Contraction of Vascular Smooth Muscle in Response to Plasma

COMPARISON WITH RESPONSE TO KNOWN VASOACTIVE AGENTS

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ABSTRACT

Addition of plasma to the physiologic salt solution in which helical strips of vascular smooth muscle were suspended caused the muscle to contract. The plasma was obtained from dog arterial blood which, extracorporeally, had been in contact only with siliconized polyethylene in the cold. The strips were from large and small vessels from several sites in rabbit and dog. The contractions were reproducible and dose dependent. Threshold concentrations for contraction ranged from 2% to 30% depending on the source of the smooth muscle. In concentrations too low to produce contraction, plasma potentiated tension developed in response to stimulation by epinephrine, angiotensin, and KCl. The response of the strips to plasma was compared with their response to the several known vasoactive agents of plasma: epinephrine, norepinephrine, serotonin, angiotensin, vasopressin, bradykinin and histamine. Important differences were found. It is possible that in normal plasma there is an as yet undefined vasoactive component which in situ may play a role in the maintenance of vascular tone.

ADDITIONAL KEY WORDS plasma vasoconstrictor vasopressin plasma potentiation of vasoconstriction angiotensin serotonin vascular tone adrenergic blockade catecholamine vascular smooth muscle from dogs and rabbits

The ability of normal plasma to enhance the contractility of cardiac and vascular muscles has long been recognized. Publications describing these effects have recently been reviewed for cardiac muscle by Nayler et al., and for vascular smooth muscle by Wurzel. The nature of the plasma component involved and the physiological importance of these actions have not been satisfactorily defined.

It is the purpose of the current study to characterize the action of plasma on vascular smooth muscle in an isolated bath of physiologic salt solution, and to determine whether this action is similar to that of any known vasoactive agent contained in plasma.

Methods

Helical strips of vascular smooth muscle from large and small vessels (3 mm down to .25 mm o.d.) from several sites in two species were studied: rabbit aorta and vena cava (strips approximately 1.5 x 7 mm); dog aorta, vena cava 1.5 x 7 mm), small subcutaneous artery and vein, and coronary artery (all approximately .15 x 3 mm). The strip was mounted in a bath of physiologic salt solution (PSS) at 37°C; the composition of the PSS in mmole/liter was: NaCl, 119; KCl, 4.7; KH2PO4, 1.18; MgSO4, 1.17; NaHCO3, 14.9; dextrose, 5.5; sucrose, 50; CaCl2, 1.6; and calcium disodium versenate, 0.026. The versenate is added to chelate possible trace amounts of heavy metals which catalyze the auto-oxidation of catecholamines. Plasma has this same chelating action. It was necessary to equilibrate the PSS with 95% O2, 5% CO2 before adding it to the bath because of frothing that develops with
bubbling in a bath after plasma has been added. Control studies indicate that a reduction in the partial pressure of oxygen in the PSS, from 600 to 100 mm Hg (which is a greater reduction than that which would be expected from the introduction of large volumes of nonaerated plasma) does not appreciably alter the responsiveness of vascular smooth muscle. pH of both plasma and PSS was approximately 7.35. Contractions were monitored by recording isometric tension with a Grass displacement transducer (FTO3) and a Grass polygraph.

Plasma was obtained from mongrel dogs, usually fasted for 18 hours to avoid a milky plasma. Fasting did not alter vasoactivity of the plasma. Large polyethylene tubing (PE 280) was siliconized and inserted in the femoral artery of a dog anesthetized with sodium pentobarbital. Blood was allowed to flow freely into siliconized polyethylene test tubes containing heparin (5 mg/100 ml blood), immediately cooled to 0° C, and centrifuged at 4° C at high speeds (10,000 X g for 30 min) to remove formed elements, including platelets. The responses to be described were obtained with plasma from the first few milliliters of blood drawn from the animal and from the last few milliliters of a rapid bleeding to death. There was greater activity in plasma obtained near the end of bleeding, but this additional activity in "late" plasma differed from the activity of "early" plasma in that it could be blocked by dibenzyl-

**Results**

**ACTION OF PLASMA ON ISOLATED SMOOTH MUSCLE**

**Stimulation of Contraction**

Plasma in a concentration of 30% in the PSS bath produced contraction of each type of vascular smooth muscle studied. We have described this phenomenon in rabbit aorta, rat aorta, and in dog mesenteric artery, coronary artery and subcutaneous artery and vein. Contraction begins within 2 to 15 sec, reaches its peak within 15 sec to 30 min depending on the particular smooth muscle studied and on the plasma concentration, and usually persists as long as the plasma is left in the bath (this was followed for 1 hour). The configuration of the initial part of the response of the dog coronary artery to plasma is shown in Figure 1. The degree of persistence of the response depends to some extent on the source of the smooth muscle stimulated;

**FIGURE 1**

Upper record: Responses of dog coronary artery (500 μ o.d.) to 20% plasma (0.4 ml plasma added to a 1.6-ml bath of PSS). The response does not show tachyphylaxis. Lower record: Responses to plasma at concentrations of 5% to 60% in the bath. The response is dose dependent. Responses are shown as percent of maximum tension developed by the same strip when stimulated with epinephrine. In absolute values, 10% of maximum tension = 10 mg; R = rinse with warmed PSS equilibrated with 95% O₂, 5% CO₂.
the response of rabbit aorta is always persistent, whereas that of dog coronary artery may decline in the continued presence of plasma.

