Tissue extracts from the wall of the aorta of man and rabbit, and especially from the internal portion of the wall, contain a substance having thromboplastic activity. The physiological significance of this substance is unknown, but evidence to be given below indicates that epinephrine, in physiological concentrations, will release a substance having thromboplastic activity from the walls of the perfused aorta. In a previous paper it was shown that administration of epinephrine to intact rabbits induced an immediate edematous reaction in the arterial wall, a platelet and leucocyte-sticking reaction, and a shortening of clotting time. Electron microscopy of the endothelial walls suggested that vesicles might discharge substances from the endothelium into the vessel lumen under the influence of epinephrine. Moreover, pretreatment of the animals with a monoamine oxidase inhibitor prevented the edematous reaction as well as the reduction in clotting time.

In order to determine whether or not the thromboplastic active substance is released from the arterial wall directly into the vessel lumen by epinephrine, a series of in vitro perfusion experiments was performed, using aortas from rabbits. This paper reviews 1. the special effect of epinephrine in physiological dosage which releases thromboplastic active substances into the vessel lumen from the arterial wall, 2. the preventive effect of the monoamine oxidase inhibitor nialamide, and 3. the ineffectiveness of norepinephrine as well as of larger doses of epinephrine.

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Methods

Operative Procedures

Seventy-five male rabbits weighing 2.0 to 2.7 kg were used. The animals were anesthetized with urethane 1.2 g subcutaneously. The chest was opened quickly and the aorta was ligated directly above the aortic valve. A plastic tube connected to a bottle containing oxygenated 0.9% NaCl at 37°C was inserted into the aorta and the aorta was perfused under a pressure of 100 mm Hg thus preventing reversion of perfusate from the tissue into the aortic lumen. Thereafter the main branches of the aorta, and the subclavian, carotid, hepatic, renal, and femoral arteries were ligated on both sides. In ligating these branches, especially the carotid artery, special precaution was taken not to exclude branches extending to the vasa vasorum which irrigate a large portion of the aortic wall as shown in figure 1. Thus a portion of the perfusate, entering the vasa vasorum, irrigates the aortic vessel lumen whereas another portion of the perfusate which irrigated the aortic vessel lumen but did not perfuse the vasa vasorum. All perfusions were completed within 60 minutes.

Calcium Clotting Time

Samples of perfusate flowing at a rate of 1.5 ml/min were collected in glass tubes from the femoral artery. They were colorless and contained no blood corpuscles. After centrifugation and filtration through cotton wool they were tested for thromboplastic activity at 37°C by the method of Biggs and MacFarlane. To 1 ml of the perfusate was added 0.1 ml fresh rabbit citrated plasma containing in siliconized glassware at 37°C. For the production of citrated rabbit plasma, 6.0 ml of blood were drawn by heart puncture using a siliconized syringe and to the blood in the siliconized glassware were added 4.0 ml of sodium citrate solution, containing 3.13 g of sodium citrate/100 ml. The citrated blood was centrifuged at 2,000 rev/min for 10 minutes, and three ml of the clear supernatant, containing almost no observable platelets, were used. To the mixture of perfusate and citrated plasma, which had been kept by thermostat at 37°C for two minutes, was added 0.2 ml 1/40 M CaCl2 at the same tempera-
RELEASE OF THROMBOPLASTIC SUBSTANCE

ture. The time which elapsed before the plasma solidified was measured. The mixture was kept in the thermostat at 37° C and the abrupt appearance of fibrin was taken as the endpoint of the calcium clotting time. The determination was repeated until the two measurements agreed to within 10.0 seconds; the mean of the two was taken as the calcium clotting time. The endpoint was clear-cut and the variation in tests on the same sample was less than nine seconds.

ADDITION OF DRUGS

Single injections were made in 1 ml containing 0.0005, 0.005, 0.05, and 0.5 μg epinephrine (Sankyo Co.) or norepinephrine (Levophed). The calcium clotting time of the perfusate was measured before and every minute after the injections. In other experiments, the aortas were perfused continuously for 15 minutes with 0.9% NaCl containing epinephrine at a concentration of 0.005 μg/ml. The effects of continuous perfusion with epinephrine could then be compared with effects of single injections.

Nialamide (Niamid, Chas. Pfizer Co., Ltd.) was administered orally in a dose of 5 mg/kg two hours before sacrificing the animals. Nialamide was also used in the perfusate at concentrations of 0.05, 0.5, and 5 mg/ml.

PERFUSION WITH TYRODE'S SOLUTION

0.9% NaCl was first used for perfusion because the endpoint in the determination of calcium clotting time of the perfusate was clear and the procedure caused no dissociation of endothelial cells as revealed by electron microscopy. However, we felt it possible that perfusion with a calcium-free solution might have some effect on intercellular substance of the aortic endothelium as found by Zweifach with capillary endothelium.

