Some Effects of a Cardioactive Fraction Isolated from Human Blood Plasma on the Peripheral Circulation of the Dog

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Methods

Types of preparations were used, viz.,
dogs on heart-lung bypass, and perfused hind limbs of dogs.

1. HEART-LUNG BYPASS PREPARATION

For the dogel dogs weighing 12 to 15 kg were placed on heart-lung bypass using a KayCass disc oxygenator. Anesthesia was induced with sodium thiopentone, (20 mg/kg iv), and maintained with nitrous oxide, oxygen mixture, as delivered from a respirator at a rate of 2 litres/min. Small supplementary doses of thiopentone were given from time to time to maintain a surgical level of anesthesia. Blood flow through a particular organ or vascular field was measured, when required, by clamping off the outflow from a wide-bore (6 cm) graduated cylinder placed in circuit between the venous outflow from the particular organ or vascular field and the oxygenator, as described below.

Dissections

Through a midline incision in the neck, a common carotid artery was dissected out and prepared for the insertion of the arterial cannula from the heart-lung machine. The thorax was entered through a sternal split, the pericardium opened, and tapes placed around the inferior and superior vena cavae. The vena azygos was ligated at its junction with the superior vena cava and a tape placed around the pulmonary artery. The abdominal portion of the inferior vena cava was then exposed through a midline incision in the abdominal wall and further dissected out so that ligatures and tapes could be
placed around it immediately below the liver and immediately below the entrance of the renal veins.

**Cannulation**

Immediately before cannulation, the animal was heparinized (2 mg/kg iv). For measurement of arterial blood pressure the right internal mammary, or occasionally, the right internal carotid artery, was cannulated and connected to either a P23 Dst Statham strain gauge or a mercury manometer. The output of the strain gauge amplifier was displayed on an ultraviolet light photographic recorder, type 5124 (Consolidated Electrodynamics Corporation). This point in the circulation is so close to the entry of the blood flow from the continuous flow pump that no systolic/diastolic fluctuations occurred and the recorded pressure is therefore taken as a static value.

The common carotid artery was transected and the tip of the cannula being placed in or near the aortic arch. The superior vena cava was cannulated through the proximal segment of the vena azygos. The thoracic segment of the vena cava was cannulated via the right atrioventricular appendage so that the catheter tip was located immediately above the diaphragm. The aorta was then placed on total body perfusion with the heart-lung machine having been primed with 11 litres of equal parts of 5% aqueous solution of dextrose and 0.9% aqueous solution of NaCl. Esophageal temperature was monitored with a thermistor and was maintained at 37°C by means of a heat exchanger placed in the arterial line. Oxygen, 100%, was supplied to the oxygenator; preliminary experiments showed that this did not produce hypoxemia for the blood PCO₂ was maintained at 35 to 40 mm Hg throughout the duration of these experiments.

When satisfactory perfusion was established, as indicated by the stabilization of blood volume in the pump, the abdominal segments of the inferior vena cava were cannulated. The ligature around the inferior vena cava immediately below the liver was tied and an incision made below it. A metal cannula (1 cm diameter) was placed so that its tip lay just above the entrance of the renal veins; this cannula was held in place with the previously positioned tape, which now was tightened. The inferior vena cava was then ligated immediately below the level of the renal veins and a third cannula placed in position. Thus the original inferior vena cava cannula now drained the liver only, the second cannula the renal veins only, and the third cannula the hind limb and pelvis. Another cannula was inserted into the distal end of the vena azygos, to drain the chest wall, the first right intercostal tributary being excluded by passing the cannula distal to its entrance. Finally, a cannula was inserted into the cavity of the right ventricle and the pulmonary artery tape tightened. The total coronary venous return to the right heart was drained through this cannula. A diagram of these cannulations is shown in figure 1. During cannulation the venous return from each segment was interrupted for less than one minute; occasionally satisfactory placement of cannulae was aided by reduction of the total perfusion rate for a short interval. Once satisfactory cannulation was established, perfusion was maintained at a constant flow rate of 100 to 120 ml/kg/min. Blood collected from the various cannulae was returned through the graduated cylinder system to a reservoir and thence to the oxygenator.