The magnitude of the smooth muscle response is dose dependent (Fig. 1, lower tracing). The concentration required for threshold contraction varies greatly, depending on both the source of the smooth muscle and the potency of the plasma sample; all samples from one species of animal are not equally potent. The unequal potency is probably not related to differences in electrolyte composition of the several plasmas since vascular smooth muscle strips do not contract when the electrolyte composition of the PSS surrounding them is altered substantially. Of the smooth muscles studied, that from the subcutaneous vein of the dog has the lowest threshold and rabbit aorta has the highest. The concentration of plasma required for threshold contraction ranged from 2% to 30%. The maximum response to plasma never exceeded 50% of a maximum contraction produced by epinephrine (Fig. 1, lower tracing). Plasma was capable of producing its maximum effect right after centrifugation, and its potency did not change during a week of storage at 0°C. Incubation for 4 hr at 38°C diminished its potency by about one-half.

The effect of plasma on the contraction of rabbit aorta produced by other agents was evaluated with epinephrine, angiotensin and KCl as stimulating agents. Plasmas prepared from 11 dogs were tested in 30 experiments. Amounts of plasma were used which would make up 1/8% to 8% of the final bath volume. The plasma was mixed with the PSS of the bath 3 min before injection of a standard amount of the constrictor agent. A concentration of constrictor agent was chosen which would, used alone, produce about one-third maximum response. Test responses were bracketed by control responses to the stimulating agent in the absence of plasma (Fig. 2). Under optimal conditions, 1% plasma in the bath always potentiated responses to epinephrine, angiotensin, or KCl. The magnitude of the potentiation was concentration dependent; the threshold concentration for potentiation was 1/4% to 1% plasma; average potentiation of the presence of 2% plasma was 100% of the control response. This alteration in vascular responsiveness far exceeds that which could have been produced by a change in electrolyte composition of the bath effected by the addition of this small amount of plasma. Usually, 8% plasma itself caused no contraction of rabbit aorta. We have no direct

**FIGURE 2**

**Responses of rabbit aorta to epinephrine 1 µg/liter with and without plasma in the bath.**

Plasma potentiates the response; the effect is concentration dependent.

Circulation Research, Vol. XIX, September 1966
evidence as to whether this potentiating action of plasma is caused by the same plasma component that, in higher concentrations, causes contraction of isolated vascular smooth muscle.

**COMPARISON OF CONTRACTION PRODUCED BY PLASMA WITH THAT PRODUCED BY KNOWN VASOACTIVE AGENTS OF PLASMA**

**Catecholamines**

The possibility that the contractile effect of plasma is due to catecholamines was tested by the use of an alpha-adrenergic blocking agent, dibenzyline, which in the concentration used, causes no mechanical response of the muscle. In studies on 13 preparations of dog subcutaneous artery and 6 of dog vein, the response to plasma persisted after the response to epinephrine or to norepinephrine, in concentrations that had given control responses equal to those of plasma, was completely eliminated by the blocking agent (0.1–1.0 mg/liter) (Fig. 3). Similar failure of dibenzyline to block contraction in response to plasma has been found in dog coronary artery and in rabbit aorta. In some instances treatment of vascular smooth muscle with dibenzyline in these concentrations had no effect on the magnitude of the response to plasma; in others it produced a significant reduction in tension development. In an earlier study we observed that the potency of the plasma was increased with hemorrhage, probably reflecting increased concentrations of catecholamines. When dibenzyline failed to reduce the plasma response, the plasma probably did not contain a detectable concentration of catecholamines; when the response was reduced, some of the control response may have been due to catecholamines.

The effect of dibenzyline blockade was also studied in five experiments in which low concentrations of plasma were used to potentiate responses of dog subcutaneous arteries and veins to KCl or angiotensin. Dibenzyline blockade neither reduced responses of these agents nor altered the potentiating action of plasma. This was also true when rabbit aorta was the tissue used.

Further evidence of the dissimilarity between the responses to plasma and those to epinephrine and norepinephrine is to be found in the observation that plasma causes contraction of smooth muscle from small coronary vessels (Fig. 1), whereas both epinephrine and norepinephrine cause only relaxation. When smooth muscle from a small coronary artery is made to contract in response to plasma, adding epinephrine or norepinephrine to the bath causes partial relaxation.

