusion experiments, nor in the pressure

of the particular paw small vein recorded in the arteriovenous consecutive segmental perfusion experiments with blood in the canine forelimb (our preliminary study). Ng, et al. found similar abrupt spasms in the isolated rabbit ear artery perfused with blood or plasma (4), but in the spiral segment of blood vessels no spasms have been observed (3). From these results the effect of certain endogenous "humoral factors" in blood may be one of the causative factors for the spasms. Furthermore, "myogenic factors" of vascular smooth muscles of the vein and pulsatile property of pumped flow may also play an important role in the abrupt venospasms.

Haddy, et al. reported that serotonin had a venopressor activity in the paw small vein when injected into the brachial artery in the arteriovenous segmental perfusion preparation of the canine forelimb (5, 6). In the present study it was confirmed by venous perfusion experiments that serotonin caused an intense venoconstriction *in situ*. On the other hand, in the arterio-venous preparation acetylcholine injected i.a. causes only a venodepressor response (7, 8), but in the present venous perfusion preparation it was demonstrated that acetylcholine produced a much stronger venoconstrictor response than did histamine or bradykinin. Rice and Long showed that acetylcholine directly injected into the perfused accessory cephalic vein of the dog produced a marked rise in perfusion pressure, and the pressor response was blocked by atropine and phentolamine (9). However in the venous perfusion preparation, acetylcholine-induced venopressor response was not blocked by phentolamine (our preliminary study). In blood perfusion, acetylcholine produced a considerably strong venoconstriction in the paw small vein, whereas in Krebs-bicarbonate perfusion experiments, the action of acetylcholine was extremely weaker. Bradykinin was reported to produce no response of the isolated dog subcutaneous veins (3), and in our Krebs-bicarbonate perfusion experiments, bradykinin caused a slight venoconstrictor response. In blood perfusion preparation, however, bradykinin produced a significant venoconstrictor response in about half of the number of dogs used. From these results, responses of this venous perfusion preparation to acetylcholine and bradykinin were demonstrated to be potentiated by blood components. Hirako (10, 11) in his qualitative experiments using isolated rabbit ear found that when bradykinin was administered, the ear marginal vein was constricted under the influence of both blood and Ringer's solution, whereas the central artery was unaffected in the perfusion experiments with Ringer's solution but dilated in those experiments where blood was perfused. He also suggested from this finding that serum components except for protein participate in the difference of the arterial responses to bradykinin.

As changes in cephalic venous pressure were little in comparison with those in perfusion pressure, epinephrine- or serotonin-induced pressor effect may be attributed to an active constriction of the large vein. While, the magnitude of pressor response to bradykinin, histamine or acetylcholine was proportional to that of a fall in systemic arterial pressure caused by each agonist, as shown in Fig. 4, and the changes in both pressures occurred at the same time, suggesting that the rises in cephalic venous pressure produced by bradykinin, histamine and acetylcholine were caused by the falls in systemic arterial blood pressure, pos-

sibly via arterio-venous shunts in the forelimb or due to sympathetic reflex (in part, catecholamines released from the adrenal glands) induced by hypotension.

In the state of extremely high vascular tone, histamine and bradykinin produced a prolonged venodilatation in the preliminary study. In the state of normal vascular tone, the agents in high doses, which altered the systemic arterial blood pressure, caused only a venoconstrictor response in the canine paw small vein. Rowley proposed that the mediators released in the inflammatory process might enhance vascular permeability by increasing the intracapillary or intravenous hydrostatic pressure due to dilating the arterial side and constricting the venous side (1). The present findings show that bradykinin and histamine have relatively weak venoconstrictor activities in the paw small vein, whereas acetylcholine, which has been said not to enhance the permeability, caused much stronger venoconstriction than did either bradykinin or histamine. Therefore, even if the potency of the vasodilating activities in arterial side (preliminary study) is taken into consideration, the enhancement of permeability cannot be accounted for only by Rowley's proposal (1). It is a generally accepted fact that the primary trigger required to form edema is the direct action of mediators to the endothelial cells of fine blood vessels. In this context, Majno (12) suggested that in the experiments on carbon leakage, hydrostatic pressure alone cannot mimic the venular leakage induced by histamine and other mediators. Furthermore he also reported (13) that in ultrastructural investigations, deformations of the rat vascular endothelial nuclei were observed under the influence of histamine, bradykinin and serotonin, and suggested that these agents stimulate an active endothelial contraction which leads to the pulling apart of venular endothelium with the consequent increase in venular permeability.

Northover found that anti-inflammatory drugs such as aspirin, phenylbutazone, fenclazic acid, indomethacin, meclofenamic acid and flufenamic acid inhibited the uptake of calcium by unfractionated membranes derived from the human umbilical venous endothelial cells (14). This finding suggests that anti-inflammatory drugs would inhibit the uptake of outer medium-calcium which in turn would lead to the deformation of the endothelial cells of capillary or venular walls. However, it can reasonably be assumed that once inter-endothelial gaps are formed, both the entrance of massive blood volume into the capillary vessels due to arterial dilatation and the blockade of drainage of venous blood due to venoconstriction, result in an extremely high pressure in the fine blood vessels, thus facilitating the enhancement of vascular permeability. Northover reported that in the isolated anterior mesenteric vein and artery of rats and guinea-pigs, nonsteroidal anti-inflammatory drugs inhibited epinephrine-, histamine- and serotonin-induced constrictor responses nonspecifically (2). In the present study it was confirmed in *in situ* experiments that nonsteroidal anti-inflammatory drugs in anti-inflammatory doses showed nonspecific, depressive effects to the venoconstrictor responses to vasoactive agents in the dog paw small vein perfusion. Furthermore, the drugs tended to dilate the small vein. In our venous perfusion preparations in preliminary experiments, nonsteroidal anti-inflammatory drugs also inhibited the venoconstrictor responses to prostaglandin E_2 or $F_{2\alpha}$.

Prostaglandins are thought to be one of the important mediators of inflammation and

inflammatory actions of the aspirin-like drugs are attributable for the greater part to the interference with endogenous prostaglandin biosynthesis (15, 16). It is suggested that anti-inflammatory effects of these drugs may be based, in part, on the activities which inhibit the secondary facilitation of vascular permeability due to the change in hydrostatic pressure.

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