![Diagram of cannulations used for heart-lung bypass preparations. S.V.C. refers to superior vena cava.](http://circres.ahajournals.org/FIGURE1.png)
CARDIOACTIVE PLASMA SUBSTANCE

Measurement of Blood Flow

Blood flow through a particular organ or vascular field was measured by determining the time required for 100 ml of blood to collect in a graduated cylinder placed in circuit between the relevant venous return cannula and the heart-lung machine. During collection of the 100 ml blood sample the connection leading from the cylinder to the reservoir attached to the oxygenator was clamped; after 100 ml of blood had collected in the cylinder the clamp was released and the accumulated blood allowed to drain by gravity into the venous reservoir. If the blood flow from any organ or field was low then smaller volumes of blood were collected. The graduated cylinders which were placed just below the level of the animal, were fitted with inflow and outflow sidearms; the diameter of the cylinders was such that the height of the column of blood collected in them, during measurement of blood flow, did not exceed 5 cm. Preliminary experiments showed that pressures in the cannulated venous segments did not change during the collection procedure. Changes of flow due to the opposing pressure head developed during the collection of blood in the collecting cylinders were considered therefore as being negligible for the purposes of these experiments. Using this procedure, with the aid of observers, as many as six successive determinations could be made each minute.

On some occasions dogs which had been previously either nephrectomized or adrenalectomized were used. The technique used for nephrectomy was as follows: Through a midline abdominal incision the peritoneum of the posterior abdominal wall was incised lateral to the inferior vena cava over the right renal vein and artery. This incision was extended and the kidney mobilized, the renal artery and vein and ureter were identified, and ligated individually as close to the kidney as possible. The kidney was then removed. The left kidney was removed in a similar manner by incising the peritoneum lateral to the aorta.

Adrenalectomy was performed as follows: Through a midline abdominal incision the peritoneum over the right adrenal gland was incised. The superior adrenal vein was ligated and the mass of tissue containing the right adrenal gland carefully mobilized from the inferior vena cava, using multiple ligation technique. Mobilization was continued downwards towards the renal pelvis and finally a large ligature was placed around the connective tissue and vessels attached to the adrenal gland and tied. The adrenal gland was then removed. On the left side, where mobilization was less difficult, a similar technique was used. At the conclusion of the dissection all obvious adrenal gland tissue had been removed.

Drugs

Drugs were injected through the arterial connection of the pump oxygenator via a stopcock and needle. Arterial blood pressures were measured before and after the injection of solutions containing the cardioactive fraction.

B. "ISOLATED" HIND-LIMB PREPARATION

Healthy mongrel dogs were anesthetized, given artificial respiration and heparinized as above. The right or left common carotid artery was dissected out and the largest possible Bardic catheter inserted until its tip was estimated to lie in or near the aortic arch. This served as the source of inflow of blood to a Sigmamotor finger pump. A femoral artery was dissected out for a short distance below the inguinal ligament and all exposed branches were ligated. The largest possible metal cannula fitted with a sidearm, was inserted into this artery distal to a ligature and connected to the output side of the pump which was immediately started and adjusted to deliver a constant flow sufficient to maintain in the artery of the limb a perfusion pressure similar to the systemic arterial pressure. Once adjusted, the pump output was not changed. The pump had been tested for constancy of flow and maintained within ±5% over an input range of 20 to 200 mm Hg and an output range of 0 to 300 mm Hg. Using P23 Db Statham strain gauges the systemic blood pressure was measured from the proximal stump of the femoral artery, and the pressure in the test hind limb through the sidearm of the inflow cannula. The output of the strain gauge amplifiers was recorded on the ultraviolet photographic recorder.

A different preparation, in which vascular isolation was more complete than in the above preparation, was used on some occasions. In these experiments the arteries and veins of the pelvis were dissected out on both sides from the level of the inguinal ligament to the bifurcation of the abdominal aorta and all branches of the main vessels were ligated. The venous drainage from the test limb was returned to a Travens disposabale bag oxygenator primed with aqueous 5% dextrose solution (35 ml/kg) and with blood obtained from the experimental animal. The outflow from the oxygenator was returned to the femoral artery by the pump already described. Complete vascular isolation may not have been achieved because of the possible occurrence of a collateral circulation via the abdominal wall. During the experiment any blood losses were made up from the systemic circulation.