For this reason oxygenated Tyrode's solution was used as an alternative to 0.9% NaCl in five experiments. In the determination of calcium clotting time, 1 ml of the perfusate of Tyrode's solution and 0.2 ml of 1/40 M CaCl₂ were kept at 37° C for two minutes and mixed in a test tube. 0.1 ml of platelet-poor plasma was added and the time measured until the fibrin appeared. The platelet-poor plasma, which was preserved at 4° C was taken out immediately before the test and used after keeping the plasma in the thermostat at 37° C for two minutes.

Results

CONTROL EXPERIMENTS

The calcium clotting time of the mixture of citrated plasma and 1 ml of 0.9% NaCl was 172.0 ± 5.9 sec and of the perfusate of all 75 cases before each challenge was 160.5 ± 4.9 sec. Addition of epinephrine to the fluid did not alter the clotting time. During perfusion of six control preparations with oxygenated saline, the calcium clotting time of the perfusate exhibited a slight but continuous prolongation from a starting value of 135.2 ± 10.6 sec to 155.7 ± 9.7 sec one hour
FIGURE 2
Calcium clotting times (means and SE) of samples from six aortic perfusions used as controls.

FIGURE 3
Calcium clotting times (means and SE) of aortic perfusates before and after injection of epinephrine as follows: A. 0.0005 μg (3 aortas); B. 0.005 μg (7 aortas); C. 0.05 μg (5 aortas); D. 0.5 μg (5 aortas).

FIGURE 4
Calcium clotting times (means and SE); three aortas perfused with 0.9% NaCl containing 0.005 μg/ml epinephrine.

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After the beginning of the perfusion as shown in figure 2.

EFFECTS OF EPINEPHRINE

As shown in figure 3A, the calcium clotting time of the perfusate showed no significant change, after the injection of 0.0005 μg of epinephrine, while the calcium clotting time after the injection of 0.005 μg of epinephrine shortened dramatically (fig. 3B) and the shortening continued for one to three minutes, returning to the control value without any prolongation except in two cases. In these cases the calcium clotting time shortened at first and then was prolonged by 28.0 and 28.3 seconds. The shortening of the calcium clotting time at one and two minutes after the injection of 0.005 μg of epinephrine was statistically significant (P < 0.01). In five cases the epinephrine injection was performed within 30 minutes after beginning perfusion with oxygenated saline, but in the remaining two cases it was performed at 45 and 50 minutes after perfusion started. However, all cases responded well to this challenge.

A second injection of 0.005 μg of epinephrine was performed 30 minutes after the first injection in all experiments. In two cases the calcium clotting time shortened more than 20 seconds one or two minutes after the second injection. In the remaining five cases the second challenge was ineffective.

Shortening of the calcium clotting time was also observed after injection of 0.05 μg epinephrine (fig. 3C). However, the shortening was slight and of doubtful significance in comparison with the case of 0.005 μg of epinephrine. In one case the initial shortening
was followed by a transient prolongation of calcium clotting time four to nine minutes after the epinephrine. This time was 49 seconds longer than that of the perfusate before the epinephrine injection.

In the case of 0.5 μg of epinephrine no shortening of the calcium clotting time was found (fig. 3D). In one case the prolongation was found three minutes after the epinephrine injection.

In three experiments the aortas were perfused continuously with saline containing 0.005 μg/ml epinephrine. The calcium clotting time shortened continuously without prolongation, and after the exchange of the epinephrine solution with physiological saline, the calcium clotting time of the perfusate recovered slowly. This phenomenon was repeated successively by the second challenge as shown in figure 4.

As shown in figure 5, in five preparations the infusion of physiological saline was performed without oxygen for 30 minutes and thereafter 0.005 μg of epinephrine was injected. There was no appreciable change in the calcium clotting time.

EFFECTS OF MONOAMINE OXIDASE INHIBITOR, NIALAMIDE

Figure 6 shows that following injection of epinephrine in doses of 0.0005, 0.005, 0.05, and 0.5 μg into the preparations from 20 rabbits pretreated with five mg/kg of nialamide, no shortening of calcium clotting time was produced.

On the other hand, in the preparation perfused directly in vitro by the nialamide-saline solution in a concentration of 0.05 mg/ml in three cases and 0.5 mg/ml in two cases, the injection of 0.005 μg of epinephrine induced a significant shortening in the calcium clotting time of the perfusate one minute after the epinephrine injection (P < 0.05). Recovery took place three to five minutes thereafter, i.e., the same as in the case of the preparations without the nialamide infusion shown in figure 7. The direct perfusion of the aortic
Calcium clotting times (means and SE); five aortic preparations perfused directly by nialamide-saline solution before and after injection of 0.005 μg epinephrine.

Calcium clotting times (means and SE); five aortic preparations perfused by Tyrode’s solution before and after injection of 0.005 μg of epinephrine.

Preparation with nialamide, i.e., the application of nialamide in vitro, was found not to prevent the appearance of the thromboplastic active substance in the perfusate. High concentrations of nialamide, such as five mg/ml prevented clotting of the test plasma itself.