Responses of dog subcutaneous artery (500 μ o.d.) to epinephrine, serotonin and plasma, before and after treatment with dibenzyline (1 mg/liter for 6 min). The response to plasma persists after alpha-adrenergic blockade; those to epinephrine and serotonin are respectively eliminated and greatly diminished.

*Figure 3*
**PLASMA VASOACTIVITY**

**Serotonin**

The possibility that the active factor in plasma is serotonin was ruled out by the observation that the response to serotonin is eliminated or greatly reduced by dibenzyline, whereas the response to plasma is not. The persistence of the response to plasma after the response to serotonin had been blocked (Fig. 3) was observed in 8 dog subcutaneous arteries and 1 vein. In an earlier study, we reported that lysergic acid diethylamide (LSD) inhibits the response to serotonin, but potentiates the response to plasma. Serum has approximately 10 times the constrictor potency of plasma; presumably the basis for this greater activity is the serotonin released by platelets during the clotting process. The effect of serum on isolated vascular smooth muscle is almost blocked by dibenzyline.

**Angiotensin**

The response to angiotensin differs from that to plasma in that angiotensin fails to sustain a contraction of most types of isolated vascular smooth muscle (rabbit aorta is an exception). This is true of vascular smooth muscle from dog subcutaneous (Fig. 4, second tracing from top), coronary and mesenteric arteries. In these preparations the response to angiotensin reaches a peak within 1 min, then, while the angiotensin is still in the bath, the muscle relaxes completely. In contrast, the response to plasma tends to remain near maximum as long as the plasma is left in contact with the muscle. The presence of a small amount of plasma does not alter the transient nature of the response to angiotensin.

The mechanism that is responsible for the evanescence of the effect of angiotensin may also account for the rapid and complete tachyphylaxis to angiotensin that is seen in most isolated vascular smooth muscle. In five experiments using dog subcutaneous artery, the response to plasma persisted during the period of complete tachyphylaxis to angiotensin (Fig. 4). The tachyphylaxis usually lasted from 1 to 2 hours before responsiveness to angiotensin returned. Responses to repeated administrations of plasma, on the other hand (as shown for coronary artery in Fig. 1, upper record), frequently did not diminish in magnitude, and where there was some diminution in the magnitude of contraction with repeated administrations, the response was never completely abolished as was the response to angiotensin.

In studies of the effect of low concentrations of plasma on the epinephrine response, no diminution in the potentiating activity of plasma was observed when it was used as many as six times on the same tissue.

**Vasopressin**

The possibility that vasopressin is responsible for the contractile action of normal plasma was studied using 9 dog subcutaneous arteries. The response of these vessels to vasopressin resembles that to plasma. It seems unlikely, however, that vasopressin can be the factor responsible for the contractile activity of plasma. Its presence in normal plasma is estimated to be in the neighborhood of 10 micro units/ml, to be responsible for the observed effects of whole plasma on subcutaneous arteries in the isolated bath, it would have to be present in a concentration many hundred times this. The vasopressin content of the plasma used may well
FIGURE 5
Responses of strips from dog subcutaneous artery (300 μ o.d.) and vein (500 μ o.d.) mounted in a common bath, to vasopressin and to plasma. Vasopressin has a greater effect on the artery than on the vein; the reverse is true for plasma.

FIGURE 6
Relaxing effect of histamine when added during response of dog subcutaneous artery (400 μ o.d.) to plasma.

have been higher than that of normal plasma, but no values approaching that necessary to account for these contractions have been reported for shed blood. Substantiating this quantitative evidence that the plasma contractile factor is not vasopressin is the observation that, whereas vasopressin has a greater effect on smooth muscle of the artery than on that of the vein, the reverse is true for plasma; it has a greater effect on the vein than on the artery (Fig. 5).

Bradykinin
Even in high concentrations (1 mg/liter), bradykinin does not produce a response of dog subcutaneous arteries or veins, or of rabbit aorta.

Histamine
Even in high concentrations (5 mg/liter), histamine fails to produce contraction of dog subcutaneous artery; however, when smooth muscle from this vessel is made to contract with plasma, the addition of histamine causes some relaxation (Fig. 6).