Neurological isolation was obtained in these
experiments by complete section of the spinal cord at the level of T10 using a thoracic approach through the intervertebral disc space, section of the sympathetic trunks at the same level, and section or crushing of the trunk at the level of L2.

C. PREPARATION OF CARDIOACTIVE PLASMA FRACTION

The cardioactive fraction used in these experiments was isolated from pooled human blood plasma as described previously. The inotropic activity of each freshly prepared solution of the plasma fraction was equated with that of noradrenaline (Levophed, Winthrop Laboratories) using the changed amplitude of isotonic contractions of toad (Bufo marinus) ventricles as described elsewhere.

Results

Aliquots of the stock solution containing the cardioactive plasma fraction were diluted with 0.9% NaCl solution until 0.1 ml of the diluted solution contained an estimated 0.4 to 0.8 μg of cardioactive substance. Immediately before the start of each experiment the positive inotropic action of this solution on an isolated toad ventricle preparation, was confirmed.

A. EFFECTS OF CARDIOACTIVE PLASMA FRACTION ON THE PERIPHERAL CIRCULATION OF DOGS ON HEART-LUNG BYPASS

Preliminary experiments established that flow through particular vascular fields of dogs on total heart-lung bypass, as described above, remained approximately constant; throughout a two-hour period variations in blood flow did not exceed ±5% and no systematic variations were detected. The

![Diagram](http://circres.ahajournals.org/)

**Arterial pressure (mm Hg) measured in the right internal mammary artery, and blood flow (ml/min) through the splanchic, femoral (hind limb), renal and coronary vasculature before and after the injection, at arrow, of 0.1 ml of cardioactive plasma fraction into the arterial line of a dog on heart-lung bypass. Estimations were made at the points indicated.**

Circulation Research, Vol. XVIII, January 1966
reactivity of vessels in the regional fields of flow, as indicated by their response to sympathetic stimulation and to known vasoactive drugs, including isoproterenol, was fully preserved throughout the period of perfusion.

The injection of 0.1 ml of diluted cardioactive plasma fraction into the arterial line of dogs on heart-lung bypass increased immediately the systemic arterial pressure. The time required for onset of the pressor response was the same as that required for the passage of the injected solution from the site of injection to the animal, calculated by reference to the rate of blood flow and volume of the inflow tubing. Figure 2 shows the pressure recorded from the right internal mammary artery of a typical preparation before and after the intra-arterial injection of 0.1 ml of the cardioactive fraction. Table 1 lists the pressures recorded from the internal mammary or the internal carotid artery before, and the maximum pressure recorded after, the injection of 0.1 ml of the cardioactive fraction into the arterial line of five other dogs on heart-lung bypass. In all preparations the pressor response was characterized by a rapidly appearing brief peak followed by a slow decline to the control level. The peak response was reached in approximately two minutes and had declined to 50% by approximately eight minutes. No decline in the level of activity was detected during repeated injections of the fraction into any particular dog.

The data summarized in table 2 show that adrenalectomy, nephrectomy and prior treatment with atropine sulfate (Hermette, David Bull and Company, 0.1 to 0.2 mg/kg) or pentolinium tartrate (Ansolysen, May and Baker, 2 mg/kg) did not abolish the pressor response of dogs on heart-lung bypass to the isolated cardioactive plasma fraction.

The pressor response described above was accompanied by changes in the distribution of blood throughout the peripheral circulation. Thus blood flow through the coronary and splanchnic circulations increased whilst that through the renal and hind limb (femoral) circulations decreased. Typical changes in blood flow, caused by the injection of 0.1 ml of the cardioactive fraction, are displayed in figure 2. Blood flow through the vena azygos, draining the chest wall, and the superior vena cava remained relatively constant, as is shown in table 3. Similar changes were observed in dogs that had been pretreated with pentolinium tartrate.