PERFUSION WITH TYRODE’S SOLUTION

Five aortas were perfused with oxygenated Tyrode’s solution. Following injection of 0.005 μg of epinephrine the calcium clotting time was again shortened significantly (P < 0.05), (fig. 8).

NOREPINEPHRINE

In doses of 0.005 μg in three cases, 0.05 μg in three cases, and 0.5 μg in three cases, no shortening was found in the calcium clotting time of the perfusate, (fig. 9A, B, and C).

Discussion

The most important finding in these experiments is the release of a thromboplastic active substance directly from the vessel wall of the aorta into the vessel lumen. This appears to be induced specifically by a physiological dose of epinephrine.

The ineffectiveness of a larger dose of epinephrine as well as of norepinephrine, together with the preventive effect of pretreatment of animals with a monoamine oxidase inhibitor, suggests that release of thromboplastic active substance might originate from mechanisms related to the edematous vascular reaction. This edematous vascular reaction is induced in the rabbit aorta as an immediate reaction by the single injection of a physiological dose of epinephrine such as 0.1 to 1.0 μg/kg but not by a dose of epinephrine larger than 10 μg/kg nor...
by norepinephrine. The ineffectiveness of a larger dose of epinephrine was found to originate from its vasoconstrictive effect on the vasa vasorum. This was shown by the authors, who employed a histological technique using India ink. The edematous vascular reaction has also been found to be prevented by pretreatment of animals with the monoamine oxidase inhibitor, nialamide. The fact that release of thromboplastic active substance by epinephrine does not take place during perfusion with non-oxygenated saline, suggests that we are dealing with a biological phenomenon that can occur in living animals. Also, the well-known enhancement of blood coagulability induced by epinephrine, may be considered to originate from the same mechanism, though perhaps not completely.

The second important finding in these experiments stems from failure of nialamide, added directly to the perfused aorta, to prevent release of thromboplastic active substance by epinephrine. This is in contrast to the effectiveness of nialamide when it is injected into the animal before removing the aorta. We know also, from the work of Thompson and Tickner, that monoamine oxidase is normally present in the walls of blood vessels. These facts, taken together, suggest that nialamide exerts its inhibitory action only if it can reach the site of monoamine oxidase in vivo via the vasa vasorum and thus prevent the edematous vascular reaction.

During the period of approximately 100 years since Virchow, it was thought that arterial walls were supplied with nutrients not only from the vasa vasorum but also from the vessel lumen. However, except for the presence of pinocytotic vesicles in the cell membrane of endothelial cells, there is no direct evidence which supports the hypothesis of Virchow. Moreover, we could not find any evidence indicating increased uptake of substances by endothelial cells when the artery exhibited an edematous reaction following administration of epinephrine or cholesterol. On the contrary, our electron microscopic observations indicated a possible release of some substances from the endothelial cells under these conditions.

Cannon considered that enhancement of blood coagulability after administration of epinephrine might be due to release of some potent substance from the liver. Tocantins found that epinephrine induces shortening of one-stage prothrombin time and considered that some hyperglycemic effect of epinephrine might be involved in the previously mentioned effect. In preliminary experiments, we have found that epinephrine seems to release certain substances which are capable of shortening the one-stage prothrombin time in the perfusate from perfused, isolated hind leg preparations of rabbits. This finding suggests that release of an active thromboplastic substance by epinephrine takes place not only in the liver but also in the isolated hind leg preparation.

Finally, we should discuss the prolongation of calcium clotting time, following large doses of epinephrine. Presumably, this indicates release of an anti-thromboplastic heparin-like substance. This is especially interesting because it is well known that the arterial wall contains heparin. However, the dose of epinephrine required to elicit this response was so high that the physiological significance of the phenomenon is difficult to evaluate.

Summary

Aortic preparations of 70 rabbits including the vasa vasorum were perfused in vitro by oxygenated saline under a pressure of 100 mm Hg. The main branches of the aorta were ligated and the perfusate, which irrigates the aortic lumen, but not the vasa vasorum, was sampled every minute. The samples were assayed for thromboplastic activity by means of the calcium clotting time. The calcium clotting time was shortened significantly in samples taken one, two, and three minutes after injection of 0.005 μg epinephrine. The response was present in perfusing with Tyrode’s solution as well as with 0.9% NaCl but was absent if oxygen was withheld from the solution. Larger doses of epinephrine (0.05 μg) caused an initial shortening of the
calcium clotting time, followed by a prolongation in two out of five cases. Still larger doses (0.5 \( \mu g \)) or much smaller ones (0.0005 \( \mu g \)) were without effect.

In the aortas of rabbits which were pre-treated by 5 mg/kg of ni-lamidé two hours before removing the aorta, the previously mentioned effect of epinephrine was prevented. However, direct perfusion of the aortic preparation by a ni-lamidé mixture in vitro did not prevent the release of thromboplastic active substance by epinephrine.

Norepinephrine in doses of 0.005, 0.05, and 0.5 \( \mu g \) was found to be ineffective. These observations may explain the effects of epinephrine on the clotting time of blood originally described by Cannon and Gray.\(^5\)

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