Discussion
The current study has characterized the effect of plasma on isolated vascular smooth muscle. Most preparations of vascular smooth muscle mounted in a bath of physiologic salt solution remain completely relaxed unless stimulated by a vasoactive agent. The various vasoactive agents show striking individualities in their effects on vascular smooth muscle from different sites in the body and different levels of the vascular tree. When an unknown vasoactive material is used to stimulate isolated vascular smooth muscle from various sites, these individualities provide information about the identity of the stimulating agent. Table 1 lists our current observations, which demonstrate that the responses to plasma differ in important ways from those of the vasoactive materials known to be in plasma. The cardioactive fraction (kinekard) described by Nayler et al. has many vascular effects that resemble those observed for whole plasma in the current study. The major basis for doubting that the whole plasma effect is due to "kinekard" is that the latter appears to be a coronary vasodilator, whereas plasma causes contraction of smooth muscle from isolated small coronary vessels. Furthermore, the vasoconstrictor action of kinekard...
Comparison of Response to Plasma with Response to Known Vasoactive Agents

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<td>contraction</td>
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on limb vessels is blocked by prior administration of phenoxybenzamine.13

Our demonstration that plasma influences vascular smooth muscle contraction in a way unlike that of any known plasma component raises the following questions:

1. Are the characteristic responses produced by plasma the result of a single agent or the interaction of multiple vasoactive agents in plasma? The mere fact that plasma contains many known vasoactive agents suggests that the contraction may be due to some interaction or potentiating action of these agents. The observation that no single known agent of plasma can simulate the effect of whole plasma may indicate that whole plasma contains an as yet unidentified constrictor agent, or that the particular combination of known agents forms a resultant stimulant that is quite different from any single agent. One clue in support of the latter possibility is the marked potentiation of the epinephrine response by plasma. This relationship, however, cannot be responsible for the action of plasma because plasma causes contraction in the presence of an alpha-adrenergic blocking agent, whereas epinephrine does not. In support of the possibility that the response is caused by a single agent is the fact that whereas the known agents tend to produce markedly individual responses in vascular smooth muscle from different sources, whole plasma is relatively consistent in its tendency to produce contraction of all vascular smooth muscle studied.

2. Are the two actions studied—direct stimulation and, in low concentrations, potentiation of the action of another vasoactive agent—both caused by the same component of plasma? This question cannot be answered with the evidence at hand. The only suggestive information available is that plasmas which have shown greater power to produce direct contraction of vascular smooth muscle have also shown greater power to potentiate responses to the known constrictor agents tested (epinephrine, angiotensin and KCl).

3. Are the responses studied caused by an agent present in plasma in situ, or is the agent formed during the process of obtaining blood and plasma? Serotonin is known to be released from platelets in shed blood14 and
kinins have been found in plasma exposed to glass surfaces.\textsuperscript{15} Extreme care was taken in these experiments to minimize the production of such artifacts in the plasma studied; all surfaces with which the blood or plasma came in contact were siliconized and the blood was cooled immediately to 0°C and centrifuged to remove platelets. The degree of activity of plasma was maximal when first tested and did not change during storage for 1 week at 0°C. Further evidence suggesting that the constrictor activity is present in plasma is found in the observation that vascular smooth-muscle strips contract when the PSS of the bath is replaced by fresh arterial blood.\textsuperscript{8}

4. Is the agent in plasma that causes contraction of isolated vascular smooth muscle responsible for non-neurogenic vascular tone, in situ? Evidence can be mustered that such a humoral factor may play an important role in the maintenance of non-neurogenic vascular tone in vivo. Folkow\textsuperscript{16} and Conway\textsuperscript{17} have emphasized that after denervation vascular smooth muscle in situ remains partially contracted and is responsible for appreciable residual vascular resistance. This is non-neurogenic vascular tone. The persistent contraction of denervated vascular smooth muscle in situ may result from either of two distinct mechanisms: (1) stimulation by humoral factors in the environment of the smooth muscle or (2) an intrinsic contractile tendency of the vascular smooth muscle cell. According to Bozler's original classification,\textsuperscript{18} and subsequent studies by Prosser et al.,\textsuperscript{19} vascular smooth muscle has properties of multi-unit smooth muscle, including the absence of intrinsic myogenic activity. This is in accord with Furchgott's observation\textsuperscript{20} and our own,\textsuperscript{21} that most isolated vascular smooth muscle in a PSS bath remains completely relaxed unless it is acted upon by a stimulating agent. The observation that vascular smooth muscle loses its state of partial contraction when removed from its in situ environment to the isolated bath of PSS and that partial contraction may be restored by the addition of plasma, constitutes indirect evidence that the plasma constrictor may be responsible for non-neurogenic vascular tone in situ. This same type of support can be mustered from the observations of Waugh and Shank\textsuperscript{22} and Stainsby\textsuperscript{23} that the autoregulatory response of the kidney and skeletal muscle perfused with whole blood deteriorates when the perfusion fluid is changed to an oxygenated physiologic salt solution. The presence of plasma in this artificial solution prevents this deterioration.

\section*{Acknowledgment}
We gratefully acknowledge the able technical assistance of Miss Judith Coy of this laboratory.

\section*{References}


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Circ Res. 1966;19:593-601
doi: 10.1161/01.RES.19.3.593

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1966 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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