B. EFFECTS OF CARDIOACTIVE PLASMA FRACTION ON "ISOLATED" HIND-LIMB PREPARATIONS

Under the conditions of constant blood flow present in the hind-limb preparations described above, any variation of vascular

<table>
<thead>
<tr>
<th>Prior treatment of dog on bypass</th>
<th>Arterial Pressure*</th>
<th>Before C.A.S.</th>
<th>After C.A.S.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy, 3 hr (3 expt.)</td>
<td>75 ± 6</td>
<td>140 ± 15</td>
<td></td>
</tr>
<tr>
<td>Bilateral nephrectomy 2 days previously (1 expt.)</td>
<td>90</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Bilateral nephrectomy 1 day previously (3 expt.)</td>
<td>85 ± 8</td>
<td>145 ± 10</td>
<td></td>
</tr>
<tr>
<td>Atropine sulfate, 0.1 to 0.2 mg/kg (4 expt.)</td>
<td>85 ± 5</td>
<td>145 ± 15</td>
<td></td>
</tr>
<tr>
<td>Pentolinium tartrate 2 mg/kg (5 expt.)</td>
<td>75 ± 10</td>
<td>140 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

*Internal mammary or internal carotid artery pressure. Mean results of the multiple experiments are given.
†Pressure measured after C.A.S. refers to maximal pressure.

Circulation Research, Vol. XVIII, January 1966

TABLE 1

Effect of 0.1 ml Cardioactive Plasma Fraction (C.A.S.) on Arterial Pressure (internal mammary or internal carotid) in Dogs on Heart-Lung Bypass

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Before C.A.S.</th>
<th>After C.A.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
</tr>
<tr>
<td>1</td>
<td>110</td>
<td>165</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>145</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>140</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>160</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>135</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>163</td>
</tr>
</tbody>
</table>
resistance in the hind limb was indicated immediately by a changed perfusion pressure. Within 2.5 to 3 seconds after 0.1 ml of the cardioactive plasma fraction was injected into the arterial supply of the more completely isolated hind-limb preparation, the perfusion pressure increased, whether or not the lumbar sympathetic supply to the limb had been crushed. Such a response is shown in figure 3. Table 4 summarizes the raised hind-limb perfusion pressures recorded after injection of the cardioactive fraction into eight other hind-limb preparations. In those preparations in which the degree of vascular isolation was less complete, the raised hind-limb perfusion pressure was followed, after a period of 30 to 35 seconds, by a raised arterial pressure in the whole body. The rapidity of the response in the hind limb excludes the possibility that the response itself was due to any interaction of the cardioactive frac-

**TABLE 3**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Maximal change (as per cent of control) in blood flow*</th>
<th>Arterial press.†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coronary</td>
<td>SVC</td>
</tr>
<tr>
<td>1</td>
<td>+175</td>
<td>-9</td>
</tr>
<tr>
<td>3a</td>
<td>+181</td>
<td>-2</td>
</tr>
<tr>
<td>4</td>
<td>+202</td>
<td>+5</td>
</tr>
<tr>
<td>5</td>
<td>+182</td>
<td>-5</td>
</tr>
<tr>
<td>6</td>
<td>+210</td>
<td>+3</td>
</tr>
</tbody>
</table>

*Per cent change in blood flow calculated as \( \frac{\text{test} - \text{control}}{\text{control}} \times 100 \). + denotes increase; - denotes decrease.

†Arterial pressure refers to systolic pressure measured in the internal mammary artery.

‡S.V.C. refers to superior vena cava.

**FIGURE 3**

Hind limb perfusion pressure, in mm Hg, of hind limb preparation before and after the injection, at arrow, of 0.1 ml of cardioactive plasma fraction. Paper speed, 2.5 mm/sec.
TABLE 4

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Maximum change in perfusion pressure (as per cent of control) *</th>
<th>Time of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+220</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>+260</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>+180</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>+220</td>
<td>2.8</td>
</tr>
<tr>
<td>5</td>
<td>+200</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>+240</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>+80</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>+140</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*Change in perfusion pressure calculated as test — control × 100 / control + denotes increase.

†Time of onset refers to time required after injection for onset of pressor response.

Discussion

The present experiments show that the relatively low molecular weight cardioactive fraction of human blood plasma has a marked effect on the peripheral vasculature of dogs. Thus the injection of 0.1 ml of plasma fraction containing an estimated 0.4 to 0.8 μg of the cardioactive substance, into the arterial inflow line of dogs on heart-lung bypass raised systemic arterial pressures repeatedly. The similar injection of this plasma fraction into the arterial supply of perfused hind-limb preparations likewise increased perfusion pressure immediately and markedly. Previously we have shown that this fraction of plasma produces a positive inotropic response from isolated mammalian and amphibian cardiac muscle and that it increases the cardiac output and raises systemic arterial pressures in intact anesthetized rabbits. That vasoactive drugs can produce their effects by changing either the distribution of blood in the peripheral circulation or cardiac output is already well recognized.

Cardioactive plasma fraction in doses of 0.1 ml was found to cause consistently a marked increase in coronary blood flow in these dogs on total heart-lung bypass. In experiments on intact animals such an increase in coronary flow could be a secondary effect, consequent upon increased work of the heart and augmented cardiac output. However, under the conditions of total body bypass, where the heart is pumping only the Thebesian and bronchial drainage (approximately 5 ml/min) it is reasonable to postulate that the augmented coronary flow described above results from a direct action of the plasma fraction on coronary vasculature.

Angiotensin, a known pressor agent, causes an immediate reduction of coronary flow, associated with increased coronary resistance, in dog heart-lung preparations. Vasopres- sin, another pressor agent, causes a decrease of coronary flow associated with coronary vasoconstriction in experiments on isolated heart-lung preparations and also on intact animals. Bradykinin and 6,7 Bradykinin and 6,7 Vasopres- sin, another pressor agent, causes a decrease of coronary flow associated with coronary vasoconstriction in experiments on isolated heart-lung preparations and also on intact animals.6,10 Bradykinin and ededoisin resemble the above isolated plasma fraction in that they cause an increase of coronary flow associated with a direct effect on the coronary vasculature; but differ from the plasma fraction in that they lower the systemic arterial pressure.

The plasma fraction caused marked changes in hind-limb blood flow. Under the present experimental conditions the flow in this field is largely that of the muscles but skin flow, and flow from the pelvic organs, is also included; as is shown in figure 2, flow in this area can involve the distribution of as much as 1100 ml blood per minute. Other workers have shown that the muscle beds are probably the most reactive as far as the sympathetic nervous system is concerned and on the presently available evidence we do not exclude the release of locally stored catecholamines as a mechanism of action for this cardioactive plasma fraction.

The mechanism underlying the peripheral effect of this particular plasma fraction has been indicated partially by these experiments, since it was found that the presence of either the adrenals, kidneys or intact sympathetic...
nervous system was not required for the initiation or maintenance of the response. That angiotensin is not involved in the response is supported by the maintenance of pressor activity in chronically nephrectomized dogs. Vasopressin and other substances of neurohypophyseal origin can be excluded from consideration as part of the mechanism because of the presence of the pressor effect in the hind-limb preparation.

It should be emphasized that the above results have been obtained from preparations far removed from a normal physiological state and caution should be exercised in extrapolating from dogs on total body perfusion and from hind-limb preparations to the normal animal. The physiological significance of this pressor component of plasma, and its relationship, if any, to the cardioglobulin system described by Hajdu and Leonard remains obscure.

Summary

Using dogs on heart-lung bypass and perfused hind-limb preparations, the effect of the relatively low molecular weight cardioactive plasma fraction on the peripheral circulation of dogs was investigated. This cardioactive plasma fraction raised the arterial blood pressure of dogs on bypass and increased the perfusion pressure in hind-limb preparations. The raised arterial pressure was found to be associated with increased blood flow through the coronary and splanchnic beds; flow through the hind limb decreased. Adrenalectomy and nephrectomy did not abolish these peripheral effects of this plasma fraction. It is concluded that the raised systemic arterial pressures recorded from intact animals following the injection of this plasma fraction may result, in part, from the effect on the peripheral circulation.

Acknowledgment

The isolation of the cardioactive substance from plasma was done by Dr. C. C. Curtain. We gratefully acknowledge the technical assistance of Messrs. J. Bremner and D. Breen.

References

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